AN ABSTRACT OF THE THESIS OF

STANLEY LEWIS PONCE II (Name) for the MASTER OF SCIENCE (Degree)

in FOREST ENGINEERING (Major) presented on June 8, 1973 (Date)

Title: THE BIOCHEMICAL OXYGEN DEMAND OF DOUGLAS-FIR NEEDLES AND TWIGS, WESTERN HEMLOCK NEEDLES AND RED ALDER LEAVES IN STREAM WATER

Abstract approved: George W. Brown III

Field studies indicate that accumulation of finely divided organic debris in the channels of mountain streams after clearcutting may be responsible for subsequent reduction in the dissolved oxygen concentration. The purpose of this study was to quantify the biochemical oxygen demand (BOD) of Douglas-fir needles and twigs, western hemlock needles and red alder leaves, ascertain the chemical characteristics of the leachate from these materials, and determine if such materials are toxic to fish.

Long- and short-term BOD and BOD rate constants were determined for the different types of vegetation. A standard temperature BOD test was run 90 days for the leaf material and 45 days for the Douglas-fir twigs. Four replications were run for the leaves and three run for the twigs. Half the leaf samples were fixed with a

nitrification inhibitor. However, later analyses proved nitrification did not occur. As a result, all the replications within a species were combined and one curve was fitted through each group of data. A BOD curve was constructed by least squares fit through the 90 days of leaf data and through 45 days of the twig data and extended to 90 days. The 90 day K_1 and the BOD_{90} were calculated from this curve and were: 0.125 and 110 mg O_2/gm (dry weight) for Douglas-fir needles, 0.056 and 110 mg O_2/gm (dry weight) for Douglas-fir twigs, 0.064 and 166 mg O_2/gm (dry weight) for western hemlock needles, and 0.046 and 286 mg O_2/gm (dry weight) for red alder leaves. Further tests showed that these 90 day values could be accurately estimated by tests of shorter duration; 20 days for Douglas-fir and western hemlock, and 60 days for red alder. The 20-day western hemlock values may, in fact, yield a more precise estimate of the actual 90-day values than the composite fit. The 20-day western hemlock projections were 0.049 for K_1 and 200 mg O_2/gm (dry weight) for the BOD_{90}.

Leachates of the test materials were analyzed with respect to composition, concentration, and rate of nutrient release. This information was to be used in the explanation of the 90 day BOD values obtained. Half the samples were poisoned with 2.7 mg/l mercuric chloride to inhibit microorganism growth while the rest of the samples remained untreated to show the effect of the

microorganisms. These data were of limited use due to fungal growths appearing after 20 days and failure of half the twig samples to be adequately poisoned. The test was terminated after 20 days.

Simple sugars and phenolic compounds were found in all the samples. Simple sugars present were arabinose, xylose, galactose, mannose, and glucose. Mean maximum sugar concentrations released in poisoned leaf samples varied from 21.5 mg glucose equivalent/gm (fresh weight) to 15.0 mg/gm (fresh weight) for Douglas-fir and red alder. The mean maximum sugar concentrations in the non-poisoned samples were much less: 6.0, 5.0, and 8.0 mg glucose equivalent/gm (fresh weight) respectively for Douglas-fir, western hemlock, and red alder. The rate of sugar release was rapid for Douglas-fir needles, slow for red alder, and intermediate for western hemlock.

The different types of phenolic compounds were not identified. The mean maximum concentration of phenolics in the poisoned samples were 0.72, 0.46, and 0.55 mg gallic acid/gm (fresh weight) respectively for Douglas-fir, western hemlock, and red alder leaves. There was little difference between mean concentrations of the poisoned and non-poisoned samples until day 10, at which time mean phenolic concentrations of the non-poisoned samples began to drop.

The Douglas-fir twigs had a slow initial sugar release, followed by an increase in the rate; the maximum concentration reached was 10.0 mg glucose equivalent/gm (fresh weight). The pattern of phenolic

release was the same with a maximum concentration of about 1.0 mg gallic acid/gm (fresh weight).

The five-day BOD of leaf material was determined under conditions of fluctuating temperature (12.8 to 35.0°C) similar to that observed in streams exposed by clearcutting. The BOD_5 values were: 202 mg O_2/gm (dry weight) for Douglas-fir, 109 mg O_2/gm (dry weight) for western hemlock, and 249 mg O_2/gm (dry weight) for red alder leaves.

The BOD_5 of samples incubated at standard temperature was much lower than those exposed to conditions of fluctuating temperature; it was 25, 42, and 24 percent of the temperature fluctuated BOD_5 for Douglas-fir, western hemlock, and red alder leaves respectively.

The toxicity of leachate extracted from 50 grams (fresh weight) per liter of water of each species was determined on guppies and steelhead fry. The 96-hr LC50 to guppies from leachate of Douglas-fir, western hemlock, and red alder was 35, 65, and 18 percent of the original concentration as opposed to 26, 7.5 and 25 percent of the original concentration for the steelhead fry. These are extremely low levels of toxicity to such a concentrated sample and pose no threat to the fish.

The Biochemical Oxygen Demand of Douglas-fir Needles and Twigs, Western Hemlock Needles and Red Alder Leaves in Stream Water

by

Stanley Lewis Ponce II

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1974

APPROVED:

Associate Professor of Forest Engineering

William G. Davis

Head of Department of Forest Engineering

Dean of Graduate School

Date thesis is presented June 8, 1973

Typed by Opal Grossnicklaus for Stanley Lewis Ponce II

ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to the U. S. Forest Service for grant P.N.W. No. 3, 1971 which made this study possible.

I am deeply indebted to Dr. George W. Brown, my major professor, for his guidance and encouragement throughout this study and for his editorial assistance in the preparation of the thesis.

Sincere gratitude is expressed to Dr. Ronald J. Miner, my minor professor, for his valuable suggestions during this research and for his review of the thesis.

I also wish to acknowledge Dr. Henry A. Froelich for his review of the thesis.

Finally I wish to thank my wife Carolyn for her continued encouragement throughout this study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE BIOCHEMICAL OXYGEN DEMAND OF DOUGLAS-FIR NEEDLES AND TWIGS, WESTERN HEMLOCK NEEDLES AND RED ALDER LEAVES IN STREAM WATER

INTRODUCTION

The Problem

The economy of the Pacific Northwest has been built around the timber and fishery resources of the region. The principal timber species of the region are Douglas-fir, western hemlock and, to a lesser degree, red alder. Harvesting this timber resource is essential. Although timber is considered the primary economic resource, the region also produces a multi-million dollar fishery resource.

The small streams originating in the timbered coastal watersheds serve as prime producers of anadromous salmon and trout. The young of these species remain in these mountain streams at least one year before they migrate to the ocean. The growth, development, and survival of these young fish are directly related to the quality of the aquatic environment in which they reside. The quality of this aquatic environment, in turn, is dependent upon the management practices applied to and the condition of the adjacent terrestrial environment.

Until recently, there has been little concern by the public of the

effect that forest harvesting practices have on the water quality of mountain streams in the Pacific Northwest. Consequently, little research has been done to quantitatively interpret the direct interaction between the aquatic ecosystem and timber harvesting practices applied to the adjacent forest environment. However, in the last eight to ten years the public has become aware of water pollution and the potential effect it may have on the fisheries resource. As a result, forest harvesting practices that were once considered "good silvicultural techniques" are now being questioned.

Because of the recent concern, forest scientists and fishery biologists have been examining the effects of various logging practices on the quality of mountain streams. The majority of research to date has dealt with the effect of logging on temperature and sediment. The result of this work has been the development of guidelines for stream protection in logging practices along with strict water quality standards. However, little attention has been given to the impact of finely divided logging debris on the quality of mountain streams.

West Coast Douglas-fir, because of its regeneration and growth characteristics, is harvested by clearcutting. Clearcutting is a harvesting practice in which an area is cut clear of all the trees, large or small. A by-product of clearcut logging is slash, which is composed of limbs, branches, and needles or leaves of trees. Often this material moves down the slope due to gravity or may be deposited

directly in the stream. In Oregon, it is mandatory by law that the logging operator remove the larger debris from the stream. Although most of the logs, large limbs and twigs are removed, finely divided material, such as splinters from limbs and logs, pieces of bark, small broken twigs, needles and leaves often remain. Federal timber harvest contracts, for example, often specify that only material greater than eight feet in length or larger than eight inches at the small end need be removed.

Finely divided material may cause some very serious water quality problems in mountain streams if it accumulates in large quantities at the right time of year. The pollutional impact of finely divided logging material depends on when the material enters the stream. Material deposited in an Oregon Coast Range stream between early fall and late winter generally causes only minor quality problems. During this period the winter freshets generally occur and the streams have the energy to flush the material through the system. However, if the material is deposited between early spring and late summer, the probability of the occurrence of serious quality problems is much greater. During this period the streams are generally at low flow and do not have sufficient energy to transport the debris.

Finely divided logging material may reduce the quality of mountain streams during the summer months in many ways. The

most apparent is the reduction in dissolved oxygen levels. When finely divided logging debris is left in a stream, the sugars and phenols it contains are readily leached out. This material, being organic in nature, is subject to biological degradation. The degradation process is one of simple oxidation by the microorganisms present. The growth and metabolism of these organisms is limited only by their ability to process this material. Since the sugars are easily oxidized, the population number and metabolic rate increases rapidly. Accompanying this increase in population and metabolic rate is an increased oxygen demand. When the demand for oxygen exceeds the stream's ability to supply it, oxygen concentration drops to levels which may effect fish life.

Fish, as all living organisms, require free oxygen to carry on their life processes. If the dissolved oxygen concentration in the water is reduced below four to five mg/l the fish present will be subject to an oxygen stress. In general, oxygen stress will inhibit growth and development of the fish. If the oxygen concentration falls below one mg/l for a prolonged period, death will probably result.

A useful test, if properly interpreted, to determine the amount of oxygen required in the stabilization of decomposable organic matter is the biochemical oxygen demand test (BOD). The BOD test is used extensively by sanitary engineers for the determination of the pollutional strength of domestic and industrial waste. However, its

application may be expanded and serve as an indicator of pollutional strength of any organic material in water, including logging debris in mountain streams.

In addition to the oxygen problem associated with logging debris, logging debris may contain organic toxins which could be released into the stream by leaching. Some studies, such as Schaumburg and Atkinson's (1969) work with logs in log ponds, indicate sugars and polyphenols are readily leached from logs and that this leachate is non-toxic. At the present, however, little is known about the composition, or toxicity to salmonids of leachates from finely divided logging debris.

Although forest scientist and fishery biologist are aware that oxygen deficits and possibly direct toxicity problems may occur when logging slash is deposited in small streams, very few quantitative studies dealing with these problems have been completed. No studies have been undertaken which seek to quantify the impact on water quality of slash from commercially valuable species typical of the forests of the Pacific Northwest.

Study Objectives

The specific objectives of this study are to quantify for Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) needles, western hemlock (Tsuga heterophylla [Raf.] Sarg.) needles and red alder (Alnus rubra

Bong.) leaves in mountain stream water:

1) Short- and long-term (90-day) BOD values and rate constants,
2) the concentration of sugars and polyphenols present in the leachate over 90 days,
3) the toxicity of leachate extracted for five days defined by 24-, 48-, and 96-hour TL50 to steelhead trout (Salmo gairdneri gairdneri) fingerlings and guppies (Poccilia reticulata),

and to quantify for Douglas-fir twigs:

1) Short- and long-term (45-day BOD values and rate constants,
2) the concentration of sugars and polyphenols present in the leachate over 45 days.

Significance of Research

The toxicity data will enable the fishery biologists to predict the acute toxicity effect of various concentrations of a five day leachate on steelhead trout fingerlings under test conditions. The leachate composition data will aid the fishery biologist in explaining why certain concentrations are lethal to guppies and steelhead trout over a given time and aid in the prediction of sublethal effects on the same fish under similar conditions.

The short- and long-term BOD values and rate constants will quantify the amount of oxygen needed by microorganisms and the rate at which they use it in the oxidation of logging debris. These values,

along with previous reaeration and debris accumulation work completed earlier, will permit the development of an oxygen depletion model. This model will enable foresters to predict on-site and downstream dissolved oxygen levels at any time after the introduction of a known amount of logging slash. The leachate composition data will serve as the basis for the explanation of the shape of the curves and values obtained from the BOD tests.

LITERATURE REVIEW

Oxygen has long been recognized as a critical water quality parameter. Its concentration plays an important role in the determination of the life present in an aquatic ecosystem. Sport and commercial fish, as well as other aquatic organisms of value to man, require high levels of oxygen. If, for some reason, all the free oxygen is extracted from the water, virtually no aquatic life survives, only a few species of facultative-anaerobic microorganisms. A common occurrence in many of our streams and rivers is the addition of organic matter. This material is subject to biological decomposition; a process which serves as a major oxygen drain on a stream or river. If the quantity of organic matter added to the system is great enough, the oxygen may be totally depleted. Because of its necessity to aquatic life and the susceptibility of organic matter to oxidation by microorganisms, scientists often use oxygen concentration as an indicator of water quality.

This literature review will deal with the importance of dissolved oxygen to the survival of Pacific Coast salmonids, with special emphasis given to the effect of logging on the dissolved oxygen (DO) level and the biochemical oxygen demand (BOD) process in a small mountain stream. A brief review of the physiochemical characteristics of oxygen and the oxygen balance will proceed the review

of the importance of DO to fish, the effect of logging on DO levels, and the BOD process of natural organic material.

Physiochemical Characteristics Controlling Oxygen Solubility

Oxygen is the earth's most abundant element. Elemental oxygen and its compounds make up 49.2 percent, by weight, of the earth's crust and as a gas about 20.9 percent, by weight, of the atmosphere (Weast, 1964). Although free oxygen is abundant in the atmosphere, it is relatively insoluble in water. The equilibrium concentration of oxygen in fresh water varies between 14.6 mg/l at 0° C to 5.6 mg/l at 50° C under 760 mm Hg pressure (American Public Health Association (A. P. H. A.), 1971).

At pressures less than four atmospheres, and room temperature, all gases obey the "ideal gas laws" of physical chemistry (Mancy and Jaffe, 1966). Physically, oxygen is a colorless gas and under natural conditions of the biosphere it obeys the ideal gas laws. Because oxygen conforms to the ideal gas laws under natural conditions, its solubility in water may be calculated. Oxygen solubility in fresh, salt free, water is a function of the pressure exerted by the gas above the water and the water temperature.

The oxygen concentration in fresh water at a constant temperature is a function of the pressure exerted by the oxygen in the

atmosphere. Application of two fundamental laws of physical chemistry, Dalton's Law of Partial Pressures and Henry's Law, allow the direct calculation of gas solubility in a liquid. Dalton's law of Partial Pressures and Henry's Law, allow the direct calculation of gas solubility in a liquid. Dalton's law of partial pressures may be stated as follows (Sawyer and McCarty, 1967):

> In a mixture of gases, such as air, each gas exerts a pressure independently of the others. The partial pressure of each gas is proportional to the amount (percent by volume) of that gas in the mixture, or in other words, it is equal to the pressure which that gas would exert if it were the sole occupant of the volume available to the mixture.

Dalton's concept of partial pressures lead to the development of Henry's law, which allows direct calculation of the weight of a gas which will dissolve in a liquid. Henry's law may be stated as follows: "The weight of any gas that will dissolve in a given volume of a liquid, at constant temperature, is directly proportional to the pressure that gas exerts above the liquid" (Sawyer and McCarty, 1967). Expressed mathematically, Henry's law may be written as the following equation:

$$C_{gas} = K_H P_{gas} \qquad (1)$$

where C_{gas} is the equilibrium concentration of the gas in the liquid in mg/l, K_H is Henry's law constant for the gas at the given temperature in mg/l-atm and P_{gas} is the partial pressure exerted by the gas in atm.

The solubility of oxygen in water as a function of temperature is not as clearly defined as for pressure. In general, oxygen is more soluble in cold water than in warm water. The effect of temperature may be expressed by an empirical equation developed by Truesdal et al. (1955):

$$C_S = 14.161 - 0.3943\ T + 0.007714\ T^2 - 0.0000646\ T^3 \quad (2)$$

where C_S is the concentration of the gas in the liquid in mg/l at temperature T in °C. At present, there is some disagreement among scientists concerning the exact values of the coefficients in equation (2). Although these disagreements exist, for the purpose of this discussion it is only important that the general relationship between oxygen solubility and water temperature is clear. Within the limits of the ideal gas laws, the solubility of oxygen in water varies inversely with water temperature.

The Oxygen Balance

The oxygen concentration in a stream at any given time is the composite effect of addition and depletion of oxygen from solution by biological and physical processes. Under natural conditions, a typical mountain stream is in a state of oxygen balance. Living aquatic animals and agents of decomposition continuously withdraw free oxygen while, at the same time, oxygen is supplied intermittently

by green plants during daylight hours, and continuously by direct absorption from the atmosphere.

The oxygen balance may be described using a mass balance approach. A volume within the stream is defined. The change in mass of dissolved oxygen within the volume is equated to the inputs minus the outputs of oxygen. Since an oxygen balance exists, there will be no change in the oxygen mass within the volume and the mass balance may be reduced and stated as: the inputs, or sources, are equal to the outputs, or drains. The oxygen balance of a mountain stream under natural conditions is illustrated diagrammatically in Figure 1. The size of the arrows between components indicates the magnitude of oxygen transfer.

A mountain stream is replenished with oxygen from three sources: the direct absorption at the water-atmosphere interface, the photosynthetic process of green aquatic plants and, to a minor extent, by influent groundwater.

The surface water is supplied primarily by direct absorption from the atmosphere. The process is one of simple molecular diffusion. In general, the rate of reoxygenation in still water is relatively slow. However, mountain streams often have steep gradients resulting in turbulent flow. Turbulence produces vertical and horizontal mixing forces which greatly enhance the rate of reoxygenation.

A second source of oxygen for the surface water is green plant

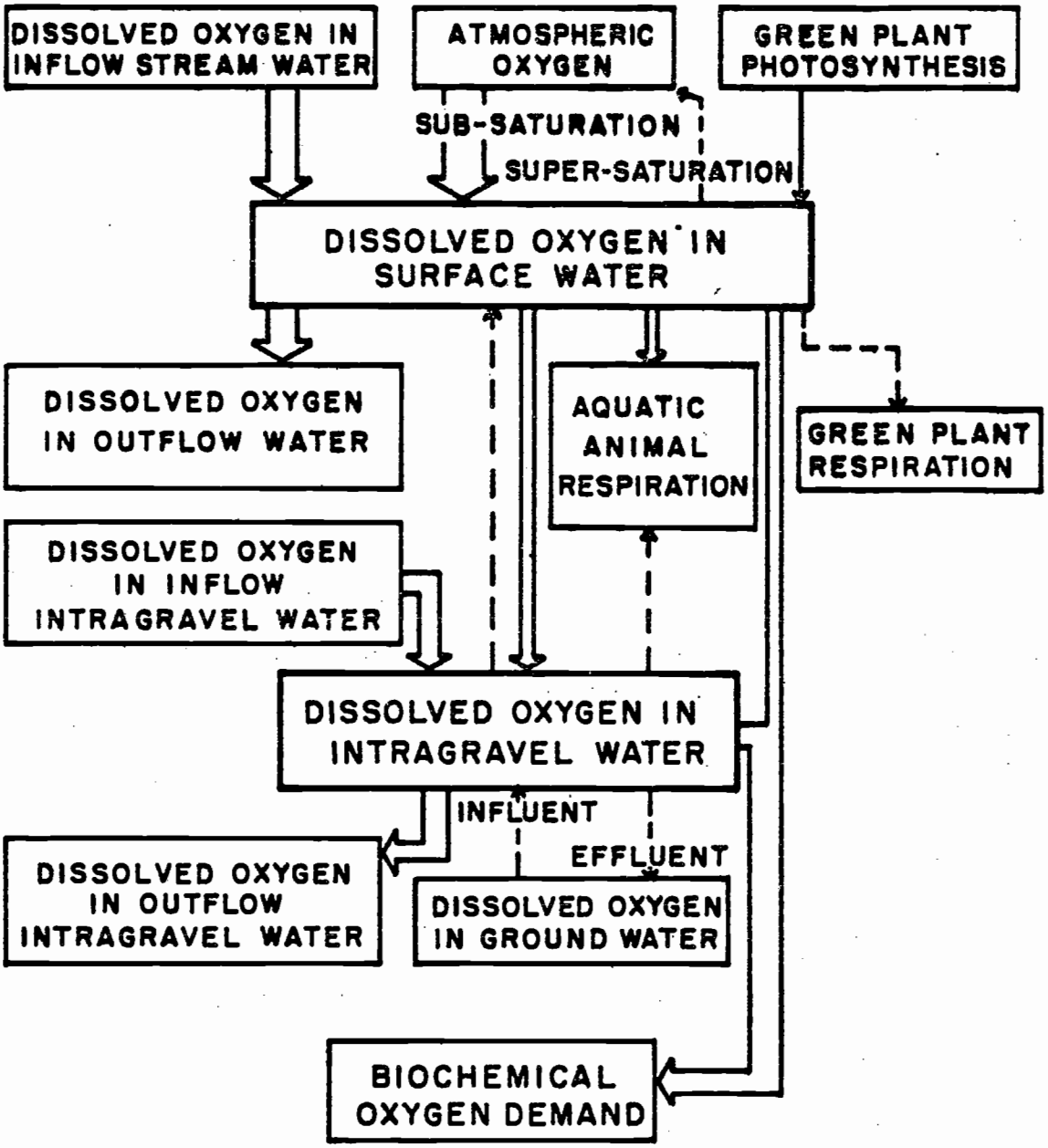


Figure 1. Inputs and outputs of dissolved oxygen in a mountain stream under natural conditions.

photosynthesis. Plankton and algae are often present in quiescent pools. During daylight hours these plants photosynthesize and produce free oxygen as a by-product. In large, low gradient streams, or lakes, photosynthesis by plants may serve as a major source of oxygen (Camp, 1965). However, in a small upland stream the direct diffusion of oxygen into the water from the atmosphere serves as the predominant oxygen source.

The intragravel water is supplied with oxygen primarily by mass transfer and diffusion from the overlying surface water. The rate of this transfer and diffusion is relatively slow. The agents of mixing present in the surface water are inhibited in the intragravel water. Water velocity through the intragravel layer is much less than the surface layer; two to five cm/hr as opposed to 500 to 1500 cm/sec (Narver, 1971).

A second, and generally very minor, input of oxygen into the intragravel water is oxygen carried in by influent groundwater (Vaux, 1962). Sheridan (1962) found in his work with pink salmon streams in Southeast Alaska that oxygen input by groundwater was very small and concluded that the major intragravel oxygen source was direct diffusion from the surface layer.

The predominate dissolved oxygen drains in an unpolluted mountain stream, both in the surface and intragravel water, are biochemical oxygen demand (BOD) and respiration by larger aquatic

life. Minor amounts will be lost to plant respiration and to the atmosphere by direct diffusion if the stream is in a state of oxygen super-saturation, while a trace quantity may be lost to effluent groundwater flow.

Biochemical oxygen demand imposes the greatest demand on a stream's dissolved oxygen supply. Under natural conditions the demand for oxygen by the microorganisms is relatively constant and well within the stream's ability to supply it. However, if a large quantity of organic matter is added, serious oxygen deficiency problems may develop downstream. This can be illustrated with a dissolved oxygen sag curve (Figure 2).

The DO sag curve is the profile of dissolved oxygen deficit along the path of water movement below the point of pollutant entry. Dissolved oxygen deficit is defined as the difference between the actual concentration and the saturation concentration of oxygen in water of a given temperature. The DO sag concept was developed by Streeter and Phelps (1925) and today is commonly applied by sanitary engineers in the calculation of permissible organic loading of receiving water.

The DO sag curve may be described mathematically as the interaction of two processes which occur simultaneously in streams: reaeration and oxygen depletion. Each of these processes, in turn, may be described by a differential equation. In the reaeration

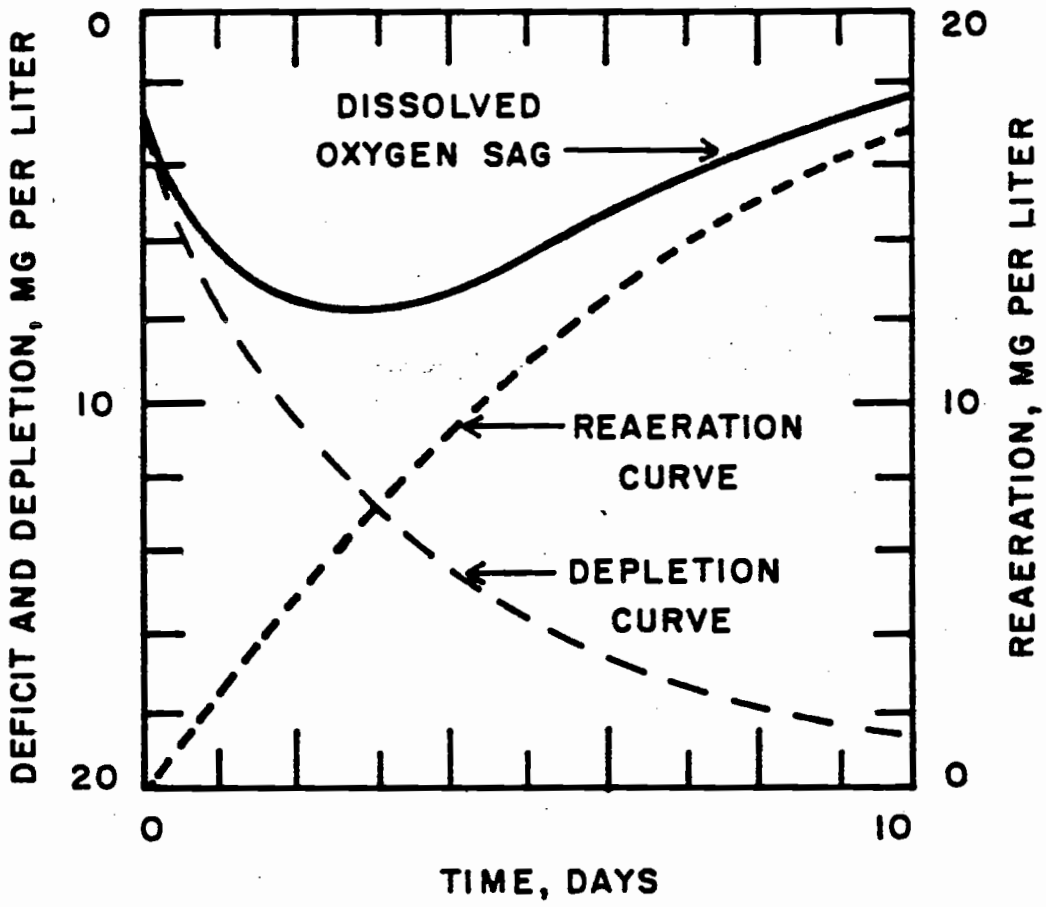


Figure 2. The dissolved oxygen sag and its components: cumulative deoxygenation and cumulative reaeration (after Fair, Geyer, and Okum, 1971).

equation, it is assumed that the rate of oxygen absorption by water is proportional to the oxygen deficit. This relation may be expressed mathematically as:

$$\frac{dD}{dt} = -K_2 D \tag{3}$$

where D is oxygen deficit in mg/l, t is time in days, and K_2 is the reaeration coefficient (base e) in units of 1/days.

In the depletion equation, it is assumed that the rate of BOD consumption due to biochemical oxidation is proportional to the amount of BOD present. This may be expressed as:

$$\frac{dL}{dt} = -K_1 L \tag{4}$$

where L is the BOD concentration in mg/l, t is time in days, and K_1 is the BOD rate coefficient (base e) in units of 1/days. Equation (4) may also be expressed in terms of oxygen deficit, D. Since BOD concentration is measured in terms of the quantity of oxygen consumed, it follows that the rate change in BOD concentration is equal to the rate of oxygen depletion. The rate of oxygen depletion may, in turn, be expressed as the rate change in oxygen deficit, as illustrated in equation (5).

$$\frac{-dL}{dt} = \frac{dD}{dt} \tag{5}$$

Substituting equation (4) into equation (5) yields equation (6).

$$\frac{dD}{dt} = K_1 L \qquad (6)$$

Equations (3) and (6) may be combined and solved for D, as illustrated in Appendix A, resulting in equation (7):

$$D = \frac{K_1 La}{K_2 - K_1} (e^{-K_1 t} - e^{-K_2 t}) + Da\, e^{-K_2 t} \qquad (7)$$

where La and Da are respectively the initial BOD and initial oxygen deficits in units of mg/l at time equal to 0, e is the base of natural logarithms (2.71828), and the remaining terms are as previously defined. Equation (7) is commonly referred to as the Streeter-Phelps equation and may be used to determine any point on the oxygen sag curve.

