BIOCHEMICAL ECOLOGY OF WATER POLLUTION

PATRICK R. DUGAN

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Patrick R. Dugan

Professor and Chairman
Department of Microbiology
The Ohio State University
Columbus, Ohio

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PREFACE

Biochemical ecology is here presented only in the context of water pollution. This is not to minimize the importance of land animals and plants in their environment or the significance of air pollution as it relates to ecology. It merely indicates that water pollution is a problem of sufficiently broad magnitude to warrant consideration by itself.

Water pollution is a problem which requires the attention of a variety of disciplines. The presentation tends therefore to follow the problem approach, as do most interdisciplinary topics. An appreciation of various viewpoints is needed among chemists, ecologists, economists, engineers, lawyers, limnologists, managers, microbiologists, and politicians, whose communications are often "hung up" in each other's jargon.

Perhaps the presentation is too elementary at times. This was done in an attempt to bridge the diverse backgrounds of those concerned with the subject. It is hoped that engineers, economists, biologists, public servants, and others will gain a greater appreciation of the interrelationship of gross observations and biological events that occur at the cellular and molecular level. Lack of such understanding is, to a large extent, the reason for our present environmental condition. At other times the presentation is perhaps too technical. This was done on the assumption that some information on chemical details may not be readily available but is desirable for an "in-depth" appreciation of the biochemical events encountered in water pollution.

The pattern of presentation is to give background information in relatively simple terms and then to support it with more detailed data. In this approach I would argue that it is the significance of reactions in the aquatic environment that is of importance. Consequently, the activities of organisms have significance to water pollution, whereas the numbers and names of the organisms are relegated to secondary considerations.

When specific reactions are discussed, it is implied that they are likely to proceed in the aquatic environment. For example, *Pseudomonas* species are very common to both soil and water. Their metabolic activities would likely be encountered in water, although they have been studied in the laboratory. I have avoided, wherever possible, using biochemical information that would not likely be encountered in the aquatic ecosystem.

In addition to the audience already mentioned, it is hoped that this monograph will be of value to both undergraduate and graduate students with an interest in the aquatic environment and to those individuals who avow an interest in the social-political-economic ramifications of an unbalanced ecosystem.

I would like to acknowledge my wife's patient assistance in the preparation of the manuscript, and to thank Dr. Jorgen Birkeland for valuable criticism and suggestions and Dr. Chester Randles for aid in preparing Chapter 11.

PATRICK R. DUGAN
September, 1971

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PART I

THE WATER POLLUTION PROBLEM

Chapter 1

SIGNIFICANCE OF POLLUTION

Water pollution is significant only when it influences living or biological systems either directly or indirectly. In a broad sense, it can be depicted as a normal consequence of the growth of organisms including man in or near the aquatic habitat. The unique physical and chemical properties of water have allowed life to evolve in it. The following quote from Szent-Gyorgyi (1958) illustrates this point of view: "That water functions in a variety of ways within a cell can not be disputed. Life originated in water, is thriving in water, water being its solvent and medium. It is the matrix of life." All biological reactions occur in water, and it is the integrated system of biological metabolic reactions in an aqueous solution that is essential for maintenance of life. This also is true of air pollutants in that their impact on organisms occurs only when the pollutants are placed in solution (e.g., in the human lung).

Water is the most preponderant chemical found within any freely metabolizing cell, and in bacterial cells the content ranges from 75 to 90 percent, with an average of about 80 percent. In addition to the importance of water in contributing to total cell mass, it is the most versatile of all chemicals in the cell by virtue of its participation in regulatory mechanisms (e.g., osmotic phenomenon and salt balance) and intermediary metabolism (e.g., hydrolytic reactions) and as a structural component (e.g., maintaining turgidity, rigidity, and tertiary structure of macromolecules).

Whether we view life at the cellular, organismic, or population levels, for our purposes all levels can be equated to the biomass of living material. In biochemical terms, the biomass requires both an energy supply to be used as fuel for carrying out the various activities of cells, organisms, or populations, and structural components for the purpose of assembling new cells, organisms, or populations. When living systems expend an energy-rich raw material for growth and activity, it is axiomatic that a lower-energy by-product or waste material will result from the process, and no species of organism can live on its own waste products. This by-product excreta or waste material constitutes a pollutant when it enters the air or water. It is therefore inevitable that living things produce pollutants and generally in proportion to the size, rate of activity, and efficiency of the biomass. It is implicit that virtually all biological and biochemical reactions are catalyzed by enzymes, and to interfere with enzymes is to interfere with life processes, although enzyme inactivation is not the only way in which pollutants interfere with or influence life processes.

Again, it is the uniqueness of water properties that in a broad sense allows pollutants to accumulate in water. To quote Revelle (1969), "the fluid character of water means that the oceans (and lakes) fill all the low places of the earth. Because of this geographical fact, the oceans (and lakes) are ultimate receptacles of the wastes of the land; including wastes that are produced in ever increasing amounts by human beings and their industries."

