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Investigations of the Air-Water Interface: A Structural Analysis of Metallic Surface
Films and Aquatic Surface Films by Comparative Microscopy

by

Randall William Smith

A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Environmental Sciences and Resources

Dissertation Committee:
Erik J. Sánchez, Chair
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Portland State University
2015

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ABSTRACT

The air-water interface is an important natural boundary layer that has been neglected as an area of environmental field research. This study establishes that comparative microscopy can be an effective environmental method, and establishes that the term metallic surface films, is a more accurate descriptor than iron oxide surface films. This research shows that surface films are complex, often with layered structure, serve as habitat for significant biota, and act as a point of mineralization to several transition metal elements including manganese, iron, copper, nickel and zinc. This study demonstrates that surface films form under several conditions and can have diverse morphology. Activity of biota, microbes, particularly diatoms, suggests that bacteria and cyanobacteria integrate into the film often in patches, represented by forms and casts. Analytical imaging is used to document and compare film morphology and structures, using scanning electron microscopy, photoemission electron microscopy and transmission electron microscopy with elemental analysis by energy dispersive spectroscopy to confirm the hypothesis. Instrument parameters and strengths are reviewed. Component layers of a copper/zinc film were used to confirm metallic layers and elemental distribution. Bacterial casts were used to confirm film interaction, and to show entrainment and enrichment of the film to incorporate autochthonous and allochthonous materials into the films themselves. Most samples were from Oregon selected sites, with some samples from Maryland and Barbados.

DEDICATION

*The best way to have good ideas is to have lots of ideas and then develop a method for
sorting the good ones from the others.*

~ Linus Pauling

This Dissertation Is Dedicated To These Family Members and Colleagues
All of Whom Had Their Own Significant Role In My Research Life:

Jon Keir Smith	(1966 - 1994)
Joel Walker Hedgpeth	(1911 - 2006)
Harvey Jackins	(1910 - 1995)
David T. Clark	(1925 - 2004)
Gertrude F. Rempfer	(1912 - 2010)

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CHAPTER ONE

SURFACE FILM RESEARCH

1.1 The Air-Water Interface and Surface Film Research

The air-water interface is a boundary layer. Before we look at any biological or chemical feature of surface waters, notice that at a boundary layer between a gas and a liquid has special properties. But, the atmosphere is not a simple gas. The atmosphere is a complex of compounds as gasses, and an atmosphere with inclusions, such as dusts, pollens, and complex molecules all suspended in the gas phase. Even the components in suspension have properties of their own. Consider also that the liquid has similar complexity, it has water, a polar molecule with special properties, one of which is the tendency to have surface tension, a polar (unequal charge) orientation, and a complex of other compounds, some complex, as we find for phenolic humic substances, or complex organics and dissolved salts, such as $\text{Na}^+ \text{Cl}^-$, metallic salts, such as Fe_2O_3 , and Fe_3O_4 etc., in solution, ionic or covalently bound, and impacted by environmental modifiers, such as carbonate-bicarbonate equilibrium, which will change pH of the liquid phase, such as estimated by the equation;

Thus, the air-water interface is not a simple environment, but a complex boundary layer condition, where compounds and organisms are able to find, attach, order, arrange or use the properties of this boundary layer, and when compounds, both

organic and inorganic, are attracted, entrained, entrapped at the air-water interface they may form a surface film, an accumulation and orientation of elements, molecules, body parts, eggs, gametes, organisms, bacteria, etc. The air-water interface is, in itself, because of surface tension, a field of weak and strong forces which do not forbid orientation, order, chirality, polarization and accumulation, which in many cases allows an organic surface film or sometimes a metallic surface film, to form. The organisms at the air-water interface are usually termed *The Neuston*, as defined and reported by Einar Naumann (1917) for the surface plankton. This concept of the neuston has sometimes been modified to include not only organisms at the interface, but also organisms in the upper 10 cm, such as for fish eggs and larvae, which partition themselves in the surface waters (Zaitsev, 1971). The concept developed by Zaitsev focused more on surface water organisms rather than surface films, but developed the term Neustonology for the study of biota in the upper 10 cm of ocean waters. But, we also have the concept of the bacterioneuston as part of the sea surface microlayer (Sieburth, 1971, 1976; Norkrans and Sörensson 1977; Norkrans, 1980; Franklin et al., 2005; Cunliffe et al., 2013).

What of the surface films themselves? For convenience I divide this into two components, the Aquatic Surface Films or ASF, also known as the Sea Surface Microlayer or (SML), and the silvery, reflective Metallic Surface Films (MSF), sometimes known as floating iron surface films, iron oxide surface films or Fe-bound surface films, as many of these films are dominated by iron oxides or mixed-valent iron oxides. I show also that these films can also include ferromanganese surface films, MnFe, or NiZn components or CuZn contributions. In summary, the term metallic

surface film or MSF can best describe the minerals, structure and nanocrystallites that can be found in these surface films.

There are two main distinctions between the organic surface slicks and films, which includes any number of polar and non-polar organic compounds, and the metallic surface films (MSF), which includes silvery patches of iron oxides and other metals and their biota. The organic films, which may include thin layers of oil spill chemicals, a thin sheen of interference colors or silvery sheen to natural organic substances, surface active substances (SAS) or natural organic material (NOM) are capable of having wave-dampening effects upon the air-water interface, an effect that is well-quantified by sea-surface films (SML) studies. Organic films and their component organic chains of molecules are able to generate a measurable film pressure against other molecules (Baier, 1970, 1972; Baier et al., 1974; Baier, 1984). This film pressure can be measured or estimated in the field by dodecyl alcohol dilutions (Adam, 1937). This is still a useful technique with a long tradition of study (Adamson, 1982). Natural surfactants are included in this collection of organic molecules, and while surfactants may dissolve in the bulk water below the interface, they are often partitioned into the air-water interface and for surface slicks and sheens noticeable because they do have wave-dampening effects on natural waters, from oceans to rivers (Garrett, 1965; Garrett 1967a; Garrett, 1967b). Wave-dampening effects are often the most noticeable field observation of organic surface films. There is rich history to surface films and slicks, which may be related to the metallic surface film formation, but is only a part of our definition for this study (Wangersky, 1976; Liss, 1974; Lion and Leckie, 1981, 1982;

Cunliffe, M., A. Engel, S. Frka et al., 2013). In general, previous studies in metallic surface films have not included organic surface film literature or made this comparison.

Unlike sea surface slicks and films, the MSF are delicate in most cases, and are often found in small patches in wetland environments, or in larger patches where reduced iron-rich waters enter surface waters (Grathoff et al., 2007). Since Oregon coastal systems are rich in iron and some related transition elements, mixed-valent iron oxides tend to dominate, but are not exclusive components of metallic surface films. Unique situations and environments can produce both patches of unique biofilms, such as CuZn or NiZn, but interesting patches of MSF found within an iron oxide surface film are reported here.

Compared to the organic surface films, the metallic surface films are points of interfacial concentration, enhancement and mineralization, even though they may have a large organic component. The structures are complex, but they do not develop film pressure and are not fluid molecules, or a fluid mosaic film at the surface, as organic molecules are often described. Even our cell membranes are described as a fluid mosaic membrane because of the molecular movements that cell membranes can do as a non-crystalline state (Singer and Nicolson, 1972). Cell walls, on the other hand, are polymeric constructions that offer rigid protection to the bacterial or eukaryotic cell, and are not usually described as a liquid or fluid mosaic phase. Cell walls, bacterial, cyanobacterial, algal are complex structures with their own history of evolution (Popper et al., 2011).

A surface film is, by definition, at the air-water interface. It may be simple or complex, visible or transparent, organic or metallic, forming in the light or the dark, sometimes lifted and transported by tides for coastal areas or a feature of flowing waters in rivers and streams. McIntyre (1974b) described the upper millimeter of the ocean as the largest surface area on the planet, something that should stay in our viewpoint for any global process we are investigating.

In some sense this is comparable to the fluid mosaic model of cellular membranes that was presented in 1972 for cell membrane theory, and is a good comparison although not identical. A fluid mosaic state is represented as a gross structural organization for molecular components. This model is still consistent with aquatic and hydrodynamic equilibrium states, and restrictions imposed by thermodynamics or ionic equilibration. But, in natural surfactants, surface active substances (SAS) and SAS ionic structures, we do have amphipathic molecules, groups with ionic, chiral and polar structures, some of which are hydrophilic and some hydrophobic and will either become attached to the hydrophilic layer or reorient away in the fashion of any hydrophobic molecule or that portion of the molecule. There may also be molecules that become globular with a hydrophobic interior exposing the more hydrophilic ends of the molecule to water. While these interactions are not studied here, it is a consideration for evaluating what kinds of surface films form and what their alterations are over time and condition.

The metallic surface films represent a mineralization, perhaps an oxidation at the air-water interface (Grathoff, et al, 2007). An examination by comparative

microscopy demonstrates small spherules that are consistent with other mineralization, where hydrated, amorphous phases of CaCO_3 have also been examined in situ (Nielsen et al., 2014a; Nielsen et al., 2014b).

1.2 A Brief History And Introduction

This dissertation presents the following concepts: Why are surface films important, and what is the history of both concept and research for these features of the air-water interface? And, for those reflective, metallic surface films: How are they similar or dissimilar from other surface films in structure and components? How can structure as revealed by comparative microscopy revise our knowledge of surface films? It would seem like a simple question, and one could certainly answer it by saying “*Because it is there*” (Mallory, 1923), one interested in seeking answers. Yet, not only do surface films have an interesting history, but also these films encompass a diverse set of research programs that have gone on their own way without much reference to each other. This work reports a focus on one piece of this research, the metallic surface films, but in doing so must show the convergence of methods and ideas that bring us to this point, a comparison and link to other surface film studies.

Surface films and characteristics of the air-water interface are significantly important because they have processes, properties and functions that are poorly understood. Surface film studies are often absent from environmental surveys, environmental impact studies or environmental assessments, thus depriving both biologists and regulators of the biological, ecological and geological information that is found at the air-water interface. Both aquatic surface films and metallic surface films

have structures that are unknown or poorly known. For a concept related to our general understanding of air-water interaction we must consider gas transfer, nutrient biogeochemistry and even our understanding or concept of what constitutes '*habitability*', the possibility of the origin of life on any planet, not just our own. It is a curious thing to see an unanswered question in physical form right in front of you. It is a far-reaching thing to suggest a conceptual change in environmental work. But, more importantly, this odd bit of science has the prospect of answering many other questions about the air-water interface and surface film formation, transport, elemental enrichment or sequestration and loss. What a curious habitat this might be? It certainly relates to many other features of the air-water interface, but what can it tell us about the functional properties of surface tension, of light, of structure, or of function?

1.3 The surface of natural waters

There are several lines of research on the air-water interface that have focused on the several kinds of studied surface films. One is the sea-surface microlayer or SML on oceanic slicks and films and it's companion research, the aquatic surface films or ASF. They are often the somewhat thicker coastal, estuarine and freshwater organic films, slicks and scums associated moreso with wetlands, seeps, springs and ponds. Another grouping are the metallic surface films, sometimes called the floating iron films, the mixed-valent iron surface films or the iron-bearing surface films, formed also in relation to hydrology, mineral composition, wetlands and other factors.

Using environmental microscopy, we find that a description of iron-bearing surface films limits the description of metallic mineralization at the air-water interface,

and the interaction of metallic elements with biota and humic substances, and that a broader area of research on metallic surface films or MSF, is a better description of this area of work. Here, we show the structure of other metallic elements in the development and description of these films from several environments.

The surface of natural waters is often viewed as a reflection of the weather and the sky and not as a structure of the body of water under study. It is simultaneously our reflective mirror of the sunrise and the sunset and the many colorful observations given to humankind and our mind's reflection upon life near the water. There is great biology, chemistry, geology and physics to be found here. It is all too common for biologists, or even the chemists and the hydrologists to 'splash away the scum of the surface so that you can get a good, clean sample of the water', when in fact you have splashed away a good portion of the critters, algae, minerals and 'good stuff' that your sample might represent. This includes humic substances, adsorbed cations and the enrichment of metallic substances that are common to the air-water interface. Certainly you might exclude the cyanobacterial filaments that tend to float at the surface, their protozoa cruising in and out of their structures, or even the transparent extracellular particulates that also tend to be buoyant (Alldredge, Passow and Logan, 1993). Many of these substances are contributions from the pollens and dusts deposited from the atmosphere, as well as the bacterioneuston contacting the surface from below. Indeed, it is often the 'neuston', that poorly known set of organisms that inhabit the air-water interface, that struggle and contend for space at the surface. This viewpoint is presented here.

We have the metallic surface films, those silvery, reflective bits, are often mistaken for a bit of oil contaminant, also floating in or near wetland environment. Sometimes these have the silvery appearance of any thin, structural film capable of internal bouncing of light at the partial wavelength scale to even give us some iridescent colors as internal reflection can give, the well-know interference colors, not reviewed here. In some sense, the algal and bacterial attachment to each other or the bacterial attachment to this bit of metallic surface qualifies our natural environment as an aquatic biofilm, and these biofilms have diversity of structure as well as a diversity of colonizers.

It has often been said that surface films, scums and foams cannot be a good or useful habitat as they are heavily irradiated by sunlight, even by UV light which will be quickly attenuated by the water making it safe for the other plankton. But, metallic surface films clearly demonstrate that they are a reflective shelter for some micro-organisms and are actively colonized by bacterial, algae, cyanobacteria often to be grazed by protozoa and rotifers, who may contribute to their more mature structure. As with most aquatic systems, surface films are subject to disruption by wind, wave, rain, tide, natural flow, splash and currents, but then they become part of the coarse or fine particulate matter of the system itself, and become part of the several biogeochemical materials and cycles of nutrients.

Certainly, the surface film, or particularly the metallic surface films, serve as a place of metal enrichment and a nutrient source for those organisms that can feed or utilize the nutrients by enzymatic methods. The diatoms, requiring iron for

photosynthesis will certainly take advantage and colonize the films. But where magnesium, calcium, manganese, copper, nickel and zinc are around to be bound or complexed in a multitude of processes, organisms are suited to acquire the enrichment of metallic and organic compounds that occurs in both aquatic and metallic surface films. In most natural waters and their biota, biota of all kinds, can and do integrate multiple external and internal signals to successfully colonize, synthesize and develop a morphological structure that is adapted for survival at the air-water interface, as can they utilize the many pollens, dusts, insect parts and autochthonous material that becomes entrained and enriched at the surface. That is what the surface does. Not only is the air-water interface a habitat of its own, but also gives us some clues to habitability where water is part of the environmental structure. Most importantly, that structure is basically unknown. But '*Habitability*' is a current question in biology, astrobiology and exobiology, important in developing new hypotheses on the possibilities that define the origins of life and of living systems, no matter what planet we are studying. Thus this research expands the concept of floating iron films to include structure and biota, to include other transition metal elements in its definition. The term 'Metallic Surface Films' or MSF, is a more accurate descriptor of these surface film formations at the air-water interface.

In a recent review of aquatic surface films, or alternatively, aquatic surface microlayers, Cunliffe et al., 2013; Cunliffe et al., 2008) described these as "unique microbial ecosystems found at the air-water interface of all open water bodies." The same can be said for metallic surface films. Indeed, the focus of this hypothesis is to

expand our knowledge base about the presence of several kinds of metallic surface films other than iron, and thus to expand the concept of mineralization at the air-water interface and the many roles of humic materials, particulates and biota.

This research is about the air-water interface and the structure of the surface films found there, particularly the silvery metallic surface films. This research is also about analytical microscopy, or in the context of natural waters, environmental microscopy. It is about a few particular features of the air-water interface to help us obtain new information about structures, properties and processes at the air-water interface to overcome some gaps in knowledge using analytical imaging and comparative microscopy. This is a structural and instrumental approach to environmental science as it pertains to current environmental and scientific problems on earth, and by extension gives us information about life, habitat and even habitability that might occur on other planets. Thus, we have both a narrow scope on surface films at the surface as well as a broad scope to processes that might apply to the origin of life and life on other planets.

1.4 Historical context

As with most research, we owe a significant debt to researchers of the recent past who have guided scientific research and discovery. For the air-water interface this research has been much divided between disciplines, and there have been too few efforts to bring these disparate pieces into one discussion. I will fill some of these gaps and bring the several areas of work into a more precise focus in order to understand processes and structures at the air-water interface.

Surface film observation and research has a long history, particularly the wave-dampening effects of organic chemicals at the air-water interface. Pliny the Elder is attributed to have noted the wave dampening effects of olive oil on the sea, sometimes for the purpose of making the bottom more visible for sponge divers. In 1774, read at the Royal Society, London, Franklin reported:

“...But recollecting what I had formerly read in Pliny, I resolved to make some experiment of the effect of oil on water, when I should have opportunity... ..At length being at Clapham where there is, on the common, a large pond, which I observed to be one day very rough with the wind, I fetched out a cruet of oil, and dropt a little of it on the water. I saw it spread itself with surprising swiftness upon the surface; but the effect of smoothing the waves was not produced; for I had applied it first on the leeward side of the pond, where the waves were largest, and the wind drove my oil back upon the shore. I then went to the windward side, where they began to form; and there the oil, though not more than a teaspoonful, produced an instant calm over a space several yards square, which spread amazingly, and extending itself gradually till it reached the leeside, making all that quarter of the pond, perhaps half an acre, as smooth as a looking-glass.”

In 1791, noting the wave dampening effects of grease thrown off sailing vessels from the cook's pots, Benjamin Franklin recalled his experiments of a 'teaspoon of oil' onto the surface of Clapham Common Pond and noted that the wave-dampening effects extended for acres of pond water. In 1891, John William Strutt, the Lord Rayleigh, published the letter of Agnes Pockels, who demonstrated surface molecules in a tin trough, (Pockels, 1891). This was very similar to the later apparatus of Irving Langmuir and Katherine Blodgett.

No surface film research would be complete without acknowledging the significant work of Katherine Blodgett (1934) and the research of N. K. Adam (1937),

soon followed by the continuing works of Katherine Blodgett and Irving Langmuir. Indeed Langmuir-Blodgett Films form an initial focus on the discrete sampling of surface films with examination by scanning electron microscopy. These were important studies on chemical films and behaviors, giving us a much better knowledge of the molecular orientation and movement as well as the weak molecular forces that molecules at the air-water interface can be measured in meaningful ways. The voice of Arthur W. Adamson (1982) brought many of these early works into current focus.

Similarly, no film study would be complete without acknowledging the curiosity of one Benjamin Franklin, who, as noted above, in the interest of the fledgling U. S. Navy, tested oil films on Clapham Pond, where from a teaspoon of oil, wave-dampening effects of molecules on surface tension was noted. While his interest was in the wave-dampening effects of oils and not biological or chemical formation of a wave-dampening surface film, it did suggest to later oceanographers that chemical analysis and study of surface films was an area of research in need of study. In this way, we acknowledge the pioneering works of Blanchard (1963), Harvey and Burzell (1972) and Garrett (1965; 1967a; 1967b; 1974) in developing basic concepts of ocean studies regarding slicks, films, bubbles and processes that are now basic to any surface study of natural waters.

For biologists, chemists and oceanographers the founding work of Duce (1972) and Hardy (1973, 1982) linked surface film chemistry to the phytoneuston as well as to the structural development of surface microlayer films. Indeed, the term 'Microlayer' linked the structures found by chemistry to the increasing load of surface enrichment or

biomagnification at the air-water interface of anthropogenic substances and pesticides as well as the concentration or enrichment of heavy metals including copper, iron, cadmium, mercury and lead cations. With these foundations, increased understanding was provided by the coastal studies of Duce et al., (1972), Hunter and Liss (1977) and many others stimulated in surface film research at this time.

For microbiology, we owe a significant role to Sieburth (1971, 1976) and to Norkrans and Sörensson (1977) and Norkrans (1980) in bring our attention to the surface microbes, the neuston as defined first by Einar Naumann (1917), but in the modern sense, the bacterioneuston of surface films, both aquatic surface films (ASF) and now to metallic surface films (MSF) as well. It was the American bacteriologist, A. Henrici, who presented further work and interest on the bacteria of natural waters and not those of pathology (Henrici, 1933), which was the emphasis for microbiology at the time. Ocean microbes were hardly recognized until the efforts of Claude Zobel (1943) came to be published. Now, ocean microbes and bacterioneuston are leading ocean research as a more comprehensive physicochemical air-water interface as an integrated whole of key components (Cunliffe et al., 2008; Cunliffe et al., 2013).

1.5 Particulate materials and surface films

There has been considerable work on the place of particulate materials in oceans and aquatic systems. Gordon (1970a; 1970b) reported a microscopic study of organic particles in the North Atlantic. Alldredge and Silver (1988) reported on the characteristics, dynamics of marine snow, a fine particulates study. Alldredge (1991) reported the analysis of marine snow and fecal pellets in marine particles, and

Allredge, Passow and Loban (1993) reported an Alcian Blue stain for non-living particulate organic matter (POM), and what is now known as transparent exopolymer particles (TEP). A broader concept of fine particulates developed with the TEP studies, and improved the broad concept of marine particulates in general. Particulates are often difficult to classify, but in this study particulates revealed by photoemission electron microscopy are new to the PEM image of metallic surface films and their biota.

Inorganic particulates, such as dusts and anthropogenic contaminants have been the focus of several studies. This study included Saharan dust as an iron nutrient study on the colonial cyanobacterium *Trichodesmium*, a sea-surface organism (Rueter, et al., 1992). Identification of dusts and iron particulates were included in early effort to define SEM analysis in relation to surface film structure (Smith & Dash, 1989). This will be reported further in a later chapter.

The initial concepts of sea surface film or microlayers (SML) has also undergone some conceptual changes. Wurl and Holms (2008) and Wurl, Miller and Vagle (2011) hypothesized and demonstrated the importance of surface active substances (SAS), and the buoyant effects of transparent polymeric particles (TEP), and the resulting measure of total dissolved carbohydrates (TDC). Azetsu-Scott and Passow (2004) pointed out that high TEP content in diatom aggregates could lead to an upward flux of material through positive buoyancy, and thus could act as an important vehicle in transporting particle-reactive compounds and metallic elements toward the air-water interface.

This is consistent with some freshwater studies that also showed for the aquatic surface films (ASF) that humic substances binding cations could facilitate elemental enrichment and the air-water interface, and possibly allow for biogeochemical binding of elements, making them unavailable in some circumstances as sequestered elements, and bioavailable to neustonic organisms as foods allowing for enrichment of metallic elements at the air-water interface (Tipping, 2002).

Another area of applied environmental work that is directly related to surface film research is that of oil and chemical spills. There is a large body of work on anthropogenic slicks, sheens and spills, for both oil and chemicals involved in contaminant response, damage assessment and environmental restoration. In general, this is not reviewed that here, but the application of methods to response and assessment as well as to environmental monitoring and planning, is consistent with this work on aquatic and metallic surface films, morphology and structure, and the role of humic substances in both sequestering metallic elements in some circumstances, and enhancing or enriching the level of metallic elements in the surface films in others (Hardy et al., 1988). Additional oil spill planning was reported by Sutherland, Jones and Smith, (1983), and guidelines by Smith and Pavia (1983).

1.6 Metallic surface films

While sea surface films or microlayers enjoyed the funding encouragement offered for oceanography and marine biology in the 1970s-1980s, the metallic surface films were largely unrecognized, often as incidental work to other research. Since iron oxides were the main constituent, these reflective films were often named as floating

iron films (Grathoff et al., 2007), iron oxide films, mixed-valent surface films, schwimweisen [floating iron] (Grathoff, et. al., 2007), something that has continued in more recent work of Karlsson & Persson (2010), Karlsson and Persson (2012), and Kleja et al., (2012).

The previous studies of metallic surface films have focused on the role of iron, as it is usually the most common transition metal for most films. Grathoff et al (2007) and Gray (2008) reviewed the few studies that explored the chemistry of Fe-bearing films, Cornell and Schwertmann (2003), Konhauser and Ferris (1994; 1996), Konhauser (1996) Schwertmann, Friedl and Stanjek (1999), and biofilms (Tazaki et al., 2002). The unpublished thesis by Gray (2008) also focused on the iron species both in natural films and artificial films cultured in the laboratory.

Instrumental analysis done here fills gaps in knowledge where scanning electron micrographs tended to jump from low magnification images, to transmission electron micrographs that were limited in finding organisms, bacterial and algal casts or the capabilities of the transmission electron microscope to address the question of amorphous structure and ordered mineral states at the atomic level. In addition, photoemission electron microscopy was used to image the several features of not only bacterial activity, but also the presence of layers and particulates, unspecified organic materials, that are quite able to emit electrons for imaging. Even if the compounds cannot be identified, the photoemission of the materials documents presence and interaction with the films and organisms themselves.

There is actually little work done on the mineralization of metallic surface films themselves. For this I take the recent work of Nielsen et al. (2014a; 2014b) on *in situ* TEM imaging of CaCO_3 nucleation, suggesting the coexistence of direct and indirect mineralization pathways. This research shows a similar possible division of process for metallic surface films. Building upon this research, much of it done at PNNL, Richland, Washington, perhaps we can say that in natural waters and under temperate aquatic and estuarine or marine conditions, mechanisms of nucleation for biological and environmental minerals are a curious feature of life on earth and the biogeochemical cycling of elements. Indeed, even for known electrolyte solutions, nucleation of mineral species have been discussed and debated for more than a century with results that are inconclusive for hydrated, amorphous minerals, such as those found in aquatic ecosystems, wetlands and at the air-water interface. Yet, these are critical to understanding air and water thermodynamics, transfer and related matters of weather and climate. The recent paper by Nielsen et al., (2014a, 2014b) finally shows that even the common mineralization of CaCO_3 , studies have been inconclusive, and that several pathways to nucleation may occur. One of these states is the presence of hydrated, amorphous states as an important precursor in defining metastable and stable phases or means of establishing unambiguous phase identification for common calcium mineralization. And calcium carbonate mineralization has been studied *ad nauseum* for generations, particularly for the importance in shell calcification and coral reef formation. Other recent studies, such as Wolf et al. (2008), hypothesized that CaCO_3 precipitation was not a phase of the solution/air interface. Wolf et al. (2008) pointed to

3000 papers in the last 10 years, but confirming only now the facilitation by organic molecules to develop a formation of hierarchically ordered inorganic-organic hybrid states and structures. Wolf et al. (2008) and Nielsen (2014a) in separate studies now used *in situ* TEM to resolve their research, and included various analytical methods of Cryo-TEM and X-ray microscopy. This suggests that the dynamic states of formation should appear in structural analysis of hydrated transition metal compounds common to aquatic systems and that biological systems of mineralization can build upon patterns of nucleation. Some of this is demonstrated here for metallic surface films.

Kleja et al. (2012) reported differences in mixed-valent iron in other studies, where some studies lacked significant amounts of Fe (II), and suggested that in Grathoff et al. (2007), where both Fe (II) and Fe (III) were present, that acidification of the sample for two-line ferrihydrite might be in error in either the application of acidification in the field versus later acidification in the laboratory. In this study high resolution TEM with EELS, electron energy loss spectroscopy, showed both mixed valent states for air-dried samples onto TEM grids for both manganese and iron. Kleja et al. (2012) suggested also that some organic matter originating from the films might bind the iron by resident organic matter or NOM in the films, electrostatically complexed, and thus not be a true component of the films. I believe that this is false, and that organic matter in the films stabilizes the film structure and possibly the oxidation states.

Here, in order to give a better structural viewpoint, surface films were examined by both light and scanning electron microscopy supplemented by photoemission

electron microscopy (PEM/PEEM) with additional supplement by hrTEM and dual-beam SEM. The corrected optics of the photoemission microscope, more accurately termed the CPEM was used to develop the use of *work function* in the information given by the images. Scanning electron microscopy with elemental analysis by energy dispersive spectroscopy (EDS) is also the primary tool supplemented by secondary ion mass spectrometry (SIMS) for additional analysis. Instrument parameters are reviewed as applied physics of instrument development.

1.7 Overview of This Surface Film Research Project

This research extends our knowledge of aquatic and metallic surface films and contributes to the technical terms and concepts of structures, processes and properties of the air-water interface. The focus is on analytical images of metallic surface films to confirm or reveal morphology and processes in natural waters and wetlands in temperate aquatic environments and on several laboratory studies and structures. This is not a field study, although samples from selected field sites are reported. It is a structural microscopical analysis using comparative methods microscopy and spectroscopy to improve our knowledge base of analytical images, structural comparisons and elemental composition of surface films, particularly for the metallic surface films sometimes called floating iron films or mixed-valent iron oxide films in the history of these studies. The terminology will be reviewed and discussed in detail. This research was undertaken because of the importance of processes at the air-water interface, the general lack of knowledge in this area of aquatic and environmental science, and as a means of improving microscopy and present and future instruments.

The instrumental focus has been on light and electron microscopy, particularly the application of scanning electron microscopy to environmental problems with elemental analysis by energy dispersive spectroscopy, and to improve the level of precision as they are applied to surface film research. Newer instruments were used and evaluated as available, as there were constraints on funding and travel. This did not deter my main objective to characterize both natural and artificial metallic surface films, but did allow us to infer the presence and role of bacteria, cyanobacteria, pollens, other microbiota and their several contributions to the structure and processes at the air-water interface.

We get a sense of the diversity of descriptions of aquatic surface films and slicks by the terms used over several decades. The same is true to a lesser extent for the metallic surface films, as the focus has been on iron-complexed minerals. By describing metallic surface films, we expand the scope of metallic, reflective films from the air-water interface to include mixed composition, biofilm modification and patchiness, and other reflective surface films, such as the copper-zinc film described below. The following Table 1–1 is a partial list of the organic surface film descriptions to demonstrate that the air-water interface has been viewed by others in several ways.

TABLE 1–1 LIST OF SURFACE FILM TERMS

Air-Sea Interface	Lion & Leckie	1981
Air-Water Interface	Henk	2004
Air-Water Interface	Wotton	2005
Aquatic Surface Microlayer	USEPA (Chesapeake Bay)	1988
Aquatic Surface Microlayer (SMIC)	Hardy, Crecelius et al.	1990

TABLE 1.1 (CONT.)

Aquatic Surface Microlayers	Södergren	1987
Boundary Layer Surface	MacIntyre	1968
Coastal Surface Films	Crow et al.	1975
Estuarine Salt Marsh Microlayer	Lion et al.	1979
Gelatinous Biofilm	Cunliffe & Murrel	2009
Gelatinous Surface Microlayer	Cunliffe et al.	2009
Microlayer	Lion et al.	1979
Microlayer	Carlson	1982a
Microlayer	Liss et al.	1997
Salt Marsh Surface Water	Harvey & Young	1980a
Sea Surface Film	Wangersky	1976
Sea Surface Microlayer	Agogué	2004
Sea Surface Microlayer	Hunter	1980
Sea Surface Microlayer.	Hardy	1982
Sea-Surface Microlayer	Piotrowicz et al.	1972
Sea-Surface Microlayer	Cunliffe & Murrell	2009
Sea-Surface Microlayer	Barker & Zeitlin	1972
Sea-Surface Microlayer	Pattenden et al.	1981
Sea-Surface Microlayer.	Hardy	1982
Sea-Surface Microlayer	Hardy, Apts et al.	1985
Sea-Surface Microlayer	Hardy, Crecelius et al.	1987
Sea-Surface Microlayer	Cross, Hardy et al.	1987
Sea-Surface Microlayer (SML)	Wurl et al.	2011
Slick Forming Materials	Garrett	1962
Surface Film (FW, AWI)	Wotton	2005
Surface Films	Carlucci et al.	1991

TABLE 1.1 (CONT.)

Surface Films (Interfacial)	McKenna	1997
Surface Microlayer	Duce, Quinn et al.	1972
Surface Microlayer (SML)	Santos	2011
Surface Microlayers	Carlson	1983
Surface Slicks	Södergren	1987

Note in Table 1–1 that there has been inconsistency in how the films have been described. It is much like a search for a proper descriptor of the processes involved.

A similar history of descriptions and terms is found for the metallic surface films, even within the same paper. In order to resolve this terminology, I have used metallic surface films (MSF) to refer to the films examined here or aquatic surface films or microlayers (ASF) to describe the organic surface films. While it is a simplification, the evidence is strong for a general descriptive term to encompass composition and structure where transition metal diversity is present. Note that there is also inconsistency and duplication in how the metallic surface films have been described. Even within the same paper or thesis, the films have been described by several terms.

Consider Table 1-2 as follows:

TABLE 1.2 PARTIAL LIST OF METALLIC SURFACE FILM TERMS

Floating Fe Films	Grathoff et al.	2007
Floating Iron Bearing Films	Kleja et al.	2012
Floating Iron Films	Gray, Z.	2008
Floating, Iridescent Films	Gray, Z.	2008
Floating Mixed-Valent Iron Films	Gray, Z.	2008
Iridescent Films	Grathoff et al.	2007

TABLE 1.2 (CONT.)

Iron in Floating Surface Films	Kleja, et al.	2012
Iron-Bearing Films	Easterly, H.	2005
Iron Oxide/Water Interface	Gunnarsson	2002
Mixed-Valent Fe Films	Grathoff et al.	2007
Mixed-Valent Fe-Bearing Films	Grathoff et al.	2007
Schwimmeisen	Grathoff et al.	2007
Thin iron oxide films	Schwertmann & Friedl	1998
Fe-Rich Biofilms	Tazaki at al.	2002

Partial List of Metallic Surface Film Terms By Author

Easterly, H.	2005.	Iron-Bearing Films
Grathoff et al.	2007	Floating Fe Films
Grathoff et al.	2007	Iridescent Films
Grathoff et al.	2007	Mixed-Valent Fe Films
Grathoff et al.	2007	Mixed-Valent Fe-Bearing Films
Grathoff et al.	2007	Schwimmeisen
Gray, Z.	2008	Floating, Mixed-Valent Fe Films
Gray, Z.	2008	Floating Iron Films
Gray, Z.	2008	Floating, Iridescent Films
Gunnarsson	2002	Iron Oxide/Water Interface
Kleja et al.	2012	Iron in Floating Surface Films
Kleja et al.	2012	Floating, Iron Bearing Films
Tazaki et al.	2002	Fe-Rich Biofilms

Tables 1–1 and 1–2 summarize the diversity of descriptors used to describe the metallic surface films, with a focus on iron. The hypothesis proposes a general change.

1.8 The working hypothesis

A working hypothesis was developed around existing publications, particularly those of Grathoff et al. (2007), the MS thesis of Easterly (2005) and the MS thesis of Gray (2008), in comparison to other surface film studies for aquatic surface films or microlayers, the oceanographic sea surface microlayer (SML), and what is learned from oil and chemical spills that affect the air-water interface (Smith, 1983; Smith, 2007c). This work departs from the chemical analysis of water to focus on the ability of analytical and environmental microscopy to develop a structural answer to remaining questions about surface films, and to focus on discrete or point sampling for accuracy.