The BOD process is discussed in detail in the section on "BOD of Natural Vegetative Material."

A relatively constant drain is the oxygen required by larger aquatic life present. Although a mountain stream may appear to be relatively free of larger aquatic life, it generally supports a multitude of organisms, such as snails, insect nymphs, crayfish and fish. All these organisms require oxygen to produce the energy needed for growth, reproduction, and motion. The rate of oxygen removal by these organisms is a function of the species present and their environmental conditions. Under natural conditions, the rate of oxygen

supply by the stream is much greater than the demand exerted by these larger organisms.

The oxygen balance is a very important water quality concept. Alteration of any of the sources or drains may, in turn, have a pronounced effect on the aquatic life present.

Importance of Dissolved Oxygen to Fish

Adequate levels of dissolved oxygen (DO) are essential for the well being and survival of fish. Fish require oxygen to metabolically produce energy required for growth, reproduction, and motion. The amount of oxygen required by fish varies with the environmental conditions and the species. Beamish (1964), from his work with fish respiration, reported that under standard pressure (O_2 partial pressure of 160 mm of Hg) standard oxygen consumption of brook trout at 15° C was 90 mg/kg-hour, carp at 20° C was 50 mg/kg-hour and goldfish at 20° C was 42.5 mg/kg-hour. As the partial pressure of oxygen was reduced from saturation, the oxygen consumption rate remained constant until the partial pressure of oxygen dropped below 105 mm Hg for all three species. This raises the question: what is an adequate dissolved oxygen level?

Presently fishery biologists feel the only adequate level, or standard, which would afford "complete protection" to the fishery resources under all conditions is no reduction in the natural level

(Doudoroff and Shumway, 1967). However a standard such as this, especially where the multiple use concept for water is being applied, is both unrealistic and unnecessary. A "rule of thumb" standard which has been widely accepted by fishery biologists for a long time and is often recommended is that DO levels should at no time drop below 5 mg/l for fish in warm water habitats or below 6 mg/l for fish in cold water habitats.

Often fish are exposed to DO concentrations well below 5 mg/l for prolonged periods of time. DO concentrations between 5 and 2.5 mg/l are generally considered sublethal to fish. When exposed to such conditions fish experience an oxygen stress, and if the exposure is for a prolonged period of time their activity, growth, and reproduction may be reduced.

Several responses to oxygen deficiencies by fish within the surface water and by fish eggs and embryos in the intragravel water have been reported. Shellford and Allee (1913) studied the avoidance reaction of 16 species of fish common to America to different concentrations of oxygen, nitrogen, ammonia, and carbon dioxide. They reported that there was a definite effort by the fish to avoid water deficient in oxygen. Jones (1951) ran a similar experiment with stickleback, minnows, and trout fry. At temperatures near 20° C all three species reacted violently and retreated rapidly when they swam into water containing 0.5 to 1.0 mg O_2/l. At a concentration

of 3.5 mg O_2/l the reaction was again one of rejection, but occurred at a much slower rate. Avoidance tests with juvenile chinook and coho salmon, large mouth bass, and bluegill were carried out by Whitmore, Warren, and Doudoroff (1960). The test was performed in a channeled avoidance tank. In general, the results supported those of earlier studies. They found marked avoidance by the chinook and coho to water containing less than 4.5 mg O_2/l, and some avoidance by coho to concentrations of 6 mg O_2/l. The bass and bluegill responded similar to the chinook salmon.

Davison et al. (1959), working with dissolved oxygen requirements of cold water fishes, reported that at a temperature of 18°C young coho salmon survived for a period of 30 days at a DO level of 2.0 mg/l. During this period they observed that the fish took in little food and lost weight. At a higher DO level, near 3.0 mg/l, the fish took in more food and had a positive weight gain. However, this gain was much less than that of similar fish in oxygen saturated water. The influence of oxygen concentration on growth and food consumption of juvenile coho salmon was further examined by Herrmann, Warren, and Doudoroff (1962). They found at 20°C that both growth and food consumption over a prolonged period of time declined gradually as the oxygen level dropped from 8.3 to about 5.5 mg/l. The decline was rapid as the oxygen level dropped from about 5 to 1.8 mg/l, and death often occurred at levels below 1.0 mg/l.

The fish consumed very little food and lost weight at oxygen levels of 2 mg/l and less.

It is evident from these studies that, in general, fish in the surface water attempt to avoid areas significantly deficient in oxygen and when exposed to such water for a prolonged period of time, their growth and food consumption rates decrease. The avoidance reaction increases as the oxygen level approaches the lethal limit, while the food consumption and growth rates decrease.

The value of high oxygen levels in the intragravel water is often overlooked. However, for Pacific Coast salmonoids it is critical. The female salmonoid digs a hole 10 to 12 inches in the gravel and deposits her eggs. The male fertilizes the eggs and they are covered with gravel. The eggs hatch and the embryos develop for approximately three months before the fry emerge into the surface water (Lantz, 1971). Continuously high oxygen levels during the period of embryo development are very important. If oxygen becomes deficient, the percent egg survival, rate of embryo development, and quality of fish produced may be reduced significantly.

Shumway, Warren, and Doudoroff (1964) examined the influence of oxygen concentration and water movement on the growth of steelhead trout and coho salmon embryos. In their experiment, embryos raised from fertilization to hatching were exposed to different concentrations of DO ranging from 2.5 to 11.5 mg/l and water velocities

ranging from 3 to 350 cm/hour under a near constant temperature of 10° C. They found that fry produced from embryos raised at oxygen levels less than 4.0 mg/l hatched later and were smaller in size at hatching than fry from embryos raised at oxygen levels near saturation. They also reported that reduced water velocities affected the fry in much the same manner as reduced oxygen levels, although the effect was not as pronounced.

Garside (1966) did a similar experiment in which he examined the effects of oxygen and temperature on brook and rainbow trout embryo development. The embryos of each species were exposed to oxygen concentrations of 2.5, 3.5, and 10 mg/l at each of four temperatures, 2.5, 5.0, 7.5, and 10.0° C, from the time of fertilization to late development. The rate of development became slower and the length of hatching period increased for both species of fish as temperature and oxygen levels declined.

In view of this evidence, it is apparent that adequate oxygen levels in the surface and intragravel water are necessary for the survival and well being of fish and embryos present in a stream. Under natural conditions a stream maintains an oxygen balance which supplies the fish with an adequate supply. This balance may be interrupted by man's activity and the fishery resource may suffer as a result. There has been recent evidence that logging practices may, in fact, affect the DO balance.

Dissolved Oxygen and Logging

Clearcut logging, typical of Pacific Northwest forests, affects DO levels in two ways; indirectly by increasing temperature and directly by supplying organic material for biochemical oxidation. The adverse effects of clearcutting on stream temperature have been known for a long time. Clearcutting, when done up to the stream channel, removes all the shade protection and exposes the stream to direct solar radiation. Recently, a study evaluating the effects of two different methods of clearcutting on stream temperature was carried out by Brown and Krygier (1970) in Oregon's Coast Range. Two watersheds, one completely clearcut and one patch-cut in three small clearcuts (about 25 percent of the area cut) with buffer strips 50 to 100 feet wide left adjacent to the stream, were compared to each other and to a third unlogged control watershed. Summer flow from the streams draining these watersheds was quite low, ranging from 0.01 to 0.2 cubic feet per second (cfs). The results of this study were that average monthly maximum temperatures increased by 14° F (7.8° C) and annual maximum temperatures increased from 57 to 85° F (13.9 to 29.4° C) on the completely clearcut watershed one year after cutting. In terms of oxygen decrease, due only to temperature fluctuation, the saturation concentration would have decreased from about 10.4 mg/l at 57° F to 7.8 mg/l at 85° F, or a twenty-five

percent reduction. Temperature levels in the stream draining the watershed which was patch-cut with vegetation left along the stream showed no significant changes due to clearcutting and maintained dissolved oxygen levels near those of the control watershed.

A second problem, and one of major concern in this study, is the effect of logging slash on DO levels. This residue from clearcut harvesting often moves down the slope by gravity or is deposited directly in the stream. Since it is organic, it is subject to biological oxidation. The problem of BOD caused by logging debris has received little attention and to date there have only been a few related studies examining this problem. This problem and the previous work are discussed in detail in the following section.

BOD of Natural Vegetative Materials

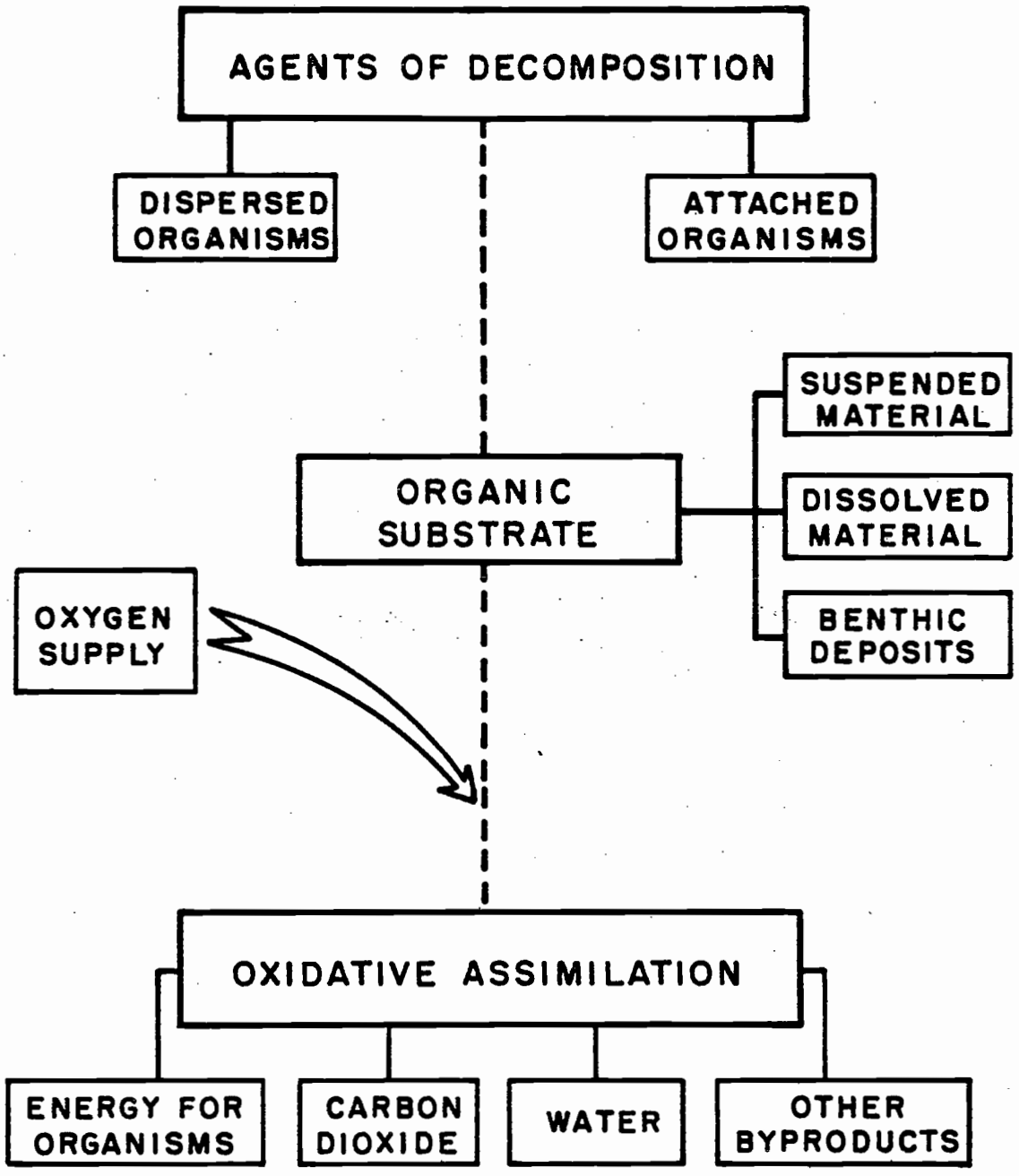
The BOD test is widely used by scientists to determine the bio-oxidizable content of organic material placed in surface waters. The BOD test may be defined as a test which measures the amount of oxygen required by microorganisms for the oxidative decomposition of organic material in water (Warren, 1971). Under natural conditions the BOD process is beneficial to the stream. The decomposers recycle nutrients by extracting elements contained in dead organic matter and dissolved nutrients. They break down complex compounds into substances required for growth and reproduction and CO_2 used by

green plants. These organisms and plants may in turn serve as food for small animals.

The BOD process in a mountain stream is illustrated diagrammatically in Figure 3. The agents of decomposition may be separated into two classes: the dispersed or attached organisms. The dispersed organisms flow freely within the stream while the attached organisms remain stationary, attached to rocks and other fixed objects. Both groups of organisms exert an oxygen demand. In a mountain stream, where the gradient is high and the flow turbulent, the dispersed organisms generally predominate. In streams where the gradient is not as great and there are a number of quiescent pools present, the attached organisms may exert a significant demand. In general, the decomposers are comprised primarily of bacteria, protozoa, fungi, and, to a lesser extent, aquatic insects.

The substrate, or food source, is suspended material, dissolved material, and benthic deposits. The mode of substrate intake by the microbes is, in general, direct absorption of dissolved materials, while the insects may obtain the material by ingestion. The dissolved material predominates in upland streams under natural unpolluted conditions.

The assimilative process is one of wet oxidation within the decomposers (Sawyer and McCarty, 1967). This process may be expressed by the following reaction:



$$\text{Substrate} + O_2 \xrightarrow{\text{Organism}} CO_2 + H_2O + \text{Energy} + \text{Other By-Products}$$

Figure 3. The biochemical oxygen demand process in a mountain stream.

$$\text{Substrate} + O_2 \xrightarrow{\text{Organism}} CO_2 + H_2O$$

$$+ \text{Other By-Products} + \text{Energy} \qquad (8)$$

In this process, the decomposers utilize oxygen to break down the substrate to produce carbon dioxide, water, other by-products which depend on the composition of the substrate, and energy for growth and reproduction.

Problems arise when large quantities of organic matter are placed in the stream. The decomposers possess the capacity to rapidly increase in number when material containing their basic nutritional needs is added in excessive amounts. Corresponding to an increase in decomposers is an increased demand on the stream's oxygen supply. A serious problem results when the oxygen demand is greater than the stream's capacity to replenish it. Recent studies indicate that natural vegetative material may be directly responsible for serious dissolved oxygen deficiencies in small streams.

Chase and Ferrullo (1957) studied the effect of autumn leaf fall on the oxygen concentration in lakes and streams. They examined the quantity of oxygen demand exerted by maple, oak, and pine leaves and needles stored under water in carboys for 386 days under aerobic conditions. Although their methods are open to question, their results indicate the magnitude of oxygen demand exerted by natural vegetative

material. They reported that after one year maple leaves demanded about 750 mg O_2/gm of initial dry weight or 75 percent of their initial dry weight, while oak leaves and pine needles required about 500 mg O_2/gm of initial dry weight or 50 percent of their initial dry weight. The oxygen uptake was relatively rapid; by day 100 maple had achieved about 70 percent, while oak and pine had achieved about 56 percent of the demand exerted by 386th day.

McHugh, Miller, and Olsen (1964), in their studies of the ecology and naturalistic control of log pond mosquitoes in the Pacific Northwest, examined the chemical composition of water in 80 log ponds. Log ponds are a common means of log storage in the Northwest. During the storage period many substances leach out of the logs. Although DO results were not reported because the workers felt the readings were inaccurate due to interferences present in the water, a number of other chemical test results were reported. Of particular interest are the soluble carbohydrate (CHO) results. The log pond waters sampled ranged from slightly to highly polluted and the amount of soluble sugars reported varied from 2.0 to 10.5 mg/l. Tests run on source waters for the ponds resulted in no soluble carbohydrates present, verifying the source as log leachate. The presence of simple sugars has a significant influence on the rate of BOD because they are readily oxidized by the decomposers commonly present in streams.

Slack (1964) reported changes in water quality resulting from

natural addition of organic matter during autumn leaf fall on the Cacapon River, West Virginia. Three different environments were studied: a riffle within a river, a slightly colored backwater channel, and an isolated highly colored pool. Many different species of deciduous trees bordered the stream. Discharge during October, well after leaf fall had begun, averaged about 60 cfs. On October 30, dissolved oxygen concentrations were determined by the Alsterburg (Azide) modification of the Winkler titration method. The highly colored pool averaged 0.09 mg/l DO, the slightly colored pool averaged 1.9 mg/l while the main stream averaged 10.3 mg/l. The oxygen concentration reported in the highly colored pool may be too low because of ferrous iron interference, but these values do indicate the ability of natural organic material to extract DO.

Slack and Feltz (1968) further examined the effect of leaf fall on quality changes in small streams. During the autumn, when the stream flow is generally low in the East and leaf fall high, they measured several water quality parameters on a small Virginia stream. They reported a decrease in dissolved oxygen and pH, and an increase in water color, specific conductance, iron, manganese, and bicarbonate concentrations as the rate of leaf fall increased. The relation between leaf fall rate and DO concentration was very apparent. There was no significant change in oxygen consumption due to leaf fall as the rate increased from 0 to 2 gm/m^2/day.

However, as the rate increased from 2 to 12 gm/m^2/day there was a corresponding drop in oxygen concentration from eight to less than one mg/l. During the period mid-September to late October, the stream flow remained relatively constant, varying between 0.3-0.5 liters per second (lps). Early in November the stream flow increased to 280 lps, flushing the stream. The dissolved oxygen responded by climbing to a level greater than 11 mg/l.

Graham and Schaumburg (1969), examining pollutants leached from selected species of wood in log storage water, further support the presence of oxidizable organic matter from natural materials. They submerged cross-cut sections of Douglas-fir and ponderosa pine logs for 40 days in water poisoned with mercuric chloride to inhibit microbial action. They reported chemical oxygen demands (COD) of the 40 day fir and pine leachates of about 6000 and 7000 mg per square foot of exposed surface area respectively.

Hall and Lantz (1969) reported the effects of logging on the habitat of coho salmon and cutthroat trout in coastal streams. Two small watersheds were treated in the following manner: one was completely clearcut and one was patch-cut with three small clearcuts that covered about 25 percent of the area. Buffer strips 50 to 100 feet wide were left along the stream in the patch-cut watershed. A third watershed having similar physical and hydrologic characteristics as the two treated watersheds was left untreated and served as a

control. Felling on the clearcut watershed began in mid-March 1966 and continued until mid-July. Timber was felled along the stream and logs yarded uphill by cable across the stream to landings. This practice resulted in the accumulation of a considerable quantity of debris, limbs, twigs, needles, and bark in the channel which restricted flow and was responsible for the formation of pools. The larger material was cleared from the stream channel, permitting free flow, in early September 1966. In October 1966, the entire watershed was burned to prepare it for reseeding.

A substantial reduction in the DO concentration was observed in the surface and intragravel waters of the clearcut watershed. The DO concentrations from late June 1966 through most of July 1966 were too low to support salmon and trout. Concentrations as low as 0.6 mg/l were reported, which produced complete mortality in less than 40 minutes to samples of juvenile coho salmon contained in live-boxes placed within the stream. During this period, the oxygen concentration of the control stream and the stream draining the patch cut watershed remained at oxygen levels near saturation. Upon removal of the larger debris from the channel and establishment of free-flowing conditions the dissolved oxygen level rapidly responded to near pre-logging concentrations in the surface water. However, intragravel oxygen concentrations remained about three mg/l lower than the pre-logging concentrations for the next two years and continued to decline

over the next four years to levels less than two mg/l at some permanent stations.

It is apparent from the preceding discussion that finely divided logging material may be responsible for severe oxygen deficits within a small stream system. It is also evident that while such changes have been observed, no quantitative information describing the potential for oxygen extraction through decomposition of logging slash has been reported.

METHODS

Sampling Methods

Stream Water

The stream water used in this study was obtained from Oak Creek in McDonald Forest. The sampling site was located about one mile up-stream from the Pacific Cooperative Water Pollution Laboratory. Water samples were collected in carboys and used in an experiment the same day they were collected.

This water was considered to be typical Oregon Coast Range stream water. It drained from a watershed supporting primarily Douglas-fir and, to a lesser extent, red alder trees. It was assumed this water supported established microorganism populations that would consume the test material.

Vegetation

Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) needles and twigs, western hemlock (Tsuga heterophylla [Raf.] Sarg.) needles and red alder (Alnus rubra Bong.) leaves were used in this study.

Consultations with plant physiologists at the Oregon State University Forest Research Laboratory and a review of the literature (Lavender and Carmichael, 1966) indicated that the chemical

composition of needles and leaves within a species may vary. It was therefore necessary to determine whether or not BOD varied among different groups of needles and leaves in order to avoid sampling bias.

Variance in oxygen depletion by Douglas-fir and western hemlock needles was examined with respect to vertical crown position, crown aspect, and age and by red alder with respect to vertical crown position and crown aspect. Current and third year Douglas-fir and western hemlock needles and current year red alder leaves were collected from the north and south crown aspects of the top and lower one-third of the crown from three mature trees of each species. One-half gram of vegetation from each age, crown aspect and vertical crown position was placed in separate one-gallon, dark brown glass jugs containing 3.5 liters of water. The water used in this experiment was composed of a mixture of 14.3 percent stream water and 85.7 percent dilution water prepared according to Standard Methods (A.P.H.A., 1971). Three replications of each age, aspect, and elevation were run for each species along with four controls. Oxygen depletion in each jug was measured daily for two weeks with a polargraphic oxygen sensor calibrated in fresh air and checked periodically by the Azide modification of the Winkler titration method. When the oxygen concentration in a jug approached 1.5 mg/l, the jug was reaerated with compressed air for 30 minutes. After reaeration the jug was allowed to stand for

20 minutes to obtain an oxygen equilibrium before a new initial oxygen concentration was measured.

Variance in oxygen depletion within Douglas-fir and western hemlock vegetation was analyzed by a three factor analysis of variance while variance within red alder vegetation was analyzed by a two factor analysis of variance, all at the 0.05 level of significance. Results of this test are presented in Tables 1, 2, and 3. These results may be summarized as follows:

1) The variation in oxygen uptake with respect to vertical crown position was not significant for all species.

Table 1. Douglas-fir position x crown aspect x age analysis of variance.

Source	D.F.	S.S.	M.S.	F.
Position	1	1.40	1.40	3.39
Crown Aspect	1	2.28	2.28	5.53*
Age	1	25.63	25.63	62.06**
Position x Crown Aspect	1	0.01	0.01	0.04
Position x Age	1	0.00	0.00	0.00
Crown Aspect x Age	1	0.11	0.11	0.26
Position x Crown Aspect x Age	1	0.17	0.17	0.40
Error	16	6.61	.41	

Table 2. Western hemlock position x crown aspect x age analysis of variance.

Source	D. F.	S. S.	M. S.	F.
Position	1	0.00	0.00	0.00
Crown Aspect	1	3.52	3.52	8.38*
Age	1	15.36	15.36	36.50**
Position x Crown Aspect	1	0.33	0.33	0.76
Position x Age	1	0.24	0.24	0.57
Crown Aspect x Age	1	1.50	1.50	3.56
Position x Crown Aspect x Age	1	0.17	0.17	0.39
Error	16	6.73	0.42	

Table 3. Red alder position x age analysis of variance.

Source	D. F.	S. S.	M. S.	F.
Position	1	1.61	1.61	7.42
Age	1	3.63	3.63	16.70
Error	1	0.03	0.03	.14
Total	11	7.20		

2) The variation in oxygen uptake with respect to crown aspect and age was significant for Douglas-fir and western hemlock needles, while not significant for red alder. As a result of this test, the following needle and leaf sampling procedures were developed.

All vegetation was taken from the lower one-third of the crown. The Douglas-fir and western hemlock needle samples were each

composed of a mixture containing an equal number of needles from the north and south crown aspects of three different trees in proportion to their average number in each age class (Table 4). The red alder leaves were taken at random from three different trees. These leaves were cut into two centimeter squares in order to fit the experimental instruments used later.

Table 4. Average percentage of needles retained by Douglas-fir and western hemlock (Smith, 1970).

Species	Needle Age in Years					
	Current	1	2	3	4	5
Douglas-fir	30	26	20	13	8	3
Western hemlock	46	41	11	1	0	0

Although no experiment was run to measure the variance in oxygen depletion rate between age, crown aspect, and vertical crown position of Douglas-fir twigs, they were also taken from the north and south crown aspect of the lower one-third of the crown from three different trees. Twigs of different ages were not mixed, but instead first, third, and fifth year age classes were formed. However, twigs within each age class from different trees were mixed. In other words, twigs of different age classes were not mixed, but twigs from different trees in the same age class were mixed. The twigs within these sample groups were cut into segments two centimeters long

and quartered longitudinally.

The Douglas-fir and red alder vegetation was taken from trees located in McDonald Forest, while the western hemlock vegetation was taken from trees on Marys Peak. The form of vegetation used in the following experiments was the same as that used in the above preliminary vegetation experiment, i.e., whole Douglas-fir and western hemlock needles, red alder leaves cut into two centimeter squares, and Douglas-fir twigs cut into two-centimeter segments and quartered longitudinally.

Analytical Methods

The COD Test

The purpose of this test was to determine the chemical oxygen demand of Douglas-fir needles and twigs, western hemlock needles, and red alder leaves. The COD test determines the total quantity of oxygen required for the complete oxidation of organic matter by strong oxidizing agents under acid conditions.

Three replications each were run of 10 mg samples, fresh weight, of Douglas-fir and western hemlock needles, red alder leaves, and Douglas-fir first, third, and fifth year twigs. The "rapid COD method" was used to determine the COD values. The analytical procedures for this method are described in detail by Jeris (1967).

Dry weight values of the sample vegetation were determined by placing three one-gram, fresh weight, samples of each material into a 70° C oven for three days.

Biochemical Oxygen Demand Tests

Two methods of BOD determination were used in this study: the Hach manometric technique and the standard dilution technique. The Hach manometric technique was used to determine all the short- and long-term standard temperature BODs while the standard dilution technique was only used to determine the fluctuating temperature BODs.

A Hach manometric BOD unit and a single cell are illustrated in Figures 4 and 5. The principle of operation is quite simple. The brown glass mixing bottle is partially filled with a measured volume of water containing the pollutant. The bottle is then connected to the closed-end mercury manometer and placed on the magnetic stirring plate. The stirring plate agitates the magnetic stirring bar contained in the bottle which, in turn, results in continuous mixing of the sample. As the microorganisms oxidize the organic matter, they remove dissolved oxygen from the water. Above the water is a volume of air containing about 21 percent, by volume, oxygen. Thus, the water is rapidly replenished with oxygen by diffusion from the air above it in the closed sample bottle due to the continuous mixing

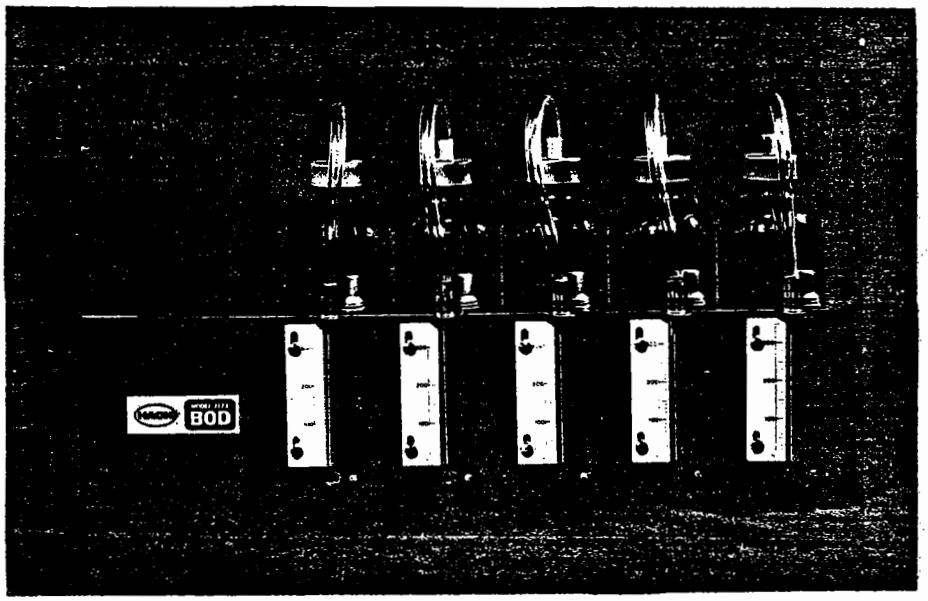


Figure 4. A complete Hach manometric unit.