Although pollution is produced by the activities of organisms, it is usually recognized only when it adversely affects other living organisms; e.g., fish are killed, algal growth is enhanced to bloom proportions which then insult human aesthetic values, people contract a disease. Generally, the reference point for identifying and assessing pollution is the impact it has on human interests. However, we have become sophisticated enough to realize that anything which indirectly influences man's well-being is as important as man himself and that pollution which ultimately influences man's well-being, although indirectly, is also a matter of survival. It is the myriad of diverse organisms acting in concert which allows the continuing recycling of the finite amount of each chemical element available on earth. When one or another of the elements is prohibited from recycling by the elimination of species of organisms responsible for a particular biochemical link in the system, it will accumulate in a particular chemical form and put the ecosystem out of balance. Herein lies the ecological significance of the pollution problem. The sum total is that pollution puts the human environment out of balance, and the scheme of nature is such that it will react to re-establish a balance. Human activities which put the ecosystem out of balance and endanger species, etc., represent part of a normal continuously changing ecosystem and therefore a natural consequence of human life. Regardless of the philosophical point of view, we can predict that newly established balances will be markedly different from life and surroundings as we know them today, if rampant polluting is allowed to continue at its present pace.

There are many intermediary stages in which various groups of organisms act upon the by-products or wastes of other organisms. What is one organism's waste or poison is another's food. Genetic, nutritional, and metabolic processes function as a natural selection process and allow shifts of populations in response to alterations in the environment and indeed exert an altering effect on the environment. Individual ecosystem components, as we know them, depend on or require waste products (pollutants) from another segment of the ecosystem. This is the way in which living systems evolve and maintain the balance. Therefore, once the nutritional energy balance relationships have formed, these segments of the ecosystem are dependent upon the pollutants from others. If the flow of pollutants at this level is stopped, this segment of a dynamic ecosystem would come to equilibrium and cease to exist. Complicated interactions in the environment are difficult to predict or evaluate and have provided a stimulus for enlisting the techniques of systems analysis.

It is the ability of certain organisms to utilize the nutritionally rich polluted environments, such as domestic sewage, that is exploited by man in biological waste treatment processes. In this regard, microbes are well suited for exploitation because of their high metabolic rates per unit cell mass. However, microbial activity, acting on sewage as nutrients for further growth, would proceed in a stream or lake if the microbes were not confined to the waste treatment plant by engineering design.

Specific chemicals, other than general poisons, are not readily definable as pollutants. Rather, it is the quantity or concentration of specific compounds in an isolated situation that must be considered in relation to an observed effect. For example, phosphate is an absolute necessity for all life, yet it is of primary concern today as a water pollutant when it is a growth-limiting nutritional factor, because the excessive amounts which have been deposited in the environment promote excessive growth of algal cells in our lakes. Pollutants then are akin to our concept of weeds—they are chemicals out of place. It would seem to follow that pollution as a state of the environment must be discussed in specific terms related to individual circumstances and, contrary to popular opinion, should not be over-generalized. However, water pollutants for the most part are chemicals dissolved or suspended in water which elicit an environmental response that is objectionable. Some pollutants are physical factors and not chemicals; e.g., heat and radiation are physical factors and exert a marked effect on biochemical reactions.

Part of our difficulty in assessing the significance of pollution is due to our lack of present knowledge of limits of tolerance. There has been a tendency to make judgments on the basis of acute toxicity values such as fish kills. It is now evident that accumulative threshold levels of combinations of pollutants must be considered as well as sublethal chronic effects. For example, it is known that air pollutants influence susceptibility of humans to upper respiratory virus infection, age has a direct bearing on nutrition of individuals, and prior exposure to detergents has a decided effect on the susceptibility of fish to chlorinated pesticides.

Another mistake we have made is in overestimating the carrying capacity of water and air for pollutants. Water acts as a solvent for pollutants, which is the reason it is used in many industrial processes as well as for disposal of domestic wastes. For these reasons, it is important to consider in greater detail the solvent properties of water in addition to its ability to mechanically carry suspended particles from agricultural fields, etc.

Finally, I would argue that the physical and chemical properties of water have a direct bearing on pollution and its effects. One such situation involves water density–temperature relationships, which control turnover of deep lakes. This has far-reaching implications in lake eutrophication because of the role of bottom particulates as surfaces for increased biochemical activity and also because of redistribution of nutrients.

Chapter 2

POLLUTIONAL CONCERNS, CAUSES, AND CONCEPTS

2.1. DISEASE PRODUCTION

There have been five or six general areas of concern over the years which have preoccupied individuals concerned with water contamination and its ecological ramifications.

One was the transmission of disease via the water route as the result of contamination by pathogenic bacteria and protozoans originating in the human intestinal tract. Epidemics of typhoid, dysentery, and other gastrointestinal diseases from "sewage" contamination resulted in the emergence of sanitary microbiology as a discipline in the late 1800s. Sanitary engineering is a technology devoted to doing something about the problem, and its development paralleled that of the science of sanitary microbiology. In recent years, we have come to realize that poliomyelitis and infectious hepatitis as well as several types of intestinal, respiratory, and other virus-caused diseases are also transmitted via the water route.