The surface films, both aquatic surface films and metallic surface films were imaged to improve our concept of surface, structure and the presence of biota. Both the air-film interface and the water-film interface were imaged in most cases. Our focus was to seek simple methods of collection that could be applied over a wide range of circumstances as discrete or point samples representative of conditions and thus form a more coherent picture of the film structures. This study improves the structure of both amorphous states and ordered states found in the same sample, and we document the presence of other transition metals in film formation.

This study establishes a working basis for the microscopic evaluation of metallic surface film structure and to establish that iron oxides, while considerably important are only one part of surface film structure. More importantly, this work establishes the role of other metallic elements, such as manganese nickel, copper, zinc and tin in metallic surface film and biofilm structure and formation. This expands the concept of metallic

surface films to embrace a larger view of components and structure, as found by example in nickel/zinc biofilm and copper/zinc surface films, and in doing so, forms a base not only for bacterial and algal biota, both as colonizers in surface films as habitat, but also conceptually to demonstrate features of structure and morphology.

This working hypothesis and the research history is outlined so that aquatic surface films (AFS) and metallic surface films (MSF) can be defined and distinguished from one another, and that discrete sampling is used to resolve differences in structure, elemental composition and the presence of biological materials. The taxonomy of the bacteria, algae, pollen are not essential at this point, but the identification of the ways in which they attach or are integrated into the films are features of structure. The data, images and spectra support the hypothesis that while iron may be dominant in many cases, the term 'Metallic Surface Film (MSF) is the preferred descriptor based on metallic content of manganese, nickel, zinc and copper in several films or components examined. The working hypothesis states that surface films are variable in structure, show interactions with algae and bacteria, and can form layers possibly to separate properties and processes, and that many features of the surface films appear as small alterations and patches. Patchiness, particularly for biota and biofilms were found to be a significant part of identifying structure. Improved documentation of mineralization is also a focus of this study, and gives us a firm basis to address the question of habitat and habitability as they apply to the origin of life and planetary exobiology.

As part of the hypothesis, methods and instruments are described so that future students will have some guidance about how to obtain the best sample and sampling

conditions for imaging and analysis. Analytical imaging is based on a suitable sample for the instrument available, or the instrument to be proposed. Individual instruments will show you some information, but will not show you the entire picture. Knowledge of the instrument capabilities and operation are essential to obtaining the most accurate and precise data and an image that consistently informs the researcher and the scientific community. In this way, instrument improvements, advancements and developments can be identified and new instruments proposed.

1.9 Optical images of neustonic biota

The following DIC images (Figures 1–1, 1–2, and 1–3) were done on a Olympus confocal system with DIC optics. In the DIC mode, contrast is obtained by Nomarski optics, prism realignment of the image path, creating good contrast for transparent material, which would not be seen in normal brightfield light microscopy .

Figure 1–1. MSF with Rotifer, 200 x.

Figure 1–2. 600 x. Cells are about 10 μm .

The neuston are the biota of the air-water interface (Naumann, 1917). In an early study, a wet, sealed mount of surface film was imaged by differential interference

contrast micro-scopy (DIC). It gave us a significant clue to the diversity of organisms that use the surface film as habitat, and thus a means of evaluating the structure for habitability. Figures 1–1 and 1–2 are demonstrations of abundant biota and suggestion of structure. Phase contrast microscopy will often give a similar recovery of the image by a different optical method, but also yields features of small transparent filaments, which would not be seen by brightfield light microscopy. The rotifer is about 0.3 mm, and the algal cells about 15 μm in these images. For metallic surface films definitions of habitability are being evaluated for their application to exobiology and the several concepts of how life might evolve on any planet, not just our own, and the air-water interface is such a location and habitat. Further enlargement of the diversity of neuston is shown in Figure 1–3.

Figure 1–3. Enlarged image to demonstrate resolution of fine filaments, and filamentous nature of the MSF.

Although Figures 1–1, 1–2 and 1–3 are without scale bars, they are adequate for the purpose of demonstrating what might be expected under SEM. These images revealed that there was considerable structure to the metallic surface films, some dark dots that could not be explained and an abundance of microbes, bacteria, cyanobacteria and filaments. Algal cells and diatom frustules are roughly 10-15 μm , and cyanobacterial filaments could be seen. In Figure 1–3, an enlargement reveals the fine structure of the surface film, and the fine filaments of a branching organism. As a test, this was sufficient to show a diversity of organisms in the sample a preparation for the SEM imaging to come.

1.10 Images of metallic surface films

The following image is of a metallic surface film, taken at a 30 deg. angle to gain some depth perspective of the surface features of cracking and folding. At 100 μm few surface features can be distinguished. However, with practice, there is some evidence of layering of the film, features of a more mature film. The cracks and eruptions suggest that there is more material underneath that is hidden in this view. Analytical images are not a single image, but a qualitative or quantitative use the instrument go gain a higher level or information as well as resolution.

The many images of metallic surface films and the inclusions or biota presented throughout the dissertation document the diversity and variety of film structure. The literature suggests that few important features have been the focus of attention, which is important to analytical imaging and increasing attitudes to comparative microscopy. This is an important contribution toward future research on surface films.

Figure 1–4. A metallic surface film taken at an angle to demonstrate the view of a flat plane that falsely gives the impression of little activity or patchiness until examined closely.

1.11 Surface morphology

Basic surface image and structure, the gross morphology of metallic surface films is distinctive and diverse. No two surface films are alike! Published literature would suggest that they are silvery, iridescent or with reflective features, somewhat uniform. These observations and the surface features will be reviewed with most of the separate chapters, reflecting the types of samples examined. What is significant to notice is that a film like Figure 1–4 is difficult to characterize because of topography. The topography and morphology suggests that other processes are at work, some mineralogical, some biological, which may not be defined by chemical analysis alone.

The analytical imaging done here resolves some of those characteristics by providing new viewpoints or perspectives on film image and structure.

1-12 Orientation and connections.

Since some samples are lifted to show the air-film interface and others the water-film side of the surface film, it is important to note the orientation of the film.

In the following four examples, a methylcellulose filter pad was used in the collection, and thus the pollens were in the water below the metallic surface film. It is logical to assume that the pollen and dust might be on the air-side of the interface, but that is not always the case. In these four examples the pollen is used to demonstrate orientation of the sample. It is important to outline the orientation of the samples. For methylcellulose pads, we are lifting the sample that has been held to the filter pad and acquired water. This means that an SEM study of that sample will observe the water-film surface, the undersurface of the film. To help this orientation, this is called the water-film interface or WFI. Note that the metallic surface film is thin, somewhat in strands, but still shows that there are some particles or structure within the film. It was the suggestion of surface film structure that lead to a further analysis of the film structure itself as well as the way biota were found or integrated into the metallic film structure. Much of the literature does not report whether the air-side of the film or the water-side of the film was recorded or examined. In this study, the orientation and method of collection is stressed to obtain better information about structure, components and composition as well as inclusions and deposits entrained in structure, or attached at one of the surfaces.

Figure 1–5. Pollen on water-side of film. Scale bar is 50 μm . Notice that the grain is significantly larger than the film itself.

Figure 1–6. Pollen on water-side of film. Scale bar is 50 μm . Notice that the grain is larger than the film but extends filaments.

Figure 1–7. There are surface features to the film that suggest internal bodies or nanocrystallites within the film.

Figure 1–8. Distinct features, such as this circular feature, although unknown, can give some distinct character to each sample.

1.13 Interim conclusions

We can conclude that there is a uniqueness to each sample, and that important and distinguishing characteristics appear often as patches, where the iron oxide film may serve as a substrate, other metallic surface films occur as unique bodies, mineralization and structures of their own. This expands the concept of metallic films

at the air-water interface to be inclusive of a much broader range of physical and environmental processes as well as a clue that many revealing features may occur as small patches of activity and structural change. These samples raise the question of both habitat and habitability as the air-water interface is subject to considerable insolation, possibly high irradiation with near-UV light, but also with the prospect of some increased reflection from the metallic film surface. As a habitat, there is considerable and active colonization, so one might expect several geological and biogeochemical processes to be either assisted or inhibited by sunlight. How best can analytical imaging and structural analysis be an effective tool for this type of study? Clearly, by increasing our knowledge of the fine structure of all surface films and by documenting the diversity of film inclusions and use by biota, particularly bacteria and cyanobacteria, algae and other materials, we expand the concept of what aquatic and metallic surface films contribute to environmental conditions, or where they act as active habitat or reservoirs of nutrient compounds or elements. In this study elemental structure is the dominant comparative feature, but morphology plays a significant role in this research. Morphology can often be a guide to properties and processes involved in film formation, and thus can guide future investigation and refinement of future working hypotheses.

CHAPTER TWO

ANALYTICAL IMAGING BY SCANNING ELECTRON MICROSCOPY

In analytical imaging, we want to establish parameters that will yield the most accurate and precise results for the materials being examined. In one sense this has always been easier for solid materials and industrial components, where properties and conductivity are well known. Biological materials are less well-known, and for both aquatic surface films and metallic surface films, where we have a combination of biological and other heterogeneous materials, imaging can be more difficult. In part it is that the electron beam can do damage to the sample from high currents or concentrated beam exposure over a period of time. In this study, the objective is to demonstrate some of the imaging parameters and conditions.

Improvements in electron microscopy in general and scanning electron microscopy in particular, with energy dispersive spectroscopy as the primary elemental spectrometry make instrumental analysis essential in environmental work. In this report, we demonstrate the differences in imaging quality based on knowledge and skills necessary to bring out important features of biological and mineral substances, particulates from the air-water interface. Full calibration is necessary to get the best results from any modern SEM with EDS or WDS spectral analysis. In most cases a copper strip or grid is suitable to calibrate the instrument. This is not one simple analysis, but a systematic regime that repeats the analysis under several instrument parameters. We show results regarding surface features of gold particles and diatoms. Instrument parameters are critical to successful analytical imaging.

Gold particles were examined at several parameters. Shown here is a 5 kV image at spot size of 3.0, and on the right 5 kV at a larger spot size of 5.0. Even this small variation shows differences in image performance. On the left surface lines of structure can be seen (Figure 2–1). These are lost in the second image (Figure 2–2), but surface spotting not seen in image one are a prominent feature of image two. At a magnification of 73 KX these are nanometer size particles, yet comparison shows important differences in our view of structure.

Figure 2–1, At 5 kV Gold Particles to show ridges or layering.

Figure 2–2, at 15 kV, Gold Particles now show spotting with only a hint of ridges.

2.1 Electron interaction with the specimen

Scanning electron microscopy includes a high degree of interaction with the sample. this is most easily shown in solid, material samples, but the same occurs with biological material. There is signficiant penetration of the electron beam to allow for x-rays and electrons to be emitted to either the objective lenses or as x-rays to the detector. This is a simplified view, but necessary to explain the analytical imaging that follows. Some of these are outlined in Figures 2–3, and 2–4, as interactions.

Figure 2–3. A Diagram of electron and x-ray emission. Diagram courtesy of U of Alberta, Canada, ~ccwj Teaching/Microscopy. Secondary electrons are referenced as SE; backscattered electrons as BSE. Auger electrons often referenced as E_{auger} or AE,

Figure 2.4 Interaction Volume.

Figure 2–4. A Diagram of Electron Interaction Volume, Auger electrons are referenced as AE or E_{auger} . Diagram courtesy of AAPG. Secondary electrons (SE) and several emission possibilities are shown. This is based on a *Monte Carlo* simulation.

Figures 2–3 and 2–4 demonstrate that there is always significant interaction between the electron beam and the sample, including penetration into the sample. In these diagrams, the basic interaction is described to assist discussion.

A field sample will normally be much more complex in structure than gold particles used in calibration, and several views may be necessary to reveal structure of surface film material. One setting or magnification in a series will not detect the structures shown in bacterial cases or in patchiness of some features. Thus, several images with different settings may be necessary to reveal both surface and interior structure, and interaction between biota entrapped during formation

Figure 2–5. *Stephanodiscus* sp. Note how the interaction volume combined with a longer working distance gives an impression of depth to the structure. Because of the silicon substrate to the diatom frustule, we are still able to image the very fine EPS.

In Figure 2–5, *Stephanodiscus sp.*, I have the general effect of specimen interaction in that it resolves some aspects of structure to give a 3D impression, and a depth partly due to a longer working distance, a feature of the scanning electron microscope. The silicon substrate provided by the diatom frustule and its structure assists contrast in the image. At 20 kV there is significant penetration of the material and the possibility of specimen damage. It may not be obvious during instrument analysis, but requires review of the image and a revision of settings to get the best results, that are better representatives of surface and hidden structure. This machine was an ISI SS40, thermionic emission based SEM. Even without sub-nm resolution, notice that we can still resolve very fine structures, the Extracellular Polymeric Substances or EPS. These fine secretions can form very delicate web structures in the diatom environments, and are a good visual mark of living organisms when examined.

2.2 Imaging, instrument settings and calibration

In imaging, care must be given to the calibration of the instrument and instrument settings. In most cases, several different settings must be used to resolve different aspects of structure or to improve dimensional resolution. In some cases working distance may be adjusted to accommodate EDS analysis on one hand and 3D and 4D imaging on the other. While the above images were done on fixed or air-dried structures, other instrumentation is used to resolve wet structures or to provide contrast only available in wet mounts. It is expected that microscopy of wet, living structures will predominate in the near future. Comparison of fix and dried material with wet structures are necessary to develop a more complete model of surface film dynamics

and structure including temporal aspects of mineralization and to get a grasp onto the several influences on the many dynamic features of change. This was shown recently in an examination of calcium mineralization (Nielsen et al., 2014a), which may be a guide to *in situ* examination of films at high resolution. This will not replace the dimensional image provided by scanning microscopies. The small details of patchiness in surface film structure become evermore important to define metallic surface film processes and interactions.

2.3 The instruments and methods

As a guideline for future work, the capabilities of the instruments are reviewed. Most instruments are unique in some way, and familiarity with the instrument is essential to achieve the best results. An analytical instrument will tell you something, but not everything. It may give you the right answer as well as the wrong answer, so complete familiarity with the instrument design and operation are essential. In many cases you will need an experienced operator familiar with the parameters of the machine. These images are not just pictures, they are analytical images, using the best capabilities of the instruments to bring new knowledge to your subject.

Part of the future work on metallic surface films will include high-resolution structural analysis, what and where elements are located and the mechanisms that brought them into place. Some of this may be direct mineralization from highly reduced species, as in high levels of ferrous iron, but others may be biological mediated or carried as cations bound to humic substances. A summary of instruments and

technical details of their capabilities are given here. Many of these capabilities were not necessary for this project, but may apply to future work

This is actually a good set of examples of how the instrument must fit the hypothesis you have. An improperly set instrument might image more from the inside or at depth, rather than on the surface. When you interact or treat your sample deeply, some delicate surface features would be lost. (Review Figure 24). There might be high resolution, but it might be sensitive to topography and variation of the sample. But, in the end, consideration must be given to the type of analysis that can be done and which instrument is best for that purpose, as well as knowing the experience of operation.

2.4 Instrument technical parameters

This and future work depends on understanding the instruments and what they are capable of giving. A good instrument will give you something, but not everything, and the capabilities of the instrument are in flux. In most cases, a user should be well trained, but also may need the assistance of operators who are familiar with the technical operation of the instruments. In most cases this is a two-way education, as most operators come from a materials or engineering background and need some training themselves in biological or environmental samples.

Both light and electron microscopes have advanced tremendously over the past few decades. For electron microscopes, it is not only the final resolution that is important, but also the other developments that have made improvements possible. One of these is the source of the electrons, the emitter. Developments in emitter technologies and computer adaptations to the machines the systems have achieve an

amazing level of resolution and analytical capabilities. In addition to electron microscopes, ion microscopes in the form of focused ion beams (FIB) have also made a large impact in in several fields of nanotechnology, one of which is an emerging nanobiology. The ion or dual-beam instruments, such as the FIB used in this work, both destructive and nondestructive image and analysis allow the user to image (albeit while removing the surface layers) and mill into a sample to elucidate the structural and chemical identification within a sample with relative ease. This is the focus of chapter eight. Although these microscopes are very capable as standalone systems to get a full picture of what the sample is made of, both inside and on the surface, with high precision the usage of several different tools is advised.

The electron microscopes, whether of transmission (TEM) or scanning (SEM) type have had a niche for some time for being the primary imaging mode of research. Technological advancements have allowed 0.050 nm resolutions to be achieved with TEMs and sub-nm resolutions with SEMs. Often, this is achieved through correction of known aberrations of the magnetic lens systems. Electron optics or lens technologies have improved considerably in recent years. Another aspect, which allows such precision in imaging, is due simply to better system designs through computer modeling as well as availability to certain advanced materials and sub-component technologies. Although resolution is extremely important in determining which system to use, one must be aware of other important factors, which are critical for analyzing a sample. Technical capabilities, such as contrast, beam current, detector sensitivity, type of detector etc., are also very important in finding and using the right machine for the

hypothesis being examined. Many of these issues are related to each other in terms of what is available on the machine and how one uses certain imaging parameters.

For this study several different electron microscopes were used. I will focus first on the scanning electron microscopes that were available for my initial sample analysis. Today's highest resolution scanning electron microscopes usually a type of emitter which allows the smallest spot to be utilized on the sample such as a Schottky or Field Emission (F or FE) cold field emitter (CFE). The thermionic emitter was used in the early studies for its convenience and availability at that time. Although not used in the latest work presented in this study, thermionic emission systems still serve a useful purpose in imaging research.

Thermionic emitters have always been a workhorse in electron microscopes, however they do not achieve a very high-resolution image to the nature of the emission. These machines do however have a niche for fairly decent current and good contrast. The beam current allows for fairly good EDS x-ray signal to be generated for x-ray analytical needs. The thermionic emitters need replacing often, but they are very inexpensive compared to the field emission units, which can easily exceed several orders of magnitude higher cost. Due the low cost of the emitter and simplicity of usage it is much simpler to gain access these types of microscopes as of late. However due to the popularity of field emission microscopes due to the much higher resolution, they are becoming much more commonplace over the past decade.

The necessity for much higher resolution has been very much pushed by both industrial and research needs as nanoscale based systems such as semiconductors and

other nanofabricated components have advanced. This has allowed for further development of microscope resolution, a competition between manufacturers. The researcher is able now to access amazing microscopes with resolutions and abilities only dreamed of decades ago. In terms of commercially available scanning electron microscopes, this study mainly utilized an older CFE SEM (JEOL JSM-6300F) and a newer Schottky field emitter, FW SEM (JEOL JSM-7800F).

The modified JEOL JSM-6300F field emission microscope is a (CFE) system capable of roughly 3 nm resolution. It is equipped with an energy dispersive spectrometer (EDS) and capable of capturing large size images. This microscope was used due to its free availability, although it has no special features in terms of imaging compared to newer machines it is still important to mention the specifications and characteristics in being able to image samples. The cold field emitter is a useful form of electron source due to small energy spread available since heating the tip for imaging is not necessary. Heated emitters, whether thermionic or field emission, have a larger beam energy spread that leads to blurring of the spot size a focus. This is analogous to having more colors of light in a focused beam and thus a chromatic aberration at the focus. The cold field emitters have a very small energy spread but suffer from low electron emission as well as stability problems and expense. The JEOL JSM-6300F has a very stable emitter through implementing a special electrostatic lens near the emitter that measures the instability of emitter and uses this information to stabilize the current. The images are fairly high resolution but the low extraction current (8 μA) thus low beam current range (10^{-12} to 10^{-10} A) makes the images fairly grainy and the EDS analysis

requires a longer time for discernible peaks. That being said it still allows for fairly nice imaging. Typically, the EDS system in the older units required a liquid Nitrogen cooled detector. Newer EDS systems operate at room temperature.

The system has a modified digital imaging system capable of getting 4096 x 4096 pixel² images. The advantage with this imaging of large pixels involves being able to take images at low magnifications and provided that the focus was very accurate, the image can be used later for zooming in and analysis of data without having to zoom in. In the past this level of pixel resolution was rare, but machines today have pixel resolutions up to 32 K x 32 K pixels². (Since pixels are a dimensional square or rectangular feature, they are represented as pixels²). Several initial images were taken for this body of work with the JSM-6300F, and these images were useful, however the Schottky emitter system provided better contrast and image resolution, due mainly to the technology advancements made in the column and detector designs. That being said, after attending many microscopy vendor shows recently, it can be seen that companies are starting to look more into the idea of adapting CFE gun designs for better resolution, but the systems still seem to be dominated with Schottky emitter-based microscopes.

The JSM-7800F has a very stable Schottky field emitter, which is placed in-lens with the capability to image nanoscale morphologies up to 1,000,000X magnification with sub-1 nm resolution. In the studies reviewed in chapter twelve, not all capabilities were used, but in the future metallic surface films examined at a higher resolution will occur. A special lens allows for angular control of the emission in such a manner that the machine can achieve 200 nA of beam current in a very small spot size (sub-100 nm)

which allows for high contrast images with high resolution. Typically the better resolutions come from being able to achieve a small spot size which raster scans an area on a sample to make the final image.

The small spot sizes usually require very low currents which in turn makes EDX difficult as well as high contrast imaging. The low current generates very few x-ray photons so time integrations take a lot longer to get defined peaks. In terms of contrast the low current generates very grainy images, unless the electron beam can subtend a larger angle thus a larger numerical aperture and smaller spot with more current present in a the smaller spot. The 7800F manages to get a large amount of current in a small spot due to have more control of angles of the electrons in the pathway of the electron column. In addition the electron optics perform a special beam deceleration which allows for nonconductive samples such as the biological components in the metallic films to be imaged without serious charging problems. This also has the effect of reducing lens aberrations of the nonconductive samples, which allow for high resolution imaging.

The system has many useful attachments capable of performing tasks such as collection of large area Electron Backscattered Diffraction Analysis (EBSD) maps at low magnifications without distortion due this angular control aspect. The system also has the ability to image at low kV while performing precise analysis on fairly magnetic samples. The special hybrid objective lens allows for minimal magnetic fields to be present at the sample. Unusually in scanning or transmission electron microscopes magnetic samples can often disappear from the sample inspection area of the system

due to being “sucked” into the objective lens. My films oftentimes have a magnetic component and it is something to worry about of the working distance is short and the lens strength is high (strong focus). The magnetic component of metallic surface films was not always present, and the reason for this is not yet known. It could be the amount of organic matter in the film or the other components, such as manganese. Since electron microscopes tend to be temperamental around magnetism, it may be a good technical study, but it will also tell us something about surface film formation, in the future. Metallic surface films, if magnetic, can actually be collected in the field by magnet, a useful observation, but not part of this study.

Table: 2–1. JEOL JSM-7800F. Operational specifications.

Description	Specifications
Secondary Electron Imaging Resolution	1.0 nm at 15 kV
	0.8 nm at 15 kV, GB mode*
	1.5 nm at 1.0 kV
	1.2 nm at 1.0 kV, GB mode
	3.0 nm at 100 V, GB mode
	3.0 nm, 15 kV, 5 nA, EDS WD of 10 mm
Accelerating Voltage	10 V – 30 kV
Magnification Range	25 X to 1,000,000 X

*GB (Gentle Beam) Mode is a proprietary imaging mode from JEOL.

Table 2–1. Description and specifications of the JEOL JSM7800F, 2014.

Another Schottky emitter based microscope that was used for this study was the Zeiss Sigma VP FESEM. It differs greatly from the previously used systems due to the variable pressure (VP) imaging mode, which is beneficial for samples, which might be nonconductive or have a low water content, like the metallic films in this study. The imaging mode lends to a very nice contrast mechanism for biological samples. Its

original purpose for acquisition was to have a great tool for geological and biological samples and to have a Wavelength Dispersive Spectroscopy (WDS) detector.

In order to image difficult samples (nonconductive or wet) the Zeiss VP utilizes a water pressure, which is introduced into the chamber which causes a cascading effect for the secondary electrons through the vapor in the chamber. The SEM has the ability to image with 1.3 nm resolution at 20 kV. There is also a gas release, which may affect the image as it is an adjustable parameter.

I should include a note that there are proprietary systems with each manufacturer. Some are a similar product with a marketing name, but essentially the same feature, but some are unique to the company and a benefit for specific situations.

Table 2–2. Zeiss Sigma VP FESEM operation specifications.

Description	Specifications
Secondary Electron Imaging Resolution	1.3 nm at 20 kV
Accelerating Voltage	100 V~30 kV
Magnification Range	30 X~1,000,000 X

Table 2–2. Zeiss™ Sigma VP SEM. Description and specifications, 2014.

The Zeiss VP SEM has the ability to get very clear large area images of biological samples due to the use of an advanced in lens detector. The large chamber design can handle large samples (250 mm diameter and 145 mm tall). Although not used in this study, there may be situations where a much larger surface film sample could be collected and analyzed over a longer period of time for specific patches of interest.

The photoemission electron microscope, the PEM or PEEM, will be described separately in chapter nine. The PEEM is a somewhat unique electron microscope

instrument that was used for imaging the metallic surface films. This system is a prototype and will be discussed in detail later. The advantage of its imaging ability lies in obtaining very fine detail of the biological samples. In particular one is able to see details of the cells and bacteria not possible with the scanning electron microscopes, such as small tendrils and cellular wall details. The sample also can be uncoated and still get approximately 5 nm resolution. Another type of important instrument used in imaging the metallic films was a single beam and dual-beam FIB.

The single beam FIB refers to a system with only a dedicated FIB, over a decade ago, they were fairly commonplace but as the need for smaller scale manipulation without destructive imaging grew, manufacturers added an electron column. The electron column allowed for nondestructive imaging at high resolution as well as the ability to perform other analytical techniques, which involve the use of an electron beam.

A prototype system which is being developed in the Sánchez NanoDevelopment Laboratory is the 3D NanoTomography system. Although the system is not complete it presently has the ability to image and mill utilizing an ion column using gallium ions as well as perform Secondary Ion Mass Spectroscopy (SIMS) analysis. When complete the system will have an integrated Atomic Force Microscope (AFM) with large scan ranges $13 \times 13 \text{ mm}^2$ (coarse) and $100 \text{ }\mu\text{m} \times 100 \text{ }\mu\text{m} \times 60 \text{ }\mu\text{m}$ (X, and Z respectively). The large Z range will be useful for rough surfaces such as geological samples with $\pm 30 \text{ }\mu\text{m}$ height differences. Most Scan systems have 3-7 μm vertical range which limits your ability to image samples like metallic films unless they are very flat, this is one reason

an AFM wasn't used in this study. In the end all the subcomponents in the machine will allow the user to obtain a very precise (0.5 nm topographic, 50 nm spectroscopic) 3D spatial mapping of the isotopes present in a sample with a fair amount of topography present. For this work only the milling feature was utilized.

Access was given to a very new dual-beam FIB available from TESCAN (Lyra 3). This machine has very high resolution in its ion column (2.5 nm with a Cobra model column). TESCAN Lyra 3 specification are given below in Table 2–3.

Description	Specifications
Electron Mode -	
Secondary Electron Imaging Resolution	1.2 nm at 30 kV
	2.5 nm at 3 kV
Electron probe current	2 pA to 200 nA
Accelerating Voltage	200 V to 30 kV / 50 V to 30 kV (OPT)
Maximum Field of View	17 mm at WD 30 mm
Magnification Range	1 X~1,000,000 X
Ion Mode-	
Ion Columns (two possible models)	Canion / Cobra
Resolution	<5 nm at 30 kV / <2.5 nm at 30 kV (1 pA)
Magnification	150 X ~ 1,000,000 X
Accelerating voltage	0.5 kV to 30 kV
Ion Source	Ga Liquid Metal Ion Source
Probe Current	1 pA to 40 nA / 1 pA to 50 nA

Table 2–3. TESCAN Lyra 3 operation specifications.

TESCAN is a European company that is known for its large sample stages in use with the electron microscopes (i.e. model - Mira 3 AMU). The large field view offered by TESCAN, (FOV), have been implemented in some of the large design TESCAN systems with very little distortion, which is very useful for looking at large sample areas without having to stitch them together. Imaging with an SEM can be frustrating at times due to not being able to find your way around on a sample since

your field of view is usually very small, it can be like looking for a needle in a haystack. Large FOVs allow the user to more easily find the area of interest. This may have good application for larger geological and biological samples, but for now has found good uses in archaeology, one of which was the large terracotta tomb soldiers in China.

For imaging thick samples it can oftentimes take a very long time to mill though to the point of interest. Normally, systems employing Ga emitters have limitations on the maximum amount of beam current in the range of 10's of nanoamps (nA). At this current range, the imaging is very bad compared to 1 pA, which is used normally for imaging. A new technology being employed recently, which would be ideal for quickly milling very thick samples such as thick metallic films is the gaseous ion column. This type of column can achieve up to microamps (μA) of current with fairly good imaging.

2.5 Interim conclusions

There will be a much greater access to structural information in the near future. Some of this is a direct result, or a technology transfer of materials science work on chip manufacture. Industry, such as INTEL, reports that as components get smaller and smaller, their demand on the instrument design and parameters is ever higher. Even though the biological and geological work may lag behind a bit, this instrumental change will support structural work on metallic surface films and other biogeochemical detection.

As an example, for much finer detail of milling and making videos of the metallic film layering discussed in this work, images being encountered during milling the gallium beam is useful due to its slower speed of milling. New instruments might

mill approximately 50 times faster than a gallium emitter type column. This does not mean it is useful for natural film examination. Industry might demand a faster, deeper milling process, but the most delicate films, shown in chapter eight, might be lost from detection if you milled too quickly and too fast. It is an important point to fit the instrument with your working hypotheses so that the instrument is appropriate to the task.

The instruments are reviewed in detail because analytical imaging depends on developing a knowledge base of what instruments can and can't do. For metallic surface films and the related aquatic surface films, much of what is presented here is a new application of the instrument, where improvements in imaging are expected to occur over time. This is particularly true for future instrument development, where the biological applications or environmental applications are often secondary to the material science applications. It is a funding issue, as support for instrument development has lagged behind other scientific funding.

CHAPTER THREE

STUDIES ON IRON PARTICULATES AND SURFACE FILMS

The objective of this study was to establish that iron particulates could be detected and identified at the air-water interface and with biota in the aquatic surface film. In particular, it was found that the neustonic cyanobacterium, *Trichodesmium*, possibly incorporated iron-bearing dusts into the extracellular polymeric substances, the mucus that holds the colony together. As part of a larger study, a sample of surface film was taken at Havre de Grace, Maryland, and used to document the potential methods (Smith and Dash, 1989). Samples of *Trichodesmium* were taken from local waters off Barbados and air-dried, transported to Portland State University for examination by the ISI SS40 SEM with an Oxford™ Link™ EDS system.

It first appeared that iron-containing dusts and iron particulates might be the first effort in surface film studies. It is known that cyanobacteria, the blue-green algae, which are actually ancient bacterial forms, required iron in their metabolism and were often successful in iron-poor environments over other algal species that also required iron. A survey of several cyanobacterial species, including *Aphanizomenon flos-aquae* indicated that they acquired dusts in their surface mucus or extracellular polymeric substance (EPS). *Trichodesmium sp.* samples from Barbados inshore waters were collected and analyzed for Saharan dusts as part of my research and reported. (Rueter et al., 1992)

Preliminary studies on surface film formation were done to resolve some basic structures with both light microscopy and scanning electron microscopy, and to speak

of the air-water interface as a representative boundary layer condition of interest to biologists, chemists, environmental scientists, geologists and physicists. The presence of so-called iron-oxide surface films, which are more accurately called metallic surface films, as a feature of wetlands, springs, seeps and dunes, as well as contaminated surface waters, provide a unique habitat with structural features to be resolved by applied environmental microscopy (Smith and Sánchez, 2011). In order to develop and to emphasize that environmental microscopy as an applied imaging procedure was an important part of documenting basic structures and relationships that could be better obtained by discrete sampling of the environment as opposed to bulk samples that disrupted structural features and patterns (Smith and Dash, 1989; Smith, 1999). Basic structures were resolved by scanning electron microscopy and subsequently with high-resolution transmission electron microscopy and photoemission electron microscopy. However, the organic components, which may yield reliable biosignatures in iron-rich environments, cannot be resolved by the electron microscopies. Particulate iron, however, was a good place to begin. Due to the color, stained slides shown separately as a supplement and greyscale images are shown for structural comparison. Samples were collected on standard glass slides, glass coverslips and for comparison Millipore™ methylcellulose filterpads and air-dried as reported by Smith & Dash (1989).

Locations: Susquehanna River at Havre de Grace, Maryland

 39° 32' 45.6" N; 76° 4' 39.5" W

 Barbados, Offshore

 13° 6' 30.7" N; 59° 39' 26.7" W

3.1 Sampling and Analysis of the Air-Water Interface

The structure of the air-water interface forms a boundary layer that involves biological, chemical, geological and physical processes in its formation. Freshwater and sea surface microlayers form at the air-water interface and include a diverse assemblage of organic matter, detritus, microorganisms, plankton, neuston, terrigenous materials and heavy metals (Hardy 1982). The sampling of the air-water interface and the examination of components is presently a significant area of study because of the input of anthropogenic materials and their accumulation and enrichment in surface films (Hardy et al., 1988). More recently Cunliffe et al. (2012) confirmed the complex structure of organic materials at the air-water interface and the formation of sea surface microlayers, and reestablished the importance of surface films of all kinds for studies at the air-water interface. There are now several reports on the multitude of methods that have been used to sample the air-water interface and the surface films (Hardy, 1988; Agoguá et al., 2004; Santos, et al., 2011).

Baier (1972) and Gucinski et al. (1980) reported the use of Langmuir-Blodgett film methods for the collection of natural organic surface films on germanium prisms. This study confirmed the usefulness of discrete samples collected on glass (Smith & Dash, 1989). The research on the Chesapeake Bay confirmed that discrete samples for both aquatic surface films (AFS) and metallic surface films (MSF) is a preferred method for obtaining surface structure at microscale and nanoscale structure and important or unique features, which may distinguish different areas of collection, formation and properties of the films and their inclusions.

3.2 Chesapeake Bay

Summer research under a NORCUS* grant to study at the Pacific Northwest National Laboratories (PNNL) marine station at Sequim, Washington included the opportunity to obtain aquatic surface film or microlayer samples in the upper estuary of Chesapeake Bay, Maryland. Samples of surface microlayer films were taken as discrete or point samples at Havre De Grace, Maryland, USA, the upper estuarine reaches of Chesapeake Bay, Maryland at the Susquehanna River entrance as part of a larger surface microlayer study. The purpose was to document both pollens and diatoms and organic and inorganic iron particulates or dusts in relation to the surface features.

*(NORCUS: Northwest College and University Association for Science).