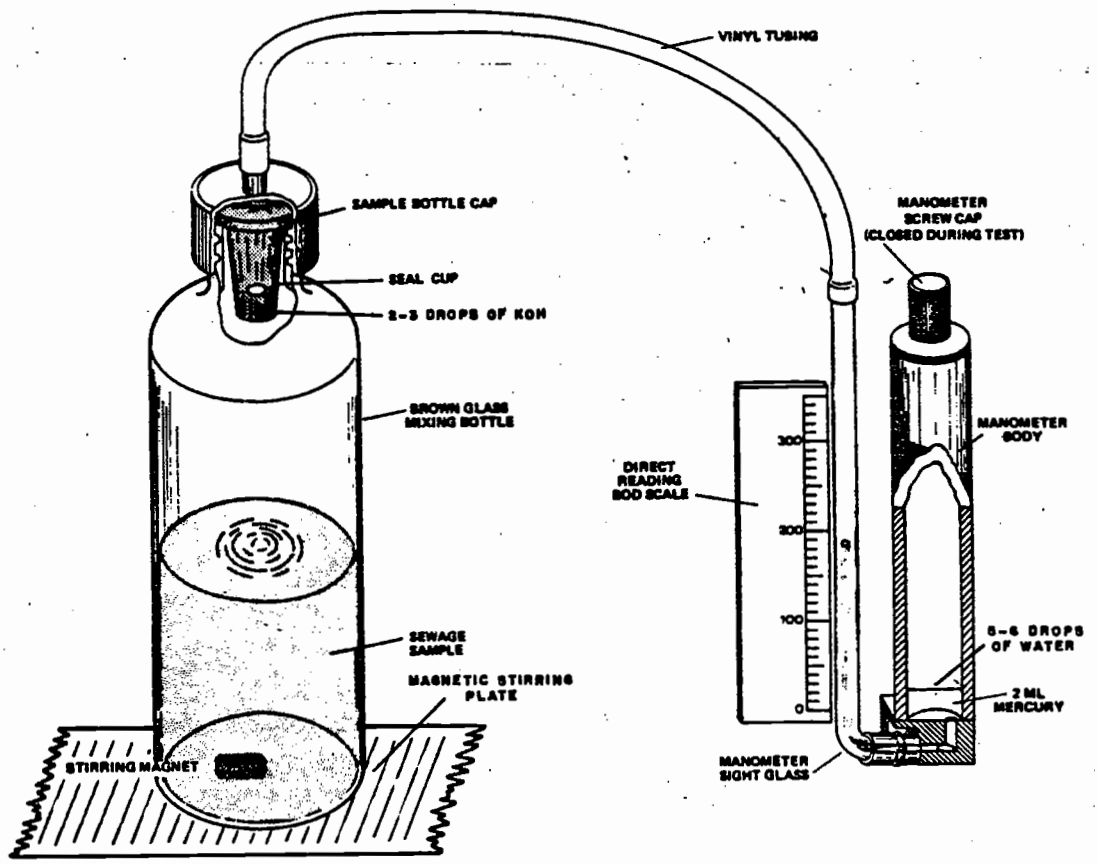


Figure 5. A single cell of the Hach manometric unit (After Hach Chemical Company, 1971).

given the sample. This results in an air pressure drop within the bottle which is registered on the mercury manometer and may be read directly as mg/l of BOD. During the oxidation process CO_2 gas is given off. To prevent a positive pressure build-up, two or three drops of 45 percent potassium hydroxide solution is placed in the seal cup in the bottle to absorb this gas.

There were three major advantages in using the Hach manometric technique to determine short- and long-term standard temperature BOD's in this study.

1) It simulated actual stream conditions more closely than any other standard temperature BOD test available; the solution is continuously in contact with oxygen and the CO_2 given off is removed.

2) The material could be handled without dilution of the stream water.

3) It was excellent for determining long-term BODs; periodic oxygen replacement is performed merely by breaking the seal between the manometer and bottle.

The standard dilution technique is explained in detail in Standard Methods (A. P. H. A., 1971). This method has one serious limitation. Due to the relatively low solubility of oxygen in water, the waste water samples must often be diluted. In accordance with standard procedure, the sample must deplete at least 2.0 mg/l of O_2 during the incubation period and leave at least 0.5 mg/l of O_2 at the end of this

period (Sawyer and McCarty, 1967). Consequently, waste with a high oxygen demand must be highly diluted. Serious errors in the calculated BOD of these types of waste may result due to a slight error in a DO reading. No matter how much analytical care is taken, the accuracy of this BOD test is no greater than the accuracy of the DO test used.

Five- and ninety-day standard temperature BOD test. The purpose of this test was to determine the five- and ninety-day BOD (BOD_5 and BOD_{90}) of Douglas-fir needles, western hemlock needles and red alder leaves at standard temperature. Three Hach manometric units, one for each species, were used. All the bottles were partially filled with 244 ml of stream water. One-gram, fresh weight, of each species was added to each of the first four bottles of each unit, while the fifth bottle served as a control. Into two of the bottles containing vegetation on each unit and into one of the three control bottles an equivalent of 10 mg/l of N-Serve, 2-Chloro-6-(Trichloromethyl)pyridine, was added to inhibit nitrification. Three additional one-gram, fresh weight, samples were taken from each species sample and oven dried at 70° C for three days to determine dry weight values. The manometric units were placed in a 20° C incubator, allowed to set for 15 minutes in order to reach the standard temperature, and then sealed. Readings were recorded daily for the first five days and at least once every three days during the following

85 days.

Periodically the oxygen in the bottles required replenishment. The following procedure was used to replenish the oxygen supply. The bottle was carefully opened and the seal cup containing the 45 percent KOH removed. The bottle was allowed to stand open to the atmosphere for about five minutes. The spent KOH solution was removed from the seal cup and replaced with two or three drops of fresh 45 percent KOH. The seal cup was carefully replaced, the bottle placed in the incubator and allowed to standardize for 15 minutes, and then sealed. The manometer scale was then readjusted to zero.

Five-day fluctuating temperature BOD test. The purpose of this test was to determine the five-day BOD (BOD_5) of Douglas-fir needles, western hemlock needles, and red alder leaves under the condition of fluctuating temperature. The extreme temperature fluctuations often observed in small streams during the mid-summer months following clearcutting was simulated. The temperature range selected was 12.8 to 35.0° C over an eight hour period, followed by a drop from 35.0° to 12.8° C over the next 16 hours. The temperature range and cycle were determined from values reported by Brown and Krygier (1970) following clearcutting in a small stream in the Oregon Coast Range.

Standard 300 ml glass BOD bottles with ground glass stoppers

were used in this experiment. There were three replications plus two controls for each species for each of the five days the experiment was run. Each bottle, except the controls, contained 20 mg, fresh weight, of the respective vegetation sample. Three additional one-gram, fresh weight, samples were taken from each species sample and oven dried at 70° C for three days to determine dry weight values. All the bottles were completely filled with a water mixture containing five percent stream water and 95 percent dilution water prepared according to Standard Methods (A. P. H. A., 1971).

A water bath, 122 x 61 x 46 cm, was used to fluctuate the sample temperatures between 12.8 and 35.0° C. The bath was equipped only with a variable temperature cooling unit and contained no means of heating the water above ambient air temperature. The heating problem was resolved by discharging hot water at 38.5° C, into the bath at a constant rate of one liter per minute. Two electric submersible pumps were placed in the bath to assure complete mixing at all times. Water was continually removed from the mixed bath at a rate equal to the input of hot water. As a result, the volume of the bath remained constant and the overall water temperature gradually rose from 12.8 to 35.0° C in eight hours. At the end of this eight-hour period, the hot water input and mixed water output were shut off and the cooling unit turned on. The water cooled from 35.0 to 12.8° C in about four hours and remained there until the start of

the eight hour heating period. The mathematical solution of the heating problem is given in Appendix B.

The initial oxygen concentration of the water mixture placed in the bottles was determined by the Azide modification of the Winkler titration method. The bottles were water sealed and submerged to their necks in the bath. The bath was covered with a black plastic tarp to exclude light. Each day three bottles from each species and two controls were removed from the bath and their respective DO concentrations determined by the Azide modification of the Winkler technique and recorded.

Douglas-fir twig BOD test. The purpose of this experiment was to determine the five- and forty-five day BOD (BOD_5 and BOD_{45}) of first, third, and fifth year Douglas-fir twigs. The first three bottles of a Hach manometric unit contained one-gram, fresh weight, of first, third and fifth year twig samples respectively, while the fourth bottle contained two-grams of the third year twig sample and the fifth bottle served as a control. This test was replicated three times. Dry weight values were determined for the twig samples by placing three one-gram samples from each age class into a 70° C oven for three days.

Periodically, the air in the sample bottles required changing. The same procedure described in the five- and ninety-day standard temperature BOD test was used. Results were recorded daily the

first five days and at least once every three days thereafter.

Concentration Dependency Test

The purpose of this test was to determine if the BOD tests run at standard temperature using one-gram samples were mass-concentration dependent. The determination was made using Douglas-fir needles and twigs. The effect of increasing concentrations of needles was determined using three Hach manometric units. The first four bottles of each unit contained 0.5, 1.0, 2.0, and 4.0 gms, fresh weight, of Douglas-fir needles, while the fifth bottle served as a control. Dry weight values were determined by oven-drying one-gram samples in the manner previously described. The units were placed in a 20° C incubator and the test run for 10 days.

The effect of the twigs was determined using data obtained from the Douglas-fir twig BOD test described earlier. In the Douglas-fir twig BOD test, bottles two and four on each of three Hach units contained one and two grams of third year twigs. The BOD results of these bottles were compared over the first ten days the experiment was run.

Ultimate BOD Exerted, $L_{e(u)}$, and BOD Rate Constant, K_1, Determination

The BOD of any organic material can be described as a first

order decay process. This can be expressed mathematically as:

$$\frac{dL}{dt} = -K_1 L \tag{4}$$

where L is the BOD concentration in mg/l, t is time in days, and K_1 is the BOD rate constant (base e) in units 1/days. Equation (4) may be solved for K_1 as follows:

$$\frac{dL}{L} = -K_1 dt \tag{9}$$

$$\int_{L_a}^{L_t} \frac{dL}{L} = -K_1 \int_{o}^{t} dt \tag{10}$$

$$\ln L_t - \ln L_a = -K_1 t \tag{11}$$

$$K_1 = \frac{\ln L_a - \ln L_t}{t} \tag{12}$$

or

$$k_1 = \frac{\log L_a - \log L_t}{t} \tag{13}$$

where L_a is the initial BOD concentration at t = 0, L_t is the BOD concentration at time t, t is time in days, K_1 is the BOD rate constant (base e) in units of 1/days, and k_1 is the BOD rate constant (base 10) in units of 1/days. Since BOD is measured as the amount of oxygen used in the oxidation process, L_a may be redefined as the ultimate BOD exerted, $L_{e(u)}$. This is illustrated by Figure 6. Equation (12) may then be restated as:

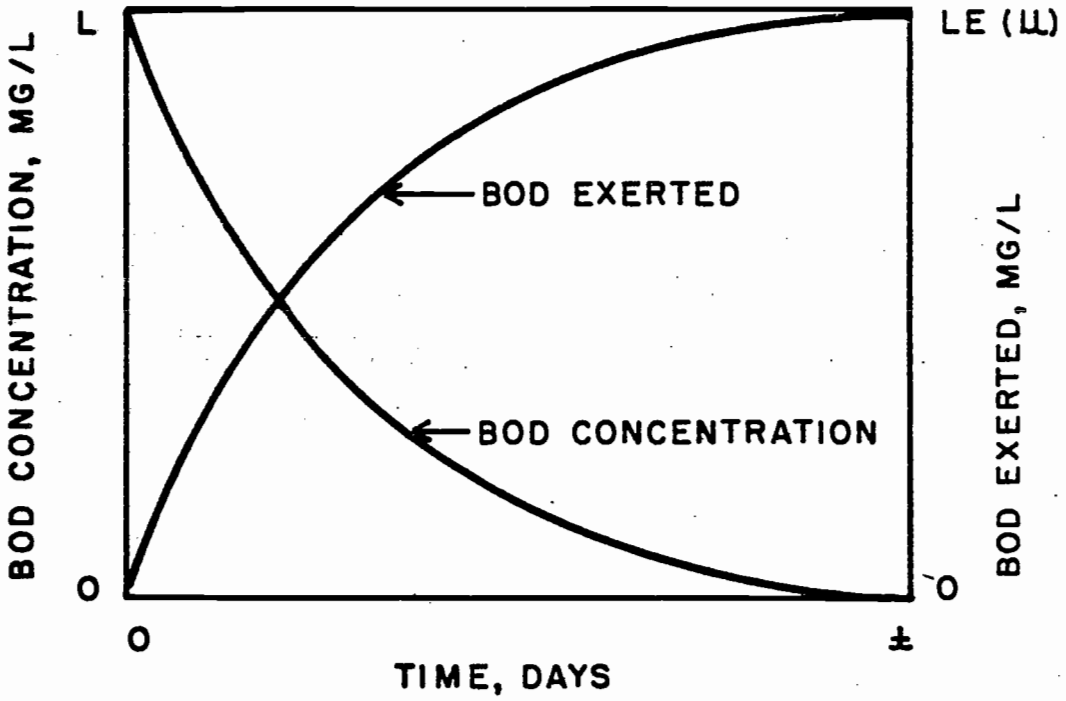


Figure 6. BOD concentration and BOD exerted curves over time.

$$K_1 = \frac{\ln L_{e(u)} - \ln L_t}{t} \qquad (14)$$

The 90-day BOD value, BOD_{90}, was chosen as the ultimate BOD exerted value, $L_{e(u)}$, because it is representative of the average time interval between spring runoff and the fall freshets. This is the period when logging debris accumulation is most likely to occur in small streams of logged watersheds in the Pacific Northwest. The BOD_t and BOD_{90} values used in the calculation of K_1 for each species were obtained from a first order curve of best fit through the replications for that species. These values were then used to calculate the 90-day K_1 values using equation (15).

$$K_1 = \frac{\ln L_{e(90)} - \ln L_t}{90} \qquad (15)$$

BOD rate constants were also determined for shorter time periods: 5, 20, 45, and 60 days for the needles and leaves; and 5, 20, and 45 days for the twigs. The same basic procedure as above was used to calculate each K_1; however, the first order curve of best fit was determined using only data for the time interval being considered. The ultimate BOD was obtained by projecting the curve to 90 days.

All the first order curves of best fit and the K_1 values for these curves were determined using a least squares fit program

for the CDC 3300 computer at Oregon State University.

Test for Nitrate-Nitrogen

The purpose of this test was to determine if nitrification occurred in any of the standard temperature BOD experiments. Nitrification is considered to be a BOD test interference and can be responsible for erroneously high results. Nitrifying bacteria oxidize nitrogenous substances in the form of ammonia to nitrites and nitrates. The nitrification process is illustrated by the following relations:

$$NH_3 + 1\tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrosomonas}} HNO_2 + H_2O + \text{Energy} \qquad (16)$$

$$NO_2^- + \tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- + \text{Energy} \qquad (17)$$

It is evident from these relations that if nitrification occurred, nitrate (NO_3^-) would be present in the closed BOD bottle.

The Brucine method was used to determine the nitrate-nitrogen concentration in all the standard temperature test samples. This method is explained in detail in Standard Methods (A. P. H. A., 1971). The nitrate-nitrogen concentration of the bottles containing vegetation were compared to those of the controls and hypothetical concentrations calculated from the nitrogen content of the vegetation used in the test.

Leachate Composition Test

The purpose of this test was to ascertain under aerobic conditions the quantity and type of sugars and the quantity of phenolics released into the stream through microorganism activity and leaching by a specified mass of Douglas-fir needles and twigs, western hemlock needles, and red alder leaves and to relate this to time. The information obtained from this experiment will aid in the interpretation of the BOD and toxicity results.

The experiment utilized both poisoned stream water and non-poisoned stream water in order to separate leaching from microorganism activity. The poisoned stream water contained an equivalent of 2.706 mg/l of $HgCl_2$ to prevent biological decomposition of leached substances (Schaumburg and Atkinson, 1970). Using this procedure, the amount of sugars and phenolics available to organisms and the amount they used could be quantified.

The leachate was prepared by the following procedure. Into a clean 300 ml glass BOD bottle, 100 ml of poisoned or non-poisoned water was added. Then one-gram, fresh weight, of material, either needles or leaves, was added to each of three bottles of each water treatment for each species. All the samples were placed in a standard temperature (20° C) room and covered with a black plastic tarp to exclude light. The bottles were left uncapped so oxygen could

enter freely. The same general procedure was followed with the Douglas-fir twigs except one-gram of first, third, or fifth year twigs was put into 125 ml glass bottles.

Dry weight equivalents of the material used were determined by placing three one-gram samples from each material sample into a 70° C oven for three days.

On days one through five, ten, 15, and 20 following the beginning of the leaching process, three poisoned and three non-poisoned samples of each species were removed from the standard room and prepared for sugar and phenolic analysis. The non-poisoned samples were handled in the following manner. About 50 ml was decanted into a plastic vial for sugar analysis and about 50 ml decanted into a glass bottle for phenolic analysis. The poisoned samples were handled differently. All the leachate was decanted into a clean 250 ml flask. An equivalent of 5 gm/l of Chelex 100 reagent (Schaumburg and Atkinson, 1970), a chelating agent, was added to each flask to remove the HgCl from solution. The flasks were placed on an Eberback shaking machine and shaken for 30 minutes. They were removed and allowed to stand five minutes so that the chelating compound could settle to the bottom. About 50 ml of the supernatant was decanted into a plastic vial for sugar analysis and 35 ml into a glass bottle for phenolic analysis. The prepared samples, both poisoned and non-poisoned, were frozen until the sugar and phenolic analysis

could be performed.

The analysis for sugar and phenolic content was contracted to the Forest Products Department of the School of Forestry at Oregon State University. Total sugar concentration was determined by the Somogy Micro-Copper method for reducing sugars (Hodge and Hofreiter, 1962; Somogyi, 1945). The types of sugars comprising this quantitative estimate of total sugar were identified by paper chromatography using the solvents and procedure outlined by Zerrudo (1973). Total phenolic concentration was determined with the Folin-Ciocatteu reagent as described by Singleton and Rossi (1965).

Leachate Toxicity Test

The purpose of this test was to determine the acute toxicity of five-day Douglas-fir, western hemlock, and red alder leachates to guppies (Poccilia reticulata) and steelhead trout fry (Salma gairdneri gairdneri). The leachate of each species was prepared in the following manner. Two stainless steel baking pans, 60 x 45 x 12 cm, were used as leaching containers. Into each pan about 20 liters of stream water treated with an equivalent of 2.706 mg/l of $HgCl_2$ (Schaumburg and Atkinson, 1970) and an equivalent of 50 gm/l of vegetative material was added. The pans were placed in a standard temperature room at 20° C for five days. The pans were covered with a black plastic tarp to exclude light during the leaching process. After five days the

leachate was decanted into a carboy and the $HgCl_2$ removed with Chelex 100 (Schaumburg and Atkinson, 1970). The chelated leachate was decanted into a second carboy and transported to the toxicity test site. In order to check the possibility of mercury poisoning, a Douglas-fir and western hemlock leachate using 50 gm/l of vegetation was also prepared with non-poisoned water and its toxicity tested in the same manner as the treated water samples.

The toxicity test was contracted to the Pacific Cooperative Water Pollution Laboratory. They determined 24-, 48-, 72-, and 96-hour median lethal concentration (LC 50) of the leachate on guppies and steelhead fry by the "Static Bioassay Method" described in Standard Methods (A. P. H. A., 1971). The guppies weighed about 0.01 gm each while the steelhead fry weighed about 2.0 gms each. The pH was adjusted from relatively acidic to normal with NaOH and, due to the high oxygen demand of the leachate, oxygen was bubbled in the chambers to insure adequate oxygen concentrations at all times. The toxicity tests were begun the same day the leachate was removed from the leaching container.

RESULTS

COD Test Results

The chemical oxygen demand (COD) test provides a quantitative estimate of the oxygen equivalent required for the complete oxidation of an organic sample by a strong oxidizing agent. The results of the COD test are summarized in Table 5. The table presents the COD values of the three replications followed by the mean for each vegetation type.

Table 5. COD of Douglas-fir needles and twigs, western hemlock needles, and red alder leaves in mg O_2/gm (dry weight) vegetation.

Vegetation Type	Replication Number 1	2	3	Mean COD
	---------- mg O_2/gm ----------			
Douglas-fir needles	460	415	487	454
Western hemlock needles	635	478	596	570
Red alder leaves	835	935	876	882
Douglas-fir twig: first year	759	999	677	812
Douglas-fir twig: third year	888	742	917	849
Douglas-fir twig: fifth year	1117	1196	1227	1180

The red alder leaves have a significantly greater mean COD than the other leaves tested. The range is from 0.45 gm O_2/gm of Douglas-fir to 0.88 gm O_2/gm of red alder. The fifth year Douglas-fir twigs have a significantly greater mean COD than the other twigs tested. The twig COD mean range for 1.18 gm O_2/gm of fifth year twigs to 0.81 gm O_2/gm of first year twigs.

The average COD for first, third, and fifth year twigs is 0.95 gm of O_2/gm (dry weight) of twig. This value is about twice as large as the mean value for Douglas-fir needles.

BOD Test Results

Five- and ninety-day Standard Temperature BOD Test

The results of the BOD_5 and BOD_{90} standard temperature BOD test for selected days are summarized in Tables 6 and 7. The complete set of results are given in Table 20 of Appendix D.

Table 6 summarizes the results of the samples not treated with N-Serve. Several trends are apparent upon examination of the mean values of the two replications. The Douglas-fir and western hemlock needles exert a 90-day oxygen demand 39 and 71 percent less than the red alder leaves over the same period. Within each species, the BOD_5 values represent only 59, 18, and 28 percent of BOD_{90} values for Douglas-fir, western hemlock, and red alder respectively.

Table 6. Cumulative standard temperature BOD exerted in mg O_2/gm (dry weight) by Douglas-fir, western hemlock, and red alder leaves in untreated stream water.

Vegetation Type	Bottle No.	Time in Days 5	10	20	45	60	90
		mg O_2/gm					
Douglas-fir needles	1	71	74	74[a]	80	82	82
	2	59	81	120[a]	128	133	145
mean	-	65	78	97	104	108	110
Western hemlock needles	6	34	76	132	192[b]	197	202
	7	40	124	175	194[b]	197	197
mean	-	36	100	154	193	197	200
Red alder leaves	11	77	108	148	220	266	282
	12	81	152	216	278	278	278
mean	-	79	130	182	249	272	280
Controls	5	0	0	5	8	10	10
	15	0	5	8	10	22	22

[a]Value interpolated between day 19 and 22 values

[b]Value interpolated between day 44 and 46 values

Table 7. Cumulative standard temperature BOD exerted in mg O_2/gm (dry weight) by Douglas-fir, western hemlock, and red alder leaves in stream water treated with N-Serve.

Vegetation Type	Bottle No.	Time in Days 5	10	20	45	60	90
		mg O_2/gm					
Douglas-fir needles	3	71	106	134[a]	140	144	150
	4	50	51	78[a]	82	82	82
mean	-	60	78	106	111	113	116
Western hemlock needles	8	40	70	99	170[b]	176	178
	9	30	68	68	79[b]	79	79
mean	-	39	69	83	125	128	128
Red alder leaves	13	79	121	156	216	262	278
	14	79	123	178	278	289	309
mean	-	79	122	167	247	276	293
Control	10	0	5	5	10	10	10

[a]Value interpolated between day 19 and 22 values

[b]Value interpolated between day 44 and 46 values

However, the BOD_{45} within each species represents about 90 percent of BOD_{90} value.

Table 7 summarizes the results of the samples treated with N-Serve to inhibit nitrification. In general, the relations between mean values of the treated samples are the same as those for the untreated samples. Douglas-fir and western hemlock needles exert a BOD_{90} 40 and 44 percent of the red alder BOD_{90} value, while within each species, the BOD_5 represents only 52, 30, and 27 percent respectively of the Douglas-fir, western hemlock, and red alder BOD_{90} values. The BOD_{45} of Douglas-fir and western hemlock represents about 90 percent of their BOD_{90} values, while the red alder BOD_{45} is only about 85 percent of its BOD_{90} value.

By comparing the mean BOD_{90} values among species in Tables 6 and 7, the effect of nitrification may be examined. There is little difference among the Douglas-fir and red alder BOD_{90} values. Although the differences are small, it is important to note that the samples treated with the inhibitor have higher mean values than the untreated samples indicating that nitrification did not occur. There is a significant difference, 72 mg O_2/gm, between treatments of the western hemlock mean BOD_{90} values. This difference may be attributed to the effect of nitrification. However, upon examination of the samples involved, bottle 9 seems to be atypical. The BOD_{10} and BOD_{20} values are the same, as well as the BOD_{45}, BOD_{60},

and BOD_{90} values. Comparing the means of bottles 6, 7, and 8 only, the difference between treated and untreated samples is much less, and may in fact, be due to natural variation, not nitrification. Although there is evidence that nitrification may have occurred in one of the test bottles, analyses described in a later section show that nitrification did not, in fact, occur.

Five-Day Fluctuating Temperature BOD Test

The results of the five-day BOD experiment in which the temperature fluctuated between 12.8 and 35.0°C daily are given in Table 8. The red alder leaves again exerted the greatest demand for oxygen over five days, followed closely by the Douglas-fir needles. In five days, the red alder leaves exerted an oxygen demand equivalent to 23.7 percent of their initial dry weight, while the Douglas-fir needles exerted a demand equivalent to 19.0 percent of their initial dry weight. Over the same time period, western hemlock needles required a quantity of oxygen equivalent to 9.7 percent of their initial dry weight.

Table 8. Mean cumulative BOD exerted in mg O_2/gm (dry weight) by Douglas-fir, western hemlock and red alder under conditions of temperature fluctuation.

Vegetation Type	Time in Days				
	1	2	3	4	5
	mg O_2/gm				
Douglas-fir needles	51	67	131	187	202
Western hemlock needles	29	60	88	104	109
Red alder leaves	77	136	191	219	249
Controls	5	5	7	12	12

Douglas-fir Twig BOD Results

The Douglas-fir twig BOD test results for selected time periods are presented in Table 9. The complete set of results is given in Table 21 in Appendix D.

The first year twigs exerted the greatest mean BOD over 45 days, slightly more than 13 percent of their dry weight. Third and fifth year twigs followed with a BOD_{45} equal to 9.3 percent and 7.8 percent of their initial dry weight. The BOD_5 was equivalent to about 25 percent of the BOD_{45} for all age classes, while the BOD_{20} value was equivalent to about 65 percent for third and fifth year twigs, and about 90 percent for first year twigs.

Table 9. Cumulative standard temperature BOD exerted in mg O_2/gm (dry weight) by first, third, and fifth year Douglas-fir twigs in stream water.