2.2. ORGANIC POLLUTANTS

2.2.1. Oxygen Consumption

Once we learned to control water-borne disease transmission by modern sanitary practices, particularly through the use of chlorine as an antibacterial and antiviral agent, it became apparent that there was more to be concerned about in sewage. Domestic sewage consists primarily of organic excreta which can be utilized as nutrients by other organisms, particularly microorganisms in the environment. These organisms metabolize the organic components of sewage via oxidation reactions and consume oxygen dissolved in the water during the process. Because oxygen has a relatively low solubility in water, it is rapidly consumed and depleted during waste organic oxidation and the water becomes anaerobic. Once the dissolved oxygen is gone, it is not available for fish and other aerobic organisms, which then die of oxygen deficiencies. It should be pointed out that when several types of aerobic organisms such as fish and bacteria are competing in an ecosystem for oxygen and nutrients, the bacteria and other single-celled microbes are the most competitive. This is due in part to the high surface-to-volume ratio of microorganisms, and, as will be pointed out subsequently in greater detail, the oxidative enzymes are located in the surface membranes of microorganisms.

Oxygen balance in water has been and still is an area of preoccupation. Again, sanitary engineering technology has striven to cope with this problem by designing waste treatment systems, many of which utilize forced aeration in the process. Secondary waste treatment has historically been aimed at removal of oxidizable organic material, usually by employing aerobic microbiological processes (e.g., activated sludge or trickling filters) for removal of biochemical oxygen demand (BOD), and the resultant sludge is often further treated in an anaerobic digester. Success of the aerobic microbiological process depends upon conversion of dissolved organics to microbial cell mass of a type which flocculates and thereby settles. The process coincidentally also removes small particulate material from suspension. Anaerobic treatment is often used to reduce sludge volume via biological dewatering reactions, thereby making the total cell mass more amenable to manipulation and ultimate disposal.

2.2.2. Organic Nutrients

In addition to oxygen consumption, most organic compounds serve as nutrients for microorganisms and more highly evolved life forms in the aquatic habitat. In recent years, the excessive algal growth in water that receives treated sewage effluents has focused attention on the necessity for removal of algae-stimulating nutrients during waste treatment. Consideration has been given to extending treatment or instituting tertiary treatment processes for the purpose of removing the algal growth stimulants that remain after secondary treatment. This includes both organic and inorganic mineral nutrients.

Liebig's law of limiting growth factors (Odum, 1959) states that the rate of growth of an organism (or any biological reaction) is controlled by the factor (nutrient) which is limiting. If the concentration of a nutrient is increased until it is no longer limiting, a different factor becomes limiting, etc. It is then apparent that any possible permutation or combination of nutrient factors may be involved in eliciting algal growth in receiving waters, depending upon other environmental influences and the variety of algal species present.

Although the algae of pollutional significance are photosynthetic autotrophic microorganisms and are capable of growth at the expense of mineral nutrients, carbon dioxide, and light in essentially the same manner as with higher plant forms, they are also capable of utilizing organic compounds as nutrients. Many unicellular green algae (e.g., *Chlorella*) can substitute organic compounds such as glucose for light as their source of energy for growth. Blue-green algae cannot substitute organic compounds as an exclusive source of energy, but they can utilize many organics for cell synthesis in the presence of light. This may be an academic point that is irrelevant in naturally polluted water because light would be available to the cells. The significance of the B vitamins (B_{12}, thiamine, and biotin) as algal growth stimulants has been described by Provasoli (1969). B vitamins are synthesized by many species of bacteria and are present in any organic waste effluent.

2.3. MINERAL POLLUTANTS

The third general area of interest is with mineral pollution. Much of this type of pollution is related to our technological development. Several different aspects can be considered as part of this problem.

2.3.1. Mineral Nutrients

As indicated under the topic of organic pollutants, organic and inorganic molecules which stimulate biological responses are not separate ecological entities. All cells need relatively high concentrations of nitrogen, phosphate, potassium, magnesium, sulfur, calcium, and iron, in addition to traces of manganese, zinc, copper, and sodium. Plants including the algae also require boron, chloride, and molybdenum ions. They usually utilize either nitrate or ammonium ions as the nitrogen source, although organic nitrogen such as urea or amino acids will also satisfy the nitrogen requirement in most cases. The source of sulfur most commonly utilized by plant cells is the mineral sulfate, although some sulfur can be derived from sulfur-containing amino acids or sulfide.

One source of minerals is to some extent a consequence of our waste treatment technology. Some of the biochemical reactions involved in organic oxidations can be summarized according to the following summary reactions:

$$C_6H_{12}O_6 \text{ (sugar)} + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O \text{ (complete oxidation)} \quad (2.1)$$

$$\text{tryptophan (amino acid)} + 5\,O \rightarrow \text{indole} + \text{pyruvate} + NH_3 \text{ (incomplete oxidation)} \quad (2.2)$$

$$H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2 \text{ (urea)} + H_2O \rightarrow CO_2 + 2\,NH_3 \text{ (hydrolytic)} \quad (2.3)$$