The wave dampening effects of the heavy surface film was noted, and samples taken onto Millipore™ 47 mm methylcellulose filterpads. Sometimes samples on Millipore™ 25 mm methylcellulose filterpads were used. The filterpads proved to be useful as a white color comparison for photographed samples, as shown below.

Samples were air-dried on site. Subsamples were subsequently mounted to 12 mm SEM aluminum stubs and examined by scanning electron microscopy. Many early samples were coated with AuPd sputtering to an approximate thickness of 1.8 nm, but this coating often interfered with the EDS spectra

This technique documents surface microlayer structure and shows promise for determining microdistribution of metals in the microlayers. In the previous chapter, Figure 2–5 shows a surface film diatom from Chesapeake Bay neuston, taken at Have de Grace, Maryland and surface film, *Stephanodiscus* sp. Figure 3.1 shows pollen

grains, taken at the same location as *Stephanodiscus* sp. The largest pollen grains are indistinguishable from the Smithsonian Institution standard for *Ambrosia artemisiifolia*, common ragweed. Smaller, unidentified pollens are also found in this image. The wave dampening effect of surface film components is now well-known, but the presence of pollen in the surface film, as shown here in a discrete sample is not well-known. It is significant that both terrestrial (Eolian) and marine components can be documented in this way. The results indicate that the microlayer, surface film components consist of both living and dead materials and aeolian dusts and pollens entrapped or entrained into the structure of the surface film.

In Figure 3–2, EDS spectra showed not only a significant iron peak, but also a small zinc peak in several places. What is clear is that the surface film matrix contains an enrichment of iron as well as a minor amount of zinc. In other parts of the study, Organotin was also found in very small amounts (not shown). See Figure 2–5 also.

The Chesapeake Bay study began to alter our viewpoint on the presence of other metals in surface films. The samples from Havre de Grace, Maryland demonstrated a surface film consistent with large amounts of extracellular polymeric substances (EPS) as a contributor not only the surface film formation, but also as a means of trapping, entraining and transporting cations available to be captured. This capturing and transport of cations is consistent with the capture of humic substances reported in several papers and works of Tipping (2002).

Figure 3–1. Eolian presence of terrigenous pollen incorporated into the surface microlayer film. The two large pollen grains are identified as *Ambrosia artemisiifolia*, but there are also several smaller pollens. Note the character of the surface film with many very small granules, not identified in this sample. Film layers can be detected. Scale bar is set at 10 μm

In Figure 3–2, we see the two spectra related not to the pollen, but to the surface material on the diatom and the diatom surface. Note the iodine peaks in several places. It happened that this filterpad was treated with Lugol's Iodine to investigate the use of iodine, but as a tracer and as a preservative. Iodine is known to be absorbed by several organic materials and cellular components, and from this study we know it is useful both as a fixative and as a tracer. The spectra of iodine are in a different location than, say, the gold-palladium coating in this case. Iodine offers some options in developing a more complete picture of organic materials in the surface film structure.

Figure 3-2. EDS spectrum of Figure 3-1. Spot locations on the surface of the diatom, upper spectrum, and on the granule, lower spectrum. Lugol's Iodine is represented by I, and both $K\alpha$ peaks are consistent with the fixative. This is consistent with the composition of KI, potassium iodide used along with elemental iodine in the solution.

3.3 Orientation In Chesapeake Bay Samples.

Collections on filterpads, as seen in Figures 2–5 (*Stephanodiscus*) and 3–2 (Pollen) demonstrate the need to maintain a sense of the orientation of the sample. What is seen under SEM is the water-film interface, the wet side of the sample. In Aquatic surface films the entire sample appears wet, but under SEM, structures are identified, but it is important to remember that these would be ‘hanging’ from the surface film into the water. This is a consideration for the metallic surface films as for those collected on filterpads, the WFI side is usually collected, although with support you can collect the air-side as well. It is important to distinguish which side of the surface films is being examined. If the material is collected on a glass slide or coverslip, as you would expect with any Langmuir-Blodgett film, it is the air-film interface (AFI) that is imaged, unless the coverslip is ‘dropped’ onto the water surface, which works and is useful in some cases. Since there are small charges and surface tension, when a coverslip is dropped, sometimes the surface film ‘reorients’ or flips into another configuration. Usually this does not occur with methylcellulose filterpads, as some water is soaked into the filter seeming to stabilize the orientation of the film. Here are some examples.

It is important to outline the orientation of the samples in reporting on the collection of discrete samples. This is not a consideration in bulk samples, but the structural relationships of the components are lost. For methylcellulose pads, we are lifting the sample that has been held to the filter pad and acquired water. This means that a SEM study of that sample will observe the water-film surface, the undersurface of

the film. To help this orientation, this is called the water-film interface or WFI. Here are two examples from pollen found on the surface of a thin surface film.

Environmental microscopy often represents a convergence of opinion and observation related to the scientific traditions under which research programs are developed. A convergence of several approaches to the air-water interface, neustonic organisms, microlayer films and metallic-oxide surface films provide an opportunity to evaluate those traditions and perspectives. This study extends the application of correlative microscopy, and improves our concept of environmental microscopy in understanding surface film formation, structure and habitat. This study also improves the environmental applications of histological and histochemical methods and techniques, such as the use of Lugol's Iodine above. These aspects of the research helped to develop the concept of environmental microscopy as a means of increasing the presence of applied physics and microscopy to environmental concerns. It is in the application of environmental science that microscopy can play a much larger role in analysis (Smith and Sánchez, 2011).

3.4 Particulates and *Trichodesmium*

The first research was done to determine if iron particulates could be identified reliably by scanning electron microscopy in concert with a method of discrete or point sampling as opposed to bulk sampling, the typical sampling methods for surface microlayer films. Multiple methods have now been developed and reported for sampling the air-water interface for aquatic surface films or microlayers, but very little for the discrete imaging of components. Following the report by Smith and Dash

(1989), further application was done to examine Saharan dust and the neustonic organisms, *Trichodesmium*. Similar SEM with EDS identification was done and reported in the book on *Trichodesmium*, a chapter on iron nutrition. Because of the physiological role of iron, it is also a consideration for the metallic surface films in that every enrichment and transport will have some nutrient contribution to the biota or for iron cycling (Rueter et al., 1992).

Iron as a nutrient is an essential element. This is particularly true for diatoms and cyanobacteria, both of which have high iron requirements for photosynthesis. *Trichodesmium* is a neustonic, surface dwelling colony of cyanobacterial strands. It is classed as a diazotroph, where it can thrive in a nitrogen-poor environment, such as tropical oceans or nitrogen poor springs in freshwater. But, *Trichodesmium* relies on many iron-containing enzymes and proteins. The iron-dependent process in *Trichodesmium* and the theoretical amount of iron required for these processes are comparable to the measured iron content of the colonies found in the open ocean. In terms of what seems to be a limited iron source in the ocean, the amounts were problematic. *Trichodesmium* not only can take up soluble forms of iron, but also has unique mechanisms that allow it to use iron-containing particulates, such as Saharan dust particles, known to cross the Atlantic in tropical winds and reaching as far as Barbados and other outer islands. Even Columbus wondered why a red dust was found on the surface structures of his ships at sea (Columbus, circa 1543). *Trichodesmium*, as a diazotroph is the predominate nitrogen fixing organism in surface waters. It has some

buoyancy so is found at the air-water interface in substantial numbers during certain blooms.

3.5 Methods for *Trichodesmium*.

Trichodesmium is a marine filamentous cyanobacterium that is found at the air-water interface, a neustonic blue-green algal for capable of substantial blooms at sea. Since in the tropical and sub-tropical marine environment, iron is often a limiting nutrient, but in the tropical and sub-tropical Atlantic Ocean, Saharan dust is carried by surface finds over the ocean, over the Cape Verde islands and as far as Barbados. Could the previous study on surface film from the Chesapeake Bay be used to identify iron-bearing particulates, to answer the question whether iron particulates were captured or entrained by the *Trichodesmium* colonies and whether these particulates could be identified as Saharan dust? The previous hypothesis was that the iron particulates allowed for solution of the iron into the ocean and subsequent uptake as soluble or biologically available iron. This study showed that there was an active process to capture iron-rich particulates into the *Trichodesmium* colony, which often had stacks of trichomes, the individual filaments, bound together by a mucoid secretion. This process was confirmed by this study. This study confirmed that the biological availability of iron is determined by the wind-borne transport of particles, Aeolian dusts from African subcontinent. Collections done off Barbados were transported to Portland State University for examination by scanning electron microscopy (Rueter et al., 1992).

Observations on the interaction of living specimens showed active movement in the presence of dust particles. *Trichodesmium* colonies were collected and incubated

with Saharan dust. Specimens were then air-dried or fixed with Lugol's iodine solution, rinsed with water to remove salt crystals, sputter coated with Au-Pd and then examined with an ISI-SS40 scanning electron microscope. The spectra for the surface tissue and particles on the surface of the colonies could be analyzed individually. Particles were determined to be either CaCO_3 or Al-Si matrix. The aluminum silicate particles had high iron content. Some specimens showed the ordering of particles into rows in between trichomes, indicating that these particles may be "subducted" into the interior of the colony by trichome rotation, assisted by the mucoid secretion of the filaments. Specimens showed dust particles with an organic coating around them, as if the mucoid, polysaccharide layers on the cell surface were coating and trapping them, as if "sticky" to particles in the water. The individual cells of each filament were capable of secreting substantial mucoid material, even loops of secretion. These observations indicate the importance of cell surface sensation and activity, and that the trapping of iron-rich particles is an active process for each trichome bundle.

Specimens of *Trichodesmium* were collected in the field and placed on filterpads. Some were air-dried and some were fixed in Lugol's iodine. In the laboratory specimens were washed with distilled water to remove labile salts. Both washed and unwashed samples were analyzed by scanning electron microscopy with energy dispersive spectroscopy. All specimens showed some accumulation of dust particles, both at the extremities of the trichome bundle and along the surface. With the SEM we examined both dust particles on the tissue and the tissue surface alone in areas without dust particles. The Saharan dust with its characteristic spectral peaks for $\text{K}\alpha$

and $K\beta$ iron emission energy were easily found at the extremities and between the trichomes along the bundle. The tissues were examined at 20 kV beam energy at 1k – 4k magnification. The EDS unit, an Oxford™ Link™ AN10000 was used in spot mode to determine the distribution of elements with particular note of the distribution of iron in the surface particulates. This is consistent with the particulate with high iron seen in the Chesapeake Bay diatom, *Stephanodiscus* sp. from Figure 2–5.

3.6 Saharan dust

The iron-bearing materials in this study are Saharan dusts, shown here:

Figure 3–3 Saharan dust plumes originating from Mauritania and Senegal, West Africa, extending into the equatorial Atlantic . NOAA2009. MODIS RGB CH(1, 4, 3)

It should be noted for Figure 3–3 that Saharan dust has been noted historically by exploratory vessels at sea. Columbus reported deposits of red dust on the decks of his ships while at sea, the origin being unknown at the time (Columbus, circa 1543). Recent satellite images show considerable plumes of dust extending from Mauritania across in prevailing westward winds over the Atlantic. In this figure, dust plumes cross near the Cape Verde islands.

3.7 Experimental observations

As noted above, specimens of *Trichodesmium* were collected in the field and placed on filterpads. Field observations confirm what was seen by SEM analysis. In this description, *Trichodesmium* colonies were observed to be quite active. Dust particles attach extremely quickly to the colonies surface and due to the movement and rotation of the trichomes (the individual strands within a colony), particles would move from the exterior to the interior in a short period of time. On a scale that could be observed under a stereoscope, the morphology of the individual colonies would change over time, such as loosening and tightening, elongating, sometimes making small humps or fraying of the trichomes and reforming at the ends of the colony. Very rapid movements of the trichomes, often a gliding movement as there is significant amounts of extracellular product, a mucus-like material. Particularly at the ends of the colony dust particles would collect and then be enclosed as the trichomes glided in and out of the colony, and dust could be incorporated with by lodging in a groove and being covered over or by the rolling action of trichomes due to rotation (Figure 3–4) (Rueter et al, 1992).

Figure 3–4. EDS spectra and image of the surface of a *Trichodesmium* colony with Saharan dust particles. Note the elevated iron peak, and the lack of peaks in the tissue sample. This specimen is fairly dehydrated from air-drying in the field.

Buoyancy in the cyanobacteria is an important feature of being at the air-water interface (Reynolds, Oliver and Walsby, 1987). It may be a positive force for surface films as well, but that is not investigated in this study. However, many references to gas vesicles in cyanobacteria were considered in this observation in this *Trichodesmium* study. Positively buoyant colonies normally would not attach enough dust to lose buoyancy, but with the addition of dust in the laboratory, and continuing rotating movement and dust accumulation within the colony, a colony could be induced to lose buoyancy and sink. Some specimens showed dust particles with a sticky organic coating around them as if the polysaccharide layers on the cell surface were coating and trapping the particulates, making them easier to be incorporated into the colony (Rueter et al., 1992).

For both aquatic surface film samples and Saharan dust particles, the means of identifying particulates for elemental analysis is consistent with what was observed in the Chesapeake Bay study, giving encouragement that the discrete sample for analysis by SEM with EDS of microlayers was a good way to develop environmental microscopy as a supportive applied method in concert with instrument development (Smith and Dash, 1989).

CHAPTER FOUR

CHARACTERISTICS OF METALLIC SURFACE FILMS

Metallic Surface Films (MSF) have been characterized mostly as floating iron oxide films and mixed-valent iron surface films or iron oxide films (Grathoff et al. 2007; Easterly, 2008). Coastal dune systems and drainage favor high iron content, and thus those films reported have substantial Fe EDS peaks, as do some Cascade and Willamette Valley wetlands (Easterly, 2008). However, occasional patches or presence of other metallic combinations occur. It is with this background that the term metallic surface films or MSF is favored and used as a parallel description to Aquatic Surface Films (ASF) to encompass primarily freshwater and wetland occurrences of the films. The oceanic sea surface film or SML is favored under those environments and circumstances, but limited in the discussion here. Metallic Surface Film (MSF) must be the preferred term to describe all mineralizing surface films at the air-water interface. No other term is broad enough to be descriptive of the several elements, biofilms and interactive components of these features of the air-water interface.

4.1 South Slough national estuarine sanctuary

A sample of surface film was taken at Charleston, Coos County, Oregon, in a wetland of the South Slough National Estuarine Research Reserve (SSNES). The sample was collected on a Millipore™ methylcellulose filterpad and thus the water-film interface (WFI) is presented. The sample was mounted on an aluminum stub and coated with gold (Au) to an approximate thickness of 1.8 nm. The sample was viewed

with the Zeiss™ Sigma™ at normal settings, as the conductive coating was adequate to prevent charging.

The spectra showed a consistent presence of silicon, zinc iron, potassium phosphorous and calcium. Phosphorous is expected to be present, but in a gold-coated sample may be obscured by one of the gold peaks. Zinc and iron are the main transition metals in the surface film as well as small amounts of antimony (Sb). Organotin, element 50, has been reported from aquatic surface films several times, and appears to be enriched in surface films (Hardy et al., 1987), but antimony, element 51, has not. Organoantimony may occur, but is seldom reported, but it does form a trioxide, similar in structure to arsenic trioxide, and there are many arsenic sources in Oregon. In Figure 4–1 a sample spectrum shows relative peaks and composition. This sample presented some unique features that confirmed the hypothesis the multiple factors are involved.

Figure 4–1. EDS spectrum of WFI of SSNES metallic surface film. Note the concentrations of zinc, iron, potassium, phosphorous, calcium and possible antimony.

The site of the spectrum was also unique. The surface of the film showed not only some bacterial casts, as might occur, but some quartets of pits that were unusual. Although no specific organism was associated with the structures, there are several algal species and cyanobacterial species that typically have Four-Cell characteristics. In this case (Figure 4–2), the surface films seem to have collapsed into the structure, but there are still ridges associated with each cast.

Figure 4–2. Surface film casts of unknown organism, possibly a 4-Cell bacterial or cyanobacterial form that was not captured or had died. The quartets are about 1.4 μm - 1.6 μm , and are consistent with cyanobacteria such as *Gloeocapsa* or *Chroococcus*.

Not only were these quartets unusual, but also this sample showed several features that we have noticed elsewhere. Another sample site was used for basic measurements of this form also demonstrate not only a cluster or patch of these quartet structures, but also other layers, which demonstrated bacterial forms on separate layers.

Several cyanobacterial colonies fit this quartet structure, such as *Chroococcus*, *Gloeocapsa* and *Eucapsis*, but no wet samples were taken at this time. However, as an image, this suggests other attachment and interaction that could be documented at a later date or in another form. There is now increasing evidence that interactions by bacteria, cyanobacteria and algae and their casts are structural features of metallic surface films, and that layers have different biota or unique surface features in an of themselves. We have no data on timing of these formations, but that is certainly an aspect of future work.

In Figure 4–2, we see the patchiness of this group of quartets, but in layers below (Figure 4–3), we see evidence of bacterial forms in other layers of the films

In Figure 4–3, we find both a surface cyanobacterium, but also mineralizing granules and layers of metallic surface film structure.

Note the several surface morphologies of the surfaces. Even the four-cell groups tend to be in groups of four, another characteristics of cyanobacteria. Even if the main cell body is lost, these characteristics are useful ones for future identification. The following images support cyanobacterial interaction and bacterial interaction in film structure and formation.

Figure 4-4. A sub-sample to illustrate bacterial casts on lower films.

Figures 4-4, 4-5 and 4-6 illustrate not only the cyanobacterial casts in the upper layer, but also the presence of other bacterial forms incorporated into a lower film as well as some other bacterial casts and a cyanobacterial strand on the surface of the film. Again, it is important to take a moment to review orientation as this is the water-interface of the metallic surface film, now seen with layers and possible points of mineralization in a separate layer from the smooth surface.

Figure 4–5. Cyanobacteria casts and below a separate bacterial shape in the lower layer, seen as a separate feature.

Figure 4–6 Surface cyanobacterium and bacterial casts on the water-film interface.

In Figure 4–4, we again find consistent iron and zinc peaks estimated as an apparent Weight % concentration of Fe 5.71. Note the high Wt. % of zinc in this sample. This confirms that transition metals other than iron as constituents in MSF.

Figure 4–7. An EDS spectrum to accompany Figure 4–4. For a metallic surface film, high levels of both iron and zinc confirm that mixed transition elements can be present.

1 μm
Figure 4–8. The cracks demonstrate not only surface features, but also expose small spherical sub-film structures and evidence of a lower layer with suspected spirochaete-like shapes. Note the bacterial cast in center left as well as the surface cyanobacterium. Spherical bodies are consistent with amorphous mineralization.

In EDS spectrum 4–7, there is again a high level of both iron and zinc, confirming consistency with the earlier sample and confirming that other transition metals contribute to the composition of the metallic surface film. Not only do the surface casts and bacterial structures demonstrate a consistency with complex layered structures, but the consistency of both iron and zinc indicate a much broader range of components than has been offered in the past. That additional elements are part of the structure indicates that a broader range of environmental surface film sampling should be included with other water evaluation programs.

Figure 4–9. At a magnification of 129.9 kX, the larger spherical bodies, which we associate with mineralization, are approximately 100 nm, leaving the smaller spherical bits at about 10-12 nm, consistent with hydrated amorphous mineralization.

4.2 Analysis of a bacterial cast

Bacterial impressions or casts are a unique feature of metallic surface films. I have called them bacterial footprints to highlight the fact that even if we do not know the species at this time, the morphology of a coast is unmistakable, yet diverse. It is these casts that may correlate with other bacterial or cyanobacterial features found in older rocks, meteorites or mars samples that have not yet been identified. In astrobiology, such morphological features might be better defined using the metallic surface films and structures.

This case shown in Figure 4–10, and the spectrum in Figure 4–11 demonstrate a bacterial morphology in size and shape. Here, I am using the size of the bacterium at 1.0 μm in width and approximately 1.8 μm in length as a guide. More accurate measurements are seen in other figures. In this case the spectrum was used to confirm

the levels of iron and zinc at the edge of the sample in the second EDS spectrum, Figure 4-12.

Figure 4-10. A bacterial cast or collapsed film from the SSNES sample.

Figure 4-11. Spectrum of Metallic Film Surface near bacterial cast.

Figure 4–12. Spectrum of film sample on the bacterial edge of the cast. Values for iron and zinc continue to be consistent in composition with other spectra.

4.3 Interim conclusion on composition

In Figures 4–11 and 4–12, we see a general consistency of elements with the exception that a small amount of antimony is recorded near the edge of the bacterial casts. The dominance zinc and iron as transition metals supports the broader scope of inclusion to the expanded definition of metallic surface films. In general these EDS spectra confirm the presence of transition elements other than iron, particularly zinc in this case, manganese in other samples discussed in a later chapter. This forces a reevaluation of floating metallic films at the air-water interface from an iron-based film to one of a more complex composition, which is more variable than previously reported, and more consistent with metallic enrichment of the aquatic surface films (Duce, Quinn, Olney et al. 1972; Hardy, 1982; Hardy, Crecelius and Kocan, 1986).

4.4 Incorporation of insect parts into surface films

What evidence do we have for the transition of a aquatic surface film to a metallic surface film? Given the small number of samples, we have no series with which to work, but we do have structural clues to film formation. Does a film bind other materials into the film? Most metallic surface films examined show some incorporation of other materials, such as pollen grains, dusts, crustacean or insect parts, all of which may be present at the air-water interface before film formation or as Eolian deposition. They are not all deposited on the air-film side of metallic surface films. In these samples from the South Sough NES, the incorporation of these insect parts suggest that the film formed in and around the cuticular surfaces. The following figures follow terrigenous insect parts and structure.

Figure 4–13. SSNES sample, the incorporation of arthropod or insect parts into metallic surface film structure.

Figure 4–14. Enlargement of Figure 4–13 to show that the metallic surface film fills and integrate in and around the arthropod or insect carapace elements. This supports the formation after the insect parts have been entrained into the aquatic surface film. Note the bacterial pits also present.

The SSNES sample was examined for integration of the metallic surface film as a clue to timing as well as structure. We reasoned that if the metallic surface film formed after the insect parts were already entrapped or entrained into the organic microlayer or aquatic surface film, scum or other surface material, the metallic surface film would show this by including or covering or forming in and around the terrigenous material or allochthonous bodies. This turned out to be the case. In Figure 4–14 we find that the metallic surface film has, indeed incorporated the arthropod or insect parts into its basic structure. It supports the idea that at least part of the film was already in a surface film environment with some buoyancy or structure. The spines or cirri can be seen extending through the surface film. We see this again in Figure 4–15.

Figure 4–15. Incorporation of insect parts into a metallic surface film.

Not only does Figure 4–15 confirm a similar incorporation or inclusion of insect parts into the metallic surface film, but it also shows several features such as bacteria pits and included bacterial forms and a surface material which is in part above the surface, but also partly incorporated into the film structure. Most of the structure is bright, appearing whitish, which means it is emitting electrons at a higher rate than the surrounding metallic surface film. This is consistent with biological material, and also gives evidence that this material acts as a point source on the films surface. Again, this is the water-film interface, and these surfaces would extend downward into the water. As part of the water-side, this also suggests that the insect parts were present before the mineralization of the metallic surface film, an inclusion into film structure.

4.5 Interim conclusions on inclusion and formation

The sample from Coos Bay, Oregon proved to be enlightening and supportive of the working hypothesis. It yields strong documentation that bacterial and cyanobacterial casts and structures are important features to film development, and might, in the future be able to shed light on the timing for formation. Particularly useful was the incorporation of insect parts, clearly present at or before the time of metallic film formation, and that zinc was an important, even dominant transition metal part of the metallic film structure. The timing of inclusion is suggestive at this point, but is consistent with the working hypothesis that the film structures are both diverse and complex, part of which would be inclusion of terrigenous material

Further, the fact that we could discern both layers and spherical bodies at high magnification consistent with amorphous mineralizing structures matches and is consistent with a later sample, quite distinct from this one to support the idea of multiple layers of material in structure and mineralizing bodies in formation. In summary, these images support the working hypothesis in specific ways.

CHAPTER FIVE

MINERAL STRUCTURES BY TEM/STEM

Following basic structure, this microscopic study sought to examine in a preliminary way, the crystalline or fine structure to determine whether amorphous structure could be located in the same sample as ordered or mineral states. While the previous focus has been on the iron oxides, the presence of manganese was found to be most revealing that the new term metallic surface films or MSF is more comprehensive. Many terms describe floating iron oxide films or mixed-valent Fe films in the literature as a feature of the air-water interface and a surface component of wetlands, springs, seeps and tidal flats (Grathoff et al., 2007). Previous studies suggested both mixed-valent properties of iron oxides as well as amorphous mineral states. We studied a typical, thin metallic surface film from Johnson Creek watershed, Clackamas County, Oregon. Both manganese and iron nanocrystallites were observed and a possible mineral structure proposed. We believe that this is only one of several possible nanocrystallites that may occur in metallic surface films.

Recent progress in transmission electron microscopy (TEM) instrumentation allows for structural and chemical analysis in geological, environmental and geomicrobiological sciences at atomic and nanoscale levels. We demonstrate this TEM capability in application to transition metal containing substances resulted from the activities of “iron bacteria”, as their sole energy source. The substance, often appeared as silvery, water-surface film, is common for natural wetlands, ephemeral pools, springs and seeps with metalliferous sediments and weathered rocks. Depending on the type of

bacteria, concentration of organic material and dissolved oxygen in the water, bacteria may either reduce insoluble ferric oxide (Fe^{3+}) in aquifer soils to soluble ferrous hydroxide (Fe^{2+}) or convert soluble ferrous iron (Fe^{2+}) into ferric oxide. Thus, the valence state of the metals in the films allows for the elucidation of biogeochemical processes at the air-water interface and in the water. In addition, humic substances as binding agents, bind iron, manganese, copper and zinc, and allow for further interaction with the films, which are more accurately described as metallic surface films.

5.1 Methods

The samples were collected at natural sources in Oregon and prepared as described. Electron Energy Loss Spectroscopy (EELS) was employed to determine the valence state of transition metal ions. The structural and chemical analysis of the samples has been performed by both FEI™ Tecnai™ G2 ST field-emission and LaB₆ JEOL™ JEM 2010 electron microscopes (both equipped with EELS and EDS) at PSU and PNNL, respectively. When resolutions on the order of a crystal lattice dimension is needed, hrTEM fills this imaging niche. These instruments require a skilled operator, as well as a very well-prepared sample. The sample must be very thin, on the order of tens of nanometers. One may be lucky and find a thin edge, but often several samples must be tried. A typical TEM bright field image for an amorphous film is shown in Figure 5–2, as it happens a thin edge. In agreement with previous observations (Grathoff et al., 2007), amorphous in SAED patterns have been shown. However, TEM images of the same area at higher magnifications/ resolutions (Figure 5–1) show that the films also include nanocrystallites of about 2–5 nm in size in ordered states. This

suggests that some products of bacteria activity may be nanocrystallites rather than amorphous mineral. However, subsequent SEM images indicate spherical bodies consistent with amorphous mineralization, where EDS indicates that the samples contain a mixture of iron and manganese oxides, organic material and traces of other elements, particularly manganese, nickel and zinc. The EELS spectra of the samples reveal the transition metal “white lines” features, i.e. L_{2,3} edges. These L_{2,3} features correspond to transitions from the 2p_{1/2} and 2p_{3/2} states to unoccupied 3d_{3/2} and 3d_{5/2} states and are widely used for the estimation of valences of 3d transition metals.

After subtraction of the background and integration of the area underneath both EELS peaks within a 4 eV window, the ratio between the “L_{2,3} areas” yields the actual valence state. In the case of iron, this ratio was 5.2 ± 0.3 and close to the value obtained for a magnetite reference sample in which the iron oxidation state is Fe^{+2.66}. The ratio between the L shell L_{2,3} areas was for manganese 3.4 ± 0.5 . This is between the tabulated ratios for Mn₂₊ = 4.3 and Mn₃₊ = 2.3, and gives the average oxidation state of manganese as Mn^{+2.45}. The EELS results confirm the mixed valences of metal ions as suggested earlier (e.g. Grathoff et al.) and indicate on incomplete oxidation process, or possibly a more dynamic state of mineralization processes, where there may be some atomic movement within the matrix. This is possible with highly hydrated minerals, or in our case with layered metallic surface films with a complex of organic and mineral structure. This directs me to consider future studies of *in situ* TEM under more controlled conditions. The more dynamic state of oxidation is consistent with mineralization patterns shown for calcium by Nielsen et al., (2014a), and makes a

compelling case for further work using *in situ* TEM under controlled conditions. This is particularly true where the mixed-valent iron concept offered by Grathoff et al., was met with caution. The following figures demonstrate conclusively from the same sample that both amorphous states and crystalline states can be found.

In the following two figures, we compare the images for ordered states and amorphous mineral, both from the same sample. It is significant that these can be found in the same sample and that manganese was the dominant transition metal component.

Figure 5–1. High resolution TEM image of ordered crystalline structure

High-resolution studies for mineral structure are quite possible for natural metallic surface films. There is often a bias, I believe, for biologists and geologists to seek the brightest film they can find, and yet what you need to seek is the thinnest, newest edge, or some thin patch of interesting material. The brightest, thickest film is

certainly the most noticeable in the field, but cultured films may give you opportunity to find the thinnest edge. Given that we have found several interesting mineral states, there is a leading edge towards finding other structures, which I am sure exist. The lack of instrument time is a major problem, so that one is restricted to opportune moments of access. Figure 5–1, 5–2 and the spectrum at 5–3 confirms again that composition is more diverse in elemental structure.

Figure 5–2. Amorphous mineral structure at 57 kX

Figure 5–3. EDS spectrum of sample with high Mn and low Fe peaks.

In Figure 5–3, the EDS spectrum shows strong manganese $K\alpha$ and $K\beta$, iron $K\alpha$ and a small $K\beta$ peak. Manganese is a dominant transition metal in the film. Copper peaks are from the copper TEM mounting grid in this case. Further studies were done to resolve possible structural and crystalline choices. This is not a complete analysis by any means, but with the instrument time available, gave us a reasonable comparison to say that there are other mineral options other than mixed-valent iron.

The analysis offered a $MnFe_2O_4$ as a fit for the high manganese sample, given as a 227 lattice termed $Fd-3m O_2$ (227). The following supportive data were obtained (Figure 5–4, Figure 5–5 and Table 5–1):

Figure 5–4. Diagrammed structure. Fe is seen in White, Mn as pink, and Oxygen as '01' red-on-white. While this is an estimate to compare with magnetite, the standard for this material, it offers the possibility of complex mineral structures within an iron oxide matrix.

Table 5-1. MnFe_2O_4 Crystalline Structure:

N	d(nm)	2Theta	Int	(hkl)	M
1	0.4913	18.040	141.6	111	8
2	0.3009	29.660	294.1	220	12
3	0.2566	34.940	1000.0	311	24
4	0.2457	36.540	87.1	222	8
5	0.2128	42.460	229.1	400	6
6	0.1952	46.480	2.4	331	24

MnFe_2O_4 . Support for a complex mineral ferromanganese oxide mineral structure.

Figure 5–5. Supporting analysis for possible lattice structure, for an MnFe_2O_4 mineral.

In Figure 5–6 a three-image composite showing the three mineral patterns found in this analysis, A, B, and C are paired images and the diffraction set.

Figure 5-6. Three analyzed images of three possible ordered states to the sample, pairs labeled A, B, and C. Diffraction pattern on the right side.

The analysis of the sample as represented in Figure 5–6, give a better picture that several possibilities exist, and that several possibilities might exist in the same sample. This does not tell us how and where mixed valent states might be located, but suggests that many dynamic or mixed states of mineralization might be much more common than is usually presented. Possible candidates are still Magnetite $d_{220} = 2.95 \text{ \AA}$, and MnFe_2O_4 $d_{220} = 3.0 \text{ \AA}$. A radial profile from the diffraction pattern labeled as (RW0410 200 mm) was also taken, suggesting amorphous material, but peaks that were too broad to identify the phases unless more work is done to refine the sample.

5.2 Interim conclusions

It was a confirmation of the working hypothesis that both manganese and iron were found in the sample. It was an important finding that alternate mineral structures might fit the data and a structure proposed, and it was a confirming spectrum to see that manganese and iron nanocrystallites were present. This finding is only one of several possible nanocrystallites that may occur in metallic surface films, and further work could refine these initial findings. It is consistent with the working hypothesis that while iron oxide minerals are a dominant feature of the films, other transition metals may also have a dominant or co-dominant place in composition

It is a useful observation, I believe, that there is a bias for people to reach for the brightest sample, which tends to be the thickest, when, in fact, you need to seek the thinnest and newest edge that will fit the instrument's design. Only then do you get the spectra and diffraction analysis that is possible. In showing once possible alternate structure, other structures are now seen to be present and should be studied further.

CHAPTER SIX

CHARACTERISTICS OF A NEW FILM FORMATION

An opportunity to find a new film in the field is not common. In this case, a heavy shower the previous day allowed for a thin new film to form overnight at Yaquina Bay, Lincoln County, Oregon. We know that it formed approximately 12 hrs. before collection because of not only the rain but also of the tide. Unlike many other metallic surface films, this one was wispy, very thin, but otherwise remarkably undisturbed. The main collection was done with Millipore™ methylcellulose filter pads due to the thin film of water and the fragile nature of the film itself. Samples were air dried in the laboratories of the Hatfield marine Science Center and placed in small Petrie dishes. A second sample was obtained from the vacuum chamber directly onto stubs, but these samples were rejected in the end because of sand grains throughout.

The site is documented in the following photographs (Figures 6–1 and 6–2):

Figure 6–1 Yaquina Bay, Oregon, Pond.

Figure 6–2. Sample vacuum chamber.

In Figure 6–1 the grounds have a surface pond of rainwater, the sampling site is off to the right (south) on an adjacent sand flat. In Figure 6–2, the vacuum chamber with gold-coated aluminum stubs is shown open in the field on *Salicornia* marsh plants.

There was no overnight rain after the cleansing showers of the previous day.

Two figures of the metallic surface film are shown with forceps as a size marker (Figures 6–3 and 6–4). These films were very thin, typical of a new formation and consistent with the weather observed from the previous day. An outgoing tide indicated another factor in the new formation of the film. The film was very fragile, but could be retrieved on filterpads. These were air-dried in the nearby facility, the Hatfield Marine Science Center, abbreviated HMSC.

Figure 6–3. Metallic surface film near the the *Salicornia* line of the tide line. The film is nearly transparent in some places.

Figure 6–4. Another view of the same metallic surface film. The wispy, thin transparent nature of the film is evident.

The shoreline and wetland environment at Yaquina Bay, HMSC, Lincoln County, Oregon was the site of the sample. This area is unlike other coastal dune systems with persistent high iron seeps and springs. However, the marine environment has wetland sand consistent with high iron content. The flow of water in the sands is unknown, but the presence of a pond slightly uphill from the site of collection could be a major source. The samples are identified as ORYB in the following treatment. The presence of diatoms in good number is treated here as attraction to iron as a nutrient.