Twig Age	Bottle No.	Time in Days					
		5	10[a]	15[b]	20	30	45
		mg O_2/gm					
First year	1	26	44	47	61	72	78
	2	47	97	128	170	175	180
	3	42	80	92	118	134	139
Mean	-	39	74	89	116	127	132
Third year	4	9	22	34	50	65	73
	5	37	55	68	82	98	108
	6	18	33	42	54	80	97
Mean	-	21	37	48	62	81	93
Fifth year	7	11	25	34	42	55	66
	8	17	32	40	48	55	69
	9	26	36	40	53	78	100
Mean	-	18	31	39	48	63	78
Controls	13	0	0	0	0	0	0
	14	0	0	0	0	0	0
	15	2	2	2	2	2	2

[a] All day 10 values were interpolated between day 9 and 11 values

[b] All day 15 values were interpolated between day 13 and 16 values

Mass-Concentration Results

This experiment was performed to determine if the BOD exerted by Douglas-fir needles and twigs were directly related to the concentration of vegetation in the sample water. High concentrations of material may inhibit the rate of nutrient transfer by diffusion or may

be toxic to the aquatic microorganisms and thus yield erroneous BOD values. The one gram, fresh weight, per liter of water concentration used in the standard temperature experiments was compared with solutions containing more and less vegetation per unit volume.

The mean BOD of Douglas-fir needles in percent relative to the one gram per liter concentration are summarized in Table 10.

Table 10. Mean BOD of different concentrations of Douglas-fir needles as a percent of the one gram per liter concentration mean BOD.

Conc.	Time in Days				
	2	4	6	8	10
gm/l	---------- percent of the 1.0 gm/l BOD ----------				
0.5	113	48	52	54	54
1.0	100	100	100	100	100
2.0	275	148	163	129	118
4.0	413	164	133	110	104

The 0.5 gm/l concentration showed a proportionate drop in BOD. It was about one-half of the 1.0 gm/l BOD from day four to day ten. On the other hand, higher concentrations did not produce a proportionate increase in BOD. Initially, the 2.0 and 4.0 gm/l BOD's were significantly greater than the 1.0 gm/l BOD but, as time progressed, the BOD exerted by the higher concentrations approached the 1.0 gm/l values.

Although the BOD of 2.0 and 4.0 gram samples did not remain two and four times higher than the 1.0 gm/l sample, the BOD exerted by the 1.0 gm/l sample probably was not mass-concentration dependent. If the large sample sizes (2.0 and 4.0 gm/l) were toxic to the aquatic organisms, the BOD probably would have terminated abruptly rather than increasing slowly after initially high rates.

The results of the mass-concentration dependency test for third year Douglas-fir twigs are summarized in Table 11. This table represents only a portion of the data collected in the full experiment (Table 21, Appendix D). Bottles 4, 5, 10, and 12 of this original data set were selected because bottles 6 and 11 were considered to have trends atypical to those of the other two replications.

Table 11. Mean BOD of different concentrations of third year Douglas-fir twigs from selected bottles as a percent of the mean BOD of the 1.0 gm/l concentration.

Conc. (gm/l of water)	Time in Days				
	2	4	6	8	10
1.0	100	100	100	100	100
2.0	62	224	222	197	184

During the first two days of the test, the BOD was not proportional to the concentration of material. This may be attributed to the fact the microorganisms were probably still adapting to their

environment. However, for the remainder of the test period, the BOD exerted by the 2.0 gm/l sample was about twice as much as the 1.0 gm/l BOD.

Ultimate BOD, $L_{e(90)}$, and Rate Constant, K_1, Results

The following terminology for the rate constant, K_1, and BOD, $L_{e(90)}$, will be used for the balance of the discussion. The K_1 and $L_{e(90)}$ values determined from the curve fit through 90 days of data will be referred to as the "ultimate" rate constant and BOD of the material. The K_1 and $L_{e(90)}$ values determined from curves fit through the data for time periods less than 90 days but extended to 90 days, will be referred to as "projected" rate constants and BODs of the material.

The K_1 and $L_{e(90)}$ values calculated from the standard temperature results of the leaf and needle tissue BOD test in untreated stream water are given in Table 12.

The five-day projected K_1 values for Douglas-fir and western hemlock are all very small, while their projected $L_{e(90)}$ values are quite large, indicating a near straight line relation. As a result, the five-day values for both these species are very poor indicators of the ultimate values. However, the 45-day projected values of the Douglas-fir and the 60-day projected values of the western hemlock serve as excellent indicators of their respective ultimate K_1 and

Table 12. K_1 and $L_{e(90)}$ values for leaf materials computed for different time periods by a least squares analysis of the BOD results using standard temperature and untreated stream water.

Vegetation Type	Bottle No.	Time in Days									
		5		20		45		60		90	
		K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1[a]	$L_{e(90)}$[b]
		1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm
Douglas-fir needles	1	1.9×10^{-4}	7.1×10^{4}	.284	77	.274	79	.263	80	.251	81
	2	1.9×10^{-4}	5.6×10^{4}	.078	153	.107	130	.105	131	.097	134
Western hemlock needles	6	5.6×10^{-4}	1.2×10^{4}	.012	640	.038	252	.049	217	.054	208
	7	5.6×10^{-3}	1416	.022	550	.080	210	.086	202	.090	200
Red alder leaves	11	.318	96	.136	151	.069	207	.043	259	.033	296
	12	.078	253	.077	284	.073	290	.075	286	.078	281
Controls	5	--	--	--	--	--	--	--	--	.022	3
	15	--	--	--	--	--	--	--	--	.023	7

[a] Referred to as the ultimate BOD rate constant in the text

[b] Referred to as the ultimate BOD in the text

$L_{e(90)}$ values.

In contrast to the Douglas-fir and western hemlock, the projected K_1 and $L_{e(90)}$ from one sample of the red alder tissue after five days provided a relatively precise prediction of the ultimate values. The other sample, however, had a projected K_1 much too high and a projected $L_{e(90)}$ too low. Reliable predictions of ultimate 90-day values are not obtained again until day 60.

The following relations can be drawn about the ultimate K_1, and $L_{e(90)}$ values in Table 12. Variation in the ultimate K_1 values among and between species is high. A given magnitude of K_1 can not be easily associated with a specific species; the range is too great. However, a relation between the ultimate $L_{e(90)}$ values and species types does exist. The ultimate $L_{e(90)}$ values for Douglas-fir is less than the western hemlock values, which in turn is less than the red alder values.

The projected and ultimate K_1 and $L_{e(90)}$ values calculated from the results of the BOD test run on needle and leaf tissues at the standard temperature but treated with a nitrification inhibitor are given in Table 13. Again, the five-day projected K_1 values are very small and the projected $L_{e(90)}$ values quite large for the western hemlock and Douglas-fir needles. In contrast to the results obtained using untreated stream water, the red alder five-day projected K_1 values from samples treated with N-Serve are generally higher and

Table 13. K_1 and $L_{e(90)}$ values for leaf materials computed for different time periods by a least squares analysis of the BOD results using standard temperature and stream water treated with N-Serye.

Vegetation Type	Bottle No.	Time in Days									
		5		20		45		60		90	
		K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1[a]	$L_{e(90)}$[b]
		1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm
Douglas-fir needles	3	1.9×10^{-4}	7.1×10^{4}	.124	146	.140	139	.135	140	.124	144
	4	1.9×10^{-4}	4.4×10^{4}	.124	78	.107	85	.111	84	.114	83
Western hemlock needles	8	5.6×10^{-4}	1.4×10^{4}	.080	123	.040	178	.030	217	.037	191
	9	1.9×10^{-4}	3.9×10^{4}	.154	77	.154	76	.148	78	.144	78
Red alder leaves	13	..290	102	.142	161	.088	197	.054	243	.039	280
	14	..280	104	.107	192	.053	275	.043	310	.041	315
Controls	10	--	--	--	--	--	--	--	--	.035	3

[a] Referred to as the ultimate BOD rate constant in the text

[b] Referred to as the ultimate BOD in the text

the projected $L_{e(90)}$ values are low. Relatively precise predictions of the ultimate K_1 and $L_{e(90)}$ values are obtained in 20 days for Douglas-fir, 45 days for western hemlock, and 60 days for red alder. The range observed in the ultimate K_1 and $L_{e(90)}$ values for western hemlock encompasses the range of values observed for Douglas-fir.

Comparing Tables 12 and 13 it is apparent that variation is high between ultimate K_1 values of a given species, while the ultimate $L_{e(90)}$ values, on the other hand, are similar. Exceptions are the ultimate K_1 values of red alder and the ultimate $L_{e(90)}$ values of western hemlock; in each instance three values are relatively close with a fourth outlier.

The projected K_1 and $L_{e(90)}$ values of the Douglas-fir twigs are given in Table 14. For the remainder of this discussion it will be assumed that the 45-day K_1 and $L_{e(90)}$ of the Douglas-fir twigs accurately predict the ultimate values of these tissues. This assumption defines a reference for the comparison of values determined from curves fit to the data over different time intervals.

The five-day projected values for K_1 are very small and the projected $L_{e(90)}$ values are very large for all three age classes tested and thus all serve as a very poor indicator of the projected 45-day $L_{e(90)}$. In general, the 20-day projected values offer a much better approximation, but still lack in precision. The 45-day projected K_1 values of the first year twigs are closely grouped,

Table 14. K_1 and $L_{e(90)}$ values for Douglas-fir twigs computed for different time periods by a least squares analysis of the BOD results using standard temperature and stream water.

Twig Age		Time in days					
		5		20		45	
		K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$
		1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm
First year	1	.079	1.7×10^4	.091	70	.071	81
	2	3.9×10^{-4}	1.7×10^4	.007	1297	.075	197
	3	3.9×10^{-4}	2.0×10^4	.077	146	.080	146
Third year	4	3.9×10^{-4}	6341	3.9×10^{-4}	6.3×10^4	.034	98
	5	3.9×10^{-4}	1.5×10^4	.064	114	.064	115
	6	3.9×10^{-4}	8788	.038	102	.025	143
Fifth year	7	3.9×10^{-4}	4146	.010	248	.033	86
	8	3.9×10^{-4}	7805_4	.057	72	.053	74
	9	3.9×10^{-4}	1.1×10^4	.087	62	.024	146
Control		---	---	---	---	0	0

while there is a moderate variance among the projected K_1 values of the third and fifth year twigs for the same period. Although there is little deviation among projected K_1 values of first year twigs at day 45, the deviation between projected $L_{e(90)}$ values is the greatest of the three age classes tested. In general at day 45, the first year twigs have the highest projected K_1 and $L_{e(90)}$ values, followed by third and fifth year twigs.

Nitrate-Nitrogen Results

This experiment was performed to quantitatively determine if nitrification took place in the leaf and Douglas-fir twig tests run at standard temperature. Nitrification is often responsible for erroneously high results in long-term BOD studies. The theoretical oxygen demand due to nitrification by one-gram, dry weight, of Douglas-fir needles and twigs is determined in Appendix C. The result of this calculation is that 56 mg of O_2 is needed to convert 8.82×10^{-4} moles NH_3-N to 8.82×10^{-4} moles NO_3^- - N. In this calculation, it was assumed all the nitrogen present in fir needles, about 1.5 percent by dry weight (Smith, 1970), was in the form of NH_3-N.

If nitrification took place, then higher NO_3^--N concentrations should be detected in the leachate of untreated samples than in those treated with N-Serve. If all the NH_3-N was oxidized, a concentration of approximately 61.5 mg NO_3^-/l plus the amount in the controls would

be found in the leachate of untreated samples. The results of the NO_3^--N analysis performed on treated and untreated samples are presented in Table 15. In all cases, mean NO_3^--N concentrations in the control bottle was greater than the concentration observed in any of the samples whether treated with N-Serve or not. There is also very little variation between values of different treatments within a species.

Table 15. Nitrate-Nitrogen concentrations in the leachate from BOD tests on leaves using standard temperatures.

Vegetation Type	Untreated Water		Treated with N-Serve	
	Bottle No.	NO_3^- Conc.	Bottle No.	NO_3^- Conc.
		mg/l		mg/l
Douglas-fir	1	.32	3	.29
needles	2	.25	4	.29
Mean	--	.30	--	.29
Western hemlock	6	.43	8	.42
needles	7	.45	9	.40
Mean	--	.44	--	.41
Red alder	11	.24	13	.21
leaves	12	.21	14	.27
Mean	--	.22	--	.22
Control	14	.44	10	.45
	15	.47	--	
Mean	--	.46	--	.45

A test for NO_3^--N in the leachate of the Douglas-fir twig samples was also performed. No NO_3^--N was found in either the control or sample solutions.

It may be assumed from these results that nitrification did not occur in any of the samples run at standard temperature.

Results of the Leachate Analyses

These tests were performed to determine the amount of simple sugar and polyphenolic compounds that leached from leaves and twigs submerged in water and to identify some of the simple sugars present. The needle and leaf samples leached for 20 days while the twig samples leached only for 15 days. After these time periods, fungal growths appeared in the poisoned samples forcing the experiment to be discontinued.

The leachate results are presented in Figures 7 through 18. These figures include the concentration of leachate observed in individual samples together with curves showing the variation in the mean concentration over time. Half the samples were poisoned with mercuric chloride to inhibit biological decomposition of the leachate, while the other half remained untreated, allowing microorganisms to grow.

The Leaf Leachate Results

The sugar concentrations in the leachate of leaves are shown in Figures 7, 8, and 9. The highest mean concentration of sugar was observed in the poisoned samples of western hemlock (Figure 8). Mean sugar concentration peaks at 21.5 mg glucose equivalent/gm,

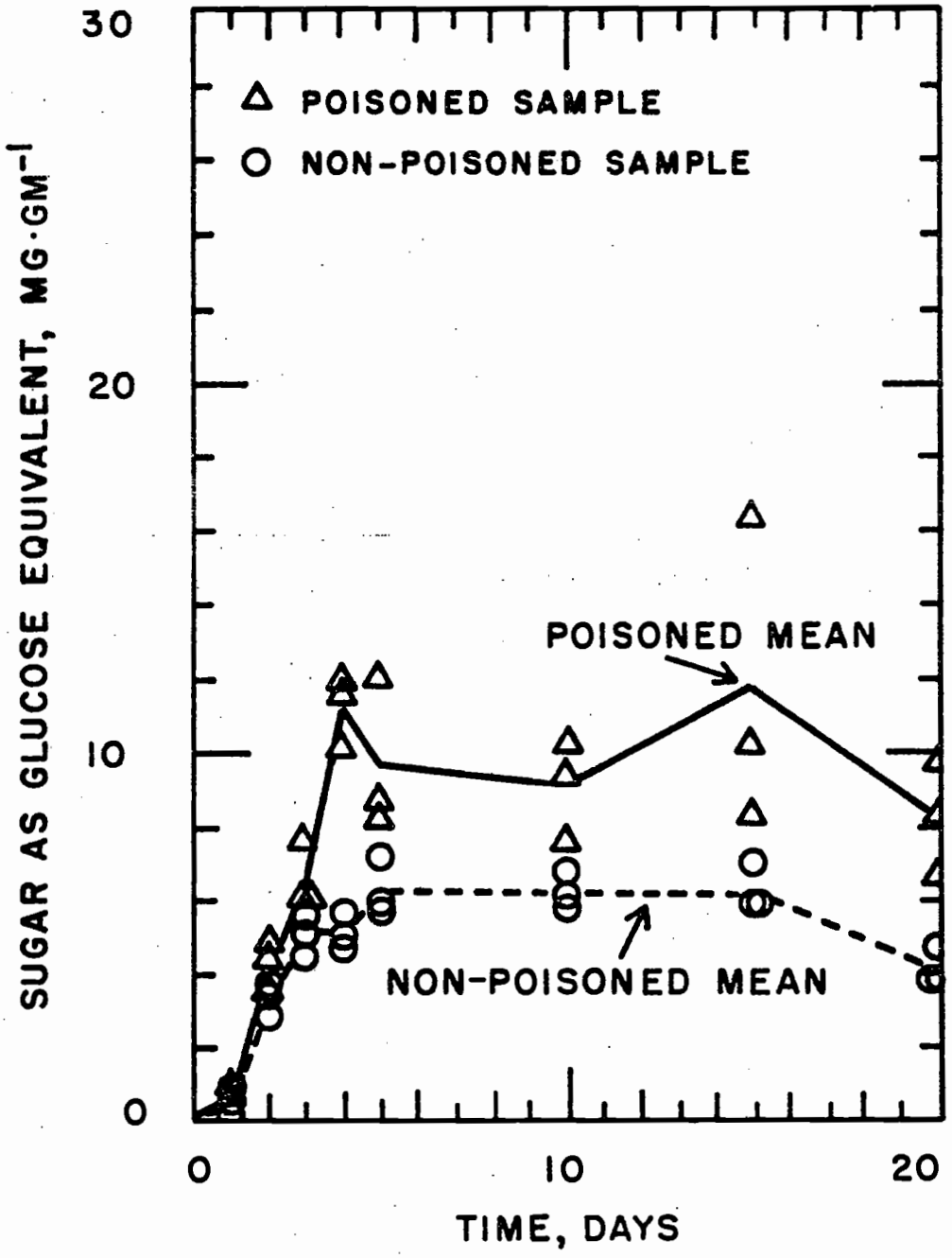


Figure 7. Sugar concentrations of the Douglas-fir needle leachate.

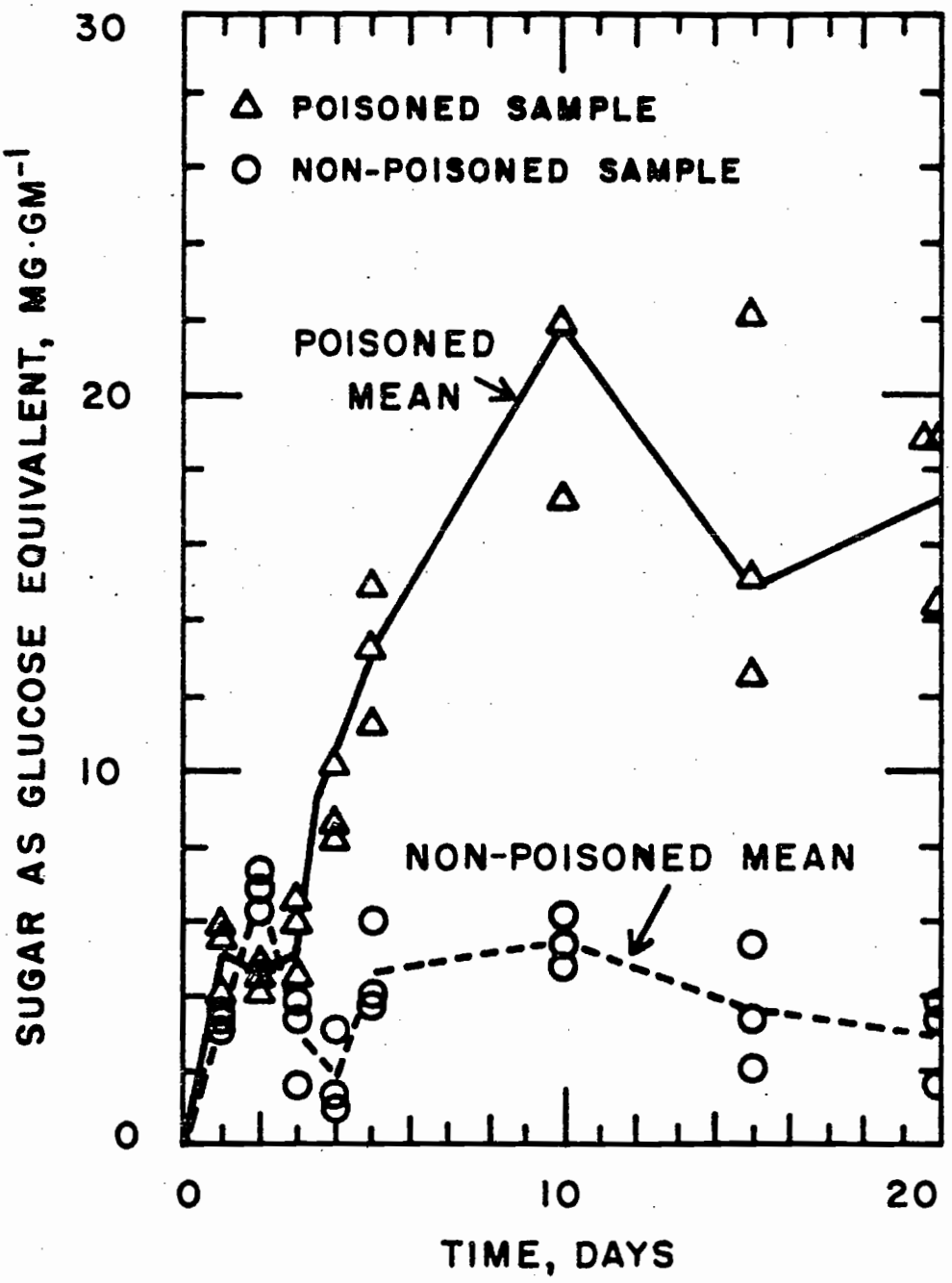


Figure 8. Sugar concentrations of the western hemlock needle leachate.

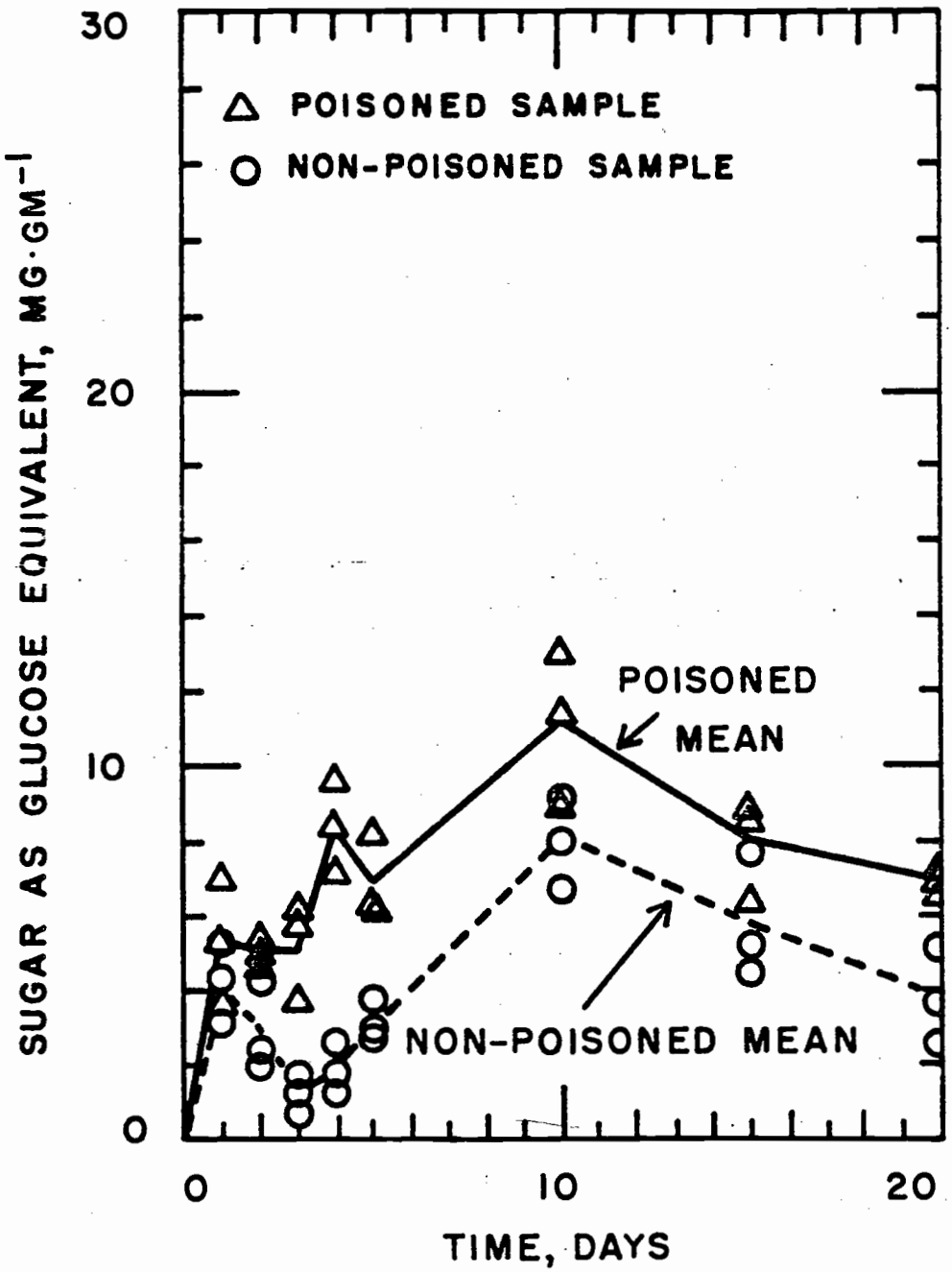


Figure 9. Sugar concentrations of the red alder leaf leachate.

dry weight, of material at day 10, and remains above 15 mg/gm through day 20. The mean sugar concentration in the poisoned Douglas-fir samples (Figure 7) peaks at day 5 and 15, while the mean concentration in the poisoned red alder samples (Figure 9) peaks at day 10. The highest mean sugar concentration for both species is about 11.5 mg/gm. As expected, the mean concentration of sugar is lower in non-poisoned samples for all species. The mean concentration of sugar in poisoned and non-poisoned samples remain about the same during the first day. During this period, leaching is occurring rapidly and the microorganism populations have not begun to utilize the sugar. After two days, the differences between the curves become apparent. This difference can be attributed to microorganism uptake. The greatest difference between mean values of poisoned and non-poisoned samples is in the western hemlock leachate, followed by the Douglas-fir and red alder samples respectively. Mean sugar concentrations in non-poisoned Douglas-fir and western hemlock samples remain relatively constant after five days. After five days, the mean sugar concentration in non-poisoned red alder samples remained parallel to, and about 3.5 mg/gm below the mean sugar concentration in the poisoned samples.

The results of the analyses of phenolic concentration in the leachate of leaves are given in Figures 10, 11 and 12. The mean phenolic concentrations are much less than the mean sugar

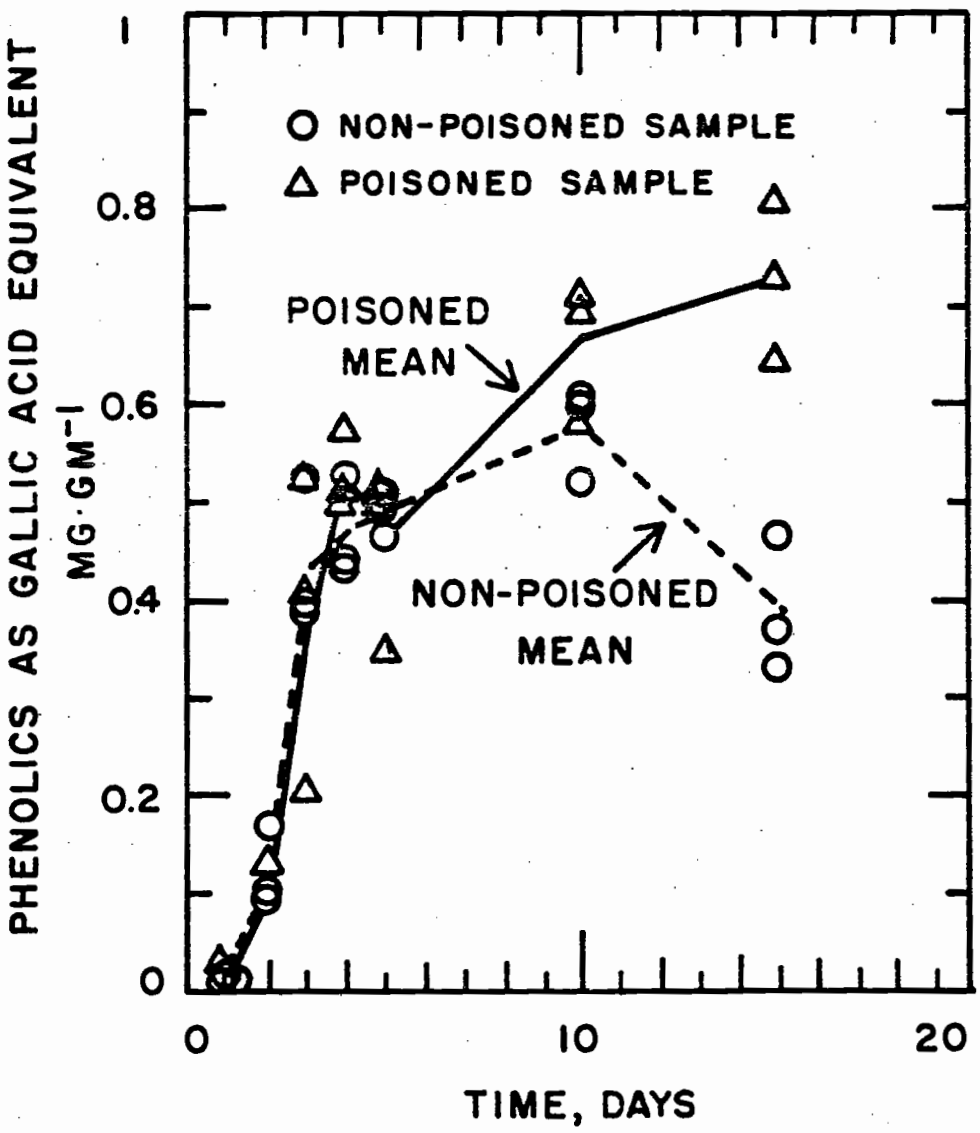


Figure 10. Phenolic concentrations of the Douglas-fir needle leachate.