The samples were examined at the Center for Electron Microscopy and NanoTechnology at Portland state University (CEMN). The diatoms were not identified to species at this point, only their presence noted in relation to the film. An FEI dual-beam system was used with an Oxford™ EDS system. The sample was coated with a thin coating of carbon to reduce charging effects as this sample was collected on a Millipore™ methylcellulose filterpad, due to the thinness of the water film over the sand at the collection site.

At low magnification, it was clear that diatoms formed a larger portion of the adjacent material (Figure 6–5). The film was thin with few distinguishing features.

Figure 6–5. ORYB, Yaquina Bay, Oregon. Diatoms and metallic surface film are clumped to the right.

However, with increasing magnification, two layers could be detected and a site chosen for examination. It was not obvious that two layers were present, and the instrument had to be adjusted for a good representation of layering. The purpose of this study was to see if a newly formed film had the characteristics of older films or layers of structure and if the layers differed in composition, and resolved these questions.

In the following image (Figure 6–6), instrument adjustment gives a good perspective, and illustrates how the SEM mode of the dual–beam can separate the layers. This was not obvious at first, and the two appeared as one layer with cracking. Once we had a good imaging separation, the layers could be analyzed separately. In Figure 6–7, the EDS spectrum in mapping mode gave a color distribution of elements.

Figure 6–6. SEM image to show two layers over filter pad substrate.

In the following three images, Figures 6–7, 6–8, and 6–9., two elements were selected to show differences in distribution between the layers, iron and nickel. In Figure 6–7 for iron, the distribution between the two layers is not large, and the layer next to the filterpad is almost indistinguishable from the upper layer. However, for nickel, we see that most of the nickel is located in the upper layer, and the distribution between the two is apparent.

Figure 6–7. Color mapping image of two layered metallic surface film.

Figure 6–8. Iron distribution.

Figure 6–9. Nickel distribution.

Figures 6–10 and 6–11. EDS distribution of selected elements from Figure 6–7:

Figure 6–10. Upper metallic surface film layer. Wt.% of Fe is 10.41. Wt.% of Ni is 0.66. There is no significant Zn peak in the upper metallic surface film. As a comparison Wt.% for K is 2.31 and Wt.% for S 1.86.

Figure 6–11. Lower metallic surface film layer. Wt.% of Fe is 3.41; Wt.% of Ni is 0.47; Wt.% of Zn is 2.84. As a comparison Wt.% for K is 0.51, and Wt.% for S is 0.88.

6.1 Interim conclusions on composition and layering

Figures 6–8 and 6–9, compared with the spectra, EDS Figures 6–10 and 6–11, show differences in elemental distribution in several respects. The EDS spectrum shown in Figure 6–11 has detectable levels of zinc, not found in Figure 6–10, and from Figure 6–9, a distinction in the levels of nickel can be shown.

The fact that ORYB was a new film yet with some unequal features again supports the working hypothesis that layers can form rapidly and that the layers can be unequal in distribution of elements. The purpose of this study was not to make an extensive comparison of the film, but to show that there was documented inequality in elemental composition in two recently formed metallic surface films. Secondly, the purpose was to show that while iron dominated one layer of the sample, other transition metals were present, in this case nickel and zinc. Mapping images Figures 6–8 and 6–9 show this very well, that in the iron map, iron is roughly in equal distribution over the two layers. This is not so for nickel, where a distinction can be made between the two layers.

EDS spectra allow us to look at calculated Wt. % of elements as an estimate of composition. The calculated Wt. % composition also show differences. In Figure 6–10, the Wt. % of Fe is 10.41, and the Wt.% of Ni is 0.66. In Figure 6–11, the Wt. % of Fe is 3.41; the Wt. % of Ni is 0.47. As a comparison of variability, in Figure 6–10, there is no significant Zn peak in the upper metallic surface film, while the Wt. % for K is 2.31 and Wt. % for S 1.86. In the lower film zinc is present at Wt. % of 2.84, while the Wt. % for K is 0.51, and Wt. % for S is 0.88.

The separation of layers suggests that there may be some separation of other physical factors, such as humic substances, small forces, such as ionic charge, and surface tension, some factors that allow for separation of film formation. These have yet to be investigated, as most charge studies, such as colloidal materials are done in bulk and not as discrete samples. Analytical imaging gives us some direction to the research and a means of developing better research questions as well as instrumental operation and design questions.

In terms of the working hypothesis, the fact that a new film already shows both diversity in composition and an elemental difference in two newly formed layers is confirming. Further, that diatoms already show some alteration, clumping or attachment to the newly formed film, suggests active participation in using metallic surface films as habitat or nutrient source, when they become available. These diatoms were not classified at this time, but diatoms of Yaquina Bay are well known, and a future identification is planned.

This field experiment is important for three reasons. One, that it is a newly formed film where maturation and Eolian inputs are minimal. Two, that even with new formation a diversity of transition elements are present in the structure. And Three, that differences between newly formed layers can be distinguished. In this case the fact that iron appeared to be uniform between both layers but that nickel presented a difference in composition between the two layers confirms that the detection and analysis by comparative microscopy and EDS spectra are useful tools to document the diversity of structural possibilities.

CHAPTER SEVEN

AQUATIC SURFACE FILMS IN FLOWING WATER

In order to show how microscopy can demonstrate both surface tension and surface film formation, an area on Johnson Creek, Clackamas County, Oregon was found to be an opportune demonstration of how partitioning of organic materials might occur in surface film formation. Flowing waters, natural waters, have special problems and characteristics. It is generally believed that stable accumulation does not occur, but this demonstration shows how an impediment or barrier can assist in surface film development and formation. This was a perfect demonstration of surface film organic structure in development to form scums and foams. It also demonstrates the ability of organic surface films to modify the air-water interface in physical ways. In this case it was not necessary to erect an artificial barrier, as the natural barrier was adequate to demonstrate the formation of a stable film pressure environment, capable of forming advanced standing wavelets to define the leading edge of organic surface film. Analysis of the visible film and scum shows elevated levels of transition metals, particularly iron oxide. We believe that these are both nanocrystallites and microcrystallites.

7.1 Surface films in flowing water

Flow in Johnson Creek was substantial following a series of rains (Figure 7-1). Here it was a warm and sunny day 29 °C. Samples on glass slides, coverslips and filterpads were taken of both thin films and scums. This visible accumulation that distinguishes transparent aquatic surface films in early stages of transparency and

formation from clean water as a film pressure barrier, quite capable of asserting film pressure, in fact enough to form resisting wavelets. Transport at the air-water interface changed dramatically from the stream speed to a very slow entrainment into the surface film. The anastomosing form of the wavelets can also be seen as stable, standing waves, a physical parameter of interaction with surface films and water at the air-water interface as a boundary layer condition.

Figure 7-1 shows the general environment of Johnson Creek at Tideman Section.

The objective of this study is to demonstrate the presence and stability of aquatic surface films in a flowing environment and the dynamic states of accumulation of the surface films. The fact that there is active resistance to the film to such a degree that there is a measurable ‘front’ as the film developed, but also that it forces the

formation of a stable set of standing wavelets in advance of the ‘front’. This front is known as the D-Line or D-Ridge and has been reported as the demarcation line for films compacted by moving water (McCutchen, 1970). Visible material on the air-water interface also demonstrates the velocity of a particle consistent with streamflow which then slows to the nearly static pace of the stable surface film, then moving slowly to the accumulating area of heavy film, scum and foam. All the surface materials then accumulate in the scum (Figures 7–2, 7–3).

Figure 7–2 shows the natural barrier and zone of surface film formation and scum/foam.

Figure 7–2 is an overview of the dynamics of active freshwater streamflow and the accumulating pool formed by the barrier. But, even at this distance to can clearly delineate the leading edge of the surface film well in advance of the line of

accumulating scum. The leading edge is a dynamic demonstration of the pressure exerted by the film at the air-water interface. This can be measured. The organic molecules and neustonic organisms seldom seen as they are widely dispersed at the air-water interface (AWI) now become entrapped and entrained into a thin and then thick surface film with its own properties. This is now stable enough to present other organic materials, nanocrystallites, insect parts, pollen grains and other small materials.

Figure 7-3 shows the standing wavelets in the center, streamflow is from left to right. The right surface is a measurable aquatic surface film leading to a layer of accumulating scum and foam. Note the wavelets and the anastomosing character of the wavelets, a resistance to the film and film pressure to the right.

In Figure 7-3, we see a closer view of the leading edge of the surface film. The demarcation between film and film pressure demonstrated convincingly that film pressure has physical attributes that can be measured. The development of wavelets in

advance of the leading edge of the film allowing the formation of stable, standing wavelets is important. What is particularly dramatic to this demonstration is the rapid movement of particles on the left as they approach the film ‘front’, and the dramatic reduction in velocity as they hit and are entrapped into the surface film itself.

This is seen again in Figure 7–4, where the surface film, or scum can be seen accumulating layers of surface material. McCutchen (1970) reported some basic features of surface film compaction and the establishment of D-Lines or Demarcation Lines regarding film edges, which is what we see here.

Figure 7–4. ORJC show the D-Line and standing wavelets and accumulating film to the right. Streamflow is coming from the left, West.

Samples were taken to the right of the D-Line, in the accumulating organic aquatic surface film, but in the transparent area, not in the scum of orange heterogeneous film. We know from sea surface film or microlayer studies, that the microlayer can be of substantial thickness, sometimes 200 μm or so. This has been seldom measured in flowing freshwater, and as not done so here, but the surface film pressures were indeed, substantial, based on previous experience.

7.2 Aquatic surface film, ORJCt at Johnson Creek.

Samples were taken at the Tideman section of Johnson Creek, Multnomah County, Oregon. Metallic surface film sites were dry at this point, so a survey for aquatic surface films were undertaken, with the unique observation of D-Line Film pressure and accompanying wavelets and surface film. The surface film at Tideman section at the above surface film had a good diatom population despite the flowing conditions. As with the ORYB samples, the diatoms and other biota are noted, but not classified because of limited sample comparison.

Figure 7–5 shows examples of the freshwater sampling materials. In this case a 6 ft. copper tube (1.82 m) was used to mount a pinching holder, which would hold each item. It could hold a single filter pad, or a 12 mm coverslip with ease, and even a 1 in. x 3 in. glass slide (25.4 mm x 76.2 mm) was stable for this collection. The objective of this set of materials is to show a set of simple standard methods, which are applicable over a wide range of sampling environments. Ease of collection is important when access itself might be difficult and even temporary for tidal or windy conditions. These

methods are also applicable for disrupted or damaged sites, such as oil and hazardous or fecal contaminated sites, where you wish to remain out of close range.

Figure 7–5. Selection of sampling materials. 1: Plain, white Millipore filter pad, 25 mm, and the blue separating paper. 2: 12 mm glass coverslip. 3: 1.0 in. edge of glass slide (Approx. 25.4 mm).

In the several trials, glass is consistent with what is known about organic films on water. Not only do the films remain connected, when there is enough natural film pressure pushing against the films. This is not the case with metallic surface films, where because of the mineralization, film pressure does not seem to be a deterrent to collecting a good sample. But, you still must be able to slowly collect the film without significant disruption. Similarly, the collection onto a filterpad seems to keep the orientation of the materials in place, and this has been consistent in sampling.

Methylcellulose filterpads have several benefits. They are a good ‘white’ color comparison. They tend to sink when wet, but do not break, as glass coverslips tend to do. Glass, however gives a very reliable discrete or point sample (Smith, 1991).

Figure 7-6. At 15 kV. Collected on a 25 mm Millipore™ filterpad. WFI, water-film interface. Note the variable thickness of the film.

In Figure 7–6, we have an example of a surface film with thin and thick areas and a couple of diatoms. There are a number of small particles consistent with dust a few unidentified materials and a thicker ridge of granular material at the lower right. In Figure 7–7, the amount of diatom frustules has increased, and there is a diversity of diatom material. The EDS spectra were taken in several locations, but the example was

selected from the aquatic surface film from this image as typical. Note that unlike metallic surface films, there are few cracks and platelets.

Figure 7–7 illustrates a diversity of materials including several diatom species of a diversity of size ranges. This is consistent with a productive diatom assemblage that may be using the iron and other minerals for nutrition.

Figure 7–8, below, is a slight enlargement of Figure 7–7 to focus on the diatom that is also covered by or entrained or entrapped into the aquatic surface film matrix. Recall the orientation, that as collected on a filterpad, this surface is the water-film interface with this surface face down into the water. All these bits are adherent to the film or part of the film and would most likely fall away upon collection if there was not some connection or adhesion to the film itself. Some are inclusive to the film matrix.

Figure 7–8.

Adhesion to the matrix. Note the diatom that is covered or included into the aquatic surface film matrix itself, as seen in center left.

The aquatic surface film matrix is, in my opinion, enriched in iron and other transition metals, particularly manganese. In the spectrum seen in Figure 7–9, while the iron peak is slightly larger than the other samples, it is consistent in that a small amount of manganese is also present. In this sample the phosphorus and sulfur peaks seem to be slightly lower than expected. Silicon is probably elevated because of the diatom frustules available. Grathoff et al., (2007) reported that silicon and iron might be related in proportion, but Easterly (2008) did not find a ratio. The abundance of diatoms suggests not only adequate silicon, but it also means natural dissolution of diatom frustules and an adequate amount of silicon ions as a source.

Figure 7–9 EDS spectrum from an area of aquatic surface film. However, notice the full peaks for iron a Wt.% value of 55.96, a very high value; manganese Wt. % estimated at 1.81, and for comparison K at 3.57, and S at 0.33.

This sample was taken away from visible diatoms, but there could be diatom fragments, so the high Si peak at 9.13 Wt. % is not unexpected. The Wt. % value for iron at 55.96 Wt. %, a very high value; manganese Wt. % estimated at 1.81, and for comparison K at 3.57, and S at 0.33. Ca has a well-defined peak and Wt. % value of 3.37. Titanium does show up in these samples at 2.53 Wt. %.

7.3 Interim Conclusions

Surface films in flowing water have some special conditions and properties in need of further work. In this case a barrier presented itself giving us one of the best demonstrations of D-Line formation I have ever seen. This is important to demonstrate as most surface films, while present, are dispersed and offer not measurable film pressure. Here, the film pressure has developed enough to form the D-Line and to

develop standing wave patterns seen in the wavelets, a characteristic of organic film at the air-water interface (McCutchen, 1970). While most collections, particularly for metallic surface films, are found in quiet wetland situations, ponds or seeps, here we have an example of enrichment in advance of scum formation and accumulation. The sample from the transparent film in advance of visible scum proved to be enriched in iron and other metals, and was colonized by a rich assemblage of diatoms of several species. This was not a diatom study, but we know that Johnson Creek is fairly well characterized, although I note that some of the species seen are apparently not on the current list, indicating that diatoms colonizing the surface film may be a different assemblage than those taken from benthic materials, the most common sampling site. Johnson Creek at Circle Ave has a site code of GS452912122291200, and the site ID at the Academy of Natural Sciences of Drexel University, the Ruth Patrick Center for Environmental Research, is Site 19119. This is a project for future work.

Aquatic surface films or microlayers are enriched in organic chemicals and heavy metals when compared to the bulk water 10 cm. below the surface (Hardy & Crecelius, 1985; Carlson, 1982). Enrichment calculated from a discrete sample cannot be made other than the representative elevation of the EDS peak for that element. It is only noted for iron, manganese and zinc for this study in comparison to some of the other samples taken. In general, this also supports the working hypothesis that in the process of film formation, several elemental enrichments are present and part of the process. Even for flowing water, the formation of a documentable surface film is seldom done, yet could yield further interesting results.

Aquatic surface films in freshwater environments even flowing waters should not be omitted from environmental studies and evaluations. The surface film can be collected and measured, and the comparison can be a useful one. If enrichment occurs, partitioning of transition metal nutrients and phosphorus are also enriched, and if surface film components are not considered, a bulk water measurement may not show the enrichment factors that are present. It may well be the cases that many minor elements are entrapped or entrained in surface film binding, such as occurs with humic substances, and thus can be reduced in bulk water samples.

Early in this study I proposed that by tradition, we have been trained to splash away the surface to get to the water, when in fact you have removed some essential ingredients from your sample. While that comparison was not part of this study, the methods developed here could expand this assessment or evaluation of surface films as another part of environmental impact, particularly for contaminated or restoration sites or for damage assessment. There are many features of the air-water interface that have applications to a wide variety of environmental processes and concerns, only a few of which are being used effectively.

CHAPTER EIGHT

FOCUSED ION BEAM MICROSCOPY AND LAYERING

The dual-beam Focused Ion Beam (FIB) is a unique tool to microscopy with many possibilities for environmental samples. Metallic surface film structure can be revealed by the focused ion beam instrument. Grathoff et al., (2007) indicates that the floating iron-oxide films are uniform in structure except for the several cracks, flocculants and some surface features, which are described there. Improvements in microscopy, such as the use of this instrument helps to resolve unique features of the metallic surface films (MSF). The sample in Figure 8-1 was taken to examine an edge.

Figure 8–1. SEM image of a metallic surface film with higher electron interaction volume to reveal the inner structure, a suggestion of particulate or other nanocrystallites. (JEOL6300F)

In this study, a sample of what was then called mixed-valent iron oxide was taken to examine the edge of the surface film, but in adjusting the instrument, found that there was a suggestion of particulate structure to the film. Following this observation revealed that there is indeed more structure to the metallic surface film. The sample was collected on a Millipore™ methylcellulose, filterpad from the ORST site, and transported to PSU for examination on the JEOL 6300F Field Emission scanning electron microscope, using 20 kV acceleration voltage to increase the interaction volume of the electron beam, which in the end revealed that there is a suggestion of particles or fine structure to the film. This particular film, ORST from South Tabor wetland soil, cultured to allow a metallic surface film to develop under quiescent circumstances so that a thin film could be collected. In order to access this structure another instrument was used, the focused ion beam.

This study used the prototype FIB discussed previously, to mill away some of the surface to reveal inner structure of the surface film. As with other samples it was collected on a Millipore™ 25 mm filter pad, a 12 mm subsample was cut into a circle and mounted to a 12 mm aluminum stub on conductive carbon film.

8.1 Description of the FIB prototype instrument

Instrument Description: Prototype Focused Ion Beam System with SIMS.

In order to mill the sample and to expose layers, a prototype FIB system in the Erik J. Sánchez NanoDevelopment Laboratory was utilized to expose the layered structure of a metallic surface film. This is partly discussed in Chapter 2 also. The focused ion beam system mills a surface of any material, solid or biological, through the use of a high

energy beam of heavy ions, gallium in this case, Ga^+ , impinging on the surface of the sample. It works on conductive as well as non-conductive samples, depending on which options are installed on the system and available to the user. In this case standard milling with a larger beam was used. When the sample is milled away and losing mass, it also releases secondary electrons, which can be captured exactly like a scanning electron microscopy, hence the images seem very similar. Although incredibly useful, Ga^{2+} ion beam typically mills very slowly, which makes it difficult to go through very thick samples. Softer materials are removed at faster rates, giving the opportunity to estimate density of the material. Sometimes a pillar forms when there is hard material on the surface and softer material between or below. It is much like a basalt cap upon soft volcanic soils, but at the microscale.

The system consists of a high-resolution gallium ion gun with software capable of assembling a series of images in frames, which can form an image stream, a movie. The energy of the ion beam is approximately 25 kV, normal incidence with 300 pA beam current, 90 ms pixel dwell, 1024 x 884 image resolution. Pixel size is approximately 32 nm, calculated. The spot size is considerably larger than a more usual 100 nm. But, using FEI™ software, *image.exe* the resulting spot is approximately 171 nm or about five pixels. We lose some resolution and gain some speed. The milling rate is approximate, but resulted in a suitable image, proving the structural analytical image. In the following images, two areas were milled away to expose the structural elements of the film. What this revealed to us was that there is indeed a very thin surface layer obscuring the more granular material below. This is consistent with the

SEM images where the surface is often seen as plain and smooth. As in Figure 8-1, by adjusting the settings and allowing structure to be revealed, microscopy allows for a more accurate view of microstructure and hopefully a better characteristic of the film itself. What this means as an analytical image is the precision gained from the instrument.

8.2 Exposure of granular layered structure

In Figure 8-2, two areas of milling exposed a more granular sub-structure. This sample was collected directly onto an aluminum stub, so that we are seeing the Air-Film interface (AWI). Several pollen grains are also shown on the surface. Pollen grains that were present in the milled area were ‘cleaned’ a bit but not damaged or moved, an indication that charging effects were minimal.

When gallium ions are scanned across the surface, in this case producing a square of surface where some material has been removed, we see that the upper surface is very thin, exposing structures not seen clearly before. The more dense structures of pollen grains were only mildly affected by the gallium ion exposure and treatment. In both Figure 8-2 and Figure 8-3, we began to see that a possible particulate or nanocrystallite structure, consistent with the structures seen in Figure 8-1, were exposed. Since we had found both amorphous and ordered mineral states in the TEM study, Chapter 5, a higher magnification of one of the milled areas was consistent with the presence of nanostructures. This is enlarged in Figure 8-3 on the right-hand milled area of Figure 8-2 to show not only the nanoparticulates, but also the edge of the very thin surface film that has been milled away, the somewhat polished area of the edge of a

pollen grain, as well as other structures and particulates that have been exposed. This sample is also consistent with the working hypothesis that there can be layered structures as part of the basic film structure including a very thin surface layer.

FIGURE 8-2. At medium magnification, dual beam milling clears the sample of the very thin upper layer. In this case two areas are treated. Several pollen grains are shown on the film surface. The long streak reveals places where the film edge is seen.

Figures 8-2 and 8-3 are consistent with the hypothesis of granularity and a more complex structure. Where some adjustment of the scanning electron microscopy revealed the possibility of granular material or nanocrystallites to metallic surface film

structure, the obscuring nearly transparent film must be thin, yet must also be metallic or some organo-metallic surface. That the FIB would reveal this difference was confirming.

Figure 8–3. An enlargement from Figure 8–2, with removal of the thin, transparent film surface exposes inner structure, but more importantly confirms the presence and thinness of the nearly transparent surface layer.

In the ion beam, exposure by gallium ions erodes the thin surface layer, showing that 1) there is a very thin layer covering the more complex surface film structure, and 2) that the inner structure can be complex. This figure also shows that entire system consists of at least two layers, the very thin upper layer, and the more complex inner layer. We get a much more precise view of how the nearly transparent, meaning

electron transparent, surface is indeed a film obscuring the more granular material, which we suspect are nanocrystallites, possible early hydrated mineralization states.

Examination of Figure 8–3 demonstrates other morphological features of useful interest. Other features exposed by dual beam treatment include the film surface rolled back on the right-hand edge. A somewhat tubular structure is also unidentified by its bright emission, but otherwise unknown. Compare this also in Figure 8–4.

Figure 8–4. An enlargement of a location where the film has peeled back or lifted from the aluminum stub surface, showing that the aluminum surface is free of particulates.

In both Figure 8–3 and 8–4 the area not milled is somewhat dark. This exposure suggests that the very thin dark layer is not stimulating secondary electrons, but that once exposed, the inner layer is brighter, indicating that secondary electrons are stimulated to form a more complete image. As a note, I could have lightened the image so that it was not so dark, and yet it is important to present that there is a reason why electron emission is reduced, even though that reason is unknown. In addition the treatment caused several perforations of the metallic surface film and expose the clean aluminum surface of the mounting stub itself, showing the morphology of the rolling edge of the entire film is indeed a very thin film overall with little structure beneath.

Figure 8–5 is also an enlargement to demonstrate the granularity of the film.

Figure 8–5. While this enlargement is a false magnification, highly pixilated, it suggests that the nanocrystallites have substructure of their own, something to be investigated.

Figure 8–5 is an enlargement, and while a magnification, highly pixilated, it suggests that nanocrystallites are diverse in size and shape, and have substructure of their own, something to be investigated further. Perhaps two features should be noted: One is the suggestion of sub-structure to the nano-crystallites, and secondly, that there is a reticulated structure to the inside of the pollen.

Note that Figure 8–4, above, includes a place where the metallic surface film has folded or peeled away from the aluminum stub surface. We get a view of not only the edge of the film that has lifted from the surface, but also that the aluminum surface below is clear of particulate materials. On the edge we see that there is the darker very thin film and underneath a thin, but brighter layer, which is the particulate, nano-crystallite layer. From this we can estimate the thickness of the film, at about 2 μm . This would put the nanocrystallites at very roughly 100-200 nm, which is consistent with other images we have found. See Chapter Four.

8.3 Interim conclusions

The FIB study was productive in confirming differences in layering structure. Not only did this experiment confirm the presence of nanocrystallite or similar particulate structures, but it also revealed the thinness of the fine surface layer. In both the SEM study and in this one, the particulates were suggested, but the near transparency, a feature of the interaction volume of the electrons, could now be revealed by milling away the thin surface layer. This turned out to be important in examining the copper surface film in another chapter.

One of the objectives of the working hypothesis was the proposal that metallic surface films had a more complex structure. At this point, evidence confirms that there are indeed possible layering and elements of substructure as the hrTEM study showed nanocrystallites as well as amorphous mineral. The next phases of the study was to confirm some higher resolution images of these conditions and structures. This study, in particular, confirmed that an instrument like the dual-beam could mill away surfaces to expose other structures even when the film itself is only 1.5 nm in thickness. These images are strong evidence that the hypothesis was correct in the initial proposal on structural diversity. This is also a confirmation that instrumental analysis, the documentation by environmental microscopy, has an important role to play in environmental evaluations of several kinds. The practical application of environmental microscopy to a wider variety of applied microscopies has both information and opportunity to contribute to our greater knowledge of environmental concerns, but it is time for a wider application of what has been learned here.

CHAPTER NINE

PHOTOEMISSION ELECTRON MICROSCOPY - CPEM

Photoemission microscopy derives from Albert Einstein's papers on the theoretical photoelectric effect, for which he was awarded the Nobel Prize in Physics (Nobel, 1921). The photoelectric effect relates to photons of the 'right' energy, being able to relieve a surface of electrons, which we call photoemission. The basic relationship for light, in terms of energy, is given as:

$$E_{\text{photon}} = h\nu, \quad (\text{Eq. 9-1})$$

where E is the photon's energy, h is Planck's constant and ν is the frequency (in Hz). Einstein theorized that a surface with the stimulation of low wavelength light (high energy), energy in excess of the photon energy would allow an electron to be liberated from the material, usually represented as the kinetic energy of the electron coming off the surface, where

$$\text{K.E.} = h\nu - \phi, \quad (\text{Eq. 9-2})$$

K.E. is the kinetic energy of the electron coming off the surface, and ϕ is the work function of the emitting material. It is known that photoelectric measurements are quite sensitive to surrounding energy, and thus require a great deal of awareness, as small changes in the surrounding materials or instruments themselves can alter the image or measurements. The photoemission electron microscope is a sensitive measurement process. The work function of a material itself is variable across a complex system as a realizable source of sensitive comparison. However, the intricate image of a bacterial surface, which this microscope makes possible, makes this a desirable research

instrument for future research, as it is able to show a high level of detail of the sample not seen by SEM imaging.

The power levels/area used are fairly large, but the cells appear robust. Cells can be damaged by too many photons. Normal sunlight received (at the equator) = 1KW/m^2 but the PEEM uses $100\text{ mW} / 100\mu\text{m}^2$. One must use care with laser power levels as we did see cell damage even in the photoemission PEM.

At Portland State University, the theory of photoelectrons used as microscopic tools was applied and developed by Gertrude F. Rempfer (1911-2010) as a true photoemission microscope, for which she was awarded the Microscopy Society scientist award in 2010. The current instruments include corrected electron optics and are usually termed CPEM rather than the original acronym PEM. PEEM is also used as a reference in the literature. In this study several samples of metallic surface film were examined for biota and extracellular materials.

The PEM was developed in cooperation with O. Hayes Griffith at the University of Oregon, Department of Biology. The productive collaboration resulted in several important works, including Griffith and Rempfer (1985); Griffith, 1987; Birrell, Habliston and Griffith, 1994; and Habliston, Hedberg, Birrell, et al., (1995), all key articles in photoemission electron microscopy.

9.1 Objective

The objective of this study was to obtain surface film data on organisms and any extracellular materials. It was unlikely that the CPEM image would have definitive information on the films themselves, but the bacteria should be evident. Several images

were obtained, and several locations on the films recorded. We used the CPEM to detect high-resolution images of bacteria, cyanobacteria and other biota and their extracellular products including lamellipodia, fine attachments, surface membrane or surface cell wall structure and related delicate materials. Extracellular matrix materials have not been imaged previously in this way.

Methods

The Corrected PEM or CPEM is illustrated as follows (Figure 9–1). What is unique about this instrument is the electron pathway, the separation of objective lenses from projection lenses in order to achieve the correction. The electron mirror is the special feature of this instrument, allowing electrons to take a double path and in doing so allow corrections to electron lens aberrations. Aberration correction is not discussed here, but referred to the more technical article.

The PEM uses a monochromatic light source at 244 nm wavelength from a Coherent™ argon ion laser. Polarization, “s” and “p” is set by waveplate. Samples were prepared as described. A metallic surface film, ORJC was collected on small, square 5 mm x 5 mm ITO coated or round chromium coated coverslips. The air-film interface or water-film interface was identified. It appeared that the images were better if the specimen was under vacuum for 24 hrs. or overnight, so a longer period was allowed before imaging. Imaging followed the procedures developed by Gertrude F. Rempfer and executed by Robert C. Word. The support of Rolf Könenkamp, is gratefully acknowledged.

Corrected PEM

Figure 9-1. Corrected PEM or CPEM schematic showing placement of electron mirror. Image courtesy of Dr. Robert C. Word.

The first PEM was developed for Dr. O. Hayes Griffith and the Department of Biology, University of Oregon, Eugene, Oregon. This was a productive collaboration and produced the first uncoated images of naked bacterial DNA, and flagellar filaments (Griffith and Rempfer, 1985). The first instrument was returned to Portland State University, the Gertrude F. Rempfer Laboratory upon the retirement of O. Hayes Griffith.

9.2 Image properties of CPEM

The photoemission electron microscope has a very small area of imaging due to a design tailored for high magnification. The sample holder is also small, (5 mm chromium coated glass coverslip), compared to the SEM stubs. The specimens reported on here were illuminated by a 244 nm argon UV laser at 10 mW power and focused to a $\sim 100\text{ }\mu\text{m}$ diameter spot. Image exposure times were typically 30 sec. Topography of the sample can be a problem for CPEM due to the nature of the photoemission process and accelerating field, which is somewhat mitigated by the use of a $30\text{ }\mu\text{m}$ aperture stop. Distortion or other image artifacts due to topography occur in several images. The monochromatic light source also produces diffraction patterns around features with significant heights.

It is almost considered unusual that images of bacteria and cellular products are so good. Cells, bacteria, algae and such are considered to be insulating dielectric materials, things that are generally problematic for electron microscopy. Yet, photoemission yield is very good and the bacterial images are bright as seen in the following micrographs.

This study focused on the presence of bacteria and the imaging of bacteria, cyanobacteria and the extracellular matrix materials, or extracellular polymeric substances (EPS), which may play a role in metallic surface film formation and structure, or perhaps with colonization and buoyancy. Both air-film interface and water-film interface were examined, but what was revealed was abundance of bacteria, and other biota on the water-film interface, primarily. Several images follow (Figures 9–2 through 9–5) to present a framework for bacterial imaging by CPEM.

Beginning with a low magnification, the abundance of bacteria on the water-film interface demonstrates colonization of the metallic surface film. In Figure 9–2 at lower magnification, the abundance of bacteria on a metallic surface film, the water film interface or WFI. These samples were taken from Johnson Creek, Clackamas County, Oregon, labeled ORJCg for site one and ORJCt for site two.

Figure 9–2. Low magnification CPEM image of bacterial density, MSF, ORJC. Image 01, Water side WFI.

The first image represents the concentration of bacteria seen on areas of the film. In general, there is patchiness in many places, and the colonization of the film is not uniform. This is consistent with patchiness observed in both structure and substrate in some cases. Images revealed an unexpected density of bacteria colonizing the water surface WFI of the surface film. At a slightly higher magnification, shown in Figure 9–3, we begin to see the features of CPEM microscopy that illustrate the usefulness of this microscopy and further characteristics of the bacterial image as well as the extracellular material. This is particularly noticeable in Figure 9–5, a slight enlargement to demonstrate the contrast and resolution obtainable by PEM/PEEM microscopy.

Figure 9–3. CPEM Water WFI. Note that surface EPS forms a considerable coat in several places, particularly on the right-side edge.

Figure 9–3 begins several magnifications of this sample because of the importance of these images and the high quality of the bacterial surface. It is seldom resolved to see the bacterial outer wall and the related extracellular material. It appears to be an important feature of the metallic surface films as habitat and the colonization of the films in short periods of time. This is the first documentation of this bacterial material on metallic surface films.

Figure 9–4 allows us not only to image several characteristics of the bacteria, but to test the unique features of photoemission electron microscopy that even if bacteria represent a dielectric material, we have a good electron yield for both the bacterial body and surrounding extracellular or delicate particulate filaments, but also that the background is not bright and thus not emitting sufficient electrons.

Figure 9–4. Enlarged image of bacteria and surface features. At higher magnification the extracellular material or bacterial cell surface can be resolved. Note the contrast of surface features of the bacteria.

It is important to stress that bacterial surfaces are not coated in some way, either artificially or naturally. The specimens imaged by PEM were not artificially treated, but that this surface appears somewhat fuzzy may be a feature of the CPEM resolution. It may also be seen as a way to increase our understanding of the work function of biological materials, or possibly chromatic aberration, and perhaps a better understanding of photoemission physics. In the following slightly higher magnification we see both dividing cells and darker surface features. The surface features suggest a core bacterium with extracellular material, some of which has the appearance of dark strands. This may be both extended material of the bacteria themselves or attached material from the surrounding water. It is possible that small charged particulates are attracted to the bacteria or that bacteria are actively seeking or attractive to dissolved or particulate material which appears here as a material that is not photostimulated to release electrons (where $h\nu < \phi$) and thus appears darker. Some inorganic material might appear darker.

In Figure 9–5, examination of the surface contrast and the features of dividing bacteria were found. First, it indicates that at the time of collection the bacterial film was alive, active and growing. There are fluorescent stains that would also give a better contrast of living and dead bacteria, but as these were air-dried in the field, the presence of dividing cells is a good indicator. This same indicator was used to describe bacterial impressions casts or ‘pits’, which also suggest active bacterial life at the time of formation. This is consistent with the working hypothesis that metallic surface films are an active, diverse habitat, something that will help develop the concept of habitability.

Figure 9–5. Bacterial Surfaces. Two appear to have recently divided.

Figure 9–5 also gives us a view to the dimensional surface of the bacteria, the delicate surface material. As noted above it would be possible to develop a stereo pair of images, but stereogrphy would also require some further instrument development, as any angular shift in alignment of either the stimulating photon beam or the emitted electrons would affect instrument performance.

9.3 The biofilm structure

Biofilms and the bacterioneuston depend to some extent on extracellular secretions, the organic surface film components in many cases. While these might be chemically defined as being present, we seldom have images and structure of both the

bacteria and the films. Extracellular matrix proteins or extracellular polymeric substances (EPS) were found to be delicate and yet complex. Images by photoemission electron microscopy raised a number of important research questions on how best to document these structures.

There is a general misconception that biofilms are just a sticky mess (Grathoff et al., 2007), when in fact bacteria tend to link up with each other, as do many fungi. In surface films observed under phase contrast microscopy, fine filaments are often seen as part of the sea-surface microlayer or aquatic surface film. These delicate strands are usually not seen in brightfield light microscopy, but only in some alternative contrast mechanism, such as phase contrast or DIC imaging.

In this PEM study we find that there are many sizes and ranges of flocculent type material, where most of the proteins and carbohydrates are delicate features often separated into distinct forms. There is a sense that many proteins and carbohydrates are linked into a gel-like structure. This is proposed for sea surface microlayer, natural organic materials (NOM), transparent exopolymer (ic) substances (TEP) and related extracellular secretions (Cunliffe et al., 2012). This links what is thought to be important structure in sea surface microlayer films or aquatic films with the metallic surface films.

For the bacteria themselves, it appears that photoemission electron microscopy is able to resolve both delicate surface features of the cell wall, but also the pads of attachment to the substrate. In the color enhancement of Figure 9–6, the color resolution of blue areas of organic material may be such an area of attachment.

Figure 9–6. False Color enhancement to add contrast to bacterial body and EPS. Use this scale for comparison with greyscale images.

In the above micrograph, color is used to add contrast to the image and distinguish between bacterial body and the strands of polymeric substances. Note that underneath each bacterium is a blue patch, which is consistent with an adhesive to the metallic surface film. Many of these flocculent structures are less than 1 μm and while some would be collected as POM, some would pass through a filter and be found as DOM in fresh-water, aquatic and estuarine waters. Wurl & Holmes (2008), Wurl et al. (2011) and Cunliffe et al. (2012) also support polymeric abundance in surface films,

and perhaps contributing to buoyancy. The color highlights features of both bacteria and extracellular products.

In the three following micrographs (Figures 9–7, 9–8 and 9–9), CPEM imaging is used to demonstrate the structure of the bacterial film environment, the biota in or near the edge of the metallic surface film and the bacterial attachments of several kinds in and around the both surfaces of the metallic surface film. That we can resolve the very fine filamentous attachments and a number of fine extracellular particulates that not only documents the favorable resolution of the instrument, but also shows the lack of beam damage to the fine structures. The presence of attached or integrating structures of the biota is imaged well with very fine filaments. Here, the metallic surface film is truly seen as an active habitat (Figure 9–7).

Figure 9–7. An MSF film edge with bacteria on all surfaces.

Figure 9–7, at a metallic surface filme edge, bacteria are seen on both sides of MSF, and lower surface also has a coating of filaments and small organic materials. Figure 9–9 is a small enlargement of filamentous attachment to film 9–8.

In Figure 9–9, a slight enlargement is used to show the several fine filaments, delicate and seemingly undamaged by sample preparation or beam damage by the instrument. This is a confirmation of the feasibility of the CPEM for environmental microscopy and possibly the first record of the delicate structures seen in and around the bacterial associated with the metallic surface film. We see a similar resolution of the fine particulate material associated with the substrate upon which the sample was collected, also an important record of delicate structures still associated with films.

Figures 9–8 (left) and 9–9 (right). Figure 9–9 is a slight enlargement of image 9–8 to show the filamentous attachment and resolution of delicate strands or lamellipodia of the cell features. Note that bacteria are present on both sides of films.

The CPEM image puts a new perspective on the location of small organic particles, both on the surface of cells and also imaging molecules on the substrate and on the films themselves. This is a unique means of detecting habitat colonization.

In Figure 9–10, color enhancement reveals differences in level of photoemission. At the same time, the small organic particles on the substrate are nicely resolved. Even the enhanced light green of the metallic surface film suggests a level of organic film, which may be related to the favorable colonization of the film itself.

Figure 9–10. Rotation and enlargement of Figure 9–8. This false color enhancement increase contrast of collection surface on coated glass. This surface should be very dark, but is a medium blue with flocculent particles. Notice the range of enhanced color in bacteria, indicating that there are still other features to be found.

9.4 Cyanobacterial filaments and flagellated spores.

With Figure 9–10, which focused on bacteria, the research shifted to images of cyanobacteria and flagellated gametes or spores, and this study is focused on images. In Figures 9–11 to 9–17, false color is used to enhance surface features, particularly for the cyanobacterial filaments. There are surface properties of cyanobacterial cells and filaments that are unknown. In highlighting some features, I present new information on possible surface morphology and physiology for future work. This is the first imaging of surface features of cyanobacterial filaments by PEM.

Figures 9–11 and 9–12. Cyanobacterial filament crossing a crack in the surface film.

In Figure 9–11, we find that a cyanobacterial strand has been pulled apart, and has some connection. This suggests that the cyanobacterium was in place, adherent to the film before the crack appeared. See the following false color enhancement (Figure 9–12), where false color enhancement of cyanobacterial filament highlights surface features, and the diffraction patterns that appear in the image. Note the width of the strand by the scale bar of 1 μm , which applies to both images.

There are similar enhancements imaged in Figures 9–13, 9–14 and 9–15. The punctate features of the cyanobacterial filament suggest other structures to be seen:

Figure 9–13. False color cyanobacterial strand. Note the partial crack in the surface film, and the punctae imaged on the cyanobacterial filament.

Figures 9–14 and 9–15 (right). Cyanobacterial strand and flagellated spore or gamete.

In Figure 9–14, we are able to image a cyanobacterial filament at length, and PEM/PEEM microscopy is able to resolve both a light and dark circular features of the filament, as a line of regular dots. These are too close together to be DNA type material, but they could be metallic features of the antenna mechanism or the buoyancy mechanisms, known in some cyanobacteria. Even if the structure is not resolved at this time, the fact that contrast can be seen consistently is important, for the structure is a positive feature for PEM/PEEM microscopy. Figure 9–15, a color enhancement of cyanobacterial strand and flagellated spore or gamete. Cyanobacterial strand has two kinds of features, the bright yellow dots and the dark circular dots. These are unknown features, but found elsewhere in the cyanobacterial strands. Note the distortion caused by topography and the accelerating field.

Figures 9–16 and 9–17. Areas with flagellated spores or gametes. Note the delicate filaments that are resolved and enhanced by the color enhancement.

Figures 9–16 and 9–17 also detail the flagellated spores, but also fine surface filaments. If these are similar to spermatozoa, the neck ring is a prominent feature.

These are very photoelectron stimulated bodies. Note diffraction patterns of particles. Note the very fine filaments from some material at lower right, and a figure-eight strand in the upper right. These figures are color enhanced to increase contrast of organic material represented by the light green over the blue coverslip surface. These are equivalent to many images obtained by SEM.

9.5 Interim conclusions.

Photoemission Electron Microscopy (PEM/PEEM/CPEM) has been most revealing as to the density and activity of bacterial habitat on metallic surface films. The images reveal an unexpectedly rich bacterial field, with evidence of extracellular polymeric substances, undefined at this point. This may be related to either the extracellular products often referred to as EPS or transparent exopolymer (ic) particles (TEP). Both EPS and TEP are playing current roles in sea surface microlayer studies (SMIC, SML) or in other aspects of aquatic surface film studies (ASF), and may be a significant part of future MSF studies. All samples were air-dried at the time of collection.

The advantage of the PEM instrument is specimen imaging without coating. Whereas the SEM and TEM use a relatively high energy for the electron beam, the CPEM uses high wavelength light or photons, where the sample is coaxed to yield electrons without coating the specimen. Without coating you get an image that is better, or more representative, than the typical SEM, but you don't have to get TEM instrument use to get good resolution. The PEM/PEEM is good at seeing the detailed surface of cells and cell components (Griffith and Rempfer, 1985).

Photoemission electron microscopy (PEM) offers some intriguing insights into surface structure. The metallic surface films, however, do not resolve the organic structure, which is believed to be present. However, on the surface, and particularly for bacterial, cyanobacterial cell bodies and extracellular materials, CPEM offers a better perspective to possible structures, as well as a means of addressing work function, and other mechanisms of photoemission, in biological materials.

Funding for instrument development is difficult if the end applications cannot be resolved in advance, and yet it is often necessary to develop the instrument first. Yet, photoemission electron microscopy, particularly if combined with other photoemission properties could include some versatility to the instrument. Since electrons have wave properties, some diffraction patterns might be captured in future instruments to take advantage of the wave properties of both high energy photons as well as electrons.

The testing of metallic surface films and attendant biota was encouraging. We addressed the application to image not only cell bodies, but also the use of the films for some yet unidentified organisms, such as the flagellated gamete or spore. There are many flagellated forms that have a similar morphology. Even though that resembles a mammalian sperm, and has all the characteristics of highly structured gamete delivery form, identification is yet to come. Do the CPEM images correlate well with the SEM studies? There are both comparable images, but also unique features yet to be resolved. But, the PEM/PEEM is a remarkable instrument for biological structural studies, as presented here. It is almost an opening window to a new area, as metallic surface films or their biota have not been imaged previous in this way.

CHAPTER TEN

BIOTA, THE BACTERIAL FOOTPRINT AND FILM INTERACTION

We now know that bacteria and cyanobacteria play an important role in colonizing and possibly interacting with the metallic surface films. It may be that iron and other elements become nutrient sources under some circumstances. We do not know if the biota form with the film or colonize the films afterward. We do know that the films are colonized quickly, as newly formed films already have biota associated with them. This was tested with the Yaquina Bay sample, which had abundant diatoms after a heavy cleansing rain and a tidal cycle, roughly 12 hrs., at most, and part of that was in the dark. Secondly, this study examined the thickness of the film for further evidence of mineralization structure.

A series of images was obtained from a wetland on the Sandy River, Clackamas County, Oregon, OROX, close to Oxbow Park, a natural area with access. This film was mounted on an aluminum stub and was examined in Zeiss VP without coating. Bacterial casts were present in several patches, and there were other features of interest to further characterize the film. The first set of images was imaged at 5 kV, a low voltage to reduce beam interaction with the specimen.

In Figure 10–1, one finds that the bacterial colony is apparently in a patch or group with several kinds of interaction. Some bacteria appear to be on the surface and collapsed. The depressions on the bacterial surface are indicative of apoptosis of the cell membrane, a dying sequence for bacteria. Some are more definite ‘pits’ or casts with a ridge around the edge. There is also evidence of recently dividing pairs.

Figure 10–1. Bacterial footprints in a patch on metallic surface film. These bacteria were recently active showing several kinds of cast formation. Bacterial size as scale.

What these images yield are the several morphologies of bacterial impressions and other impressions that form these surface structures on the air-film surface. The fact that the bacteria on the surface are torn by the cracking, indicates that they were present before the cracking occurred, and indeed one can see areas of tearing. Some bacterial casts appear to be within the matrix of the metallic surface films as if it formed around the bacterial cell, which subsequently collapsed. It is also clear from the cracking that there is structure underneath the smooth surface, and that there are fingers or projections of material at the crack or tear indicating some active process or growth process or at least some interaction (Figure 10–2).

Figure 10–2. A second patch of bacterial casts, clearly seen as a separate group, but with a chain of bacterial cases, which appear to be on top of the surface casts. 10 KX.

That the bacteria appear in definite patches is consistent with bacterial colonies in general. That there appears to be some superposition of some bacteria over others, as if a bacterium has been pulled away, leaving a cast versus bacterial formations that have collapsed. We see both kinds of structure.

In the following Figures 10–3 to 10–8 are representative casts taken from different areas of the OROX films. Some are from groups of bacteria, others isolated cells or casts. The cases appear to be within the surface matrix, not just collapsed upon it, but several casts are definitely within the metallic surface film. The junctions with the metallic surface film that one can conclude that the film formed around the bacteria.

Figure 10-3. Bacteria as surface casts or forms seeming on top of or incorporated. There are three casts as pits each with an edge or folded area and some topography.

Figure 10-4. Bacteria or bacterial pair integrated or collapsed into the film. These seem to have a prominent edge. Did the film form around the bacteria?

Figure 10-5. Bacterium that appears to have collapsed. Note the sharp leading edge.

Figure 10-6. Bacterial cast that seems to have collapsed leaving a hole or pit.

In these imaging studies, since the bacterial size is quite reliable, I have continued to use the bacterial morphology as relative scale. For environmental studies this is often used and a guide until a measured series can be done. Since each of the images has some morphological as well as structure question, that is the main focus of the supporting images and the questions they raise about biota.

Figure 10-7. A cast or pit which appears to be more of a hole extending into the matrix.

Figure 10-8. A collapsed bacterial cast in the vicinity of a surface alga, dehydrated.

There is also a characteristic morphology to the bacterial pairs, which may be recent diving cells (Figures 10-9 and 10-10. Here, too, these cells appear to have been incorporated into the film, perhaps as the films was forming. Some are surface casts, and some are seen more as pits or collapsed pairs. Figure 10-11 suggests near division, but not separated.

Figure 10-9. Two bacterial pairs, one separated.

Figure 10-10. Dividing bacteria?

Figure 10–11. Bacterial pair possible in the process of dividing. The depressions are consistent with apoptotic openings to the cell wall, and indication of bacterial cell death, and seldom imaged.

Figure 10–11 not only suggests preparation for cell division, with two centers of cell membrane collapse, but the small collapsed areas are also an indication of bacterial apoptosis, the opening of the cell wall prior to death. The collapsing structures are consistent with cell wall apoptosis, which is often diagramed, but seldom imaged.

10.1 Other morphology indicating patchiness

In one sample from the ORJCg site, Gresham, Oregon, an unusual morphology was identified. It was a small patch similar in size to the bacterial patches seen here, but with a distinct morphology. Upon EDS examination, this was a nickel/zinc biofilm. Clearly it was a modified patch and there were a couple of patches that could be seen. It was identified as a biofilm of uncertain nature, but the morphology had been seen before in other biofilms, some from the human mouth on teeth (But not with nickel/zinc) at the metallic constituent. This is consistent with the size of other bacterial patches within an iron or manganese/iron metallic surface film. The sample

was coated with gold to an approximate thickness of 1.8 nm and imaged in the JEOL 6300F with an Oxford™ EDS spectrometer. The image is shown in Figure 10–12, and the spectrum in Figure 10–13. The Au - gold peaks are the conductive coating. One notices the lack of iron.

Figure 10–12. Image of a patch of distinct morphology within a metallic surface film of a more typical iron oxide structure. This biofilm, however, is nickel/zinc as shown by the following spectrum.

Figure 10–13. EDS spectrum for nickel zinc biofilm. Notice the lack of iron, manganese or zinc. Nickel-zinc biofilms are known to occur.

This biofilm basically confirms the working hypothesis that other metallic components are part of the metallic surface film, even if these appear as an integrated patch with the film. It is especially distinct that development with a surface film is not restricted to the particular nutrient environment, but that structures do occur. This entire patch is similar in size to that of Figure 10–2, but distinct in morphology.

10.2 Other surface features indication interaction

There are several structures that indicate other interactions, such as holes, tubes and other organisms. While this not a complete list, it is a place to begin as the morphology can be recognized in other images.

Figure 10–14. A tube-like structure larger and longer than the bacteria in the sample.

Figure 10–15. A tube structure somewhat extending into the film, of bacterial size.

Structures of bacterial size are seen in Figures 10–14 and 10–15. Figure 10-14 is somewhat larger than the bacteria cells imaged in the picture, but seems to also have some surface feature in the tubular wall. Figure 10–15 is also tubular, but is also of bacterial size and seems to extend into the surface of the film.

Figure 10–14 somewhat enlarged to view the mineral structure seen around a removed circular piece of film exposing round bodies, which are consistent with hydrated mineralization structures, which will be reviewed later from another sample. Figure 10–14 reveals several important features. First it is still in the middle of the bacterial patch, exposing what appears to be layers of bacterial activity. Observe the bacterial pair or cast on top of embedded bacteria. Were these lifted away from the surface? With a lifted edge, we can again estimate the thickness of the metallic surface film as approximately 30 - 150 nm with considerable material below, which is consistent with highly hydrated mineralization on the water film interface of the film.

Figure 10–16. The lifting of a circular patch of thin surface reveals a number of round bodies that are consistent with hydrated mineral structures. These appear to compose a significant portion of the water film interface, the lower surface of the film.

Figure 10–16 demonstrates the presence of circular bodies that are consistent with hydrated amorphous mineralizing structures that allow ordered mineralization to begin within the mineral body. We see this possibility in Figure 6-17 from another area of the OROX metallic surface film where the subsurface structure is seen to be more complex but otherwise hidden by the smooth, thin surface of the metallic surface film.

Figure 10–17. Metallic surface film, OROX, at 91.6 KX to resolve what appear to be mineralizing structures in the second layer of the film. The thin upper metallic film is consistent with 30-50 μm , with the circular structures of 150-300 nm.

In Figure 10–17 we have another example or what may be amorphous mineralizing structures seen as part of the layering structure. The presence of layering

of the metallic surface film has now been seen in several samples, and seems to be a consistent piece of film formation and structure, particularly in young films. The timing of these structures is uncertain. It is consistent with mineralization processes of hydrated calcium matrix seen in *in-situ* TEM studies reported by Nielsen et al. (2014a; 2014b), who state that round, hydrated structures allow ordered mineral states to begin within the hydrated body. We see a suggestion of this in the following image, Figure 10–17, not as an enlargement, but at 91.6 kX.

The other features should not be left out of the discussion, however brief. In Figure 10–18 we have a surface, but somewhat dehydrated algal filament, attached to the metallic surface film on the surface itself with some bacterial casts nearby.

Figure 10–18. The algal filament, somewhat dehydrated.

Again, in orientation this is on the water-film interface, it must have some surface attachment, but would have extended into the water. While these images have

focused on the bacterial features and casts or integrated bacterial forms found within the metallic surface film structure, there are these colonizers, not identified at this point, but a present feature of this habitat. The following figures are somewhat curious.

In Figure 10–19, we have a worm-like structure, although very small, but with some surface folds that are consistent with muscle bands for nematodes. It appears to be entering the surface film (or exiting), at least integrated into the film body. At this early state of structural research, odd or interesting forms are likely to occur. They are not the primary focus at this point, but there is likely to be some consistency of use with the metallic surface film as habitat.

Figure 10–19. Nematode-like structure seemingly penetrating the surface of the metallic surface film, but of very small size. It could be a bacterial form, however.

10.3 Interim conclusions.

In the above studies, we have used the bacterial form as our scale. The bacterial size, previously measure is reliably about 0.8 to 1.0 μm in width and about 1.2 to 1.5 μm in length. Other features can be estimated using that scale. For a series of images on the same material this is acceptable.

The biofilm shown in Figure 10–12 biofilm is a distinct confirmation of the working hypothesis that the metallic surface film supports and integrates other metallic structures, events and colonies, even if these appear as a distinct and seemingly unrelated patch with the film. Future work is needed to find what clues, chemical or morphological, those surface signals that attract and allow colonization to occur, and to find what makes this a habit as well as a habitable environment. ‘Habitability’ is the cogent question for a future phase of research, and it is consistent with what is being asked in astrobiology. It is especially distinct that development with a surface film is not restricted to the particular nutrient environment, but that structures do occur. This entire patch is similar in size to that of Figure 10–2, but distinct in morphology. The fact that it occurred within a seeming normal film is consistent with other patches seen in these samples. There is a significant question of how bacteria can concentrate or enhance small levels of distinct elements, and that is not answered here.

There are many structural questions raised by biota and curious structures. A structural and imaging study is only the beginning of what could be a fruitful direction for analytical imaging, for instrument development and for chemical imaging, a current topic in microscopy research.

CHAPTER ELEVEN

COPPER ZINC METALLIC SURFACE FILMS:

EXPANDING THE CONCEPT OF METALLIC SURFACE FILMS

It was an unexpected find to see that there was a stable surface film located as a cooling tank metallic surface film that appeared to be a copper oxide or salt, as the appearance of the film was reflective, similar in appearance to any other surface film except for the bluish tinge to the material. It was unusual as well, as this solution was not rich in humic materials, nor was it subject to sunlight, but kept mostly in the dark. This contradicts some surface film proposals for photo-induced oxidation or transformation of the surface film. While we sure that photoprocesses are important to both the films and to the biota involved, but not at this film and nor for this site. This makes it an excellent study to confirm the hypothesis.

The cooling tank is located at Portland State University in the Department of Geology supporting the Philips X-Ray Diffraction instrumentation. A stable film had been present for some years, as I understand it without much alteration. The surface film is stable over a constant current, something noticed in the field when a surface film forms over a current. Aquatic surface films or microlayers demonstrate this stability as well.

This suggests, and even confirms that more than one pathway exists for metallic surface films to form. The discovery of a copper-based metallic surface film in a dark enclosure, gave another argument in favor of multiple pathways to mineralization and metallic surface film formation. As noted above, it is not iron based. As noted above,

it is not photoexcitation-based either, at least not in the typical sense of exposure to sunlight. It is unlikely that the periodic, intermittent and weak room light would have a stimulating effect, but that cannot be ruled out. We are again directed to the physical properties of surface tension at the air-water interface as being a prime mover in the development and formation of surface films, both organic surface films or aquatic surface films, and the more specialized metallic surface films, where transition metals appear to be the primary elements involved in this mineralization. We cannot rule out heavier elements in this process, but usually these elements are not available in sufficient concentration under natural conditions. Artificial conditions may show other possibilities, and certainly extreme environmental conditions could offer unusual mineralization in aquatic systems and at either the air-water interface or the benthic water interface, both boundary layer conditions, where properties may impart unusual conditions.

At low magnification, 3.42 kX, differences in density and patchiness are suggested by the pattern of mineralization as seen from the air-film surface. The fine pattern of cracks into platelets is also similar to other metallic surface films. And while it is not filled with dramatic features, when we examine this at 6 or 7 kX, we do find bacterial clusters, similar to the patches seen elsewhere. Two figures are given, Figure 11-2 and 11-3. Figure 11-3 has been darkened to enhance contrast of what appear to be dense areas of the cell. What is most noticeable is that the patches or clusters of bacterial cells are surrounded by an extracellular matrix. This could be a protective barrier, as a copper/zinc environment should not be the most hospitable place to grow.

Figure 11-1. Copper zinc film structure at 3.42 kX. Note density differences. The white patches are bacterial features.

As seen below, at least 6 kX is necessary to reveal the presence of these small bacteria within the copper-based metallic surface film. Two frames are shown.

Figure 11-2. Bacterial colony or cluster. Note the white, clear area, an indication of an extracellular matrix or wall. This is possibly a protection for this unusual environment.

Figure 11–3. Altered contrast of figure 11–2 to enhance the dark surface features of each cell. While of unknown structure, it is a distinctive feature that may assist morphological features and identification.

Two images of the bacteria found in the copper/zinc surface film are shown.

First, it is important to note that they were all in small patches within the metallic surface film. Secondly, there is a bright halo representing either a cell wall or extracellular matrix around the colony. It is unusual that the SEM does not image a clearer representation of the clear area or the edge of the patch. This is another case where instrument sensitivity and adjustment may be necessary to obtain a better image. In this case it was to confirm that biota were present in the film, as this tank condition lacked humic substances or a known nutrient source.

11.1 Sequential frame images to demonstrate structure

In order to get a better detail of structure, the sample was mounted on an aluminum stub and examined in the Focused Ion Beam for milling. This proved to be a

confirmation of layering, even though it was a thin sample. Selected frames are shown in the following images, but 50 frames were taken and a short video will be presented of the milling process and the revealing of layers. That this film consists of two delicate layers and one or two robust layers also supports the working hypothesis that metallic surface films have layered structures.

The frames below are being milled at a rate described in Chapter Eight. Instrument settings are also as described in chapter eight. The instrument parameters for an instrument under development a prototype FIB with altered features were used here for the examination of the copper-zinc film. The first frame is dark, but highlighted as each milling stage proceeds. What was revealing by this procedure was the fenestrated nature of the surface film once treated with Ga^+ ion beam. As noted in chapter eight, milling proves a clue to the molecular or atomic density of the material, and can be a guide to its structure. The following Figure 11–4 consists of six representative frames of the milling structural features,

Frame 2 Copper-zinc surface film

Frame 5. Copper–zinc surface film

Frame 8

Frame 14

Frame 18 Copper-zinc surface film.

Frame 21 Copper-zinc surface film.

Figure 11–4. Sic representative frames of the milling process for the copper-zinc film.

In the following image of Frame 14, shown here as Figure 11– 4, at a standard size, as demonstrates the fenestrated structure of the milled film, indicating both the thinness of the film and some differences in density. The lined structure to the lower right of center is associated with the crack in the film, indicating that even cracks have some contribution to structure, both in density and with associated attachments. A movie was prepared of 50 frames and shown at the dissertation defense. It more clearly demonstrated the presence of two thin layers on top of one or more dense layers seen in

Frames 21 – 49. Following Figure 11–5, Figure 11–6, Frame 43, shows several dense features to the milled metallic surface film. Note that to the left center, there is an unusual bacterial like body, a tubular shape within a small depression. Note also that the tubular structure at center right comes from the crack seen in the film from the beginning. It suggests that even cracks are not separations, but become an active part of the metallic surface film structure.

Figure 11– 5. Frame 14 in a series of milled copper-zinc metallic surface film. This particular figure shows the second delicate film to be milled. In a higher energy system, the delicate films might be swept away without being imaged and the structural detail lost. The fenestrated appearance is related to the density of the material as it is being milled away.

Figure 11–6. Frame 43 in a series of milled copper-zinc metallic surface film, showing increasing density in the film structure compared to earlier frames.

11.2 Interim conclusions.

The copper–zinc surface film confirmed several features and contradicts some common knowledge about surface films. In that sense it is a positive statement toward expanding the general nature of research on metallic surface films to include a much broader range of structural and biological environments. As a feature from the dark, it suggests that a broader range of light interactions and impacts influences surface film development, even though these will tend to be experimental rather than natural films from the environments. Yet, the nickel– zinc biofilm shown in the previous chapter also supports an expanded search for structures in the natural environment. In general

in means a better resolution where needed, as these structures often do not appear until you are looking at a range between 7 kX and 10 kX. It is too easy to miss the structures of interest. This supports instrument development and recognition in this area of study.

Examination of the copper-zinc films by FIB milling puts a positive light and support for the working hypothesis in every way. Not only do we have material derived from a nutrient poor, light poor environment, but also we have biota to colonize it. Not only do we have structure, but also we have layered structure of at least two very thin layers of some light density, yet fenestrated when treated, but also we have a significant amount, and perhaps two layers of more dense material and inclusions. In a higher energy system, the delicate films might be swept away without being imaged and the structural detail lost. The fenestrated appearance is related to the density of the material as it is being milled away.

In Figure 11–5 and 11–6, we also have a tracing of the crack in the film, which turns out to be more than just a separation. There is a density associated with it that also has structure, and a feature that one can follow through the milling process. It sheds a new light on the fissures and cracks, that they may yield more information than is usually considered. Cracks and fissures are often passed by as a feature, but chemical changes or alteration as a feature of the fissure itself might have useful information quite separate from the structure of the film.

CHAPTER TWELVE

METALLIC SURFACE FILMS AND THE CAPTURE OF CONTAMINANTS

The formation of a surface film, either an aquatic surface film or a metallic surface film, the capture, entrainment or entrapment of other particles is new information about environmental processes. From *Trichodesmium*, we know that Saharan dust particles are transported long distances, yet can be identified and used for iron nutrition by the local cyanobacterial colonies (Rueter et al., 1992). The enrichment of anthropogenic organic chemicals and heavy metals in the sea-surface microlayer has been reported several times (Hardy, 1982; Hardy et al., 1990). We now know that insect parts, crustacean exoskeletal parts, diatom frustules and fragments, dusts etc., can be entrained and entrapped into the metallic surface film structure.

12.1 Contaminant analysis by high resolution SEM

A high resolution JEOL™ 7800F Field emission scanning electron microscope was available for a trial. A cultured metallic surface film, with humic substances was sampled directly onto an aluminum stub, air-dried and mounted in the microscope. The sample was analyzed in the mapping mode to locate elements. The resulting analysis confirmed the usefulness of metallic surface films as a capturing method as well as a means of locating the structural features of contaminating particles. In this case Bismuth particles were identified in the sample. Bismuth is a heavy metal near lead in the periodic table and an unexpected identification. Possible sources include experiments in other laboratories at Portland State University, where microcrystals or nanocrystals of bismuth had been produced in other projects. Another possible source

was as escaping vapors from bismuth-containing solder, which had been used in the laboratory occasionally. This type of contaminant deserves further work.

No other known source for bismuth particulates was expected or was known to occur. Since this was an isolated situation, it still represented an interesting contaminant and a useful demonstration. Since it was unexpected, JEOL technical advisors were asked to review and confirmed that the calibration was accurate and the sample particles could not be lead, and that the bismuth peak was accurate.

In Figure 12-1, an image of the crystals embedded in the metallic surface film were identified as a bright area of crystals, yet unidentified. That they were bright indicated free emission of secondary electrons as well as x-ray emission.

Figure 12-1. Bismuth particles within a metallic film matrix.

Two areas were sampled, as we were restricted in beam time on this instrument as shown in Figure 12-2. The first sample area, identified as 001 is directly around the major unidentified crystalline area. The second sample area identified as 002 is in the metallic surface film matrix. Not that the metallic surface film matrix appears to enclose the bismuth crystallites, as it overshadows some areas, and appears to have some below the crystallites as well. The edges are indistinct, as if these crystallites have been bound or incorporated into the matrix, which has some indistinct edges itself.

Figure 12-2. Sample areas scanned for analysis

Consider that in the examination of a surface film expected to be Fe, O, and S detectable, we find an unusual particle. Determined by EDS to be unexpected bismuth, but essentially as a contaminant. The anion could have been CO_3^- , SO_4^- most likely, but this was not determined by this method. It was a good example of the ability of

metallic surface films to incorporate other particulates or contaminants, and even if they are rare events, are detectable and visible, a documentation for further work. An interim conclusion from this research is a recommendation that examinations of metallic surface films be complete, with several scans to check if unexpected particles or rare events are present, as a means of locating unusual elements and particulates. It is consistent with cation binding that other heavy metals may be found, even though the separation of mineralization as *de novo* particles or the incorporation of particulates as contaminants or even Eolian drift into the matrix can be identified.

12.2 Analysis of particulate inclusions

In this case, because we were only using 10 kV as the beam energy the $K\alpha$ and $K\beta$ peaks would not be available, but as a large atom, $M\alpha$, $M\beta$ and several smaller M shell x-ray emissions could be detected, confirming bismuth by consistent registration of M shell x-ray emissions. These bismuth M-shell electron emissions are absent in the adjacent matrix.

In terms of contaminant detection, it supports the idea that abiotic or artificial cultured metallic surface films may be a source of documentation for small levels of particulate contaminants. We know that we have an increasing use of nanoparticles in the environment, but typically seen only by air-filtration. The air-water interface and the entrapment or entrainment of fine dusts and nanocrystals or nanoparticulates into the metallic surface film structure, whether biotic or abiotic might be a useful and supporting tool for environmental analysis and contaminant evaluation. The following Figure 12–3 shows the spectra for the film matrix and the identified particle.

Figure 12–3. Spectrum 001, above and spectrum 002 of the matrix from Fig. 12–2.

12.3 High-resolution mineralization and layering

One test was done on the TESCAN Lyra3, dual beam, another high resolution scanning electron microscope with milling capability. In this case a high humic substances film with ferrous iron was collected directly upon an aluminum stub. This study demonstrated clearly that at least two layers are involved in a new formation. It supports not only the very thin surface layer, but also demonstrates the granular layer, most likely an amorphous, hydrated mineralizing film (Figure 12–4: a, b, c, d).

Figure 12–4 a, b, c, d. Scale bar is 3 μm , and confirms a very thin surface layer and a sub-layer of granular material, probably amorphous, hydrated mineral bodies.

12.4 Interim conclusions

An instrument like the JOEL™ 7800F represents a new line of high resolution microscopes, and from the EDS spectra we see a higher level of resolution in the elemental spectra as well. It is a logical conclusion that the formation of metallic surface films as a cultured or artificial film could be a useful detector of airborne particles as there is some tendency to capture dusts and fine particulates. The ability to detect these particles at high resolution has usefulness to both fieldwork and laboratory experiments, part of which is the binding properties of the particles to the matrix, something not investigated here.

Contamination by microparticles or nanoparticles is a timely topic of research, public interest and speculation. As increasing number of nanoparticulates become environmentally part of research and industrial or medial production, detection in the general environment becomes evermore important. Not only is this useful for detection, but also for the hazard and the interference it represents for contamination into other research programs and protocols. If these particulates are drifting around our science building, how might they affect other research?

The TESCAN™ Lyra3 is also a high-resolution instrument with other capabilities as a dual beam that we presently do not have at Portland State University. The demonstration, which also confirms the working hypothesis in terms of complex and identifying structure, suggests support for a better examination of field samples in a timely manner.

We often speak of resolution of the microscope for imaging purposes, as resolution is often considered an optical property. But in terms of precision for the instrument, resolution or the ability of the detectors, such as the EDS X-Ray detector to locate the sources of the X-Ray photons is also a conceptual part of resolution.

These two events were both supportive of the research topic, but also engaging in that confirmation that by expanding our knowledge of metallic surface films, we also broaden the scope of work to include the possibility for these structures as a part of exobiology, as a functional assessment to astrobiology, and as a link to the properties and processes of the air-water interface where water is located or may have been located in the past.

CHAPTER THIRTEEN

DISCUSSION

As this research shifted from possible fieldwork to instrumental work, the role of imaging in developing a better set of tools to determine structure was unclear. The differences in the instruments allow for differences in imaging resolution, contrast and analytical information. However, it has been a very productive endeavour to determine that it is possible to broaden a category of surface film work to encompass a much larger area. Interim discussions and conclusions have been part of each chapter due to the differences in instruments, applications and samples. It is fair to say that the working hypothesis was based on good preliminary samples and observations, and that the hypothesis that structure was an very important part of the films and a definable habitat for inclusion of many kinds of materials, some of which are entrapped or entrained at the air-water interface, and others that colonize these environments. The opportunities to locate manganese, copper, nickel and zinc in several different situations confirmed that the other transition metals must be considered and that the terms ‘floating iron films’, ‘mixed-valent iron films’ and schwimmeisen (floating iron) are less useful as descriptors of metallic surface films.