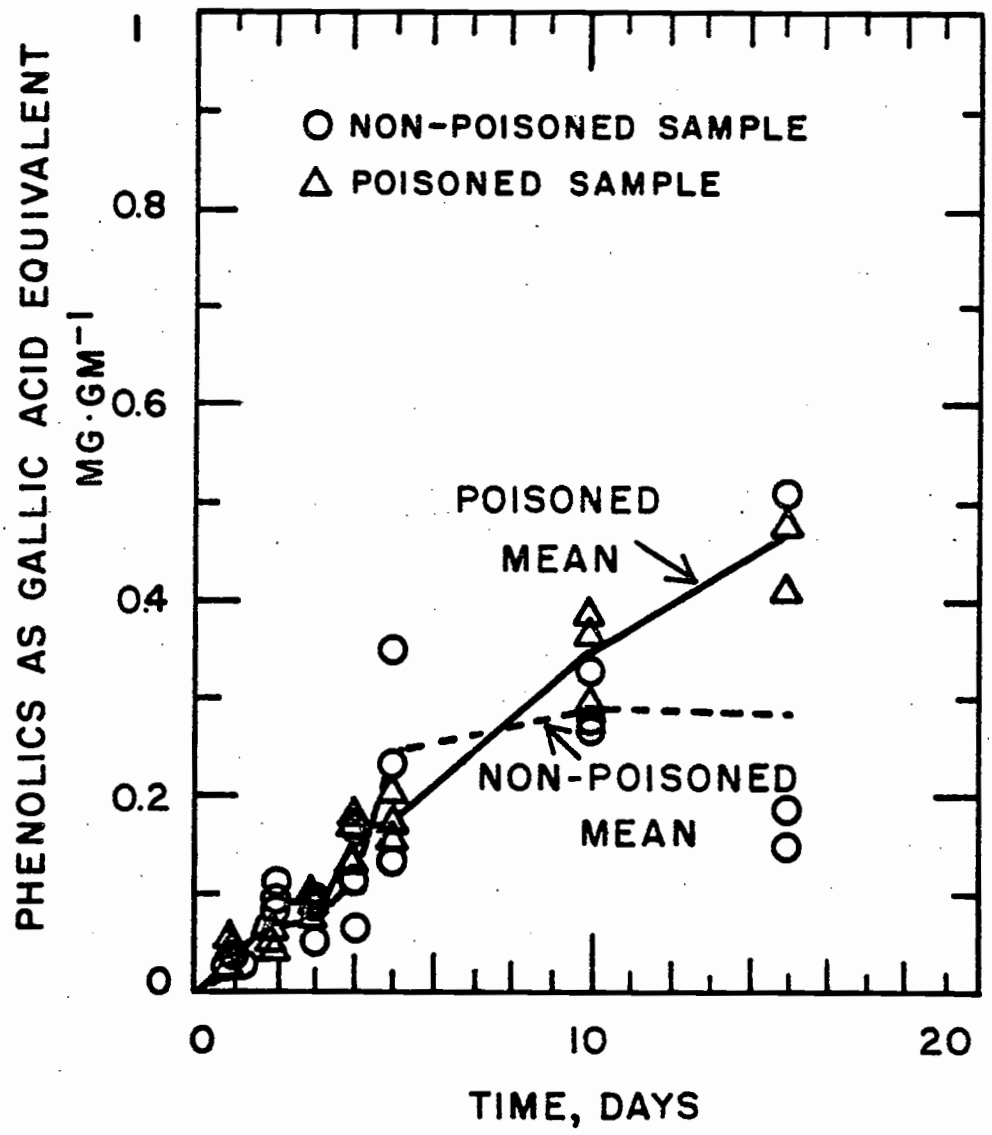


Figure 11. Phenolic concentrations of the western hemlock needle leachate.

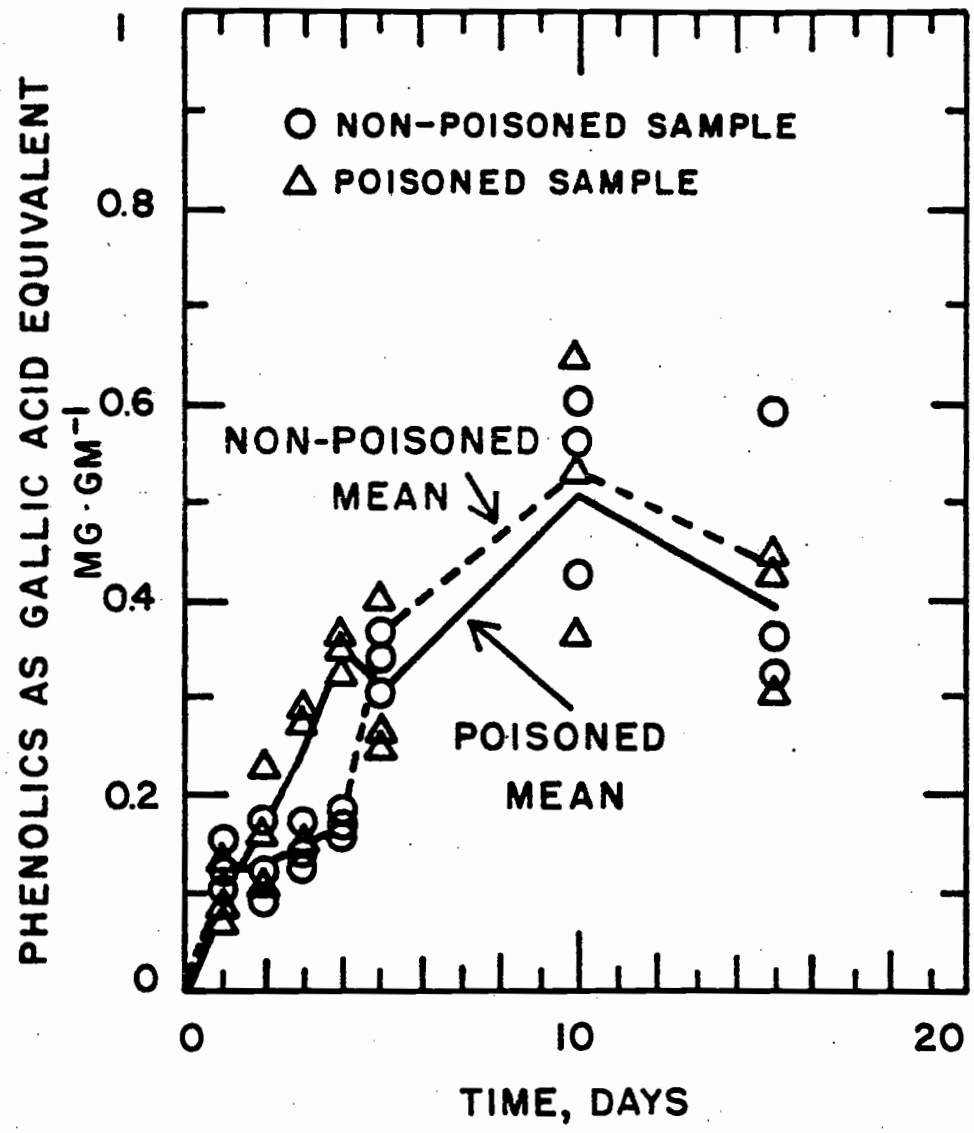


Figure 12. Phenolic concentrations of red alder leaf leachate.

concentrations and are less than 0.8 mg gallic acid equivalent/gm material. This is probably due to the greater complexity of the compounds classed as phenolics, which in turn, inhibit their rate of leaching.

In general, there is little difference between the mean concentration of phenols in poisoned or non-poisoned samples until day 10. At day 10 the mean concentration of phenols in the non-poisoned samples begins to decrease in all species. There are two possible explanations for this delayed reduction of the phenolic compounds. First, as stated earlier, the phenolic compounds are more complex chemically. As a result, it may take the microorganisms longer to oxidize them. Secondly, as was shown in Figures 7, 8, and 9, there is a large quantity of sugar available to the organisms. Since the sugars are readily oxidized they may be preferred by the microorganisms. In the presence of an unlimited food and oxygen supply, the growth of the microorganism population is limited only by its ability to reproduce. By the seventh day the population may have reached the point at which food becomes limiting. The organisms may now be forced to utilize other available substrates, such as the phenolics.

It should be noted that in Figure 12, the phenolic concentration of the red alder leachate does not respond in the same manner as for Douglas-fir and western hemlock shown in Figures 11 and 12.

Although the mean phenol concentration in the non-poisoned samples declines at day 10, the mean concentration of phenolics in the poisoned samples also drops at this time. This indicates that the poison had no effect on the organisms present. This point is further substantiated by the fact the mean concentration of phenolics is about the same in both poisoned and non-poisoned samples. Re-examination of the sugar concentration results of the same sample (Figure 9) also shows a drop in the mean concentration of sugar in the poisoned samples after day 10 and closely parallel the mean concentration in the non-poisoned samples.

Douglas-fir Twig Results

The Douglas-fir twig results are shown in Figures 13 through 18. The results of these tests were not as expected. A very thorough check was made of all the analytical procedures involved in the experiment. Samples were reanalyzed to examine the accuracy of the results initially reported. No errors were found.

Selected poisoned and non-poisoned samples from each age class were analyzed for mercury to check the possibility that poisoned and non-poisoned samples were mislabeled. The results of this test showed less than 0.002 mg Hg^{++}/l in the non-poisoned samples and a range of 0.052 to 0.335 mg Hg^{++}/l in the poisoned samples. It was concluded from these results that although the $HgCl_2$ was in solution,

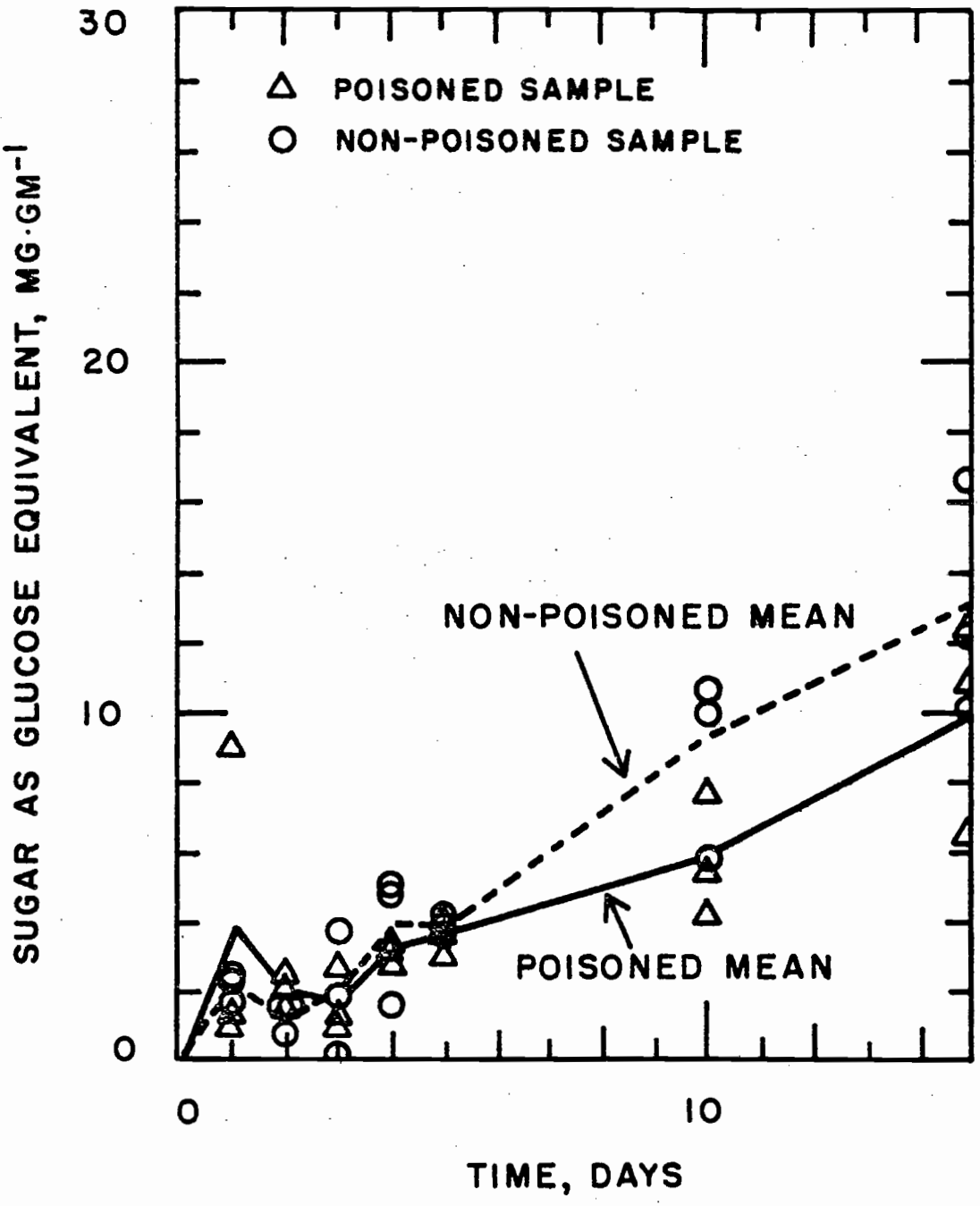


Figure 13. Sugar concentrations of the Douglas-fir first year twig leachate.

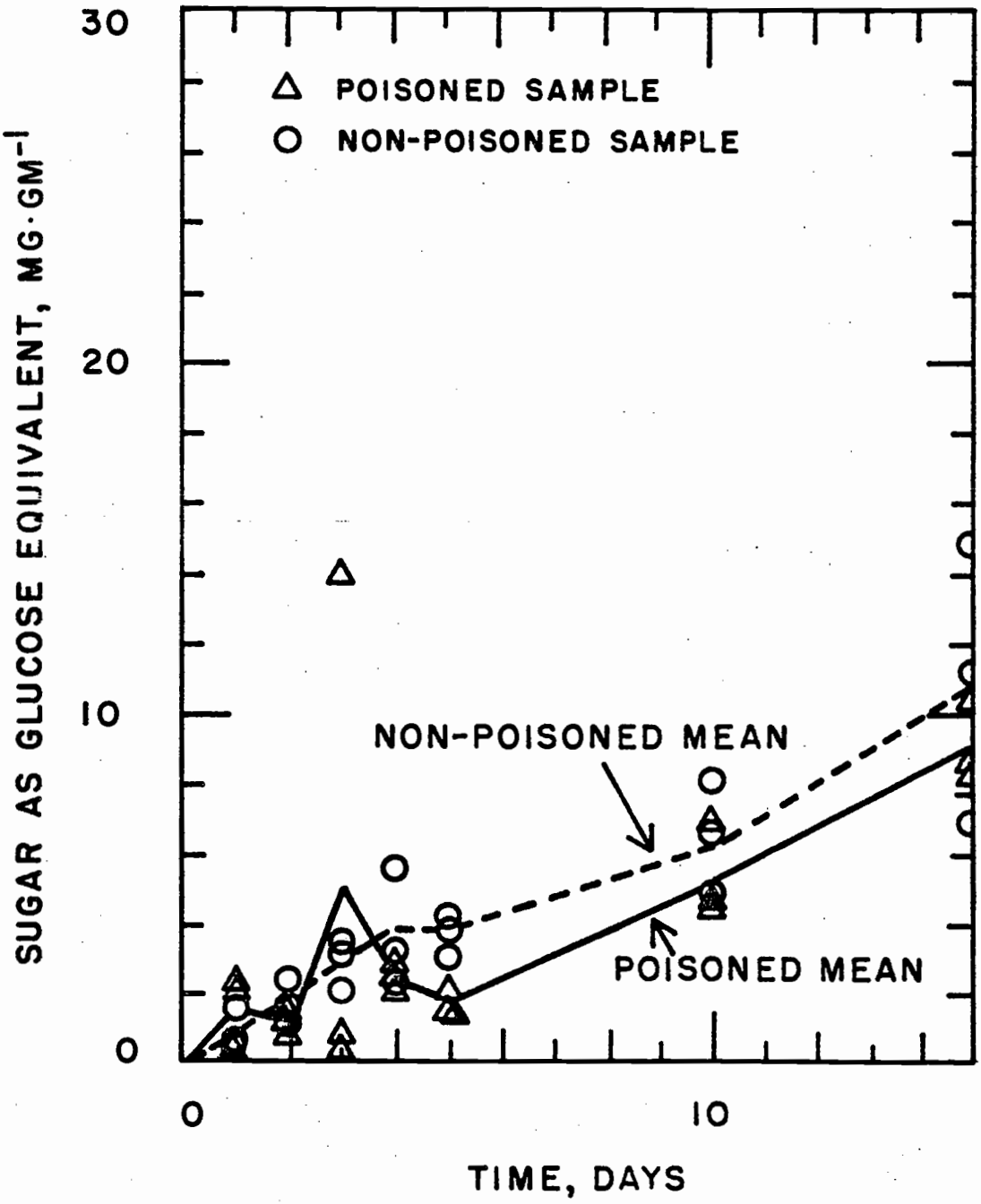


Figure 14. Sugar concentrations of the Douglas-fir third year twig leachate.

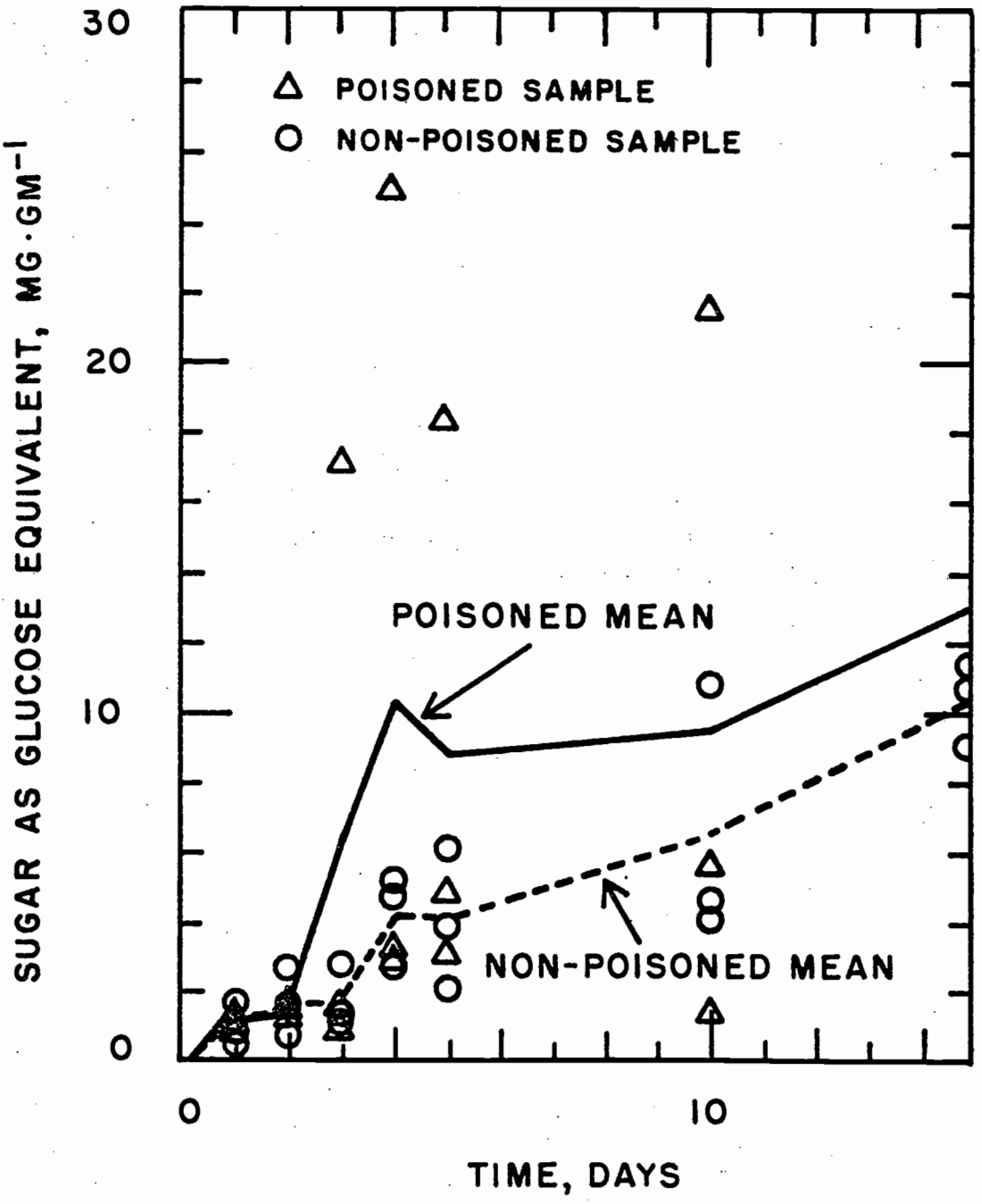


Figure 15. Sugar concentrations of the Douglas-fir fifth year twig leachate.

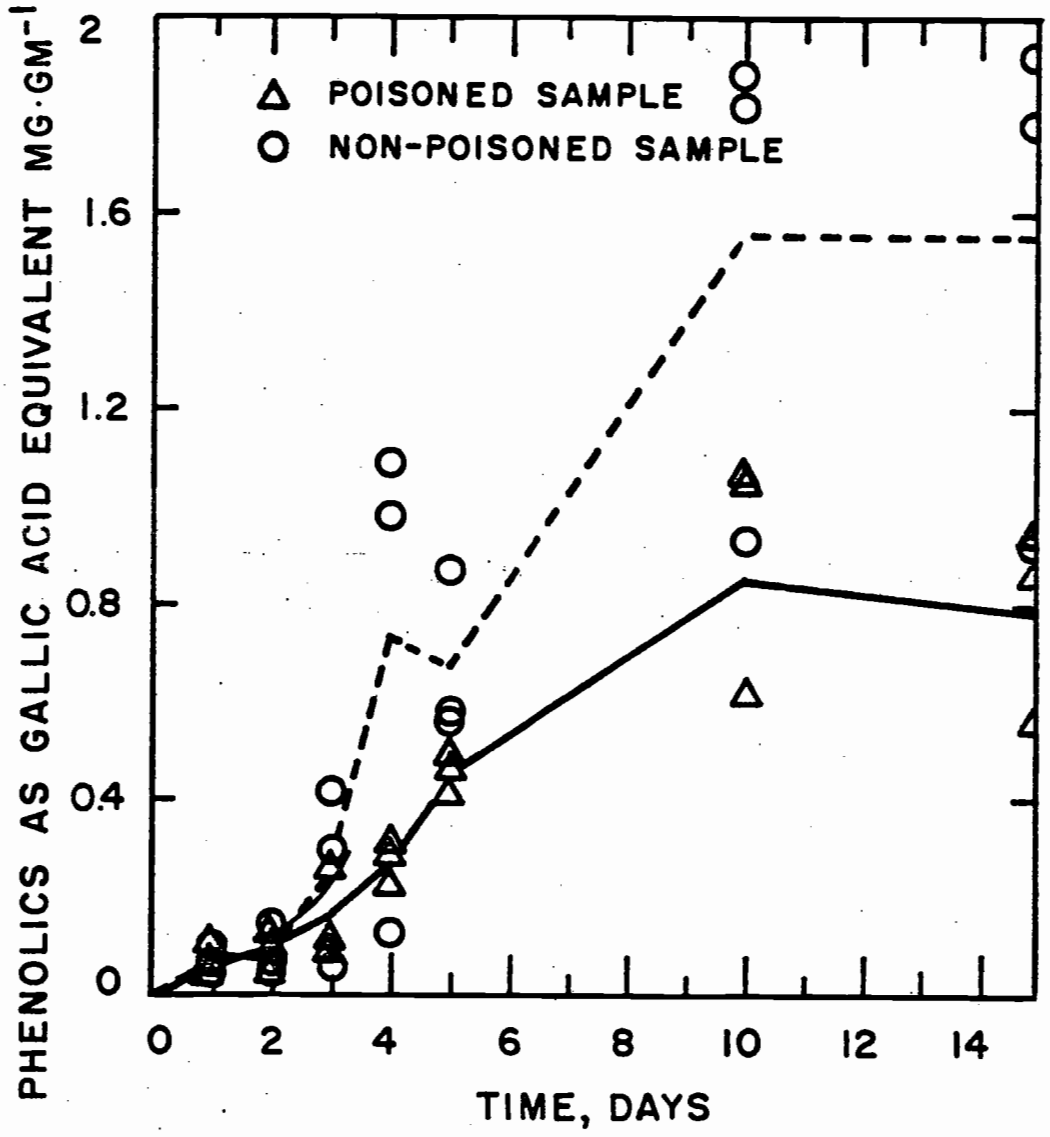


Figure 16. Phenolic concentrations of the Douglas-fir first year twig leachate.

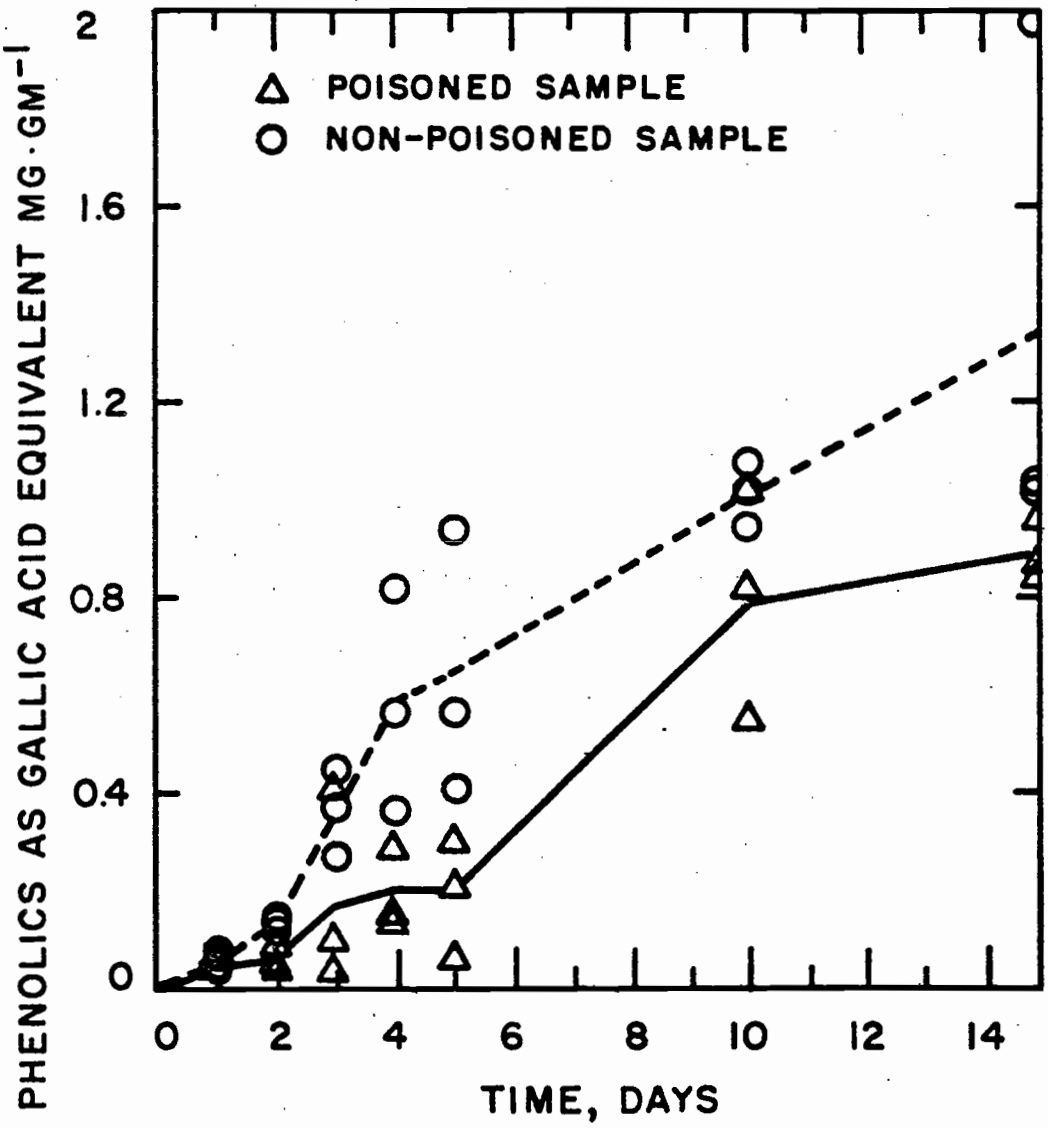


Figure 17. Phenolic concentrations of the Douglas-fir third year twig leachate.