Investigations of the air-water interface, is a broad topic. In this research my several discussions indicate that there is still a gap in perceptions of the air-water interface. In some sense, this is better described as being toward a natural history of the air-water interface, of which aquatic surface films, metallic surface films and the heavier scums and foams are individual parts. We are looking mostly on one part,

which in itself has a diversity of structure that is previously unappreciated as a research topic. It points to the fact that as a boundary layer, there are such unexpected, unusual or unique properties, as any boundary layer condition might have.

To continue as ‘a structural analysis of metallic surface films and aquatic surface films by comparative microscopy’ constricts the research to some degree but in the process omits some important water chemistry for someone else to consider. Yet, within these structures presented here, we find some knowledge and clues we did not have to work with before. This was very productive in ideas. One of these is the relationship of surface tension of water and natural surfactants, which act to allow surface films to develop. One aspect of surface films is the formation of metallic surface films, the majority of which are mixed-valent iron oxide surface films. At the air-water interface other unique conditions of surface tension and the unique ecological constructions of the biota, allow organisms to participate in the air-water interface as habitat. It is a two-way condition, and we can reasonably expect that the biota are well-adapted to this surface condition, the properties of the air-water interface and the special conditions that can be imposed by the physical nature of the environment. For example, one of these are the fine structures on the legs of water striders (Gerridae) to allow better use of the epineustonic environment. Another is the spawning behaviour of syllid polychaetes whose pheromones are dispersed at the air-water interface (Gidholm 1965; Smith, 2008)

There are several research questions that develop around these conditions. For some organisms, such as water striders, the surface tension and specialized structures

allow for the use of the surface for support and travel. This is strictly a condition of clean water with a high surface tension. A small amount of natural surfactants or anthropogenic surfactants (detergents) will make the interface uninhabitable by water striders and related organisms. This use is termed epineustonic, or on top of the water surface.

Some organisms cling to the area just below the water surface. Some small snails will support themselves at the air-water interface as hyponeustonic habitat. Here, too, a small amount of natural surfactants or anthropogenic surfactants will make this uninhabitable. There is a strong cast to include surface film studies as part of environmental assessment, environmental impact statements and damage assessments. This is particularly true for dramatic events such as oil spills, but it is also true for chronic contamination of the air-water interface, sampling of which is done rarely if at all. While I have presented only one major aspect of the air-water interface, the documentation of both aquatic surface films and their components as well as the metallic surface films and their structures, provides a useful basis for assessment and comparison, even where the background documentation is lacking. As with sea surface films, the SML, it is reasonable to expect enrichments in both anthropogenic inputs and heavy metal enrichments at the air-water interface, much of which can be transported to other sites with other impacts to be evaluated. This is particularly true for eggs and larval forms of several invertebrates as well as for the bacterial, cyanobacterial and algal components of surface films and scums.

At the air-water interface natural surface tension is a strong force. Organic molecules are forced to extend above the water, forming a hydrated layer of organic surface film, usually termed the aquatic surface film (ASF) or microlayer. Visible slicks and films are often some organic molecules, which are known to have a wave-dampening effect can be identified easily. This was demonstrated fully in Chapter 7.

In calm or quieter conditions or near seeps and springs or wetlands, some metallic oxides will form at the air-water interface. As reported by Grathoff et al. (2007) and in Gray (2008), both derived from several historic works on the iron oxides, that abiotic formation can certainly occur particularly where high iron content forces oxidation to Fe_2O_3 and precipitation (Martin, 2005). Manganese can be included both in formation and precipitation under certain circumstances (Martin, 2005). But those are not the only circumstances. Since this work does not go into solution chemistry, but in structure, the complex relationships of manganese and iron is saved for some future hydrology, but some structural basis has been presented here. In reviewing the several environments for these minimal numbers of samples, we still get a wide range of formation with high values for carbon, silicon, calcium, and potassium, far above the moderate values for manganese, iron, nickel, zinc and copper.

13.1 Surface film research in environmental science

This research on aquatic and metallic surface films has a place in biology, a place in physics, a place in microscopy and a place in geology. This work has a place in environmental science in that it brings together several divergent paths of research and assessment that are often unrecognized by one another, such as hydrology paying

little attention to particulate sequestering of nutrient metals in inorganic or organic materials. This research, this view of the world by analytical imaging has a place in science, thought, consideration, assessment, concept, analysis and documentation in order to move researchers and government policy toward new areas of research that inform a wide range of stakeholders in water and wetland use, or if damaged as a better means of assessment. It is no less rigorous to develop a viewpoint from the vantage of imaging of discrete points of structure, morphology, biota and place.

More importantly, we have here a better way of asking the burning questions of the day in a microscopic context, which is often linked directly or indirectly to the larger processes of environmental conditions. With this is the opportunity to look at the instruments we have, which are often guided by materials science and applied technology, since that is where most of the instrument development is being done. Here, we can note and advocate how these instruments could be improved for the next generation of biological and environmental work. It is only with analytical research such as this that progress is made in instrument development. It is all too often the case that biology and environmental studies 'make do' with the sweepings of industry, as the instruments are designed more for industrial use. It is in the scholarship of this work that we have been able to develop and use the photoemission electron microscope, and it was biology that drove the initial work on its construction. We thank Gertrude Rempfer for that skill, knowledge and persistence.

In Chapter 1, surface films are presented in context, and the context is broad, more so than is usually presented. In Chapters 2 and 3 the analytical basis and the

relationship to particulates is established. In the following chapters more specific examples and technical or environmental conditions were developed and instrumental design discussed. Each chapter continued specific aspects of the working hypothesis with an interim conclusion. Chapters seven through twelve developed analytical images for each of the topics presented.

It is clear from the comparison of aquatic surface films that there is integration of surface film organic components into the developing metallic surface film, as shown in Chapter 10. It is also clear from the structural analysis in Chapter 8 that the formation of layers is not only part of film formation, but may be a key to charge separation and other means of compartmentalization. Also from Chapter 8, the fact that a copper-zinc film was analyzed confirms the working hypothesis that other transition metals must be fully considered in the evaluation of metallic surface films found at the air-water interface.

In several places we could examine molecular and nanoscale structures in general support of the working hypothesis. In particular, Chapter 12 demonstrated the inclusion of analytical images to address both mineralization and nanoparticulates as contaminants. This, with instrumental improvements, is an advance in this area of research. Students should evaluate the technical details outlined in Chapter 2, as the instruments continue to offer new means of imaging. But, not all of these changes will be useful to biologists and geologists, if speed and depth wipes away the areas of interest. Be aware that fine, delicate structures as seen by CPEM are important clues to the several biogeochemical processes being imaged.

This research expands the concept of metallic surface films from an iron oxide oxidation-reduction concept to something broader. This is not to say that the reduced iron found in abundance does not dominate many situations, such as can be found along the Oregon coast, but it also says to look more broadly at how films are constructed, how elements constitute diverse structures, and what instruments will aid in this determination. This may play some role in how we look at past water evidence on other planets, or how bacterial casts might document ancient biota in some way. The bacterial casts that emerged in this study are certainly structurally formed in a possible way to be trapped in some fossil sediment or concretion. The diversity of patches of activity can also be searched for in other surface film structures as well.

13.2 Astrobiology, exobiology and the air-water interface

I have presented new knowledge and information about the formation of metallic surface films to show that for any aquatic environment surface films of several kinds can form. It is in the polar structure of water that we can expect molecular arrangements at the air-water interface to be a feature of any planet with substantial water. This does not have to be water in the present. Ancient water or water long gone from the surface will leave traces of structure or biosignatures of organic materials. If there were living forms, traces of life should be detectable. In the case of metallic surface films, it now appears that these mineralized films represent the oxidation-reduction conditions that are present as well as the metallic elements that are available for mineralization. It may be a simple combination, such as several iron oxides, or combinations of metallic elements, such as ferromanganese films, structures with nickel

and zinc or even copper and zinc films. Only a few are shown here, but I expect that as we look for them, other combinations will be found. It appears that these may be present as patches within other films. The nickel-zinc biofilm appeared as a patch within an iron oxide surface film. The bacteria within the copper zinc surface film were present as a patch, as were several of the bacterial casts and forms within the ferromanganese surface film from Coos Bay, Oregon, and estuarine environment. These should be considered for any planetary analysis, remote sensing or future sampling, as remnants of these films may support other clues for both past water and past life.

In this study, I have shown that living microbes demonstrate integration into metallic surface films in the form of impressions or casts of living forms, which I have called the 'bacterial footprint'. Fossil impressions of these structures and forms are likely to occur, as the morphology is consistent with other fossil conditions and forms, and are comparable to bacterial or cyanobacterial forms attributed to Martian meteorites. This is a different aspect of possible fossilization and geological record, not restricted to earth. Microscopical evidence of present aquatic environments and conditions, are also consistent as possible key features in defining active habitat, conditions favorable to an evolving definition of habitability, and the through examination of metallic-rich environments.

Metallic surface films represent air-water interfacial properties of an aquatic boundary layer, thus including water conditions, biotic conditions, and structural forms of mineralization. Structural analysis reveals that several mineral structures should be

included in order to locate previous or present microbial casts as an interactive part of surface film formation and use as habitat. The structural analysis of metallic surface films suggest a broader characterization of habitability to include properties of the atmospheric–water boundary layer, or the air-water interface, and the boundary layer conditions and properties that occur under whatever conditions are present. It is quite likely that if an atmosphere has a different set of gas components, the properties of the air-water interface will be different than those found on earth. Yet the basic water–gas chemical and physical properties of exchange and solution can be understood. The air-water interface is, after all, a relatively understudied environment.

CHAPTER FOURTEEN

CONCLUSIONS

In this research structural analysis and image evaluation has been used to broaden the scope and perspective of the air-water interface. Basic knowledge of the air-water interface has been somewhat restricted to selected topics of chemistry and physics, where environmental factors biological uses and climate exchange has been important, but fragmented. Environmental analyses, planning and policy have seldom included surface film evaluation and air-water interface impacts or assessments in any significant way except in the case of oil and chemical spills, and that related more to visible cleanup. Here we make a case or inclusion as part of the broad environmental landscape of ideas, perceptions, study, research and assessment.

The public perceptions of the air-water interface are distant from the actual processes that can be evaluated, and we have made a case for specific, microscopic evaluation and corresponding instrumentation to make those evaluations a reality. In practice a public talk on the 'Scum of the Earth' proved to be a receptive means of presenting a technical subject. Support for these sorts of projects depend on both student and public education in surface film studies.

Yes, only a small part has been encompassed by a structural investigation. Morphology, in the biological sense, is often more of a guide to other research. But, what is clear is that biota play a significant role in surface film structure and colonization and that it appears to be functional part of habitat, the favored environment, and the utilization of nutrients and shelter provided by these unique

habitats. But, since there is interest in exobiology and astrobiology, it serves all of us to demonstrate the broad scope of metallic surface films as they might be found on any planet or condition with water, or for any planet or condition where water once existed. And if we were to expand this, then for any planet or condition where water might be introduced. It would be a useful thing to do a fresh inundation to an environment and follow the surface film conditions as colonizers inhabit the air-water interface and as aquatic surface films and metallic surface films give some clue to the movement of transition or other elements in the hydrosphere of that place.

It is clear that manganese, copper, nickel and zinc appeared in unexpected ways, and yet there is little experience with the roles these elements play in environmental evaluations and concerns. The role of patchy development or interaction needs further investigation, but along with this the presence of these elements either in addition to iron oxides or as separate biofilm elements is revealing that other processes, yet to be discovered, are at work. The functions of cation binding to other components of the air-water interface and of enrichments of transition metal ions in surface waters and surface films require further study. Structural analysis by analytical imaging is one means of gaining these perspectives.

In doing this evaluation, I also build a firm case for the concepts of habitability, the idea that there are factors to habitat that make it desirable and possible for life forms to develop and evolve. In conclusion, we have a body of work, which can guide further research. I have established that metallic surface films are not limited only to the more dominant iron oxides, even though they are present, ubiquitous and important, but are

also places for other mineralization. In these findings, have expanded the concept of metallic surface films to a much broader base of possibilities. In investigating metallic surface films with several different instruments, several perspectives were gained. In applying these viewpoints, it made it possible to develop the hypothesis of expanding the nature of metallic surface films studies to include unique events, such as the nickel-zinc biofilm and the copper-zinc metallic surface film with biota. There are new means of identifying and presenting patchiness within a film, with the view that patchiness is a clue to the biogeochemical processes that alter biofilms and nutrient cycles. This research is a firm declaration of an expanded area of study.

This work was an instrumental study. In order to shed some new light upon metallic surface films it was necessary to investigate the applications of instruments and instrumental development to the practical concerns of environmental microscopy. The several light microscopies gave good contrast, but could not resolve fine structure. The SEMs gave reliable resolution and introduced more questions to the hypothesis, but in the end helped to define a direction for the research. The hrTEM was necessary to address the question of amorphous mineral and ordered states of mineralization. No other instrument could do that. The hrSEM and dual-beam were the only means of milling away suspected layers to prove that thin, delicate layers could exist, and that we could now get a sense of the density of metallic film structure. Back to the SEM, however, SEM combined with EDS spectroscopy is an ideal tool for determining elemental composition at different stages of surface film development. Yes, there are other tools to be used, once the structural diversity is better defined in terms of what

research questions could be asked. This piece of environmental microscopy and structural analysis has a broad application for any water–mineral situation, for any past, present or future air-water interface study, assessment or condition.

The air-water interface is a boundary layer with unique properties and conditions that make it suitable for surface films to form. In the past this was seen as mostly organic surface films, slicks or scums, with an experimental history of wave-dampening effects that could be quantified. Organic surface films or sea-surface films, the microlayers, were found to be enriched in both complex organic molecules and heavy metals. The metallic surface films are also a feature of the air-water interface, but understudied. Previously, the metallic films were thought to be variations of iron oxide mineralization, and that is still a common case. But, it must be said that metallic surface films are far more complex than previously reported, both in terms of mineral, or mineralizing structure themselves, as well as incorporation of other biota or fragments of biota, as demonstrated here. Many of these features occur in patches, or patches within a surface film, something to be examined further. These conditions and structures force us to reconsider the metallic surface films in a much broader scope that could include other metallic mineralizations and structures, that are still consistent with what we know about the air-water interface, boundary layer properties, surface tension and polar chemical structures in such environments. Analytical microscopy or comparative microscopy is shown to be a useful set of tools to further explore these structural features of aquatic environments on whatever planet where water may occur or have occurred in the past.

GLOSSARY

Aquatic Surface Films. See ASF. The preferred term to describe organic surface films, surface films or surface microlayer films. See Microlayer, SAS, SML, SSM.

Allochthonous: Particulate material from somewhere else. Can be rocks transported from some other place. In biology leaves or trees coming from some other place.

- αλλος or allos, meaning other, or different;
- κθονος or kthonos, meaning earth.

Anemoneuston: Terrigenous (earth formed) organic and inorganic material transported by wind to the surface of water and the air-water interface.

Archaeoneuston: Bacterioneuston derived from the Archaea, ancient bacteria, often using anoxic or lithotrophic nutrient sources. Chemoautolithotrophs: not using photosynthesis.

Autochthonous: Particulate material formed in the place where found. Indigenous. Buried in place, as for a fossil. Autochthonic.

ASF: Aquatic Surface Film. Surface organic film, chemicals and organisms, often restricted to freshwater environments and films, including those of ponds, wetlands, bogs etc. as well as lakes, streams and rivers. See: Microlayer and SSF.

AWI: Air-Water Interface. Any air-water interface observation, experiment, collection or activity. Includes the adjacent interface to floating metallic oxide surface film or iron oxide surface films, the traditional microlayer or ASF environments.

Bacterioneuston: Microbial organisms, the Eubacteria, Archaeobacteria and Cyanobacteria living at the air-water interface. May be freshwater, estuarine, thermal springs, mineral springs or marine. See Pleuston, which refers to marine organisms. Bacterioneuston defined by Yu P. Zaitsev in part, but Sieburth particularly.

Benthic Environment: Those environments of the “bottom”, the water-sediment, water-substrate, water-geologic interface.

Benthos: Organisms of the benthic environments. The water-sediment interface or water-substrate interface. This includes the air-sediment interface when water is or has been present, such as intertidal flats and wetland flats that still contain benthic organisms.

Biofilm: Any organic or cellular film of biological origin, typically bacterial films on surfaces, tubing, rocks, structures or benthic habitats, but not excluding surface biofilms at the air-water interface.

Biogeochemical: Any natural process, which integrates biological activity with minerals, mineralization or inorganic chemical processes or features. Often used to describe nutrient cycling, such as ‘biogeochemical cycling of iron’ as a nutrient.

Biogeochemical: Any natural process, which integrates biological activity with lithology or geological processing of rocks, including minerals, mineralization or inorganic chemical processes or features. Often used to describe weathering of minerals, such as ‘biogeochemical cycling of iron’ as a geological process.

Bioremediation: Any remedial, restorative or corrective action taken to reduce contamination by biological means, such as mineralizing bacteria, oil-feeding microbes, etc.

Biological Stain: Any number of chemical stains used to impart color and contrast to biological samples. Stains are highly regulated for use in pathology by the *Biological Stain Commission* in the United States of America, which tests products for consistency against paraffin section tissue, and certifies the stain for use in pathology.

Biosignature: Often applied in astrobiology as analyzable biotic components that remain in geological formations or soils. Remnant components of previous biological activity may serve to detect early life. See Chemical Biosignatures

Chemautolithotroph: Bacteria using chemical electron pathways instead of photosynthesis. Chemautolithotrophic. Chemautolithotrophy. See Lithotrophy.

Chemical Biosignatures: Analytical chemical detection of organic materials that may represent biochemistry or biochemical compounds representative of living systems, physiology or metabolism. May include metalloporphyrins and similar compounds.

Colloids: A dispersed phase of small particles, generally described 1.0 nm to 1000 nm or 2.0 nm to 2.0 μm . Sometimes described as dissolved rather than a suspension as the medium particles pass through a 0.2 μm filter. See DOM, POM. Organic colloids include hydrophilic polymers dispersed in water, as in a gel. Colloidal.

CPEM: Corrected photoemission electron microscopy. Aberration correction of electrostatic lenses by electrostatic mirror, as defined by Gertrude F. Rempfer. See PEM.

Cyanobacteria: Broad classification of the prokaryote blue-green algae, the ancient lineage cyanobacteria. (Cyanophyta). The Blue-Green Algae are a bacterial lineage. Many are neustonic due to the presence of vesicles, which regulate buoyancy. See *Trichodesmium* and Diazotroph. There is often an iron-dependent nutrient pathway.

Desert Varnish: Desert Varnish is a molecular surface layering, related to high solar input and heat that shifts elements, including iron and manganese to the air-solid interface.

Diazotroph: Any nitrogen fixing alga, but usually species of *Trichodesmium*, a neustonic cyanobacterium. See Rueter, Unsworth, Hutchins & Smith, 1989 in References.

DIC: In optical microscopy, Differential Interference Contrast or ‘Nomarski’ method in optical microscopy for achieving contrast by beam shearing/amplitude modification.

DOC: Dissolved Organic Carbon. Any carbon containing molecule or detritus that can pass through a 0.2µm filter. Also termed DOM, dissolved or soluble organic material.

DOM: Dissolved Organic Material. Generally, any organic molecule or detritus that can pass through a 0.2 µm filter. Distinguished from DOC usually by ash weight to calculate inorganic elements following incineration. DOM is generally defined as including humic substances, polysaccharides and proteins (Philippe and Schaumann (2014).

EDS: Energy Dispersive Spectroscopy. Elemental analysis of x-ray photons emitted by elements at the atomic level, stimulated by electrons. Measures specific energy level of the x-ray photon. Requires x-ray energy detection.

EDSX: Energy Dispersive X-Ray Spectroscopy, also known by the brand name, EDX. EDS is the preferred acronym, but EDSX seems to be gaining favor as a reference to this type of spectroscopy. See EDS.

EELS: Electron Loss Spectroscopy. An instrumental method with the capability of measuring inelastic kinetic energy scattering or loss. The amount of energy loss is measured as inner-shell electrons shift and EELS can determine atomic composition.

Electron Microscopy: Those several microscopies that generate and use electrons as their primary source of excitation and contrast to form an image or to obtain spectral information. See PEM, SEM, TEM.

EPI: Acronym for epiillumination in light microscopy. Light is passed through the objective lens to illuminate the surface of the specimen to the objective lens. As opposed to transmitted light through the specimen to the objective lens.

Epiinterfacial Surface: The upper or air or gas exposed surface of a film.

Epineuston: Neustonic organisms found above the air-water interface or using surface tension for their support. Water striders are epineustonic insects. Naumann, E. 1917.

Epipelagial: Alternative definition of neustonic organisms, particularly free-floating in ocean environments. Not in common use any longer.

EPS: Extracellular Polymeric Substances. Any extracellular product or deposit, either as part of the immediate cellular environment or excretion or as a substance attached or covering the substrate to modify the environment as a suitable habitat or increasing habitability of the immediate environment. Often used to describe secretions of biofilms or of individual diatom excretions. See TEP

Exopleuston: Positively buoyant organisms with dry upper surfaces, but with most of the organism underwater, the habitat of the endopleuston.

FeFilms: Fe-Bound surface films. Alternate acronyms for MSF or MVIF. Used particularly with reference to wetlands, surface microlayers, other surfactants and oils as ecosystem components.

Ferrolithotroph: Any organism able to mobilize or acquire iron nutrients or electrons from a mineral source. See Lithotroph. Lithotrophic. Lithotrophy.

Ferromanganese: Any mineral component containing both iron and manganese. Ferromanganese nodules are found in both freshwater and marine environments. Ferromanganese mixed valent surface films are reported here.

FIB: Focused Ion Beam. In electron microscopy, an instrumental method of accelerating elemental ions, usually gallium, upon a sample to remove or deposit layers of material, to cut or shave a sample or other invasive processes. Integrated with an SEM system typically. Sometimes occurring in combination with both EDS and SIMS spectroscopies.

Films: Any boundary condition between substrates. Here, an organic or mineral film formed at the air-water interface. There are also films formed between water-solid interface, such as biofilms upon rocks. Our definition is any structural layer formed between differences in substrate. See: Scums and Foams.

Floating Iron Films: Alternate keyword to describe iron mixed-valent films. See Metallic Surface Films, MSF.

Foams: Complex or layered films of some structural development that include bubbles or other buoyancy. Like scums, foams are usually highly visible and not transparent and may contain any number of structural components, extracellular substances and mineral contributions, insect parts, algal residue and surfactants.

FPOM: Fine Particulate Organic Matter. Fine particulates generally defined as 0.45 μ m - 1.0 mm. But some would limit FPOM as 0.45 μ m - 10 μ m as POM or CPOM is retained by a 0.2 μ m filter.

Fulvic Acids: A natural, complex of organic phenolic substances. Fulvic acids are usually defined as the non-precipitating or soluble portion of hydroxide analytical treatment.

Humic Acids: A natural, complex of organic, phenolic substances. Humic acids are usually defined as hydroxide precipitating humic substances. See Fulvic Acids.

Humic Substances: Naturally occurring phenolic substances consisting of humic and fulvic acids. Humic substances are reported as important cation binding substances. Humic substances are usually considered to consist of both humic and fulvic acids.

Hypointerfacial Surface: The lower, water or substrate exposed surface of a film, the obverse of a film collected with the air-interface uppermost.

Hyponeuston: Neustonic organisms living predominately below the air-water interface.

Ichthyoneuston: The ichthyological component of the surface film, typically neustonic fish eggs, but also stages of fish larvae feeding on the surface film. Ichthyoneuston.

Interface, Air-Water: The boundary condition between air and liquid water. The physical and chemical conditions present at this boundary layer and the physical parameters that affect conditions and properties. See *Neuston* for organisms that are present at the air-water interface or conditions such as films and scums. See AWI.

Interface, Water-Sediment: A submerged boundary-layer condition between water and the sediment or substrate. This implies permanent cover, but for the intertidal zone or for vernal or ephemeral pools and ponds, it can refer to seasonal wetlands and conditions. See Benthic Environment; Benthos.

Lacustrine: Lacustrine Paleolimnology. Having to do with analysis of lakes, lake biota and lake beds. Includes paleolimnological studies of sediments.

Light Microscopy (LM): Optical Microscopy using various sources of light, usually not including lasers using several glass or quartz optical elements in various configurations. Brightfield, Darkfield, Phase Contrast and Laser Confocal Scanning microscopies are typical members.

Limnetic: Referring to the shores, particularly to shallow waters, or as a reference to limnological work.

Limnology: The study of lakes and ponds, lake systems and historic lakes. See Paleolimnology. Limnology encompasses a large body of aquatic work including all bodies of water from ponds to reservoirs, flowing streams to vernal pools.

Lithotrophy: The condition of iron oxidizing/reducing nutrition. Particularly iron-oxidizing bacteria. Lithotroph, lithotrophic, lithotrophic environment, autolithotroph. See Chemolithotroph.

Littoral: Particularly the intertidal zone, the area subject to fluctuations in water, such as in tides, but also to reservoir shores, subject to fluctuation in shoreline exposure.

Metachromasia: The characteristic of some biological stains to have several spectral variations of color, imparted to the specimen, thus giving greater contrast to tissues and structures. Often hydration, such as added water rinse after a staining procedure, will impart a diversity of color variation. The ability of the stain to give this contrast is *metachromasia*. In some cases, metachromasia represents pH differences or anion-

cation charge, but it is a histological application of biological stains that has application to environmental histology and surface film evaluation.

Microlayers: Microlayer surface films of freshwater, estuarine or marine origin. The concept of microlayers suggests separation of physical, chemical and environmental structure and attendant organisms, the neuston.

MSF: Metallic surface Films. The mineral equivalent of surface microlayer films with reference to mineralization or iron oxide surface films. See Floating iron

Munsell Color: The Munsell system of color matching, separating Hue, Chroma and Value to obtain comparison. Developed by Charles Munsell, 1912. The Munsell color charts are a commercial product. See the Land Color System, Optical Society of America – Uniform Color Space, and the International Commission on Illumination (CIE, CIECAM02).

MVIF: Mixed Valent Iron Films. This is an alternate acronym for surface metallic films for freshwater and estuarine environments, a mix of Fe_2O_3 and Fe_3O_4 and several states of mineralization and hydration. See MSF, the preferred term.

Nanobiology: Structural elements, cytoskeletal elements, inclusions and crystals derived from biological processes and systems in the nanometer size range.

Neuston: From Einar Naumann, *Das Neuston*. Organisms of the water surface, particularly of the air-water interface of oceans, ponds and estuaries. Further divided in epineuston, hyponeuston, bacterioneuston, and exopleuston and endopleuston. Neustonic, neustont, neustic.

Neustonology: From Yu. P. Zaitsev. The study of surface water organisms and the neustonic environment at or around the air-water interface. Those organisms living, traveling, reproducing or feeding at the air-water interface. Sometimes to the upper 10 cm of water and the organisms and floating eggs in that neustonic layer.

NOM: Natural Organic Matter. Now a common term for unquantified particulate carbon or particulate organic material or a combination.

Optical Microscopy: Using glass, quartz or other crystalline optics and other refractive optics, such as prisms or reflective optics such as mirrors. Light sources usually lamps, arcs, solar or lasers. Increasingly, diode lasers are being used as the light source.

OFE: The Oxide Film Edge. The edge of films has some special characteristics or interaction that suggests a separate term.

OFM: The Oxide-Film Matrix. In this study, the iron oxide or other metallic oxide surface film matrix, the structural film matrix.

Organic Biosignature: Biosignatures with primary reference to laves or structures of carbon or organic chemical spectra or signatures.

Oxyhydroxides: The general term for different states of oxidation/reduction of several elements in aquatic situations. Ferrous and Ferric oxyhydroxides, the several mineral states for iron oxides.

Paleolimnology: Historic metabolic, sedimentary and structural states of past lake and pond systems. Includes the fossil record of sediments including both morphological remnants and biogeochemical substances. May include Biomarkers.

Palynology: The study of pollen, pollens of the air-water interface, particularly the Epiinterfacial surface component of metallic surface films as well as aquatic surface films.

Palynoneuston: Pollens of the air-water interface. Includes the entrainment of terrestrial, wind blown pollens that become common, obligative or facultative nutrient parts of the air-water interface, aquatic surface film or metallic surface film structure.

Pelagial: Recognized habitat realm of the sea surface, free-floating environments.

PEM or PEEM: Photoemission Electron Microscopy. An instrumental method using high intensity light, photons, to stimulate electrons into the lens system. Compare to TEM.

Phase Contrast Microscopy: In optical microscopy, contrast enhancement by annular rings, which produce a $\frac{1}{2}$ lambda wavelength shift.

Phototrophic: Attracted to light. Often a behavior related to feeding, mating or other reproduction. Includes diel migrations to the air-water interface. Phototrophy.

Phytoneuston: Plant life of the neuston. Typically algae, protozoa, plant or phytotrophic components of the air-water interface including spores, seeds, and pollen.

Phytotrophic: A diet of plant materials. Particularly attracted or symbiotic with algae and plant materials. Phytotrophy.

Picoplankton: Planktonic organisms, usually microbes in the small size range of 0.2 to 2.0 μm .

Planktonic: Recognized habitat realm of aquatic organisms subject to currents. May be freshwater, estuarine or marine, lacustrine, or any large body of water.

Plant Names: Recognized taxonomy of plant names and codes as defined by the Integrated Taxonomic Information System (ITIS) and cooperating databases.

Pleuston: Organisms of the marine air-water interface, particularly bacteria. See Bacterioneuston, which may include any surface environment. Overlap in definition with *neuston*, which now tends to be the more common term.

Pleustal: Recognized habitat realm of the pleuston.

PIN: Particulate Inorganic Nitrogen. Nitrogenous materials that are retained by a 0.2 μm filter.

POC: Particulate Organic Carbon. Any particulate carbon-containing material that is retained by a 0.2 μm filter. See POM, DOC. POC differs from POM as a carbon vs ash weight is determined by incineration, giving a true carbon value lost to incineration.

POM: Particulate Organic Material. See FPOM, fine particulate organic material. Sometimes called CPOM, coarse particulate organic material. There are differences in definition as POC is retained by a 0.2 μm filter. POM may be marked by stains without incineration so that the ash weight is not determined with any accuracy.

Probe Microscopy: Those several microscopies that use a sensing probe to determine structure, such as Atomic Force Microscopy (AFM), Apertureless near-field scanning optical microscopy (ANSOM) and others.

SAS: The Surface Active Substance(s). An alternate measure of surface film by electronic charge.

Schwimmeisen: Alternate descriptive term for MVIF or FeMVSF meaning ‘floating iron’. Proposed by Grathoff (2007). Not a geological classification, such as ‘hematite’.

Scums: Complex or layered films of some structural development that generally does not include bubbles or other buoyancy. Like foams, scums are usually highly visible and not transparent and may contain any number of structural components, extracellular substances and mineral contributions, insect parts, algal residue and surfactants. See Foams and Films.

Scums and Foams: Generally defined as thick surface films, often with other components such as algal filaments, bacterial filaments, and for foams, bubbles or other buoyant features. See Films.

SEM: Scanning Electron Microscopy. In electron microscopy, an instrument where the electron beam is scanned across the surface of the sample, a raster scanned pattern, to achieve excitation and release of other electrons, which are detected for analysis (e.g. secondary electron detector). Multiple detectors are common.

SIMS: Secondary Ion Mass Spectroscopy. An instrumental spectroscopy method able to separate not only elements, but also isotopes of elements. SIMS is capable of better resolution than EDS spectroscopy, and high-end SIMS can reliably distinguish isotopes of elements of similar atomic weights and structures. See FIB, Dual Beam.

SMIC: Aquatic Surface Microlayer. Older term by Hardy, Crecelius et al. 1990. See ASF, SML.

SML: The sea surface microlayer, the current and preferred term. Applied by Cunliffe et al. (2012). See also aquatic surface film or ASF, also a current term, particularly for nearshore waters and estuaries.

SSF: Sea Surface Film. Surface organic film, chemicals and organisms, often restricted to marine and oceanic environments. An alternate acronym for microlayer or sea surface microlayer film. See Aquatic Surface Films, although ASF more typically refers to freshwaters and upper estuaries. See SML, the preferred term.

STEM: Scanning Transmission Electron Microscopy. In electron microscopy, high resolution instruments where a focused beam of electrons is transmitted through a thin sample in scanning mode to form an image or to retrieve other information about the sample (e.g. crystal structure, diffraction). Greater resolution than electron lens TEM.

Taxonomy: The comprehensive definition of scientific names as cataloged by the Integrated Taxonomic Information System (ITIS) and cooperating database systems,

which include the Global Biodiversity Information System, Species 2000, Smithsonian Institution and others.

TEM: Transmission Electron Microscopy. In electron microscopy, instruments where a focused beam of electrons is transmitted through a thin sample to form an image or to retrieve other information about the sample (e.g. crystal structure, diffraction).

TEP: Transparent Exopolymer Particles. Similar to the definition of Extracellular Matrix Proteins, or Extracellular Polymeric Substances. See EPS. Transparent and buoyant constituents of microlayer films (See Cunliffe et al., 2010).

Terrigenous: Of the earth. Components derived essentially from the land, but geological and biological derivatives found in surface films, integrated into metallic surface films, or dusts at the air-water interface or as components of sea-surface microlayers. See Allochthonous.

***Trichodesmium*:** Genera of Cyanobacteria known for neustonic development in marine surface waters. See Diazotroph. See Rueter, Hutchins, Smith and Unsworth (1992), in the references as a primary SEM study in this dissertation.

Vernal Pools: Seasonal or Ephemeral ponds, pools or standing water. Usually in dry climates such as Eastern Oregon and Eastern Washington, and the site for seasonal invertebrates, especially crustaceans, brachiopods, fairy shrimp, etc.

Wetlands: Marshes, swamps, vegetated and unvegetated wetlands as defined by U. S. Department of the Interior, U. S. Fish and Wildlife Service, Wetlands and Deep Water Habitats of the United States (Cowardin et al., 1983(Rev.)). There are many independent definitions of wetlands. Most definitions include a broad reach of wetland and riparian habitats, soils, hydric soils and sediments. Generally superseded by USEPA guidelines for Wetlands, but more complete in its scope.

WFI: The Water Film Interface. Generally the underside of floating iron-oxide surface films, in intimate contact with the water, the site of most biological activity.

REFERENCES

- Adam, N. K. 1937. A Rapid Method for Determining the Lowering of Tension of Exposed Water Surfaces, with Some Observations on the Surface Tension of the Sea and Inland Waters. *Proc. R. Soc., London, Ser. B. Biol. Sci.*, 122:134-139.
- Adam, N. K. 1941(1968). *The Physics and Chemistry of Surfaces*. Dover, NY, U.S.A.
- Adamson, Arthur W. 1982. *Physical Chemistry of Surfaces*. 4th Ed. J. Wiley and Sons, Inc., NY, NY U.S.A. 664 p.
- Agogu, H, E. O. Casamayor, F. Joux et al. 2004. Comparison of Samplers for the Biological Characterization of the Sea Surface Microlayer. *Limnol. Oceanogr.*–Methods 2:213-225.
- Albright, L. J. 1980. Photosynthetic Activities of Phytoneuston and Phytoplankton. *Can. J. Microbiol.* 26:389-392.
- Albright, L. J., S. K. McCrae and B. E. May. 1986. Attached and Free-Floating Bacterioplankton in Howe Sound, British Columbia, A Coastal Marine Fjord-Embayment. *Appl. Environ. Microbiol* 51:614-621.
- Allredge, A. L. 1991. *In situ* Collection and Laboratory Analysis of Marine Snow and Large Fecal Pellets. In: *Marine Particles: Analysis and Characterization*. Geophysical Monograph No. 63. Am. Geophys. Union.
- Allredge, A. L., U. Passow and B. E. Logan. 1993. The Abundance and Significance of a Class of Large, Transparent Organic Particles in the Ocean. *Deep Sea Research I*. 40(6):1131-1140
- Allredge, A. L. and M. W. Silver. 1988. Characteristics, Dynamics and Significance of Marine Snow. *Progress in Oceanography*, 20: 41-82.
- Asher, William E., G. S. Frysiner and G. M. Korenowski. 1988. Reflected Optical Second-Harmonic Generation in the Study of Naturally Occurring Organic Films at the Ocean Surface. *J. Geophys. Res.* 93(C6):6955-6957.
- Asher, William E. and J. F. Pankow. 1989. Direct Observation of Concentration Fluctuations Close to a Gas-Liquid Interface. *Chem. Eng. Sci.* 44(6):1451-1455.

- Azetsu-Scott, K and U. Passow. 2004. Ascending Marine Particles: Significance of Transparent Exopolymer particles (TEP) in the Upper Ocean. *Limnol. Oceanogr.* 49(3):741-748.
- Baier, R. E. 1970. Surface Properties Influencing Biological Adhesion. In: R. S. Manley (Ed.): *Adhesion in Biological Systems*. Academic Press, NY U.S.A.
- Baier, R. E. 1972. Organic Films on Natural Waters: Their Retrieval, Identification, and Modes of Elimination. *J. Geophys. Res.*, 77:5062-5075.
- Baier, R. E. 1980. Substrata Influences on Adhesion of Microorganisms and their Resultant New Surface Properties. In G. Bitton and K. C. Marshal (Eds.): *Absorption of Microorganisms to Surfaces*. J. Wiley & Sons, Inc., NY
- Baier, R. E. 1981. Initial Events in Microbial Film Formation. *Symposium on Marine Biodeterioration*. Office of Naval Research, Bethesda, Maryland.
- Baier, R. E. 1984. Initial Events in Microbial Film Formation. In: J. D. Costlow and R. C. Tipper (Eds.): *Marine Biodeterioration: An Interdisciplinary Study*. Annapolis, MD Naval Institute Press.
- Baier, R. E. and V. A. DePalma. 1970. Electrodeless Glow discharge Cleaning and Activation of High-Energy Substrates to Insure Their Freedom From Organic Contamination and their Receptivity for Adhesives and Coatings. Calspan Corp., Buffalo, NY Report No. 176.
- Baier, R. E., D. W. Gouptil, S. Perlmutter and R. King. 1974. Dominant chemical Composition of Sea-Surface Films, Natural Slicks, and Foams. *J. Recherches Atmospheriques*. 8: 571-600.
- Baier, R. E. and P. O. Blantz. 1978. Characterization of Oral *in vivo* Films Formed on Different Types of Solid Surfaces. *Acta Odontologica Scandinavia* 36: 289-301.
- Bailey, C. A., R. A. Neihof and P. S. Tabor. Inhibitory Effect of Solar Radiation on Amino Acid Uptake in Chesapeake Bay Bacteria. *Appl. Environ. Microbiol.* 46(1):44-49. OTM
- Balleck, R. W. and E. Swift. 1986. Nutrient- and Light-Mediated Buoyancy Control of the Oceanic Non-Motile Dinoflagellate *Pyrocystis noctiluca*, Murray ex Haeckel (1980). *J. Exp. Mar. Biol. Ecol.* 109:175-192.
- Banse, K. 1990. Does Iron Really Limit Phytoplankton Production in the Offshore Subarctic Pacific? *Comment. Limnol. Oceanogr.* 35(3):772-775.

- Banse, K. 1991. Rates of Phytoplankton Cell Division in Iron Enrichment Experiments. *Limnol. Oceanogr.* 36(8):1886-1898.
- Barbier, M. D. Tusseau, J. C. Marty and A. Saliot. 1981. Sterols in Aerosols, Surface Microlayer and Subsurface Water in the North-Eastern Tropical Atlantic. *Oceanol. Acta* 4(1):77-84.
- Barker, D. R. and H. Zeitlin. 1972. Metal-Ion Concentration in Sea-Surface Microlayer and Size-Separated Atmospheric Aerosol Samples in Hawaii. *J. Geophys. Res.* 77:5076-5086.
- Barger, William R. Jr. 1985. Naturally-Occurring Surface-Active Material Concentrated at the Air-Water Interface. PhD Thesis. Univ. Maryland. 217 p.
- Barger, W. R., W. H. Daniel and W. D. Garrett. 1974. Surface Chemical Properties of Banded Sea Slicks. *Deep-Sea Res.* 21:83-89.
- Baylor, E. R. W., H. Sutcliffe and D. S. Hirschfeld. 1962. Adsorption of Phosphates onto Bubbles. *Deep Sea Res.* 9(March-April):120-124.
- Beil, C. R. and L. J. Albright. 1982. Bacteriological Investigation of the neuston and Plankton in the Fraser River Estuary, British Columbia. *Estuarine Coastal Shelf Sci.*, 15:385-394.
- Bendure, R. L. and R. S. Hansen. 1967. Capillary Ripple Investigations on Monolayers at Intermediate Elasticities. *J. Phys. Chem.* 71: 2889-2892.
- Betzer, P. R., K. L. Carder, R. A. Duce, et al. 1988. Long-Range Transport of Giant Mineral Aerosol particles. *Nature* 336(6199; Dec. 8): 568-571.
- Bewin, D. W. and W. A. Zisman. 1965. The Wetting of Gold and Palladium by Water. *J. Physical Chem.* 69:4238-4242.
- Bezdek, H. F. and A. F. Carlucci. 1972. Surface Concentration of Marine Bacteria. *Limnol. Oceanogr.* 17:566-569.
- Bidelman, T. F. and C. E. Olney. 1974. Chlorinated Hydrocarbons in the Sargasso Sea Atmosphere and Surface Water. *Science* 183:516-518.
- Birrell, G. B., D. L. Habliston and O. H. Griffith. 1994. Photoelectron Imaging of Viruses and DNA: Evaluation of Substrates by Unidirectional Low Angle Shadowing and Photoemission Current Measurements. *Biophys. J.* 67:2041-2047.

- Bitton, G. and K. C. Marshall. 1980. Adsorption of Microorganisms to Surfaces. J. Wiley & Sons, New York, NY
- Blanchard, D. C. 1963. The Electrification of the Atmosphere by Particles from Bubbles in the Sea. *Prog. Oceanogr.* 1(1963): 71-202.
- Blanchard, D. C. 1983. The Production, Distribution and Bacterial Enrichment of the Sea-Salt Aerosol. In: P. S. Liss and W. G. N. Slinn. *Air-Sea Exchange of Gases and Particles*. D. Reigdel Publ. Co., Dortrecht, Netherlands pp. 407-454.
- Blanchard, D. C. and B. C. Parker. 1977. The Freshwater to Air Transfer of Microorganisms and Organic Matter. In: J. Cairns, Jr. Ed: *Aquatic Microbial Communities*. Garland Publ. Co., Inc., New York, NY. pp. 625-658.
- Blanchard, D. C. and L. D. Syzdek. 1970. Mechanisms for the Water to Air Transfer and Concentration of Bacteria. *Science* 170: 626-628.
- Blanchard, D. C. and L. D. Syzdek. 1977. Production of Air Bubbles of a Specified Size. *Chem. Eng. Sci.*, 32:1109-1112.
- Blanchard, D. C. and L. D. Syzdek. 1988. Film Drop Production as a Function of Bubble Size. *J. Geophys. Res.* 93:3644-3654.
- Blanchard, D. C. and L. D. Syzdek. 19 Reply. *J. Geophys. Res.* 95:7393.
- Blanchard, D. C. and A. H. Woodcock. 1957. Bubble Formation and Modification in the Sea and its Meteorological Significance. *Tellus.* 9:145-158.
- Blodgett, Katharine B. 1934. Monomolecular Films of Fatty Acids on Glass. *J. Am. Chem. Soc.* 56(2):495.
- Boehmn. P. D. 1980. Evidence for the Decoupling of dissolved, particulate and Surface Microlayer Hydrocarbons in Northwestern Atlantic Continental Shelf Waters. *Mar. Chem.* 9():255-2xx.
- Borstad, G. A. 1982. The Influence of the Meandering Guiana Current on Surface Conditions Near Barbados - Temporal Variations of *Trichodesmium* (Cyanophyta) and other Plankton. *J. Mar. Res.* 40:435-452.
- Borstad, G. A. and L. E. Borstad. 1984. Needlelike Projectiles Observed Issuing from Lysing *Trichodesmium* (Cyanophyta) Cells. *Limnol. Oceanogr.* 29:1097-1099.

- Brockmann, U., H. H. Hünerfuss and G. Kattner. 1982. Artificial Surface Films in the Sea Near Sylt. *Limnol. Oceanogr.* 27:1050-1059.
- Bryers, J. and W. Characklis. 1981. Early Fouling Biofilms Formation in a Turbulent Flow System: Overall Kinetics. *Water Res.* 15:483-491.
- Buick, R. 2008. When Did Oxygenic Photosynthesis Evolve? *Proc. Trans. R. Soc. B.* 363:2731-2743.
- Burton, E. D., R. T. Bush, L. A. Sullivan and D. R. G Mitchell. 2008. Schwertmannite Transformation to Goethite via the Fe(III) Pathway: Reaction Rates and Implications for Iron-Sulfide Formation. *Geochim. Cosmochim. Acta.* 72(2008): 4551-4564.
- Cajal, S. Ramón y. See Ramón y Cajal, S. (Histologist known generally as Cajal).
- Canavan, R. W., C. P. Slomp, P Jourabchi, P. V Cappellen, A. M. Laverman and G. A. van den Berg. 2006. Organic Matter Mineralization in Sediment of a Coastal Freshwater Lake and Response to Salinization. *Geochim. Cosmochim. Acta.* 70(2006):2836-2855.
- Carlson, D. J. 1982a. Surface Microlayer Phenolic Enrichments Indicate Sea Surface Slicks. *Nature* 296:426-429.
- Carlson, D. J. 1982b. A Field Evaluation of Plate and Screen microlayer Sampling Techniques. *Mar. Chem.* 11:189-208.
- Carlson, D. J. 1982c. Phytoplankton in Marine Surface Microlayers. *Can J. Microbiol.* 28:1226-1234.
- Carlson, D. J. 1983. Dissolved Organic Materials in Surface Microlayers: Temporal and Spatial Variability and Relation to Sea State. *Limnol. Oceanogr.* 28:415-483.
- Carlson, D. J. 1985. A Field Evaluation of Plate and Screen Microlayer Sampling Techniques. *Marine Chem.* 11(3):189-208.
- Carlson, D. J., J. L. Cantey and J. J. Cullen. 1988. Description of and Results from A New Surface Microlayer Sampling Device. *Deep-Sea Res.* 35(7):1205-1213.
- Carlson, D. J. and L. M. Mayer. 1980. Enrichment of dissolved Phenolic Material in the Surface Microlayer of Coastal Waters. *Nature* 286(): 482.

- Carlucci, A. F., D. B. Craven and S. M. Henrichs. 1985. Surface-Film Microheterotrophs: Amino Acid Metabolism and Solar Radiation Effects on their Activities. *Mar. Biol.* 85(1):13-22.
- Carpenter, E. J. 1983. Nitrogen Fixation by Marine Oscillatoria (Trichodesmium) in the World's Oceans. In: Carpenter, E. J. and D. G. Capone (Eds): Nitrogen in the Marine Environment. Academic Press, New York, NY. pp. 65-103.
- Carson, R. 1951. *The Sea Around Us*. Oxford University Press, Oxford & New York.
- Chapman, P. and P. S. Liss. 1981. The Sea Surface Microlayer: Measurements of Dissolved Iodine Species and Nutrients in Coastal Waters. *Limnol. Oceanogr.* 26(2): 387-390.
- Cheng, L. 1975. Marine Pleuston - Animals at the Sea-Air-Interface. *Oceanogr. Mar. Microbiol, Ann. Rev.* 13(1975):181-212.
- Cipriano, R. J. and D. C. Blanchard. 1981. Bubble and Aerosol Spectra Produced by a Laboratory "Breaking Wave". *J. Geophys. Res.* 86:8085-8092.
- Cohen, A. L. 1977. A Critical look at Critical Point Drying -- Theory, Practice and Artifacts. *Scanning Electron microscopy, Vol. I. Proceedings of the Workshop on Biological Specimen Preparation Techniques.* IIT Research Institute, Chicago, Illinois. pp. 525-536.
- Cornell, R. M. and U. Schwertmann. 2003. *The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses.* Wiley-VCH 703 p.
- Cosovic, B., and V. Vojvodic. 1987. Direct Determination of Surface Active Substances in Natural Waters. *Marine Chem.* 22:363-373.
- Cosovic, B., V. Zutic and Z. Kozarac. 1977. Surface Active Substances in the Sea Surface Microlayer by Electrochemical methods. *Coratia Chem. Acta.* 59:229-237.
- Cowardin, L. M, V. Carter, F. C. Golet and E. T. LaRoe. 1979 (1985). *Classification of Wetlands and Deepwater Habitats of the United States.* U. S. Department of the Interior, Fish and Wildlife Service, Office of Biological Services, FWS/OBS-79/31.
- Craik, J. C. A. and S. M. Harvey. 1987. The Causes of Buoyancy in Eggs of Marine Teleosts. *J. Mar. Biol. Assoc. U. K.* 67(2):169-182.

- Crist, R. H., K. Oberholser, B. Wong and D. R. Crist. 1992. Amine-Algae Interactions: Cation Exchange and Possible Hydrogen Bonding. *Environm. Sci. Technol.* 26(8):1523-1526.
- Crosby, M. P., R. I. E. Newell and C. J. Langdon. 1990. Bacterial Mediation in the Utilization of Carbon and Nitrogen from Detrital Complexes by *Crassostrea virginica*. *Limnol. Oceanogr.* 35(3):625-639.
- Crow, S. A., D. G. Ahern, W. L. Cook and A. W. Bourquin. 1975. Densities of Bacteria and Fungi in Coastal Surface Films as Determined by a Membrane-Adsorption Procedure. *Limnol. Oceanogr.* 20(4):169-182.
- Cunliffe, M., A. Engel, S. Frka et al., 2013. Sea Surface Microlayers: A Unified Physicochemical and Biological Perspective of the Air-Ocean Interface. *Prog. Oceanogr.* 109:104-116.
- Cunliffe, M., H. Schafer, E. Harrison, et al. 2008. Phylogenetic and Functional Gene Analysis of the Bacterial and Archaeal Communities Associated with the Surface Microlayer of an Estuary. *ISME J.* 2:776-789.
- Cvrkovic-Karloci, Z., D. Krznaric, M. Seruga, and B. Cosovic. 2011. Simple Electrochemical Determination of Surface-Active Substances in Natural Waters. *Int. J. Electrochem.* 7p.
- Dahlbäck, B., M. Hermansson, S. Kjellebert and B. Norkrans. 1981. The Hydrophobicity of Bacteria - An Important Factor In Their Initial Adhesion at the Air-Water Interface. *Arch. Microbiol.* 128:267-.
- Danos, S. C., J. S. Maki and C. C. Remsen. 1983. Stratification of Microorganisms and Nutrients in the Surface Microlayer of Small Freshwater Ponds. *Hydrobiologica* 98:193-202.
- Daumas, R. A. 1974. Influence of Temperature and Planktonic Development on the Mechanisms of Accumulation of Organic Matter in the Surface of the Sea. *Mar. Biol.* 26:111-116.
- Daumas, R. A., P. L. Labored, j. C. Marty and A. Saliot. 1976. Influence of Sampling Method on the Chemical Composition of Water Surface Film. *Limnol. Oceanogr.* 21:319-326.
- David, P. M. 1965. The Neuston Net. A Device for Sampling the Surface Fauna of the Ocean. *J. Mar. Biol. Assoc. U. K.* 45(2):313-320.

- deHarven, E., N. Lampen and D. Pla. 1977. Alternatives to Critical Point Drying. Scanning Electron Microscopy/1977. Vol. I. Proceedings of the Workshop on Biological Specimen preparation Techniques. IIT Research Institute, Chicago, Illinois. pp. 519-524.
- Dempsy, M. J. 1981. Marine Bacterial Fouling: A Scanning Electron Microscopy Study. Mar. Biol. 61(4):305-315.
- DeSouza-Lima, Y. 1985. Accumulation de sels Nutritifs dans la Microcouche de Surface: Influence Possible des Fracteurs Abiotiques et Biotiques du Milieu. Oceanologica Acta 8(1):47-58.
- DeSouza-Lima, Y. and J. C. Romano. 1983. Ecological Aspects of the Surface Microlayer. I. ATP, ADP, AMP Contents, and Energy Charge Ratios of Microplanktonic Communities. J. Exp. Mar. Biol. Ecol. 70:107-122.
- Dietz, A. S., L. J. Albright and T. Tuominen. 1976. Heterotrophic Activities of Bacterioneuston and Bacterioplankton. Can. J. Microbiol. 22:1699-1709.
- Dixit, S. S., J. P. Smol, J. C. Kingston and D. F. Charles. 1992. Diatoms: Powerful Indicators of Environmental Change. Environm. Sci. Technol. 26(1):23-33.
- Dubois, M., K. A. Gillies, J. K. Hamilton, P. A. Rogers and F. Smith. 1956. Colorimetric Method for Determination of Sugar and Related Substances. Anal. Chem. 28:350-356.
- Duce, R. A. 1986. The Impact of Atmospheric Nitrogen, Phosphorus and Iron Species on Marine Biological Productivity. In: P. Buat-Menard (Ed.): The Role of Air-Sea Exchange in Geochemical Cycling. Reidel Publ. Co., Dordrecht, Netherlands. pp. 497-529.
- Duce, R. A., J. G. Quinn, C. E. Olney and S. R. Piotrowicz, B. J. Ray and T. L. Wade. 1972. Enrichment of Heavy Metals and Organic Compounds in the Surface Microlayer of Narragansett Bay, Rhode Island. Science 176(4031):14 April:161-163.
- Dutka, B. J. and K. K. Kwan. 1977. Confirmation of the Single-Step Membrane Filtration Procedure of Estimating *Pseudomonas aeruginosa* Densities in Water. J. Appl. Environ. Microbiol. 33:240-245.
- Dutka, B. J. and K. K. Kwan. 1978. Health-Indicator Bacteria in Water-Surface Microlayers. Can. J. Microbiol. 24(2):187-188.

- Easterly, H. 2005. Characterization of Iron-Bearing Films Found On Ephemeral Pools, Central Coast, Oregon. MS Thesis, Portland State University, Department of Geology, Portland, Oregon. 110 p.
- Elzerman, A. W. and D. E. Armstrong. 1979. Enrichment of Zn, Cd, Pb, and Cu in the Surface Microlayer in Lakes Michigan, Ontario and Mendota. *Limnol. Oceanogr.* 24:133-144.
- Elzerman, A. W., D. E. Armstrong and A. W. Andren. 1979. Particulate Zinc, Cadmium Lead and Copper in the Surface Microlayer of Southern lake Michigan. *Environ. Sci. Technol.* 13(6):7200-7xx.
- Estep, K. W. J. S. Maki, S. C. Danos and C. C. Remsen. 1985. The Retrieval of Material from the Surface Micro Layer with Different Sampling Devices and it's Implications for Partitioning of Material Within the Micro Layer. *Freshwater Bio.* 15(1):15-19.
- Fletcher, R. L. and R. W. Baier. 1984. Influence of Surface Energy on the Development of the Green Alga *Enteromorpha*. *Mar. Biol. Letters* 5:251-254.
- Fogg, G. E. 1986. Picoplankton. *Proc. Roy. Soc. Lond., Ser. B. Biol. Sci.* 228(1):1-30.
- Franklin, B., W. Brownrigg and Rev. Mr. Farish. 1774. Of the Stilling of Waves by Means of Oil. Extracted from Sundry Letters between Benjamin Franklin, LL.D., F.R.S., William Brownrigg, M.D., F.R.S., and the Reverend Mr. Farish. *Phil. Trans. Roy Soc.* 64:455-460.
- Franklin, M. P, I. R. McDonald, D. G. Bourne, et al. 2005. Bacterial Diversity in the Bacterioneuston (Sea Surface Microlayer): The Bacterioneuston Through the Looking Glass. *Env. Microbiol.* 7(5):723-736.
- Fritz, L. W., L. M. Ragone and R. A. Lutz. 1990. Biomineralization of Barite in the Shell of the Freshwater Asiatic Clam *Corbicula fluminea* (Mollusca: Bivalvia). *Limnol. Oceanogr.* 35(3):756-762.
- Gallagher, J. L. 1975. The Significance of the Surface Film in Salt Marsh Plankton Metabolism. *Limnol. Oceanogr.* 20(1): 120-123.
- Garrett, W. D. 1965. Collection of Slick-Forming Materials from the Sea Surface. *Limnol. Oceanogr.* 10:602-605.
- Garrett, W. D. 1967a. The Organic Chemical Composition of the Ocean Surface. *Deep Sea Res.* 14:221-227.

- Garrett, W. D. 1967b. Damping of Capillary Waves at the Air-Sea Interface by Oceanic Surface Active Material. *J. Mar. Res.* 25:279-287.
- Garrett, W. D. 1974. The Surface Activity of Petroleum and It's Influence on the Spreading and Weathering of Oil Films at Sea. *J. Res. Atmos.*, 13:55-??.
- Goering, J. J. and D. W. Menzel. 1965. The Nutrient Chemistry of the Sea Surface. *Deep Sea Res.* 12: 839-843.
- Gordon, D. C. 1970a. A Microscopic Study of Organic Particles in the North Atlantic Ocean. *Deep Sea Res.* 17:175-185.
- Gordon, D. C. 1970b. Some Studies on the Distribution and Composition of Organic Particulates in the North Atlantic Ocean. *Deep Sea Res.* 17:233-243.
- Graham, D. E and M. C. Phillips. 1979a. Proteins at Liquid Interfaces. I. Kinetics of Adsorption and Surface Denaturation. *J. Colloid Interface Sci.* 70:403-414.
- Graham, D. E and M. C. Phillips. 1979b. Proteins at Liquid Interfaces. II. Adsorption Isotherms. *J. Colloid Interface. Sci.* 70:415-426.
- Graham, D. E and M. C. Phillips. 1979c. Proteins at Liquid Interfaces. III. Molecular Structures of Adsorbed Films. *J. Colloid Interface. Sci.* 70:427-437.
- Grathoff, G. H., J. E. Baham, H. R Easterly, et al. 2007. Mixed-Valent Fe Films ('Schwimmeisen') on the Surface of Reduced Ephemeral Pools. *Clays and Clay Minerals* 55(6):635-643.
- Gray, Z. N. 2008. The Characterization and Hydrogeochemistry of Floating, Mixed-Valent Iron Films. MS Thesis, Portland State University, 127 p.
- Griffith, O. H. 1987. Photoelectron Imaging and Photoelectron Labeling. *Ultramicroscopy* 24(2-3):299-312.
- Griffith, O. H. and G. F. Rempfer. 1985. Photoelectron Imaging in Cell Biology. *Ann Rev. Biophys. Biophys. Chem.* 14:113-30.
- Gucinski, H. 1982. The Distribution of Organic Matter at the Sea Surface. NATO Adv. Study Inst., Durham, NH. Poster.
- Gucinski, H. 1984. Correlation of Biophysical Surface Characteristics with hydrodynamic Properties of Adhesive Biofilms. PhD Dissertation. State Univ. of NY, Buffalo. 183 p.

- Gucinski, H and R. E. Baier. 1983. Estuarine Bacterial Slime Films: Relation of Biophysical Properties to Hydrodynamic Drag. Int. Estuarine Res. Fed. Symposium, Virginia Beach, VA.
- Gucinski, H, R. E. Baier, A. E. Meyer, M. S. Fornalik and R. W. King. 1984. Surface Microlayer Properties Affecting Drag Phenomena in Seawater. Proc. 7th Int. Congress on Marine Corrosion and Fouling. Athens, Greece.
- Gucinsky, H. D. W. Goupil and R. E. Baier. 1981. The Sampling and Composition of the Surface Microlayer. In: S. Eisenreich (Ed.) Atmospheric Pollutants to Natural Waters. Ann Arbor Science Publishers, Ann Arbor, MI. 512 p.
- Habliston, D. L. K. K. Hedberg, G. B. Birrell, G. F. Rempfer and O. H. Griffith. 1995. Photoelectron Imaging of Cells: Photoconductivity Extends the Range of Applicability. Biophys. J. 69:1615-1624.
- Hansen, H. P. 1977. Photodegradation of H7drocarbon Surface Films. Rapp. P. V. Reun. Cons. Int. Explor. Mer. 171:101-1??.
- Hardy, J. T. 1971. Ecology of Phytoneuston in a Temperate Marine Lagoon. PhD Dissertation, University of Washington, Department of Botany. 160 p.
- Hardy, J. T. 1973. Phytoneuston Ecology of a Temperate Marine Lagoon. Limnol. Oceanogr. 18(4):525-533.
- Hardy, J. T. 1982. The Sea-Surface Microlayer: Biology, Chemistry and Anthropogenic Enrichment. Prog. Oceanogr. 11(1982):307-328.
- Hardy, J. T. and E. A. Crecelius. 1981. Is Atmospheric Particulate Matter Inhibiting Marine Primary Productivity? Env. Sci. Technol. 15:1103-1105.
- Hardy, J. T. and C. W. Apts. 1984. The Sea Surface Microlayer: Phytoneuston Productivity and Effects of Atmospheric Particulate Matter. Mar. Biol. 82(2):293-300.
- Hardy, J. T., J. A. Coley, L. D. Antrim, and S. L. Kiesser. 1988. A Hydrophobic large-Volume Sampler for Collecting Aquatic Surface Microlayers: Characterization and Comparison with the Glass Plate Method. Can. J. Fish. Aquatic Sci. 45(5):822-826.
- Hardy, J. T., E. A. Crecelius and R. Kocan. 1986. Concentration and Toxicity of Sea-Surface Contaminants in Puget Sound. Battelle, Pacific Northwest National Laboratory, PHL-5834.

- Harvey, G. W. and L. A. Burzell. 1972. A Simple Microlayer Method for Small Samples. *Limnol. Oceanogr.* 17:156-157.
- Harvey, R. W. and L. Y. Young. 1980a. Enrichment and Association of Bacteria and Particulates in Salt Marsh Surface Water. *Appl. Environ. Microbiol.* 39:894-899.
- Harvey, R. W. and L. Y. Young. 1980b. Enumeration of Particle-Bound and Unattached Respiring Bacteria in the Salt Marsh Environment. *Appl. Environ. Microbiol.* 40:156-160.
- Harvey, R. W., L. W. Lion and L. Y. Young. 1980c. Transport and Distribution of Bacteria and Diatoms in the Aqueous Surface Microlayer of a Salt Marsh. *Estuarine Coastal Shelf Sci.* 16:543-547.
- Hasslett, J. P. and M. A. Anderson. 1979. Association of Hydrophobic organic Compounds with Dissolved Organic matter in Aquatic Systems. *Env. Sci. Technol.* 13(12):1526-1529.
- Hedgepeth, Joel W. (Ed.). 1957. *Treatise on Marine Ecology and Paleoecology, Volume I: Ecology*. Waverly Press, Baltimore, MD.
- Hedgepeth, Joel W (Ed.). 1978. *The Outer Shores*. Edward F. Ricketts: Parts 1 and 2. Mad River Press, Eureka, California 128 p. and 182 p, respectively.
- Hedgepeth, Joel W. 1996. *Marine Biologist and Environmentalist: Pycnogonids, Progress and Preserving Bays, Salmon and Other Living Things,* An Oral History conducted in 1992 by Ann Lage, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, California.
- Henrici, A. T. 1933. Studies of Freshwater Bacteria. I. A Direct microscopic Technique. *J. Bacteriol.* 25:277-286.
- Henrici, A. T. and D. E. Johnson. 1935. Studies of Freshwater Bacteria. II. Stalked Bacteria, a New Order of Schizomycetes. *J. Bacteriol.* 30:61-92.
- Hermansson, M. 1990. The Dynamics of Dissolved and Particulate Organic material in Surface Microlayers. In: R. Wotton, Ed.: *The Biology of particles in Aquatic Systems*. CRC Press, Boca Raton, Florida, USA Ch7, pp. 145-159.
- Hermansson, M. and B. Dahlbäck. 1983. Bacterial Activity at the Air-Water Interface. *Micro. Ecol.* 9:317-328.

- Hermansson, M., S. Kjelleberg and B. Norkrans. 1979. Interaction of Pigmented Wild Type and Pigmentless Mutant of *Serratia marcescens* with Lipid Surface Film. FEMS Microbl. Lett. 6:129-137.
- Hermansson, M., S. Kjelleberg, T. K. Korhonen and T. -A. Stenström. 1982. Hydrophobic and Electrostatic Characterization of Surface Structures of Bacteria and Its Relationship to Adhesion at an Air-Water Surface. Arch. Microbiol. 131:308-317.
- Hodge, V., S. R. Johnson and E. D. Goldberg. 1978. Influence of Atmospherically Transported Aerosols on Surface Ocean Water Composition. Geochemical J. 12(1):7-20.
- Hood, D. 1970. *Organic Matter in Natural Waters*. Alaska Inst. Mar. Sci. Occas. Publ. 1.
- Horrigan, S. G., A. F. Carlucci and P. M. Williams. 1981. Light Inhibition of Nitrification in Sea-Surface Films. J. Mar. Res. 39:557-565.
- Hudson, R. J. M., and F. M. M. Morel. 1989. Distinguishing Between Extra- and Intracellular Iron in Marine Phytoplankton. Limnol. Oceanogr. 34(6):1113-1120.
- Hunt, A., D. L. Johnson, J. M. Watt and I. Thornton. 1992. Characterizing the Sources of particulate lead in House Dust by Automated Scanning Electron Microscopy. Environm. Sci. Technol. 27:807-822.
- Hunter, K. A. 1980. Microelectric Properties of Natural Surface-Active Organic Matter in Coastal Seawater. Limnol. Oceanogr. 25:807-822.
- Hunter, K. A. 1983. On the Estuarine Mixing of Dissolved Substances in Relation to Colloid Stability and Surface properties. Geochem. Cosmochim. Acta 47(3):467-473.
- Hunter, K. A. and P. S. Liss. 1977. The Input of Organic Material to the Oceans: Air-Sea Interactions and the Organic Chemical Composition of the Sea Surface. Mar. Chem. 5:361-367.
- Hunter, K. A. and P. S. Liss. 1981a. Organic Sea Surface Films. In: E. K. Duursma and R. Dawson (Eds.): Marine Organic Chemistry. Elsevier, Amsterdam, Neth. pp. 259-298.
- Hunter, K. A. and P. S. Liss. 1982. Organic Matter and the Surface Charge of Suspended Particles in Estuarine Waters. Limnol. Oceanogr. 27(2):322-335.

- Hühnerfuss, H. 1981a. On the Problem of Sea Surface Film Sampling: A Comparison of 21 Microlayer, 2 Multilayer-, and 4 Selected Subsurface-Samplers - Part I. *Sonder. Meerestech.* 12(5):137-142.
- Hühnerfuss, H. 1981b. On the Problem of Sea Surface Film Sampling: A Comparison of 21 Microlayer, 2 Multilayer-, and 4 Selected Sub-surface-Samplers - Part II. *Sonder. Meerestechnik* 12(6):170-173.
- Hühnerfuss, H., W. Alpers, W. L. Jones, P. A. Lange and K. Richter. 1981. The Damping of Ocean Surface Waves by a Monomolecular Film Measured by Wave Staffs and Microwave Radars. *J. Geophys. Res.* 86:429-438.
- Hutchinson, T. E. and A. P. Somlyo. 1981. *Microprobe Analysis of Biological Systems*, Academic Press. New York, NY 427 p.
- Jannasch, H. W., O. C. Zafiriou and J. W. Farrington. 1980. A Sequencing Sediment Trap for Time-Series Studies of Fragile particles. *Limnol. Oceanogr.* 25(5):939-943.
- Jarvis, N. L., W. D. Garrett, M. A. Sheiman and c. O. Thompson. 1967. Surface Chemical Characterization of Surface Active Material in Seawater. *Limnol. Oceanogr.* 12(1):88-97.
- Johnson, K. M., C. M. Burney and J. McN. Sieburth. 1981. Enigmatic marine Ecosystem metabolism measured by Direct Diel CO₂ and O₂ Flux in Conjunction with DOC Release and Uptake. *Mar. Biol.* 65(1):49-60.
- Johnson, P. W. and J. McN. Sieburth. 1979. Chlorococcoid Cyanobacteria in the Sea: A Ubiquitous and Diverse Phototrophic Biomass. *Limnol. Oceanogr.* 24:928-935.
- Johnson, P. W. and J. McN. Sieburth. 1982. The Utilization of Chlorococcoid Cyanobacteria by Marine Protozooplankters by Not by Calanoid Copepods. *Ann. Inst. Oceanogr. Paris* 58(Suppl.): 297-308.
- Johnson, P. W., H. -S. Xu and J. McN. Sieburth. 1982. In Situ Morphology and Occurrence of Eucaryotic Phototrophs of Bacterial Size in the Picoplankton of Estuarine and Oceanic Waters. *J. Phycol.* 18:318-327.
- Joint, I. R., N. J. P. Owens and A. J. Pomroy. 1986. Seasonal Production of Photosynthetic Picoplankton and Nanoplankton in the Celtic Sea. *Mar. Ecol. Prog. Ser.* 28: 251-258.