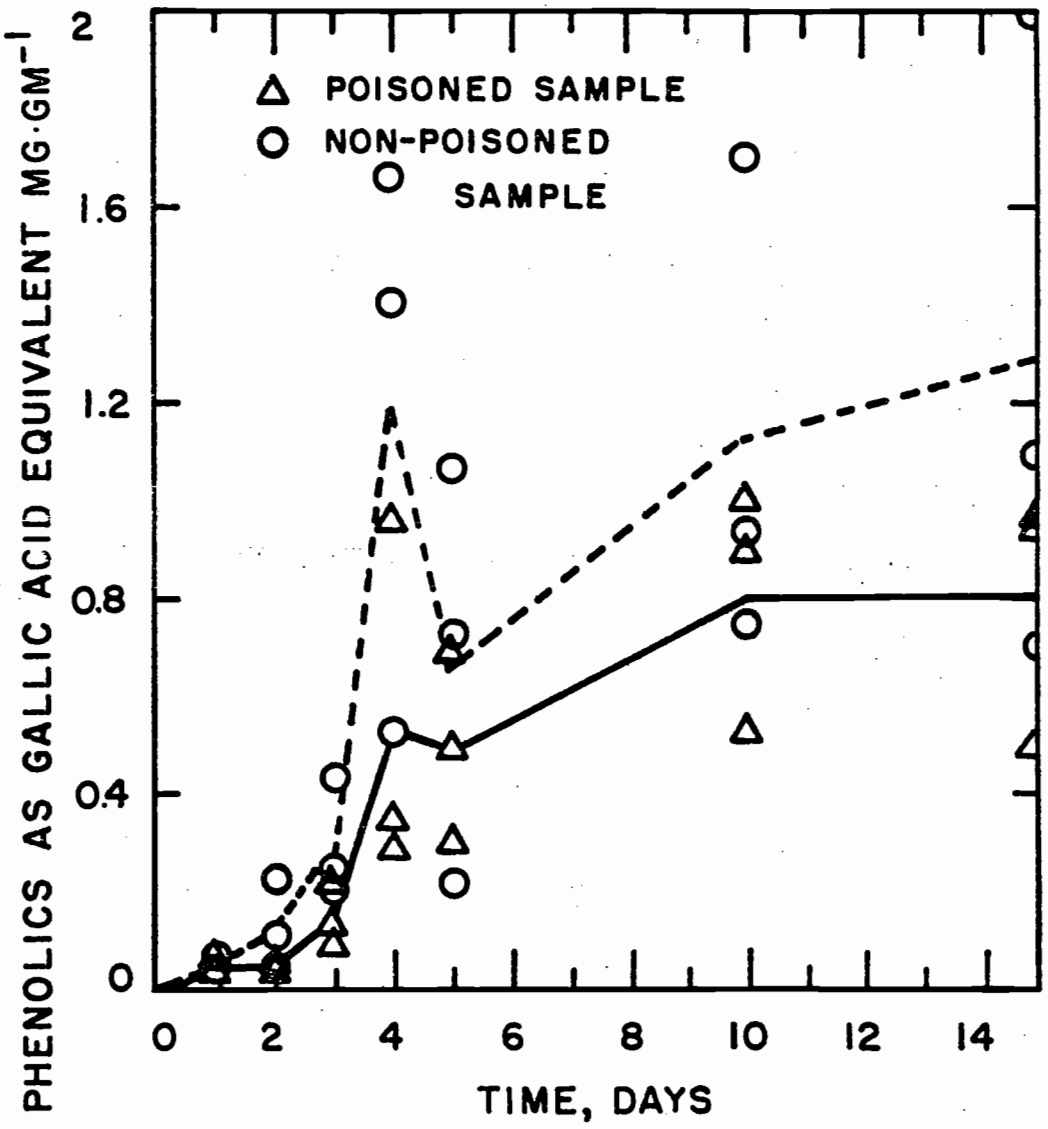


Figure 18. Phenolic concentrations of the Douglas-fir fifth year twig leachate.

it was not toxic to the microorganisms. This conclusion is further supported by the data. There are no pronounced breaks in mean concentrations of sugars in the twig leachate as were present in the leaf leachates. As a result, the results of the twig leachate test will be discussed in very general terms with respect to the release trends of the leachates.

The sugar concentration results are shown in Figures 13, 14, and 15. In general, the change in mean concentration with time is the same for all three age classes, i.e., a relatively slow initial release, followed by a steady increase to near 10 mg glucose equivalent/gm, dry weight, material at day 15. This concentration is about the same as observed for the leachate from Douglas-fir needles over the same time period.

The phenolic results are shown in Figures 16, 17, and 18. The release trend is basically the same as the sugars, i.e., slow at first followed by a gradual increase. In general, the quality of phenolics released by the twigs is about 1.0 mg gallic acid equivalent/gm, dry weight, material, or about 0.2 mg/gm greater than the needles.

Types of Sugars Present in the Leachate

The simple sugars present in a Douglas-fir needle leachate sample were identified by paper chromatography. Two pentoses,

arabinose and xylose, and three hexoses, galactose, mannose, and glucose were found. All these sugars are readily oxidized by aerobic bacteria.

Leachate Toxicity Test Results

The bioassay results for guppy and steelhead trout fry are given in Tables 16 and 17.

Table 16. LC50 for guppies exposed to various dilutions of leachate extracted from 50 gms of leaves per liter of water.

Vegetation Type	Time in Hours			
	24	48	72	96
	----LC50 as percent of 50 gm/l solution -----			
Douglas-fir needles	78	66	65	65
Western hemlock needles	59	42	35	35
Red alder leaves	30	18	18	18

The concentration of leachate necessary to kill half the initial fish population (LC50) from a 50 gm (fresh weight) sample of vegetation placed in a liter of water was determined by bioassays. The LC50 value observed at 24 hours changed only slightly for all three species of vegetation and both species of fish over the test period. In general, there was no change in the LC50 after one day. The

guppies were more tolerant to the three leachates than the trout; the 96-hr LC50 for guppies in Douglas-fir, western hemlock, and red alder leachate was 35, 65, and 18 percent by volume as opposed to 26, 7.5, and 24 percent by volume for the trout.

Table 17. LC50 for steelhead trout fry exposed to various dilutions of leachate extracted from 50 gms of leaves per liter of water.

Vegetation Type	Time in Hours			
	24	48	72	96
	---LC50 as percent of 50 gm/l solution ---			
Douglas-fir[a] needles	26	26	26	26
Western hemlock[a] needles	7.5	7.5	7.5	7.5
Red alder leaves	27	24	24	24

[a]These samples were not treated with HgCl

The Douglas-fir and western hemlock leachates in the steelhead trout bioassay were not treated with $HgCl_2$ in order to examine the possibility of mercury poisoning (Table 17). It can be concluded from these data that sufficient mercury was removed from the leachate of red alder by the chelating compound. Toxicity values for Douglas-fir and western hemlock are in the same order of magnitude as for red alder. In addition, the fish in the poisoned alder leachate would have been killed very quickly if mercury had been present in sufficient

quantities. Since the Douglas-fir and western hemlock leachates were not treated with $HgCl_2$ to inhibit microorganism decomposition of the leached substances, the LC50 values may be high.

Although it has been shown that the leachates of Douglas-fir, western hemlock, and red alder leaves can be toxic to guppies and steelhead fry, it is unlikely that any problems will occur from direct toxicity of these materials in mountain streams. The concentration of material needed to produce these toxic effects is very high; so high, in fact, that the demand for oxygen would probably be responsible for death.

DISCUSSION AND CONCLUSIONS

The primary objective of this study was to quantify the impact of leaves and needles of selected tree species on the quality of mountain stream water. Emphasis has been given to the biochemical oxygen demand of these materials and the rate at which the oxidation reaction proceeds.

Biochemical Oxygen Demand Relations of Douglas-fir, Western Hemlock, and Red Alder

The initial BOD results were presented earlier. Since nitrification of the standard temperature leaf and twig samples did not occur, the four replications within each species of the leaf samples and the three replications within each age group of Douglas-fir twigs were combined. Composite BOD curves were then constructed using 90 days of data and also using data for shorter time intervals, but extended to 90 days. Estimation of long-term BOD using tests of shorter duration may thus be considered in much the same manner that a five-day BOD is used to estimate 70 to 80 percent of the ultimate demand for sewage wastes. The results are presented in Table 18 and shown diagrammatically in Figures 19 through 26.

The composite ultimate and projected K_1 and $L_{e(90)}$ values for selected time intervals are summarized in Table 18. Figures 19 through 26 illustrate the 90-day BOD curves and a BOD curve fit

Table 18. Computed K_1 and $L_{e(90)}$ in mg O_2/gm of leaf and twig vegetation by a least squares fit to the composite standard temperature BOD results in stream water over different time periods.

Vegetation Type	Time in Days									
	5		20		45		60		90	
	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1	$L_{e(90)}$	K_1[a]	$L_{e(90)}$[b]
	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm	1/days	mg O_2/gm
Douglas-fir needles	3.9×10^{-4}	2.9×10^{4}	.140	106	.137	107	.132	108	.125	110
Western hemlock needles	5.8×10^{-4}	1.3×10^{4}	.049	200	.064	166	.063	167	.064	166
Red alder leaves	.237	113	.109	193	.070	239	.055	268	.047	286
Douglas-fir twigs	4.8×10^{-4}	9512	.043	130	.056	110	--	--	--	--

[a] Referred to as the ultimate BOD rate constant in the text

[b] Referred to as the ultimate BOD in the text

through the data which accurately predicts the 90-day, or ultimate BOD.

Each BOD curve is bounded by lines representing a 10, 20, or 30 percent envelope. This provides an indication of the variability of oxygen uptake within a species. Whenever a process is evaluated using a bioassay technique, such as a BOD test, several sources of variability exist. The BOD curves obtained in this study were the result of a multitude of complex interactions, such as leaf and twig chemistry, the rate at which compounds leached from the material, and microorganism populations. In view of these interactions, the composite curve was considered to be a good representation of the process of oxygen uptake if all the data plots fit into the 30 percent envelope at day 90.

It is evident from Table 18 that the 5-day projected K_1 and $L_{e(90)}$ values for leaves are very poor indicators of the ultimate BOD. However, the ultimate BOD can be accurately predicted from the 20-day value of Douglas-fir, the 60-day value for red alder, and the 45-day value of western hemlock. These relationships are illustrated in Figures 19 through 24. Examination of the Douglas-fir (Figures 19 and 20) and alder (Figures 21 and 22), composite BOD curves show the predictions of the ultimate BOD to be relatively accurate. At day 90, most of the data for Douglas-fir are included within the 30 percent envelope even using only 20 days of data, while

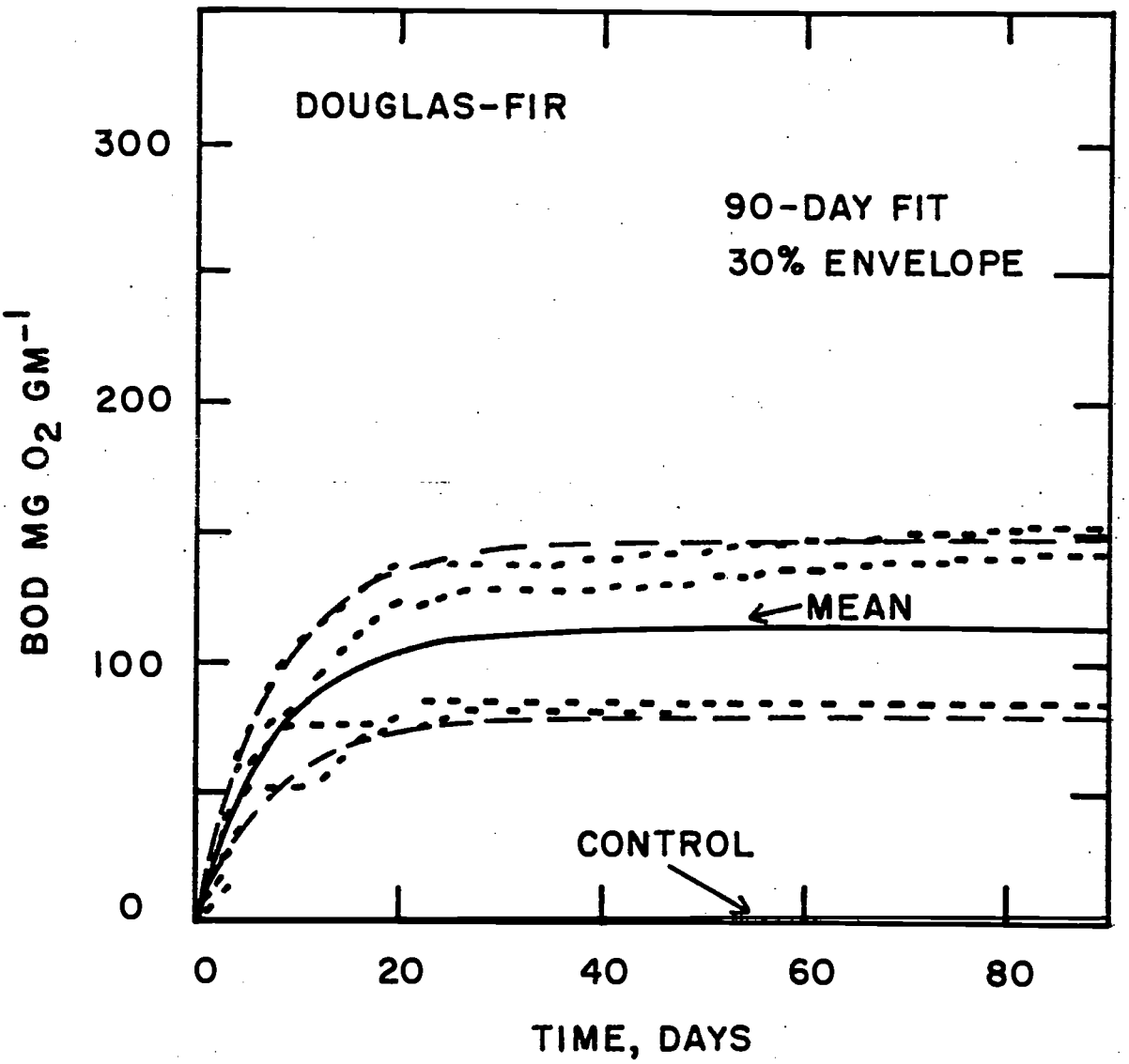


Figure 19. The best-fit BOD curve with a 30 percent envelope fit over 90 days through the Douglas-fir composite data.

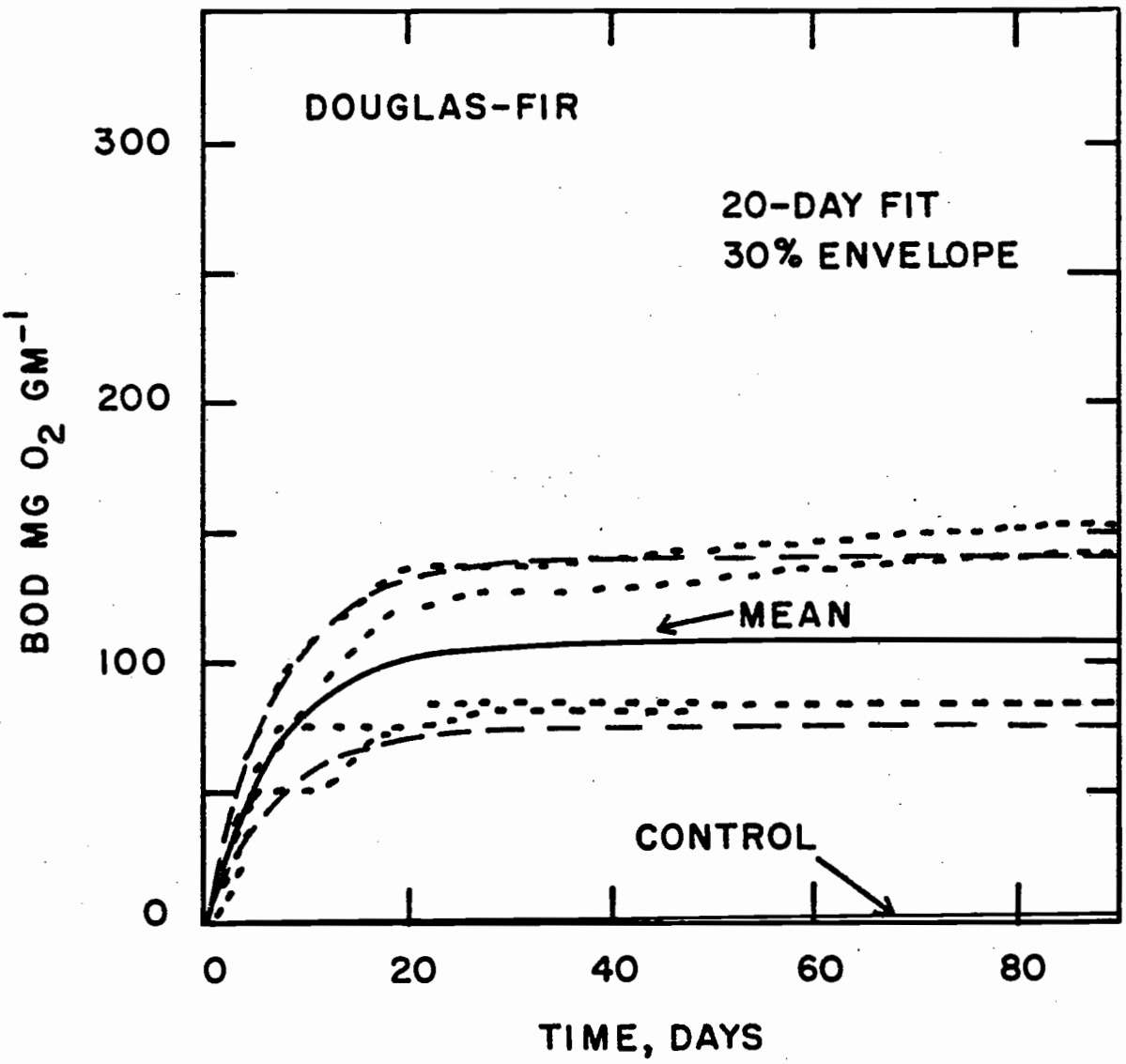


Figure 20. The best-fit BOD curve with a 30 percent envelope fit over 20 days through the Douglas-fir composite data.

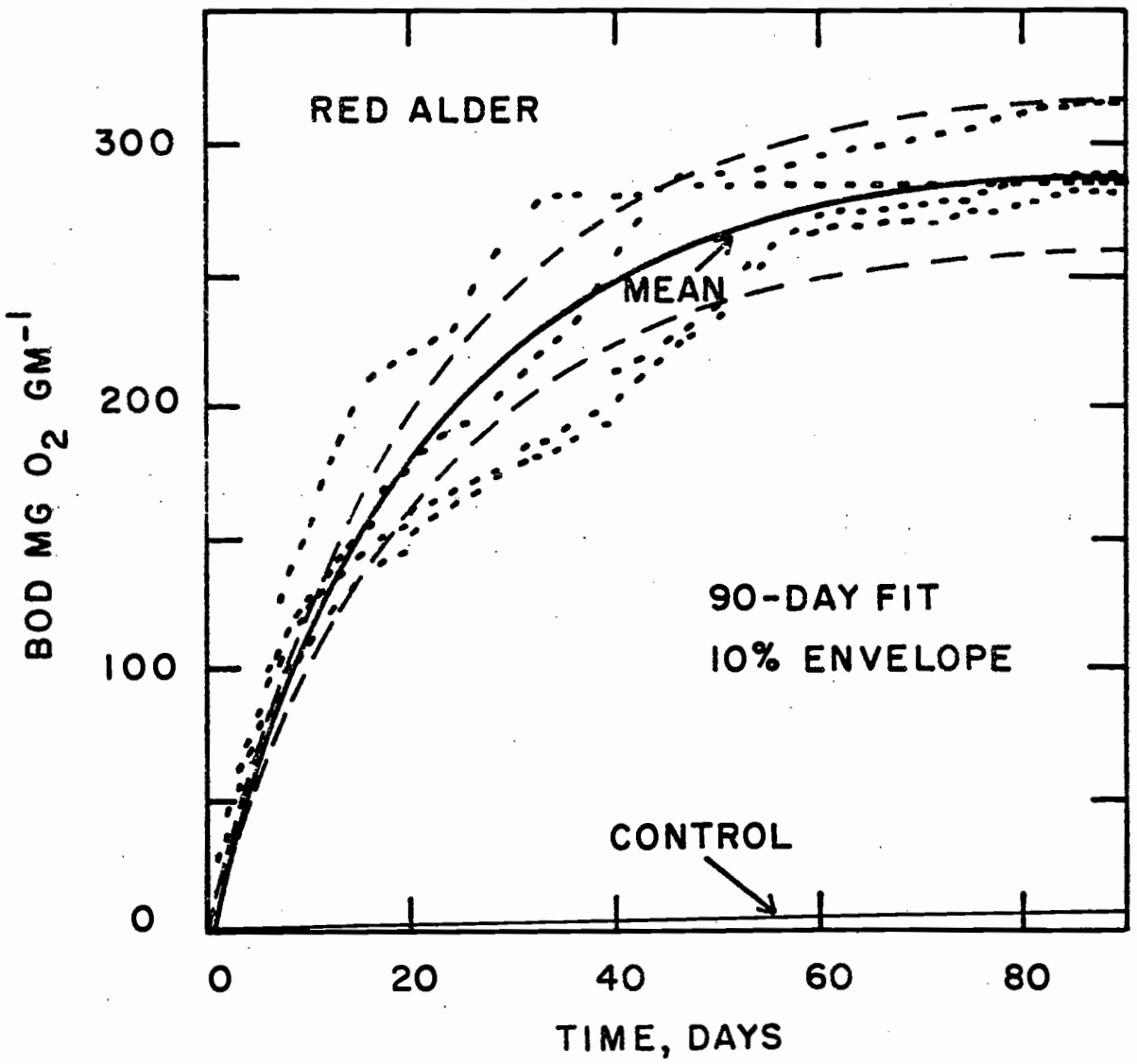


Figure 21. The best-fit BOD curve with a 10 percent envelope fit over 90 days through the red alder composite data.

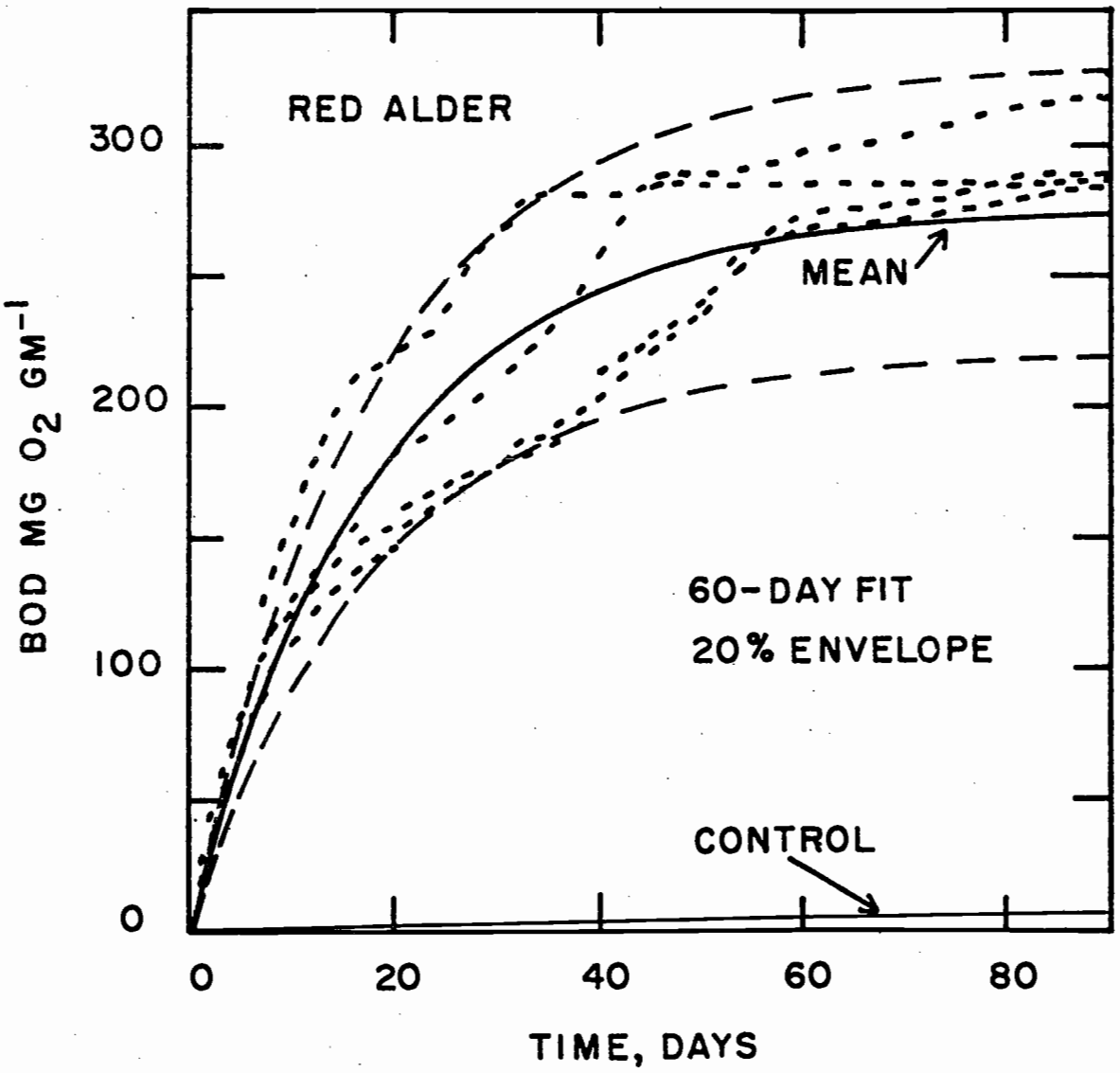


Figure 22. The best-fit BOD curve with a 20 percent envelope fit over 60 days through the red alder composite data.

all the red alder data are within the 20 percent bounds using 60 days of data. All of the western hemlock data (Figures 23 and 24) are not included in the 30 percent envelope at day 90 using either 90 or 45 days of data. The reason is that one sample exerted a BOD much lower than the other samples. In this sample, the BOD was nearly satisfied by day 10 and was completely satisfied by day 40. The remaining western hemlock samples exhibited a much different pattern of oxygen demand. There is little deviation between these three samples; all three are included in the 30 percent envelopes about the composite curves.

A different, and probably more precise estimate of the ultimate BOD for western hemlock was obtained by using only the first 20 days of data to construct the composite curve (Figure 25). There was close agreement between all four samples during this time period; the deviation of the fourth sample did not begin until after day 10. The composite curve projected from the 20-day period estimated the ultimate BOD as 200 mg O_2/gm; the three similar samples were included in a 10 percent envelope about the composite curve.

It may be concluded from these results that the red alder exerted the greatest oxygen demand over 90-days, while at the same time having the lowest rate constant. The Douglas-fir needles have the lowest oxygen demand and the highest rate constant. Western hemlock was intermediate, both in total oxygen demand exerted and the rate of the

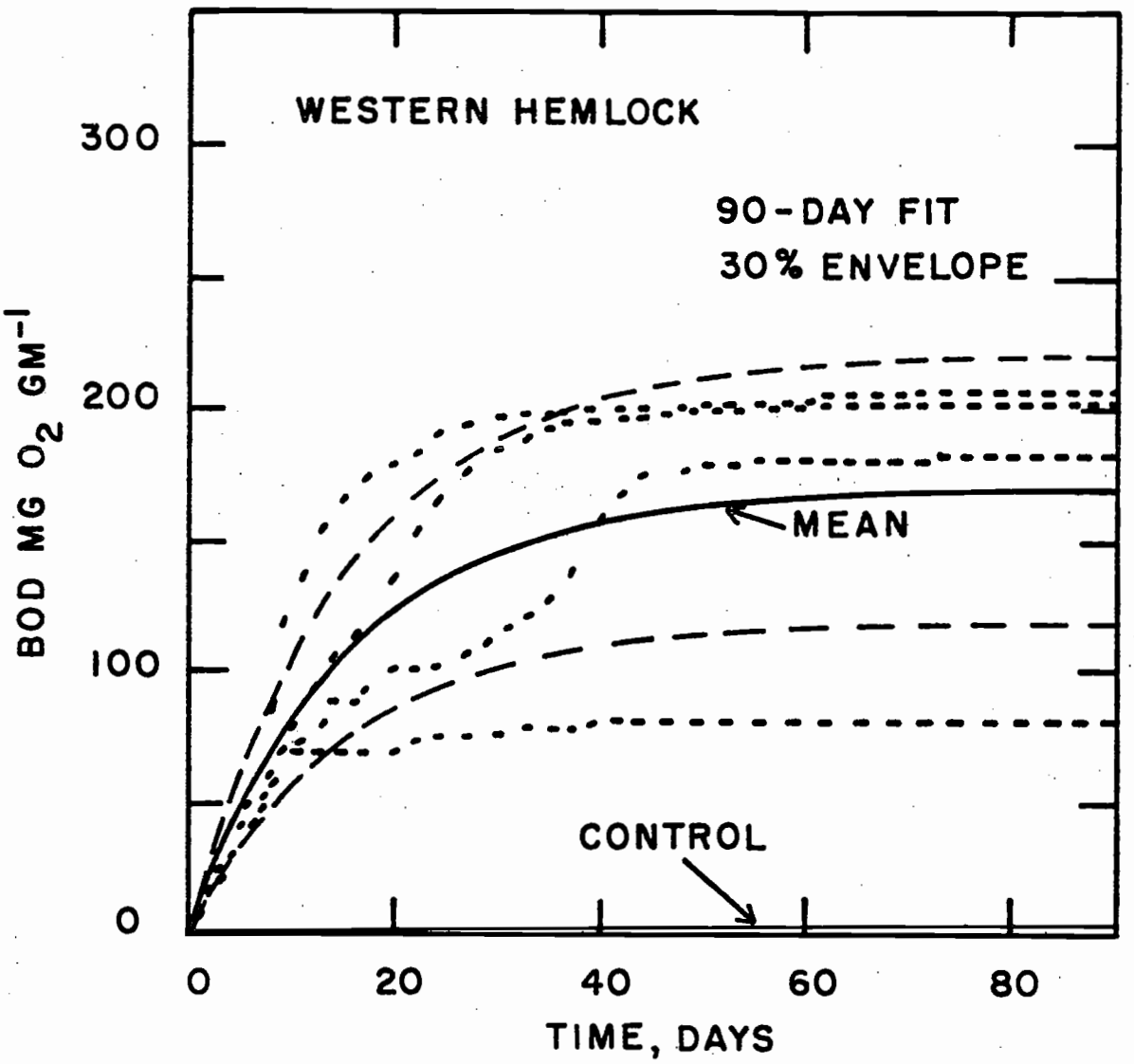


Figure 23. The best-fit BOD curve with a 30 percent envelope fit over 90 days through the western hemlock composite data.

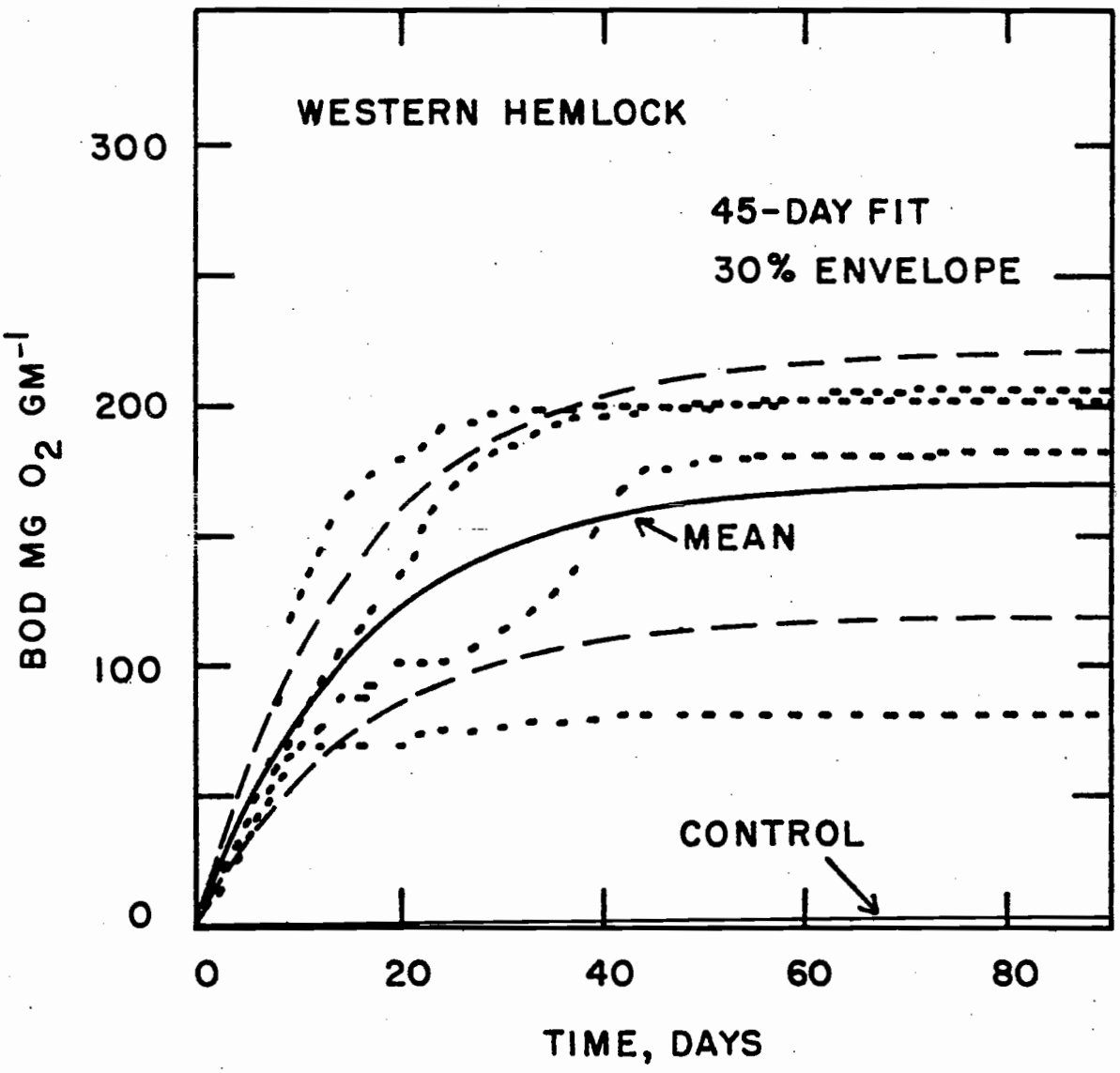


Figure 24. The best-fit BOD curve with a 30 percent envelope fit over 45 days through the western hemlock composite data.

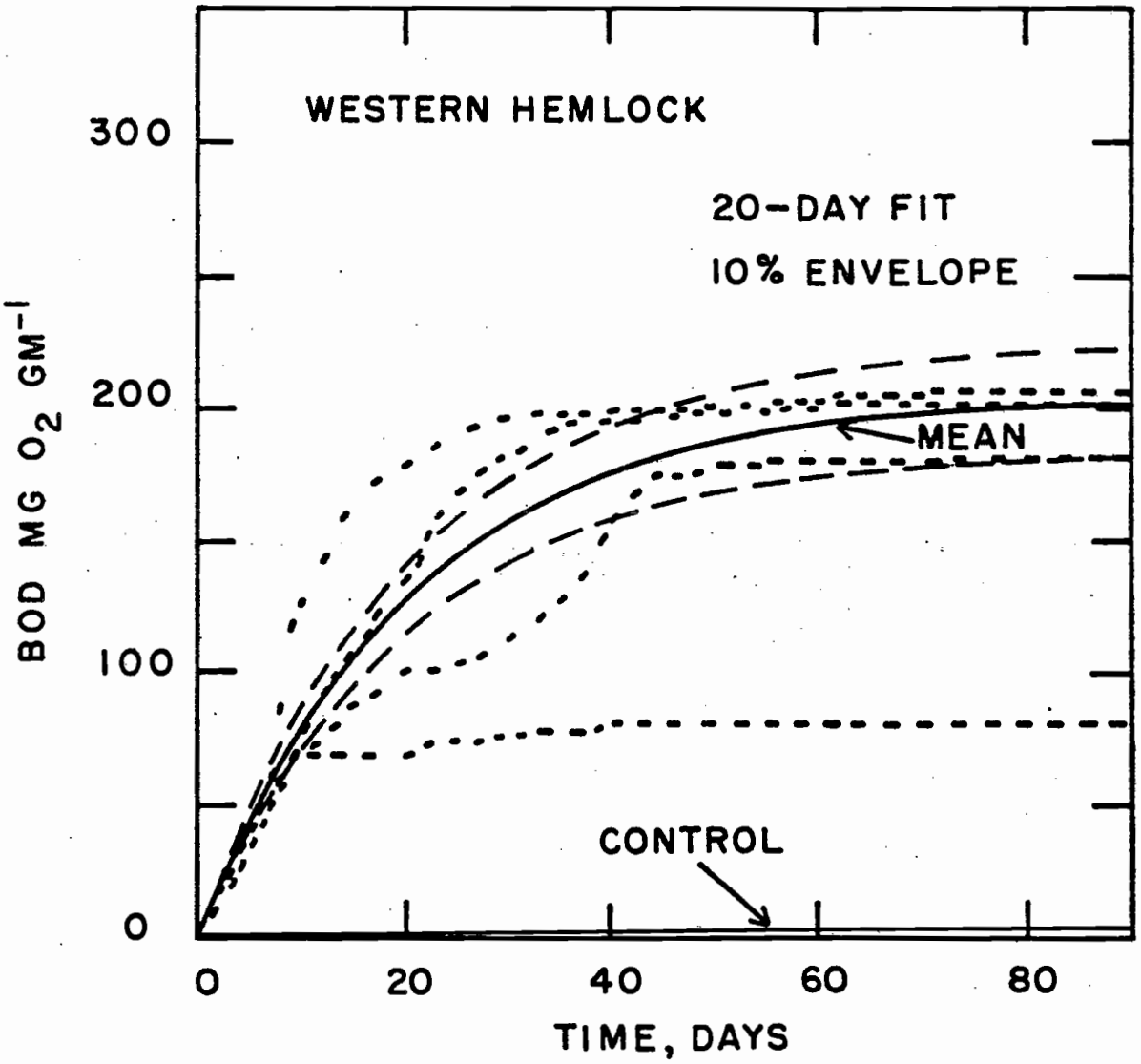


Figure 25. The best-fit BOD curve with a 10 percent envelope fit over 20 days through the western hemlock composite data.

BOD reaction. In general, the composite Douglas-fir twig curve is similar to the composite leaf curves.

From Table 18 it is evident that the 5-day projected values for Douglas-fir twigs are poor indicators of the 45-day predictions of the ultimate BOD. However, the projected $L_{e(90)}$ value is similar to the projected value for Douglas-fir needles over the 45-day time period. The twigs have a much lower K_1 value, indicating that their demand will be exerted over a longer time period.

The composite curve for Douglas-fir twigs is illustrated in Figure 26. There is a high degree of variation among samples; four of the nine samples are not included in the 30 percent envelope. Seven of the nine samples are closely bunched and dominate the shape of the curve. However, the ultimate demand predicted using the 45 days of data may be slightly high due to the effect of the two high samples.

Leachate and BOD Rate Constant Relations

The rate at which the BOD reaction proceeds is a function of the incubation temperature, the microorganisms present, and the substrate available to the organisms. Since the temperature was constant and the same stream water was used in all the samples, the difference in rate coefficients may be explained by the different type of substrate present. It was the intent at the outset of the study that the leachate results would be used to describe the

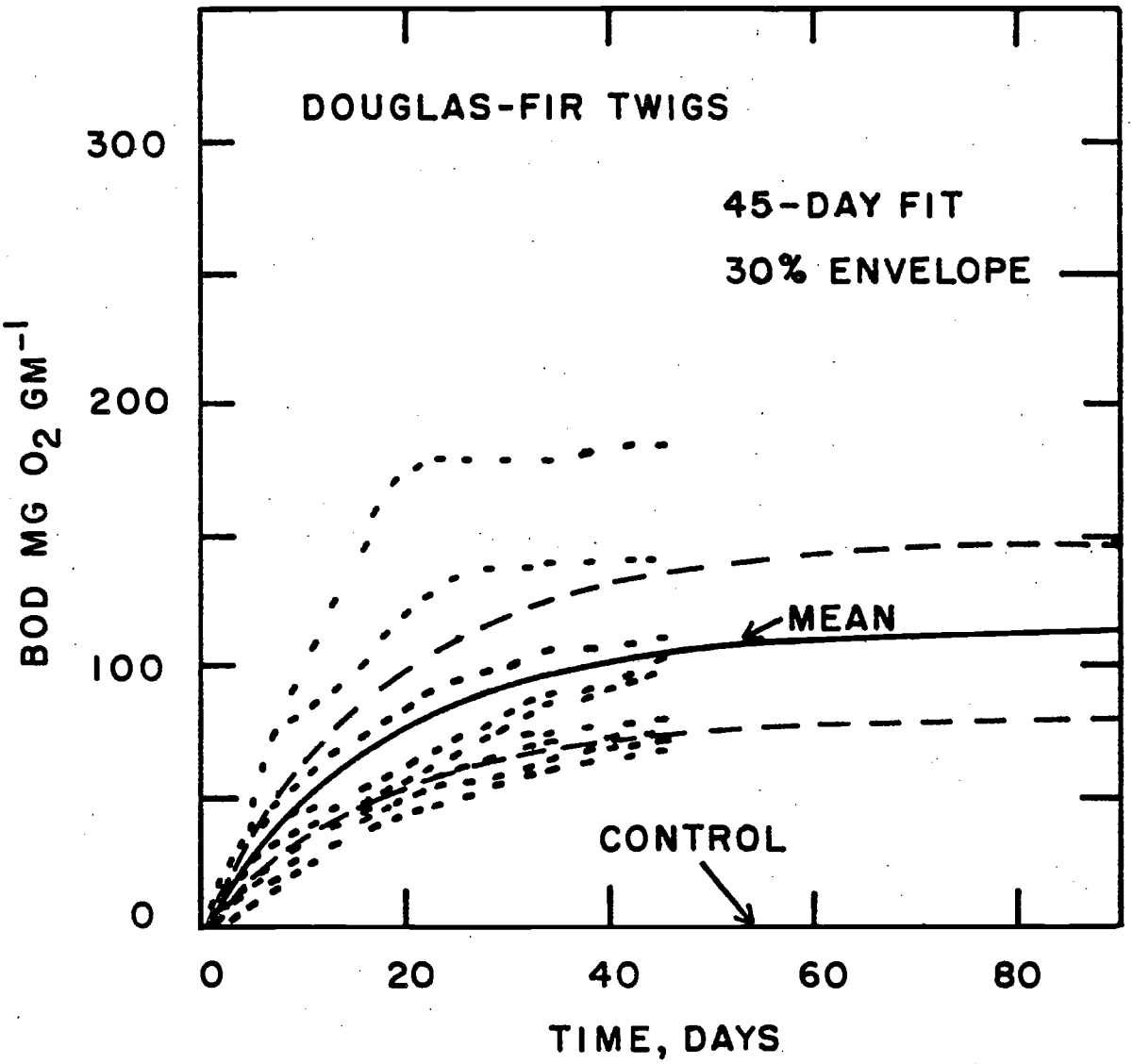


Figure 26. BOD best-fit curve with a 30 percent envelope fit over 45 days through the Douglas-fir twig composite data.

differences in K_1 between species. However, the leachate test was terminated after 20 days due to fungal interference. Therefore, the results can only be applied to the initial portions of the curve.

The 20-day rate coefficients, K_1, for Douglas-fir, red alder, and western hemlock are 0.140, 0.109, and 0.049 respectively. The Douglas-fir and red alder K_1 values may be explained by the leaching pattern for sugars and phenolics shown in Figures 7 and 10, and 9 and 12. The Douglas-fir has a very rapid sugar and phenolic release; most of the simple sugars are leached out in five days. The sugar and phenolic release by red alder is not as rapid, taking at least 10 days. However, the amount released is nearly the same for both species. This is why both species have relatively high 20-day K_1 values and why the red alder is slightly less than the Douglas-fir value.

Western hemlock has a relatively slow release of sugars and phenolics during the first three days followed by a very rapid and highly concentrated release during the next seven days. However, in contrast to the Douglas-fir and red alder K_1 values, western hemlock has a relatively low K_1 value. This is probably due to the high sugar concentration present. With such a high concentration of sugars released, conditions may have been created that were unfavorable to the microorganisms, forcing them to require more time to adapt. The phenolic release by western hemlock and uptake by

microorganisms is similar to that of fir and alder.

Since half the twig leachate samples were not adequately poisoned with $HgCl_2$ the actual concentration and rate of sugar release over time is not known. As a result the relation between twig K_1 values and twig sugar and phenolic leaching rate can not be defined quantitatively.

Standard and Fluctuated Temperature BOD Relations

Neither K_1 nor $L_{e(90)}$ values were determined for the BOD data collected under conditions of fluctuating temperature. However, some general conclusions can be drawn by comparing the mean BOD_5 values obtained using fluctuated temperature with those obtained under standard temperature conditions. The BOD_5 values using standard temperature were taken from the best fit BOD_{90} curve through the composite data. These results are presented in Table 19. The mean BOD_5 of leaves exposed to conditions of fluctuated temperature were much greater than those exposed to standard conditions; 75 percent higher for Douglas-fir, 58 percent higher for western hemlock, and 76 percent higher for red alder.

It is apparent from these comparisons that Douglas-fir, western hemlock, and red alder leaves exposed to conditions of temperature fluctuation comparable to those observed in a small stream exposed by a clearcut, have a much higher oxygen demand in five days than

the same vegetation incubated at standard temperature.

Table 19. Five day BOD in mg O_2/gm of Douglas-fir, western hemlock and red alder leaves exposed to different temperature treatments.

Temperature Treatment	Douglas-fir	Western Hemlock	Red Alder
	mg O_2/gm	mg O_2/gm	mg O_2/gm
Standard	51	46	60
Fluctuated	202	109	249

BOD/COD Relations

The BOD/COD ratio is often used by sanitary engineers in sewage treatment plant management. If a sample is composed of compounds that are oxidized by both the BOD and COD procedures, a relationship may be established. If this relation is consistent, the COD test may be used to predict the ultimate BOD of a material. This is very advantageous since the COD only takes about 3 hours to perform. However, if the relationship between BOD and COD is inconsistent, predicted ultimate BOD from the COD test are likely to be erroneous. In general, materials containing high concentrations of cellulose have inconsistent BOD/COD relation.

The relation of BOD to COD is not consistent for leaf and twig tissue. There is high variation among the BOD exerted within species

as shown by Figures 19 through 26, as well as the determined COD as shown by Table 5. However, the BOD/COD ratio was computed to give a general estimate of how completely the microorganisms oxidize the material.

Specific BOD values for each species were taken from the best fit curves through the composite data while the mean COD values were used. The ratios were found to be 0.24, 0.29, 0.32 and 0.12 for Douglas-fir needles, western hemlock needles, red alder leaves and Douglas-fir twigs respectively. These ratios indicate that about one-fourth of Douglas-fir needles, three-tenths of western hemlock needles, and one-third of red alder leaves can be oxidized by aquatic microorganisms in 90 days while only one-eighth of Douglas-fir twigs can be oxidized over the same period under standard conditions.

Again, it is emphasized that these relations are only very general relations drawn from samples with high variability.

SUMMARY

The results of this research have provided several important facts about the BOD of needles, leaves, and twigs, the nature of the substances leaching from these materials that cause this BOD, and the toxicity of these leachates to fish.

1. The BOD exerted by Douglas-fir and western hemlock needles varies depending on their aspect in the crown and their age. The BOD exerted does not vary significantly with elevation in the crown. The BOD exerted by red alder leaves does not vary significantly by crown position.
2. Nitrification did not occur in any of the standard temperature BOD tests.
3. The ultimate BOD or 90-day BOD and rate constant (K_1) was 110 mg O_2/gm and 0.125 for Douglas-fir needles, 110 mg O_2/gm and 0.056 for Douglas-fir twigs, 200 mg O_2/gm and 0.049 for western hemlock needles, and 286 mg O_2/gm and 0.047 for red alder, based on dry weight.
4. Further analysis showed that 90-day values for BOD and K_1 could accurately be estimated by tests of shorter duration: 20 days for Douglas-fir needles, 20 days for western hemlock needles, and 60 days for red alder leaves. The standard 5-day BOD was a poor estimator of the ultimate BOD and associated K_1.

5. The standard temperature BOD samples of Douglas-fir needles and twigs were not mass-concentration dependent.
6. The concentrations of simple sugars were followed over a 20 day period. Half the samples were poisoned with 2 mg/l $HgCl_2$ to inhibit microorganism growth. Mean maximum concentrations of sugar varied from 21.5 mg glucose equivalent/gm in western hemlock to about 15.0 mg/gm for Douglas-fir and red alder leaves in those samples that were poisoned. Mean maximum concentrations of sugar in the non-poisoned samples were 6.0 mg/gm for Douglas-fir needles, 5.0 mg/gm for western hemlock needles, and 8.0 mg/gm for red alder leaves. Mean maximum concentration of sugar for the Douglas-fir twig samples was 10.0 mg/gm. Values are based on fresh weight.
7. A paper chromatography analysis showed that the sugars present in the leachate from needles, leaves, and twigs consisted of arabinose, xylose, galactose, mannose, and glucose.
8. The concentrations of phenolics were followed over a 15 day period. Mean maximum concentrations of phenolics were 0.72, 0.46, and 0.55 mg gallic acid equivalent/gm for Douglas-fir needles, western hemlock needles, and red alder leaves in those samples that were poisoned. There was little difference between mean concentration of phenolics in the unpoisoned and poisoned samples until day 10, when the mean concentrations of phenolics

in the unpoisoned samples decreased. The Douglas-fir twig samples had a maximum mean concentration of phenolics of about 1.0 mg/gm.

9. The five-day BODs of leaf material exposed to conditions of fluctuating temperature (12.9 to 35.0°C) were determined. Samples exposed to standard temperature conditions exerted a BOD_5 of 25, 42, and 24 percent of the temperature fluctuated BOD_5 for Douglas-fir needles, western hemlock needles, and red alder leaves respectively.

10. The mean COD was 454 mg O_2/gm (dry weight) for Douglas-fir needles, 947 mg O_2/gm (dry weight) for Douglas-fir twigs, 570 mg O_2/gm (dry weight) for western hemlock needles, and 882 mg O_2/gm (dry weight) for red alder leaves.

11. The toxicity of a leachate extracted from 50 gm (fresh weight) per liter of water of each species was determined on guppies and steelhead trout. The 96-hour LC50 to guppies from leachate of Douglas-fir, western hemlock, and red alder leaves was 35, 65, and 18 percent of the original concentration, while steelhead trout fry had 96-hour LC50's of 26, 7.5 and 24 percent of the original concentration for the same species.

Suggestions for Further Research

This research has been directed towards quantifying the short- and long-term BOD of logging debris and the rate at which this oxidation process proceeds. The purpose of this research has been to provide the oxygen depletion parameters of a model which will predict the impact of logging debris on the dissolved oxygen concentration at the site of accumulation and downstream. Earlier research has provided models which predict the reaeration in small mountain streams, and presently research is being carried on at Oregon State University which seeks to quantify potential debris accumulation at a site.

Although it is beyond the scope of this study, a model which simultaneously evaluates the effects of BOD and reaeration with respect to debris accumulation potential needs to be developed. In its present form, equation (7) may be used to predict the combined effects of BOD and reaeration. Equation (7) may be used to predict the oxygen deficit at any point downstream from the point of debris entry. Of particular interest is the maximum oxygen deficit caused by the debris under given conditions and the point at which it occurs. It is important that such a model be completed in order to more clearly relate logging and water quality.

It has been shown that finely divided logging debris exposed to

conditions of fluctuating water temperature exerts a much higher oxygen demand than samples exposed only to standard temperature. Further evaluation of the BOD of debris under such conditions is recommended. An alternative to the approach used in this study is the actual placement of samples in a stream.

It was also shown that the BOD rate coefficients were a function of the type of leachate present. It would be valuable to have reliable leachate results for the entire ninety-day period. These results could be used not only in BOD rate coefficient evaluation, but also by fishery biologist in the prediction of chronic effects of the debris on fish or in evaluating the impact of leachate composition on primary production.

Specific concentrations of given leachates were found to be toxic to fish. However, the bioassay test performed only measured the effect of the leachate on fish. Under natural conditions an oxygen stress may be created due to the presence of debris. More extensive tests should be carried out which examine the acute and chronic effects of various leachate concentrations at different oxygen levels on fish.

BIBLIOGRAPHY

American Public Health Association, Inc., Standard methods for the analysis of water and waste-water, 13th ed., Washington, D. C., 874 p., 1971.

Beamish, F. W. H., Respiration of fishes with special emphasis on standard oxygen consumption, III. Influence of oxygen, Canadian Journal of Zoology, 42, 355-366, 1964.

Brown, G. W., and J. T. Krygier, Effects of clear-cutting on stream temperature, Water Resources Research, 6(4), 1133-1139, 1970.

Camp, T. R., Field estimates of oxygen balance parameters, Proceedings of The American Society of Civil Engineers, 91, No. SA5, 1, October 1965.

Chase, E. S., and A. F. Ferullo, Oxygen demand exerted by leaves stored under water, Journal of the New England Water Works Association, 71, 307-312, 1957.

Davison, R. C., W. P. Breese, C. E. Warren, and P. Doudoroff, Experiments on the dissolved oxygen requirements of cold water fishes, Sewage and Industrial Wastes, 31, 950-966, 1959.

Doudoroff, P., and D. C. Shumway, Dissolved oxygen criteria for the protection of fish, In: A Symposium on Water Quality Criteria to Protect Aquatic Life, ed. by E. L. Cooper, American Fisheries Society, Special Publication No. 4, p. 13-19, 1967.

Fair, G. W., J. C. Geyer, and D. A. Okum, Elements of water supply and waste water disposal, Second Ed, Wiley and Sons, Inc., New York, 752 p., 1971.

Garside, E. T., Effects of oxygen in relation to temperature on the development of embryos of brook trout and rainbow trout, Journal of the Fisheries Research Board of Canada, 23, 1121-1134, 1966.

Graham, J. L. and F. D. Schaumburg, Pollutants leached from selected species of wood in log storage waters, Paper read at the 24th Purdue Industrial Waste Conference, Lafayette, Indiana, May 6, 1969.

Hach Chemical Company, Instructions for the Hach model 2173 monometric BOD apparatus, Ames, Iowa, 13 p., 1972.