- Joint I. R. and R. K. Pipe. 1984. An Electron Microscope Study of a Natural Population of Picoplankton from the Celtic Sea. *mar. Ecol. Prog. Ser.* 20:113-118.
- Jones, J. G. 1979. A Guide to Methods for Estimating Microbial Numbers and Biomass in Freshwater. *Freshwater Biol. Assoc. Sci. Publ.* No 39.
- Karlsson, T. and P Persson. 2012. Complexes with Aquatic Organic matter Suppress hydrolysis and Precipitation of Fe (III). *Chem. Geol.* 322-323:19-27.
- Karlsson, T. and P. Persson. 2010. Coordination Chemistry and Hydrolysis of Fe(III) in a Peat Humic Acid Studied by X-Ray Absorption Spectroscopy. *Geochim. Cosmochim. Acta.* 74:30-40.
- Katsaros, K. B. 1980. An Aqueous Thermal Boundary layer. *Boundary-layer Meteorology* 18(1980): 107-127.
- Katsaros, K. B. 1981. Convection Patterns in a Pond. *Bull. Am. Meteorol. Soc.* 62(10):1446-1453.
- Katsaros, K. B., W. T. Liu, J. A. Businger and J. E. Tillman. 1977. Heat Transport and Thermal Structure in the Interfacial Boundary Layer Measured in an Open Tank of Water in Turbulent Free Convection. *J. Fluid Mech.* 83(2):311-335.
- Kattner, G. G. and U. H. Brockman. 1978. Fatty-Acid Composition of Dissolved and Particulate Matter in Surface Films. *Mar. Chem.* 6:233-239.
- Kenis, P. R. 1968. Drag Reduction by Bacterial Metabolites. *Nature.* 217:940-941.
- Kjelleberg, S. 1985. Mechanisms of Bacterial Adhesion at Gas-Liquid Interfaces. In: D. C. Savage and M. Fletcher, Eds., *Bacterial Adhesion*. Plenum Publ. NY, USA Chapter 7.
- Kjelleberg, S. and T. A. Stenström. 1980. Lipid Surface Films: Interaction of Bacteria with Free Fatty Acids and Phospholipids at the air-Water Interface. *J. Gen. Microbiol.* 166:417-426.
- Kjelleberg, S., T. A. Stenström and G. Odham. 1979. Comparative Study of Different Hydrophobic Devices for Sampling Lipid Surface Films and Adherent Microorganisms. *Mar. Biol.* 53:21-2x.
- Kleja, D. B., J. W. J van Schalik, I. Persson and J. P. Gustafsson. 2012. Characterization of Iron in Floating Surface Films of Some Natural Waters Using EXAFS. *Chem. Geol.* 326-7:19-26

- Konhauser, K. O. 1998. Diversity of Bacterial Iron Mineralization. *Earth-Science Reviews* 43(1998):91-121.
- Konhauser, K. O. and F. G. Ferris. 1996. Diversity of Iron and Silica Precipitation by Microbial Biofilms in Hydrothermal Waters, Iceland: Implications for Precambrian Iron Formations. *Geology* 24:326.
- Konhauser, K. O. and F. G. Ferris. 1994. Bacterial Formation of Clay Phases in Freshwater Biofilms. In: A. K. Gupta and R. Kerrich (Eds). *The Dynamic Geosphere*. Nat. Acad. Sci., India, Allahbad. pp. 139-153.
- Konhauser, K. O. and M. M. Urrutia. 1999. Bacterial Clay Authigenesis: A common Biogeochemical Process. *Chem. Geol.* 161:399-413.
- Lal, D. 1977. The Oceanic Microcosm of Particles. *Science* 198:997-1009.
- Lalond, S. V. and K. O. Konhauser. 2015. Benthic Perspective on Earth's Oldest Evidence for Oxygenic Photosynthesis. *Proc. Natl. Acad. Sci., USA*. 112:995-1000.
- Larsson, K., G. Odham and A. Södergren. 1974. On Lipid Films on the Sea. I. A Simple Method for Sampling and Studies of Composition. *Mar. Chem.* 2(1):49-57.
- Lewin, J. and C. H. Chen. 1971. Available Iron: A Limiting Factor for marine Phytoplankton. *Limnol. Oceanogr.* 16:670-675.
- Lewin, R. A. and L. Cheng. 1989. Some Lipogenic, Eukaryotic, Picoplanktonic Algae from the Caribbean Region. *Phycologia*. 28(1):96-108.
- Lion, L. W., R. W. Harvey, L. Y. Young and J. O. Leckie. 1979. Particulate Matter, its Association with Microorganisms and Trace Metals in an Estuarine Salt Marsh Microlayer. *Environm. Sci. Technol.* 12(12):1522-1525.
- Lion, L. and J. O. Leckie. 1981. The Biogeochemistry of the Air-Sea Interface. *Ann. Rev. Earth Plant. Sci.*, 9(1981):449-486.
- Lion, L. and J. O. Leckie. 1982. Accumulation and Transport of Cd, Cu and Pb in an Estuarine Salt Marsh Surface Microlayer. *Limnol. Oceanogr.* 27(1):111-125.
- Lion, L. and J. O. Leckie. 1983. Particulate Matter at the Estuarine Salt Marsh Surface Microlayer: Particle-Size Distributions and Comparison to Bulk Suspension. *Estuar. Coast. Shelf Sci.*, 16:689-696.

- Liss, P. S. 1971. Exchange of SO₂ between the Atmosphere and Natural Waters. *Nature* 233(5318;Oct 1):327-329.
- Liss, P. S. 1974. Chemistry of the Sea Surface Microlayer. In: J. P. Riley and G. Skirrow, Eds. *Chemical Oceanography*, Vol. 2. Academic Press, NY. 193 p.
- Liss, P. S. 1981. Air-Sea Gas Fluxes in Estuarine and Coastal Zones. In: United Nations. *River Inputs to Ocean Systems*. UNEP/UNESCO, 1980. NY. pp. 370-374.
- Liss, P. S. 1983. Gas Transfer: Experiments and Geochemical Implications. In: P. S. Liss and W. G. N. Slinn, Eds. *Air-Sea Exchange of Gases and Particles*. D. Reidel Publ. Co., Dordrecht, Neth. pp. 241-298.
- Liss, P. S. and P. G. Slater. 1974. Flux of Gases Across the Air-Sea Interface. *Nature* 247(January 27): 181-184.
- Liss, P. S. and W. G. N. Slinn. 1983. *Air-Sea Exchange of Gases and Particles*. NATO ASI Series. Series C. Proc. NATO Adv. Study Inst on Air-Sea Exchange of Gases and Particles. Univ. of N. Hampshire, Durham, NH. D. Reidel Publ. Co., Dordrecht, Neth. 561p.
- Loeb, G. 1977. Adsorption of organic Films at the Platinum Seawater Interface. *J. Mar. Res.* 35(2):284-291.
- Lowry, H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein Measurement with the Folin Phenol. Reagent. *J. Biol. Chem.* 193:265-275.
- McCrackin, F. L., E. Passaglia, R. R. Stromberg and H. G. Steinbert. 1963. Measurement of the Thickness and Refractive Index of Very Thin Films and Optical Properties of Surfaces by Ellipsometry. *J. Res. Nat. Bureau of Standards - Appl. Physical Chem.* 67A:363-377.
- McCutchen, C. W. 1970. Surface Films Compacted by Moving Water: Demarcation Lines Reveal Film Edges. *Science* 170(3953):61-64.
- MacIntyre, F. 1970. Geochemical Fractionation During Mass Transfer from Sea to Air by Breaking Bubbles.
- MacIntyre, F. 1974. Chemical Fractionation and Sea-Surface Microlayer Processes. In: E. D. Goldberg, Ed., *The Sea*, Vol. 5, Marine Chemistry. J. Wiley & Sons, NY. pp. 2456-2479.

- MacIntyre, F. 1974. The Top Millimeter of the Ocean. *Sci. Am.* 320(5):62-77.
- MacRitchie, F. and A. E. Alexander. 1963. Kinetics of Adsorption of Proteins at Interfaces. *J. Colloid Interf. Sci.* 18:453-45x.
- Mallory, G. 1923. Climbing Mount Everest is work for Supermen. *New York Times*, 18 March, 1923. Interview.
- Maki, J. S., S. C. Danos and C. C. Remsen. 1984. Quantitative Changes in Fungal Colony-Forming Units in the Surface Microlayers of Two Freshwater Ponds. *Can. J. Microbiol.* 30:578-584.
- Margulis, L and D. Sagan. 1986. Origins of Sex. Three Billion Years of Genetic Recombination. Yale Univ. Press, New Haven, CT. 258 p.
- Martin, J. H., W. W. Broenkow, S. E. Fitzwater and R. M. Gordon. 1990. Yes It Does: A Reply to the Comment by Banse. *Limnol. Oceanogr.* 35(3):775-776.
- Martin, J. H., R. M. Gordon, S. Fitzwater and W. W. Broenkow. 1989. VERTEX: Phytoplankton/Iron Studies in the Gulf of Alaska. *Deep Sea Res.* 36():649-680.
- Martin, S. T. 2005. Precipitation and Dissolution of Iron and Manganese Oxides. Ch. 4. In: Grassian, V. H. *Environmental Catalysis*. CRC Press. 701 p.
- Marty, J. C. and A. Saliot. 1974. Etude Chimique Comarée du Film de Surface et de L'eau de Mer Sour-jacente: Acides Gras. *J. Res. Atmos.* 8(3-4).
- Marumo, R., N. Tuga and T. Nakai. 1971. Neustonic Bacteria and Phytoplankton in Surface Microlayers of the Equatorial Waters. *Bull. Plankton Soc. Japan* 18(1):36-41.
- Maynard, N. G. 1968. Aquatic Foams as an Ecological Habitat. *Zeit. Allgem. Microbiol.* 8:119-126.
- Mayr, E. 1963. *Animal Species and Evolution*. The Belknap press of Harvard University Press, Cambridge, Mass. 797p.
- Mayr, E. 1979. *Evolution and the Diversity of Life. Selected Essays*. The Belknap Press of Harvard University Press, Cambridge, Mass. 701p.
- Mayr, E. 1997. *This is Biology. The Science of the Living World*. Belknap Press, Harvard University Press, Cambridge. 327 p.

- Mellchiorri-Santolini, U. and J. W. Hopton (Eds). 1971. Detritus and Its Role in Aquatic Ecosystems. Proceedings of an IBP-UNESCO Symposium. Memorie Dell'Istituto Italiano Di Idrobiologica. 29(Suppl.): 540 p.
- Morel, F. M. M. J. G. Rueter, D. M. Anderson and R. R. L. Gjillard. 1979. AQUIL: A Chemically Defined Phytoplankton Culture Medium for Trace Metal Studies. J. Phycol. 15:135-141.
- Moum, J. N., D. J. Carson and T. J. Cowles. 1990. Sea Slicks and Surface Strain. Deep-Sea Res. 37(5):767-775.
- Murrell, C. The Microbiology of the Sea Surface Microlayer: "The Bacterioneuston". Surface Ocean Lower Atmospheric Study (SOLAS). Power Point.
- Naumann, E. 1914. Beiträge zur Kenntnis des Teichnannoplanktons. I. Vorläufige Übersicht einiger Arbeiten and der Fischerei-versuchstation Aneboda in Südschweden in den Jahren 1911-1913, Biol. Zentralbl. 34(9):581-594. 37(2):98-106.
- Naumann, E. 1917. Beiträge zur Kenntnis des Teichnannoplanktons. II. Über das Neuston des Süßwassers. Biol. Zentralbl. 37(2): 98-106.
- Nielsen, M. H. et al. 2014a. In situ TEM imaging of CaCO_3 Nucleation Reveals Coexistence of Direct and Indirect Pathways. Science 345(6201):1158-1162.
- Nielsen, M. H. et al. 2014b. In situ TEM imaging of CaCO_3 Nucleation Reveals Coexistence of Direct and Indirect Pathways. Sciencemag.org 345(6201): Suppl. 16 p. www.sciencemag.org/content/345/6201/1158/suppl/DC1.
- Nishizawa, S. 1971a. Concentration of organic and Inorganic material in the Surface Skin at the Equator, 155°W. Bull. Plankton Soc. Japan 18(2):42-44.
- Nishizawa, S. 1971b. Concentration of Particulate and dissolved Organic Material at the Sea Surface Skin. (Abs). In M. Uda (Ed.): The Ocean World. Jap. Soc. Promotion Sci.
- Norkrans, B. 1980. Surface Microlayers in Aquatic Environments. In: M. Alexander, Ed.: Advances in Microbial Ecology. Vol. 4. Plenum Press. NY pp. 51-85.
- Norkrans, B. and F. Sörensson. 1977. On the Marine Lipid Surface Microlayer--Bacterial Accumulation in Model Systems. Bot. mar. 20:473-478.

- Paerl, H. W. 1974. Bacterial Uptake in Relation to Detrital Aggregation in Marine and Freshwater Systems. *Limnol. Oceanogr.* 19():966-972.
- Paerl, H. W. 1982. Interactions with Bacteria. In: N. G. Carr and B. A. Whitton, (Eds.): *The Biology Of Cyanobacteria*. Blackwell Sci. Publ., Oxford, UK pp. 441-461.
- Paerl, H. W. 1988a. Growth and Reproductive Strategies of Cyanobacteria. In: C. D. Sandgren, (Ed.): *Growth and Reproductive Strategies fro Freshwater phytoplankton*. Cambridge Univ. Press, Cambridge UK pp. 261-315.
- Paerl, H. W. 1988b. Nuisance Phytoplankton Blooms in Coastal Estuarine and Inland Waters. *Limnol. Oceanogr.* 33:823-847.
- Paerl, H. W. 1989. Regulation of N₂ Fixation in Natural Waters: An Ecophysiological Perspective. *Adv. Microb. Ecol.* 11.
- Paerl, H. W., B. M. Bebout and L. E. Prufert. 1989. Bacterial Associations with Marine *Oscillatoria* sp. (*Trichodesmium* sp.) Populations: Ecophysiological Implications. *J. Phycol.* 25:773-784.
- Paerl, H. W., and S. L. Shimp. 1973. Preparation of Filtered Plankton and Detritus for Study with Scanning Electron Microscopy. *Limnol. Oceanogr.* 18():802-805.
- Patterson, M. P. and K. T. Spillane. 1969. Surface Films and the Production of Sea-Salt Aerosol. *Quart. J. Roy. Met. Soc.*, 95:526-534.
- Pavia, R. and R. W. Smith. 1984. Development and Implementation of Guidelines for Dispersant Use: Regional Response Teams. In T. E. Allen (Ed.), *Oil Spill Chemical Dispersants*. Am. Soc. Testing and Materials, ASTM STP 840 pp. 378-389.
- Petersen, R. P. 1982. Influences of Copper and Zinc on the Growth of a Freshwater Alga, *Scenedesmus quadricauda*: The Significance of Chemical Speciation. *Environm. Sci. Technol.* 16:443-447.
- Philippe, A. and G. E. Schaumann. 2014. Interactions of Dissolved Organic Matter with Natural and Engineered Inorganic Colloids: A Review. *Env. Sci. Technol.* 48:8946-8962.
- Piotrowicz, S. R., B. J. Ray, G. L. Hoffman and R. A. Duce. 1972. Trace Metal Enrichment in the Sea-Surface Microlayer. *J. Geophys. Res.* 77(27):5213-5254.

- Pomeroy, L. R. 1984. Significance of Microorganisms in Carbon and Energy Flow in marine Ecosystems. In: M. J. Klug and c. A. Reddy, Eds. Current Perspectives in Microbial Ecology. Proc. Third Int. Symp. On Microbial Ecol. Am. Soc. Microbiol., Washington DC pp. 405-411.
- Platt, Trevor, D. V. Subba Rao and B. Irwin. 1983a. Photosynthesis of Picoplankton in the Oligotrophic Ocean. Nature 301():318-329.
- Platt, Trevor et al. 1983b. Photosynthetically Competent Phytoplankton from the Aphotic Zone of the Deep Ocean. Mar. Ecol. Prog. Ser. 10(1):105-110.
- Pockels, A. 1891. Surface Tension. Nature. 46:437.
- Pockels, A. 1892. On the Relative Contamination of the Water Surface by Equal Quantities of Different Substances. Nature. 47:418.
- Pockels, A. 1893. Relations Between the Surface Tension and Relative Contamination of Water Surfaces. Nature. 48:152.
- Pockels, A. 1894. On the Spreading of Oil Upon Water. Nature. 50:223.
- Popper, Z. A., G. Michel, C. Hervé et al. Evolution and Diversity of Plant Cell Walls: From Algae to Flowering Plants. Annu. Rev. Plant Biol. 62(2011)567-590.
- Prézelin, B. B., R. R. Bidigare, H. A. Matlick, M. Putt and B. VerHoven. 1987. Diurnal Patterns of Size-Fractionated Primary Productivity Across a Coastal Front. Mar. Biol. 96: 563-574.
- Prospero, J. M. and R. T. Nees. 1977. Dust Concentration in the Atmosphere of the Equatorial North Atlantic: Possible Relationship to the Sahelian Drought. Science 196:1196-1198.
- Raiswell, R., M. Tranter, L. G. Benning, M. Siebert, R. De'ath and P. Huybrechts and T. Payne. 2006. Contributions from Glacially Derived Sediment to the Global Iron (Oxyhydr) Oxide Cycle: Implications for Iron Delivery to the Oceans. Geochim. Cosmochim Acta. 7-(2006):2765-2780.
- Ramón y Cajal, S. 1999(1897/1914). *Advice For A Young Investigator. [Reglas y Consejos sobre Investigación Científica: Los Tónicos de la voluntad.]*. Trans. N. Swanson and L. W. Swanson. MIT Press, Cambridge 150 p.
- Rayleigh. Lord Rayleigh, John William Strutt. See Strutt, John William, the Lord Rayleigh and Pockels, A. 1891.

- Reynolds, C. S., R. L. Oliver and A. E. Walsby. 1987. Cyanobacterial Dominance: The Role of Buoyancy Regulation in Dynamic Lake Environments. *N. Z. J. Mar. Freshw. Res.* 21(3):379-390.
- Reynolds, N. 1973. The Estimation of the Abundance of Ultraplankton. *Br. Phycol. J.* 8:135-146.
- Rich, H. W. and F. M. M. Morel. 1990. Availability of Well-Defined Iron Colloids to the Marine Diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 35(3):640-651.
- Ricketts, E. F. and J. Calvin. 1962. *Between Pacific Tides. An Account of the Habits and Habitats of Some Five Hundred of the Common, Conspicuous Seashore Invertebrates of the Pacific Coast between Sitka, Alaska, and Northern Mexico.* 3d Ed, Revised. Revisions by Joel W. Hedgpeth. Stanford University Press, Stanford, CA.
- Ricketts, E. F. 1978. *The Outer Shores.* Joel W. Hedgpeth (ed.). Two volumes: Part 1: *Ed Ricketts and John Steinbeck Explore the Pacific Coast*, and Part 2: *Breaking Through.* 128p and 182 p, respectively. Mad River Press, Eureka, California.
- Rijkenbert, M. J. A., L. J. A. Gerringa, V. E. Carolus, I. Velzeboer and J. K. W. de Baar. 2006. Enhancement and Inhibition of Iron Photoreduction by Individual Ligands in Open Ocean Seawater. *Geochim. Cosmochim. Acta.* 70(2006):2790-2805.
- Roy, V. M., J. L. Dupuy, W. G. McIntyre and W. Harrison. 1970. Abundance of marine Phytoplankton in Surface Films: A Method of Sampling. In: W. G. Weist, jr. and P. E. G. Reeson (Eds.): *Hydrobiology - Bioresources of Shallow Water Environments.* Am. Water Resource Assoc.
- Rueter, J. G. 1988. Iron Stimulation of Photosynthesis and Nitrogen Fixation in *Anabaena* 7120 and *Trichodesmium* (Cyanophyceae). *J. Phycol.* 24(): 249-254.
- Rueter, J. G. and D. R. Ades. 1987. The Role of Iron Nutrition in Photosynthesis and Nitrogen Assimilation in *Scenedesmus quadricauda* (Chlorophyceae). *J. Phycol.* 23():452-457.
- Rueter, J. G., D. A. Hutchins, R. W. Smith and N. L. Unsworth. 1992. Iron Nutrition in *Trichodesmium*. In: E. J. Carpenter et al (Eds.): *Marine Pelagic*

- cyanobacteria: Trichodesmium and other Diazotrophs*. NATO Adv. Studies Inst. Kluwer Academic Publ., Dordrecht, Netherlands pp. 289-306.
- Rueter, J. G. and R. R. Petersen. 1987. Micronutrient Effects on Cyanobacterial Growth and Physiology. *N. Z. J. Mar. Freshwater Res.* 21: 435-445.
- Schnell, E. 1956. Slippage of Water Over Nonwetable Surfaces. *J. Appl. Physics* 27(10):1149-1151.
- Schrader, M. 1982. On Adhesion of Biological Substances to Low Energy Solid Surfaces. *J. Colloid Interface Sci.* 88(1):296-297.
- Schwertmann, U, J. Friedl and H. Stanjek. 1999. From Fe (III) Ions To Ferrihydrite and then to Hematite. *J. Colloid and Interface Sci.* 209:215–223.
- Seiler, W., A. Holzapfel-Pschorn, R. Conrad and D. Scharffe. 1984. Methane Emission from Rice Paddies. *J. Atmos. Chem.* 1:241-268.
- Sieburth, J. McN. 1971. Distribution and Activities of Oceanic Bacteria. *Deep-Sea Res.* 18:190-193.
- Sieburth, J. McN. 1976. Bacterial Substrates and Productivity in Marine Ecosystems. *Ann. Rev. Ecol. Syst.* 7:259-
- Sieburth, J. McN. 1979. *Sea Microbes*. Oxford Univ. Press, Oxford UK, 491p.
- Sieburth, J. McN. 1983. Microbiological and Organic-Chemical Processes in the Surface and Mixed layers. In: P. S. Liss and W. G. N. Slinn (Eds): *Air-Sea Exchange of Gases and Particles*. Reidel, Hingham Mass, USA.
- Sieburth, J. McN., H. L. Pratt, P. W. Johnson and D. Scales. 1975. *Microbial Seascapes. A Pictorial Essay On Marine Micro-Organisms And Their Environments*. Univ. Park Press, Baltimore, Maryland, USA unpag.
- Sieburth, J. McN., P. Willis, K. M. Johnson, C. M. Burney, d. M. Lavoie, K. R. Hinga, D. A. Caron, F. W. French, III, P. W. Johnson and P. G. Davis. 1976. Dissolved Organic Matter and Heterotrophic Micro- Neuston in the surface Microlayers of the North Atlantic. *Science* 19:1415-1418.
- Silver, M. W., A. L. Shanks and J. D. Trent. 1978. Marine Snow: Microplankton Habitat and Source of Small Scale Patchiness in Pelagic Populations. *Science* 210:136-139.

- Singer, S. J. and G. L. Nicolson. 1972. The Fluid Mosaic Model of the Structure of Cell membranes. *Science* 175(4023):720-731
- Smayda, T. J. 1970. The Suspension and Sinking of Phytoplankton in the Sea. *Oceanogr. Mar. Biol., Ann. Rev.* 8(1970):353-414.
- Smith, R. W. 2012. The Scum of the Earth: Science at the Air-Water Interface, from Iron-Oxide Surface Films to Neuston, Aquatic Surface Films and Oil Spills. The Plankton Group, Oregon Shores conservation Coalition and CoastWatch. (Invited).
- Smith, R. W. et al. 2008. Perspective on Cellular Analysis: Linking Quantitation to Structure and Function by Instrumental Methods and Analysis. *Microsc. Microanal* 14(Suppl. 2): 752-753. doi: 10.1017/S1431927608089034.
- Smith, R. W. 2007a Beyond Pacific Tides: The Inner Shores: Remembering Joel Walker Hedgepeth (1911-2006). *The Quarterly Rev. Biology.* 2(2):93-96
- Smith, R. W. 2007b. Genetic and Genomic Advances in the Kinetoplastid Parasites of Humans: Prospects of Vaccine Development for Trypanosomatids and Leishmaniases. *Proceedings, Parasitology in the 21st Century, Immunoecology Seminar, Portland State University, Portland, Oregon*
- Smith, R. W. 2007c. Neuston, Microlayers and Surface Films: Science at the Air-Water Interface. *Proc. Coastal Zone 07. NOAA* 5p.
- Smith, R. W. 2006. Reproductive Diversity in Syllid Polychaetes: Epitoky and the Development of K-Selected Reproductive Strategies for Fertilization, maternal Care and Larval Incubation. *Proceedings, LARVAL2006, the 7th Larval Biology Symposium, Coos Bay Oregon. Abs.*
- Smith, R. W. 2003. Fluorescence and Non-Imaging Microscopies. *Microsc. Microanal.* (9:Suppl 2): .
- Smith, R. W. 1994. Heavy Metals and Particulates at the Air-Water Interface: Environmental Contaminant in Aquatic Surface Films for the Susquehanna River and Chesapeake Bay, Maryland, USA. *Proceedings, International River Quality Symposium, March 21-25, Portland State University. Abs.*
- Smith, R. W. 1991. Using Microscopy To Develop New Models of Aquatic Surface Films. *Proceedings, Society for Environmental Toxicology and Chemistry (SETAC), 12th Annual Meeting, November 3-7, Seattle, Washington USA. Abs, (Invited).*

- Smith, R. W. and D. T. Clark. 2005. The Hitchhiker's Guide to the Inner (Cellular) Galaxies: Cytology, Cytometry, Cytomics, Cellomics and Kinomics. *Microsc. Microanal.* 11(Suppl. 2): 252-253.
DOI: 10.1017/S143192760551033X
- Smith, R. W. and J. Dash. 1989. Sampling and Analysis of the Air-Water Interface with SEM and EDS of Microlayers. *Proc. 49th Meeting of the Elect. Microsc. Soc. Am.* pp. 1005-1006.
- Smith, R. W. and R. Pavia. 1983. Dispersant Use Guidelines for Federal Regions IX and X. *Proceedings of the 1983 Oil Spill Conference*, API, Washington, D. C. pp. 3-6.
- Södergren, A. 1979. Origin of ¹⁴C and ³²P Labeled Lipids Moving to and From Freshwater Surface Microlayers. *Oikos*. 33:278-28x.
- Södergren, A. 1987. Origin and Composition of Surface Slicks in lakes of Differing Trophic Status. *Limnol. Oceanogr.* 32:130-13x.
- Staresinic, N., G. T. Row, D. Shaugnessey and A. J. Williams. 1978. Measurement of the Vertical Flux of particulate organic Matter with a Free-Drifting Sediment Trap. *Limnol. Oceanogr.* 23:559-563.
- Stocker, J. G. 1988. Phototrophic Picoplankton: An Overview from Marine and Freshwater Ecosystems. *Limnol. Oceanogr.* 33(4) (part 2):765-775.
- Stocker, J. G. and N. J. Antia. 1986. Algal Picoplankton from Marine and Freshwater Ecosystems: A Multidisciplinary Perspective. *Can. J. Fish. Aquatic Sci.*, 43:2472-2503.
- Stocker, J. G., M. E. Klut and W. P. Cochlan. 1989. Leaky Filters: A Warning to Aquatic Ecologists. *Can. J. Fish. Aquatic Sci.*, 47(1):16-23.
- Stolzenback, K. D., K. A. Newman and C. S. Wong. 1992. Aggregation of Fine Particles at the Sediment-Water Interface. *J. Geophys. Res.* 97(C11): 17,889-17,898.
- Stramski, M. and T. D. Dickey. 1992. Variability of Bio-Optical Properties of the Upper Ocean Associated with Diel cycles in Phytoplankton populations. *J. Geophys. Res.* 97(C11):17,873-17,887.
- Strutt, J. W. See Rayleigh and Pockels, A, 1891.

- Subba Rao, D. V. and P. A. Yeates. 1984. Effects of Iron on Phytoplankton Production in the Sargasso Sea. *J. Exp. Mar. Ecol.* 81:281-289.
- Susskind, L. 2008. *The Black Hole War. My Battle With Stephen Hawking To Make The World Safe For Quantum Mechanics.* Little, Brown and Co., New York, NY 470 p.
- Sutherland, G. B., I. W. Jones and R. W. Smith. 1983. Oil Spill Protection Planning for Natural Resources in Oregon. Proceedings, 1983 Oil Spill conference, API, Washington, D. C. pp183-186.
- Szekiela, K. H. , S. I. Kupferman, V. K. Emes and d. F. Polis. 1972. Element Enrichment in Organic Films and Foam Associated with Aquatic Frontal Systems. *J. Geophys. Res.* 77(27):5278-52??.
- Tazaki, K, R. Asada and Y. Ikeda. 2002. Quick Occurrence of Fe-Rich Biofilms on the Water Surface. *Nendo Kagaku.* 42(1):21-36.
- Thorpe, S. A. 1985. Small-Scale processes in the upper Ocean Boundary layer. *Nature* 318(6046; 12 Dec):519-522.
- Tipping, E. 2002. *Cation Binding by Humic Substances.* Cambridge U. P. 434 p.
- Tipping, E. 1981. The Adsorption of Aquatic Humic Substances by Iron Oxides. *Geochim. Cosmochim. Acta.* 45(2):191-199.
- Tsyban, A. V. 1971. Marine Bacterioneuston. *J. Oceanogr. Soc. Japan.* 27(2):56-66.
- Ulloa, O. and S. Sathyendrananth. 1992. Light Scattering by marine Heterotrophic Bacteria. *J. Geophys. Res.* 97:9619-9629.
- U. S. EPA. 1988. Chesapeake Bay Program. Review of Technical Literature and Characterization of Aquatic Surface Microlayer Samples. CPB/TRS 20/88.
- Uttermöhl, H. 1958. Vervollkommung der quantitativen Phytoplankton- Methodik. *Mitt. int. Ver Limnol.* 9(1):1-38.
- Van Vleet, E. S. and P. M. Williams. 1983. Surface Potential and Film Pressure measurements in Seawater Systems. *Limnol. Oceanogr.* 28(3):401-414.
- Vincent, W. F. 1980. Mechanisms of Rapid Photosynthetic Adaptation in natural Phytoplankton Communities. II. Changes in Photochemical Capacity as Measure by DCMU-Induced Chlorophyll Fluorescence. *J. Phycol.* 16:568-577.

- Vincent, W. F. 1983. Fluorescence Properties of the Freshwater Phytoplankton: Three Algal Classes compared. *Br. Phycol. J.* 18(1):2-21.
- Vogel, S. 1981. Life In Moving Fluids: *The Physics and Biology of Flow*. Willard Grant Press, Boston, MA, USA. pp.
- Wangersky, P. J. 1976. The Surface Film as a Physical Environment. *Ann. Rev. Ecol Syst.* 17(1976):161-176.
- Wheeler, J. R. 1975. Formation and Collapse of Surface Films. *Limnol. Oceanogr.* 20(3):338-342.
- Williams, P. M. 1967. Sea Surface chemistry: Organic Carbon and Organic and Inorganic Nitrogen and Phosphorus in Surface Films and subsurface Waters. *Deep-Sea Res.* 14:791-800.
- Williams, P. M., A. F. Carlucci, S. M. Henrichs, E. S. Van Vleet, S. G. Horrigan, F. M. H Reid and K. K. Robertson. 1986. Chemical and Microbiological Studies of Sea-Surface Films in the Southern Gulf of California and Off the West Coast of Baja, California. *mar, Chem.* 19(1):17-98.
- Williams, P. M., D. R. Long, C. C. Price, K. J. Robertson and E. S. Van Vleet. 1982. A Mobile Platform for Studying Sea-Surface Films. *Deep-Sea Res.* 29(5a):641-646.
- Williams, P. M. and K. J. Robertson. 1973. Chlorinated Hydrocarbons in Sea-surface Films and Subsurface Waters at Nearshore Stations and in the North Central Pacific Gyre. *Fish. Bull.* 73:445-447.
- Williams, P. M., K. J. Robertson, K. Chew and H. W. Weiss. 1974. Mercury in the South Polar Seas and in the Northeast Pacific Ocean. *Marine Chemistry* 2:287-299.
- Williams, P. M., E. S. Van Vleet and C. R. Booth. 1980. In situ Measurements of Sea-Surface film Potentials. *J. Mar. Res.* 38:193-199.
- Wilson, W. B. and A. Collier. 1972. The Production of Surface-Active Material by Marine Phytoplankton Cultures. *J. Mar Res.* 30(1):15-21.
- Wolf, S. E. J. Leiterer, M. Kappl et al. 2008. Early Homogenous Amorphous Precursor Stages of Calcium Carbonate and subsequent Crystal Growth in Levitated Droplets. *JACS* 130:12342-12347.

- Word, J. Q., L. S. Word, J. N. McElroy and R. M. Thom. 1986. The Surface Microlayer: Review of Literature and Evaluation of Potential Effects of Dredge Activities in Puget Sound. Evans Hamilton, Inc., Prepared for the U. S. Army Corps of Engineers, Seattle district. March, 1986. 134 p.
- Wurl, O. 2011. The Gelatinous Nature of the Sea-Surface Microlayer. COST Action 735 Meeting, MBA Plymouth UK, January 25-26, 2011.
- Wurl, O. and M. Holmes. 2008. The Gelatinous Nature of the Sea-Surface Microlayer. *Mar. Chem.* 110:89-97.
- Wurl, O., L. Miller and S. Vagle. 2011. Production and Fate of Transparent Exopolymer Particles (TEP) in the Ocean. *J. Geophys. Res.* 116:COO>H13.
- Wurtsbaugh, W. A. and A. J. Horne. 1983. Iron in Eutrophic clear lake, California: Its Importance for Algal Nitrogen Fixation and Growth. *Can. J. Fish. Aquatic Sci.* 40:1419-1429.
- Wurtsbaugh, W. A., W. F. Vincent, R. A. Tapia and C. I. Vincent. 1985. Nutrient Limitation of Algal Growth and Nitrogen Fixation in a Tropical Alpine lake, Lake Titicaca (Peru/Bolivia). *Freshwater Biol.* 15:185-195.
- Zaitsev, Yu P. 1971. Marine Neustonology. NOAA/NSF, Translations. Israel Program for Scientific Translations. Washington, D.C. 207 p.
- Zhengbin, Z., L. Liansheng, W. Zhijian, L. Jun and D. Haibing. 1998. Physico-chemical Studies of the Sea Surface Microlayer. I. Thickness of the Sea Surface Microlayer and Its Experimental Determination. *J. Colloid and Interface Sci.* 204:294-299.
- Zifirou, O. C. 1986. Atmospheric, Oceanic and Interfacial photochemistry as Factors Influencing Air-Sea Exchange Fluxes and Processes. In: P. Buat-Ménard (Ed.): The Role of air-Sea Exchange in Geochemical cycling. D. Reidel, Dordrecht, Netherlands. pp. 113-127.
- Zobell, C. E. 1943. The Effect of Solid Surfaces upon Bacterial Activity. *J. Bact.* 46(1):39-56.