Hall, J. D. and R. L. Lantz, Effects of logging on the habitat of coho salmon and cutthroat trout in coastal streams, In: Symposium on salmon and trout in streams, ed. by T. G. Northcote, Vancouver, University of British Columbia, p. 355-375, 1969.

Herrmann, R. B., C. E. Warren, and P. Doudoroff, Influence of oxygen concentration on the growth of juvenile coho salmon, Transactions of the American Fisheries Society, 89, 17-26, 1962.

Hodge, J. E. and R. T. Hofreiter, Determination of reducing sugars and carbohydrates, In: Methods in Carbohydrate Chemistry, Volume I., ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York, N. Y., p. 380, 1962.

Jeris, J. S., A rapid COD test, Water and Waste Engineering, p. 89-91, May, 1967.

Jones, J. R. E., The reactions of fish to water of low oxygen concentration, Journal of Experimental Biology, 29:403-415, 1952.

Lantz, R. L., Guidelines for stream protection in logging operations, Oregon State Game Commission, Portland, Oregon, 29 p., August, 1971.

Lavender, D. P. and R. L. Carmichael, Effect of three variables on mineral concentrations in Douglas-fir needles, Forest Science, 12(4), 441-446, 1966.

Mancy, K. H. and T. Jaffe, Analysis of dissolved oxygen in natural and waste waters, Cincinnati, 94 p., (U. S. Department of Health, Education and Welfare, Robert A. Taft Sanitary Engineering Center, Public Health Service Publication No. 999-WP-37), 1966.

McHugh, R. A., L. S. Miller, and T. E. Olsen, The ecology and naturalistic control of log pond mosquitoes in the Pacific Northwest, Portland, Oregon, Oregon State Board of Health, Division of Sanitation and Engineering, 87 p., 1964.

Naver, D. W., Effects of logging debris on fish production, In: Proceedings of a Sumposium on Forest Land Uses and Stream Environment, ed. by J. T. Krygier and J. D. Hall, Oregon State University, Corvallis, Oregon, p. 100-111, 1971.

Rektorys, K., Survey of applicable mathematics, Translated by Vyborny et al., English translation edited by staff of the Dept. of Mathematics, University of Surrey, M. I. T. Press, Cambridge, Mass., 1369 p., 1969.

Sawyer, C. N. and P. L. McCarty, Chemistry for sanitary engineers, Second Ed., McGraw-Hill Book Co., New York, N. Y., 518 p., 1967.

Schaumburg, F. D., and S. Atkinson, BOD_5 and toxicity associated with log leachates, Paper read before the meeting of the Western Division of the American Fisheries Society, Victoria, British Columbia, July 13-16, 1970.

Shellford, V. E., and W. C. Allee, The reactions of fishes to gradients of dissolved atmospheric gases. Journal of Experimental Zoology, 14, 207-266, 1913.

Sheridan, W. L., Waterflow through a salmon spawning riffle in Southeastern Alaska, U. S. Fisheries and Wildlife Service, Special Scientific Report, Fisheries No. 407, 20 p., 1962.

Shumway, D. L., C. E. Warren, and P. Doudoroff, Influence of oxygen concentrations and water movement on the growth of steelhead trout and coho salmon embryos, Transactions of the American Fisheries Society, 93, 342-356, 1964.

Singleton, V. L., and J. A. Rossi, Jr., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture, 1663), 114-158, 1965.

Slack, K., Effect of tree leaves on water quality in the Cocopon River, West Virginia. U. S. Geological Survey Professional Paper 475-D, p. D181-D185, 1964.

Slack, K. and H. R. Feltz. Tree leaf control on low flow water quality in a small Virginia stream. Environmental Science and Technology. 2(2):126-131, 1968.

Smith, H. G. Unpublished research on weight, size and persistance of needles of Douglas-fir, western hemlock, western red alder, and other British Columbia Conifers, University of British Columbia, Vancouver, B.C. July, 1970.

Somogyi, M., Determination of blood sugar, The Journal of Biological Chemistry, 160, 69-73, 1945.

Streeter, H. W., and E. B. Phelps, A study of the pollution and natural purification of the Ohio River, Public Health Bulletin 146, 75 pp, U. S. Public Health Service, U. S. Department of Health, Education, and Welfare, Washington, D.C., Feb., 1925.

Truesdale, G. A., A. L. Downing, and G. F. Lowden, The solubility of oxygen in pure water and sea-water, Journal of Applied Chemistry 5, 53-62, 1955.

Vaux, W. G., Interchange of stream and intragravel water in a salmon spawning riffle, U. S. Fish and Wildlife Service, Special Scientific Report, Fisheries No. 405, 11 p., 1968.

Warren, C. E., Biology and water pollution, W. B. Sanders Co., Philadelphia, Pa., 434 p., 1971.

Weast, R. C., Handbook of chemistry and physics, 45 ed, The Chemical Rubber Co., Cleveland, Ohio, 1964.

Whitmore, C. M., C. E. Warren, and P. Doudoroff, Avoidance reactions of salmonid and centrarchid fishes to low oxygen concentrations, Transactions of the American Fisheries Society, 89, 17-26, 1960.

Zerrudo, J. V., Douglas-fir bark; water soluble carbohydrates and alkaline degradation of a xylon, Ph. D. Thesis, Oregon State University, Corvallis, Oregon. 156 p., 1973.

APPENDICES

APPENDIX A

DERIVATION OF THE OXYGEN DEFICIT EQUATION

(Equation 7)

Notation:

D	= Oxygen deficit relative to saturation	mg/l
D_a	= Oxygen deficit relative to saturation at t = 0	mg/l
e	= Base of the natural logarithms (2.71828)	none
K_1	= Deoxygenation rate coefficient (base e)	1/days
k_1	= deoxygenation rate coefficient (base 10)	1/days
K_2	= reaeration rate coefficient (base e)	1/days
k_2	= Reaeration rate coefficient (base 10)	1/days
L	= BOD concentration	mg/l
L_a	= BOD concentration at t = 0	mg/l
t	= time	days

Given:

$$\frac{dL}{dt} = -K_1 L \qquad (30)$$

$$\frac{dD}{dt} = -K_2 D \qquad (31)$$

Expresssing (30) in terms of rate change in deficit over time yields:

$$\frac{dD}{dt} = K_1 L \qquad (32)$$

Combining (31) and (32):

$$\frac{dD}{dt} = K_1 L - K_2 D \tag{33}$$

Solving (30) for L

$$\frac{dL}{L} = -K_1 dt \tag{34}$$

Integrating between the limits La and L, and 0 and t, where La = L at t = 0:

$$\int_{La}^{L} \frac{dL}{L} = -K_1 \int_{o}^{t} dt \tag{35}$$

$$L = La\, e^{-K_1 t} \tag{36}$$

Substituting (36) into (33) yields:

$$\frac{dD}{dt} = K_1 La\, e^{-K_1 t} - K_2 D \tag{37}$$

Separating variables and dividing both sides by $K_1 La$:

$$\frac{d\left(\frac{D}{K_1 La}\right)}{dt} + K_2 \frac{D}{K_1 La} = e^{-K_1 t} \tag{38}$$

Equation (38) is a first order ordinary differential equation of the general form:

$$\frac{dy}{dx} + ay = ce^{bx} \tag{39}$$

where $y = \frac{D}{K_1 La}$, $x = t$, $a = K_2$, $c = 1$, and $b = -K_1$

The general solution to (39) may be found in Survey of Applicable Mathematics by Rektorys (1960) on page 832.

$$Y = \left(\frac{c}{a+b}\right)^{e^{bx}} + Ce^{-ax} \qquad (40)$$

Applying the solution (40) to (38):

$$\frac{D}{K_1 La} = \left(\frac{1}{-K_1 + K_2}\right) e^{-K_1 t} + ce^{-K_2 t} \qquad (41)$$

Multiplying both sides by $K_1 La$ and letting $K_1 La \cdot C = C'$ equation (42) is obtained:

$$D = \left(\frac{1}{K_2 - K_1}\right) e^{-K_1 t} + C' e^{-K_2 t} \qquad (42)$$

To determine the value of the arbitrary constant C', solve equation (42) for C' at $t = 0$.

$$C' = D_a - \frac{K_1 La}{K_2 - K_1} \qquad (43)$$

In (43), D_a is the oxygen deficit at $t = 0$.

Substituting (43) into (42):

$$D = \left(\frac{1}{K_2 - K_1}\right) e^{-K_1 t} + \left(D_a - \frac{K_1 La}{K_2 - K_1}\right) e^{-K_2 t} \qquad (44)$$

Simplifying:

$$D = \frac{K_1 La}{K_2 - K_1} \left(e^{-K_1 t} - e^{-K_2 t} \right) + Dae^{-K_2 t} \qquad (45)$$

Equation (45) may also be expressed in terms of $\log_{10}$:

$$D = \frac{k_1 La}{k_2 - k_1} \left(10^{-k_1 t} - 10^{-k_2 t} \right) + Da\ 10^{-k_2 t} \qquad (46)$$

APPENDIX B

SOLUTION OF THE WATER BATH HEATING PROBLEM

The Problem: Determine at what temperature water entering the bath should be to raise the temperature of the solution in the bath from 12.8° C to 35° C in eight hours.

Notation:

r	rate of water into and out of the bath	l/hr
t	time	hr
T(t)	temperature of water in the bath at time t	°C
T_w	temperature of water coming into the bath	°C
v	volume of water in bath	cm^3

Assumptions:

1. The only major heat gain or loss of the water volume is from the inputs and outputs of water.
2. The water volume is completely mixed.
3. The water volume is constant with time.

Equation Derivation:

$$\begin{bmatrix}\text{Rate change of heat within the volume}\end{bmatrix} = \begin{bmatrix}\text{Rate input of heat into the volume}\end{bmatrix} - \begin{bmatrix}\text{Rate output of heat from the volume}\end{bmatrix} \qquad (18)$$

Given:

1. The rate input of heat is $T_w r$
2. The rate output of heat is $T(t)r$

3. The total heat in the volume at anytime t is vT(t). Therefore the rate change of temperature within the volume is d[vT(t)]/dt.

Substituting these relations into (18) yields equation (19).

$$\frac{d[vT(t)]}{dt} = T_w r - T(t) = [T_w - T(t)]r \qquad (19)$$

Since v is constant with time, (19) may be written as:

$$\frac{v\,dt(t)}{dt} = [T_w - T(t)]r \qquad (20)$$

Dividing both sides of (20) by v yields (21).

$$\frac{dT(t)}{dt} = [T_w - T(t)]\frac{r}{v} \qquad (21)$$

Equation (21) may be solved for T(t) by using a general solution presented in Survey of Applicable Mathematics by Rektorys (1969) on page 832.

$$\frac{dy}{dx} + ay = c \qquad (22)$$

The solution to (22) is:

$$y = \frac{c}{a} + Ce^{-ax} \qquad (23)$$

Equation (24) is obtained by algebraically rearranging (21) into the form of (22).

$$\frac{dT(t)}{dt} + \frac{r}{v}[T(t)] = \frac{r}{v}(T_w) \tag{24}$$

Applying the solution to (23):

$$T(t) = \frac{r/v(T_w)}{r/v} + Ce^{-\frac{r}{v}(t)} \tag{25}$$

$$T(t) = T_w + Ce^{-\frac{r}{v}(t)} \tag{26}$$

Solution of the Heating Problem:

Given the following initial value conditions: $T = 12.8°\,C$ at $t = 0$ and $T = 35.0°\,C$ at $t = 8$, and $v = 240\ cm^3$ and $r = 60\ l/hr$. Substituting the initial value conditions into equation (26) and solving for T_w yields:

$$T_w = \frac{35.0 - 12.8\,e^{-\frac{r}{v}(8)}}{1 - e^{-\frac{r}{v}(8)}} \tag{27}$$

Substituting the values of r and v into (27) and solving:

$$T_w = \frac{35.0 - 12.8\,e^{-\frac{60}{240}(8)}}{1 - e^{-\frac{60}{240}(8)}} \tag{28}$$

$$T_w = 38.46 \simeq 38.5 \tag{29}$$

APPENDIX C

THEORETICAL OXIDATION OF NITROGEN IN A DOUGLAS-FIR NEEDLE

Given: $$NH_3 + 1\tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrosomonas}} HNO_2 + H_2O + \text{Energy} \quad (16)$$

$$NO_2^- + \tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrobactor}} NO_3^- + \text{Energy} \quad (17)$$

Assume one gram of Douglas-fir needles contain 1.5% nitrogen (Lavender and Carmichael, 1966) in the form NH_3-N. From equations (16) and (17) it is apparent that two moles of O_2 are necessary to convert one mole NH_3-N to one mole NO_3^--N. Since 0.015 gram of NH_3-N is equal to 8.82×10^{-4} moles NH_3-N:

$$x = \frac{0.015}{17} \text{ moles } NH_3\text{-N}$$

$$x = 8.82 \times 10^{-4} \text{ moles } NH_3\text{-N}$$

The amount of oxygen needed to oxidize 8.82×10^{-4} moles NH_3-N to NO_3^--N is:

$$(8.82 \times 10^{-4})(64) = .056 \text{ gram of } O_2$$

or

$$56 \text{ mg of } O_2.$$

APPENDIX D

Tables

Table 20. Cumulative standard temperature BOD exerted in mg O_2/gm (dry weight) by Douglas-fir, western hemlock, and red alder leaves over 90 days.

Species	Bottle Number	Day Number									
		1	2	3	4	5	6	7	8	9	10
Douglas-fir	1	4.1	16.5	39.9	60.5	71.4	-1.0[a]	74.2	74.2	74.2	74.2
	2	6.9	16.5	26.1	44.0	59.1	-1.0	66.0	71.4	78.3	81.0
	3	9.6	17.9	37.1	63.2	71.4	-1.0	89.3	96.2	101.7	105.8
	4	4.1	11.0	19.2	34.3	49.5	-1.0	50.8	50.8	50.8	50.8
Control	5	0	0	0	0	0	0	0	0	0	0
Western hemlock	6	7.0	12.7	19.7	26.8	33.8	40.9	48.0	55.0	67.7	76.1
	7	8.5	15.5	24.0	31.0	39.5	46.5	60.6	84.6	115.6	124.1
	8	7.0	15.5	24.0	31.0	39.5	48.0	53.6	59.2	67.7	70.5
	9	7.0	14.1	22.6	29.6	38.1	46.5	52.2	57.8	64.9	67.7
Control	10	0	0	0	0	0	0	0	0	0	5.0
Red alder	11	27.0	44.4	59.8	67.5	77.2	81.0	86.8	98.4	102.2	108.0
	12	19.3	34.7	54.0	69.5	81.0	98.4	123.5	133.1	144.7	152.4
	13	27.0	44.4	59.8	69.5	79.1	90.7	100.3	110.0	115.8	121.5
	14	27.0	44.4	57.9	69.5	79.1	92.6	102.2	111.9	117.7	123.5
Control	15	0	0	0	0	0	2.5	2.5	2.5	5.0	5.0

[a] The symbol "-1.0" denotes a day in which the BOD was not recorded.

Table 20. Continued

Bottle Number	Day Number									
	11	12	13	14	15	16	17	18	19	20
1	-1.0	74.2	74.2	74.2	74.2	74.2	74.2	74.2	74.2	-1.0
2	-1.0	93.4	97.5	101.7	105.8	109.9	112.7	116.8	119.5	-1.0
3	-1.0	112.7	115.4	118.1	120.9	125.0	127.8	130.5	133.3	-1.0
4	-1.0	53.6	56.3	60.5	64.6	68.7	70.1	72.8	75.6	-1.0
5	-1.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
6	83.2	91.7	97.3	102.9	107.2	111.4	118.4	-1.0	-1.0	132.5
7	132.5	141.0	150.9	156.5	162.1	165.0	169.2	-1.0	-1.0	174.8
8	70.5	74.7	77.6	86.0	86.0	86.0	90.2	-1.0	-1.0	98.7
9	67.7	67.7	67.7	67.7	67.7	67.7	67.7	-1.0	-1.0	67.7
10	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
11	115.8	119.6	123.5	127.3	-1.0	133.1	137.0	138.9	140.8	148.6
12	165.9	175.6	183.3	192.9	-1.0	206.4	208.4	210.3	212.2	216.1
13	125.4	129.3	133.1	137.0	-1.0	142.8	146.6	148.6	150.5	156.3
14	129.3	133.1	138.9	144.7	-1.0	150.5	164.0	167.9	169.8	177.5
15	5.0	5.0	7.5	7.5	-1.0	7.5	7.5	7.5	7.5	7.5

Table 20. Continued

Bottle Number	Day Number									
	21	22	23	24	25	26	27	28	29	30
1	-1.0	74.2	74.2	76.9	76.9	79.7	79.7	-1.0	79.7	-1.0
2	-1.0	119.5	120.9	122.3	123.7	125.0	125.0	-1.0	125.0	-1.0
3	-1.0	134.6	134.6	134.6	134.6	134.6	134.6	-1.0	134.6	-1.0
4	-1.0	82.4	82.4	82.4	82.4	82.4	82.4	-1.0	82.4	-1.0
5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	-1.0
6	138.2	149.5	156.5	160.7	165.0	-1.0	172.0	-1.0	177.7	180.5
7	176.2	179.1	183.3	186.1	187.5	-1.0	189.0	-1.0	191.8	191.8
8	98.7	98.7	98.7	98.7	101.5	-1.0	104.3	-1.0	108.6	111.4
9	69.1	71.9	71.9	73.3	73.3	-1.0	73.3	-1.0	74.7	74.7
10	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	-1.0	-1.0
11	152.4	154.3	156.3	160.1	162.0	164.0	165.9	169.8	-1.0	-1.0
12	218.0	219.9	221.9	223.8	231.5	239.2	247.0	252.7	-1.0	-1.0
13	158.2	160.1	162.0	164.0	165.9	167.9	169.8	171.7	-1.0	-1.0
14	179.4	181.3	185.2	187.1	189.1	192.9	194.9	198.7	-1.0	-1.0
15	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	-1.0	-1.0

Table 20. Continued

Bottle Number	Day Number									
	31	32	33	34	35	36	37	38	39	40
1	79.7	79.7	79.7	-1.0	79.7	79.7	79.7	79.7	79.7	-1.0
2	125.0	125.0	125.0	-1.0	125.0	125.0	125.0	125.0	126.4	-1.0
3	134.6	134.6	134.6	-1.0	134.6	136.0	136.0	137.4	137.4	-1.0
4	82.4	82.4	82.4	-1.0	82.4	82.4	82.4	82.4	82.4	-1.0
5	5.0	5.0	5.0	5.0	7.5	7.5	-1.0	7.5	-1.0	7.5
6	180.5	-1.0	184.7	187.5	187.5	190.3	190.3	-1.0	190.3	190.3
7	193.2	-1.0	193.2	193.2	193.2	193.2	193.2	-1.0	194.6	194.6
8	114.2	-1.0	118.4	121.3	124.1	128.3	134.0	-1.0	148.0	155.1
9	74.7	-1.0	76.1	76.1	76.1	76.1	76.1	-1.0	77.6	79.0
10	5.0	5.0	10.0	10.0	10.0	10.0	-1.0	10.0	-1.0	10.0
11	175.6	177.5	177.5	179.4	183.3	183.3	-1.0	189.1	189.1	208.4
12	262.4	270.1	274.0	274.0	274.0	274.0	-1.0	274.0	-1.0	274.0
13	179.4	181.3	181.3	183.3	187.1	189.1	-1.0	192.9	-1.0	198.7
14	208.4	214.1	216.1	219.9	223.8	227.7	-1.0	239.2	-1.0	252.7
15	10.0	10.0	20.0	20.0	20.0	20.0	-1.0	20.0	-1.0	20.0

Table 20. Continued

Bottle Number	Day Number									
	41	42	43	44	45	46	47	48	49	50
1	79.7	79.7	-1.0	79.7	79.7	79.7	-1.0	79.7	-1.0	82.4
2	126.4	126.4	-1.0	126.4	127.8	127.8	-1.0	127.8	-1.0	129.1
3	137.4	137.4	-1.0	138.8	140.1	140.1	-1.0	140.1	-1.0	140.1
4	82.4	82.4	-1.0	82.4	82.4	82.4	-1.0	82.4	-1.0	82.4
5	7.5	7.5	-1.0	7.5	7.5	7.5	7.5	7.5	-1.0	7.5
6	-1.0	191.8	191.8	191.8	-1.0	193.2	-1.0	194.6	194.6	194.6
7	-1.0	194.6	194.6	194.6	-1.0	194.6	-1.0	194.6	196.0	196.0
8	-1.0	163.6	166.4	170.6	-1.0	170.6	-1.0	172.0	173.4	174.8
9	-1.0	79.0	79.0	79.0	-1.0	79.0	-1.0	79.0	79.0	79.0
10	10.0	10.0	-1.0	10.0	10.0	10.0	10.0	10.0	-1.0	10.0
11	210.3	212.2	-1.0	216.1	219.9	221.9	223.8	227.7	-1.0	229.6
12	274.0	274.0	-1.0	275.9	277.8	277.8	277.8	277.8	-1.0	277.8
13	202.6	206.4	-1.0	212.2	216.1	219.9	221.9	225.7	-1.0	233.4
14	258.5	264.3	-1.0	274.0	277.8	279.7	281.7	281.7	-1.0	281.7
15	20.0	20.0	-1.0	20.0	20.0	20.0	20.0	20.0	-1.0	20.0

Table 20. Continued

Bottle Number	Day Number									
	51	52	53	54	55	56	57	58	59	60
1	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
2	130.5	130.5	130.5	130.5	131.9	131.9	133.3	133.3	133.3	133.3
3	141.5	141.5	141.5	141.5	141.5	141.5	141.5	141.5	141.5	144.3
4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
5	7.5	-1.0	7.5	7.5	7.5	-1.0	10.0	-1.0	10.0	10.0
6	194.6	194.6	194.6	196.0	196.0	196.0	196.0	196.0	196.0	197.4
7	196.0	196.0	196.0	196.0	196.0	197.4	197.4	197.4	197.4	197.4
8	174.8	174.8	174.8	174.8	176.2	176.2	176.2	176.2	176.2	176.2
9	79.0	79.0	79.0	79.0	70.0	79.0	79.0	79.0	79.0	79.0
10	10.0	-1.0	10.0	10.0	10.0	-1.0	10.0	-1.0	10.0	10.0
11	237.3	-1.0	245.0	248.9	254.7	-1.0	260.4	-1.0	264.3	266.2
12	277.8	-1.0	277.8	277.8	277.8	-1.0	277.8	-1.0	277.8	277.8
13	237.3	-1.0	248.9	250.8	254.7	-1.0	258.5	-1.0	260.4	262.4
14	281.7	-1.0	283.6	283.6	285.5	-1.0	285.5	-1.0	287.5	287.4
15	20.0	-1.0	20.0	20.0	20.0	-1.0	20.0	-1.0	20.0	22.5

Table 20. Continued

Bottle Number	Day Number									
	61	62	63	64	65	66	67	68	69	70
1	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
2	133.3	133.3	134.6	134.6	134.6	134.6	134.6	136.0	136.0	136.0
3	144.3	144.3	144.3	144.3	145.6	145.6	145.6	145.6	147.0	147.0
4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
6	198.8	200.2	200.2	202.2	200.2	200.2	200.2	200.2	200.2	200.2
7	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4
8	176.2	176.2	176.2	176.2	176.2	176.2	176.2	176.2	176.2	176.2
9	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0
10	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
11	266.2	268.2	268.2	268.2	268.2	268.2	268.2	270.1	270.1	270.1
12	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8
13	262.4	262.4	262.4	262.4	262.4	264.3	264.3	264.3	264.3	264.3
14	289.4	289.4	291.3	291.3	291.3	293.2	293.2	295.2	295.2	295.2
15	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5

Table 20. Continued

Bottle Number	Day Number									
	71	72	73	74	75	76	77	78	79	80
1	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
2	136.0	136.0	136.0	136.0	137.4	137.4	137.4	137.4	137.4	137.4
3	147.0	147.0	147.0	147.0	147.0	147.0	147.0	148.4	148.4	148.4
4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
6	201.6	201.6	201.6	201.6	201.6	201.6	201.6	201.6	201.6	201.6
7	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4
8	176.2	176.2	177.7	177.7	177.7	177.7	177.7	177.7	177.7	177.7
9	89.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0
10	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
11	272.0	272.0	272.0	272.0	275.9	275.9	275.9	277.8	277.8	277.8
12	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8
13	264.3	266.2	266.2	268.2	268.2	268.2	268.2	270.1	270.1	272.0
14	297.1	297.1	299.0	299.0	299.0	301.0	302.9	302.9	304.8	304.8
15	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5

Table 20. Continued

Bottle Number	Day Number									
	81	82	83	84	85	86	87	88	89	90
1	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
2	137.4	137.4	138.8	138.8	138.8	138.8	138.8	138.8	138.8	138.8
3	148.4	149.8	149.8	149.8	149.8	149.8	149.8	149.8	149.8	149.8
4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4	82.4
5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
6	201.6	201.6	201.6	201.6	201.6	201.6	201.6	201.6	201.6	201.6
7	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4	197.4
8	177.7	177.7	177.7	177.7	177.7	177.7	177.7	177.7	177.7	177.7
9	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0
10	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
11	279.5	279.5	279.5	281.7	281.7	281.7	281.7	281.7	281.7	281.7
12	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8	277.8
13	272.0	272.0	274.0	274.0	275.9	275.9	275.9	275.9	277.8	277.8
14	306.8	306.8	306.8	306.8	308.7	308.7	308.7	308.7	308.7	308.7
15	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5

Table 21. Cumulative standard temperature BOD exerted in mg O_2/gm (dry weight) by first, third, and fifth year Douglas-fir twigs over 45 days. The bottles containing 2 grams third year twigs have BOD's in mg O_2/2 gm.

Twig Age	Bottle Number	Day Number									
		1	2	3	4	5	6	7	8	9	10
First year	1	6.2	11.1	17.3	21.0	26.0	28.5	31.0	-1.0[a]	43.3	-1.0
	2	7.4	8.7	9.9	18.6	47.0	59.4	71.8	-1.0	89.2	-1.0
	3	2.5	16.1	24.8	31.0	42.1	64.4	71.8	-1.0	78.0	-1.0
Third year	4	7.9	9.0	9.0	9.0	9.0	11.2	14.6	-1.0	20.2	-1.0
	5	2.2	5.6	13.5	19.1	37.1	39.4	45.0	-1.0	51.7	-1.0
	6	1.1	6.8	10.1	13.5	18.0	20.2	24.8	-1.0	30.4	-1.0
Fifth year	7	1.1	1.1	3.3	5.5	11.0	13.2	16.5	-1.0	24.2	-1.0
	8	2.2	5.5	8.8	12.1	16.5	22.0	25.3	-1.0	30.8	-1.0
	9	2.2	6.6	8.8	17.6	26.4	28.6	30.8	-1.0	35.1	-1.0
Third year[b] (2 grams)	10	3.4	11.2	18.0	22.5	23.9	29.2	29.5	-1.0	29.5	-1.0
	11	5.6	6.8	6.8	6.8	6.8	9.0	9.0	-1.0	9.0	-1.0
	12	5.6	14.6	22.5	28.1	34.9	40.5	50.6	-1.0	67.5	-1.0
Controls	13	0	0	0	0	0	0	0	-1.0	0	-1.0
	14	0	0	0	0	0	0	0	-1.0	0	-1.0
	15	2.5	2.5	2.5	2.5	2.5	2.5	2.5	-1.0	2.5	-1.0

[a] The symbol "-1.0" denotes a day in which the BOD was not recorded.

[b] The bottles containing 2 grams of third year twigs were run only for 10 days.

Table 21. Continued

Bottle Number	Day Number									
	11	12	13	14	15	16	17	18	19	20
1	44.6	-1.0	44.6	-1.0	-1.0	52.0	53.2	55.7	58.2	60.7
2	105.3	-1.0	121.4	-1.0	-1.0	142.4	154.8	162.2	167.2	169.7
3	83.0	-1.0	87.9	-1.0	-1.0	99.1	104.0	109.0	113.9	117.6
4	24.8	-1.0	31.5	-1.0	-1.0	40.5	42.7	45.0	47.2	49.5
5	58.5	-1.0	65.2	-1.0	-1.0	72.0	75.4	77.6	78.7	82.1
6	34.9	-1.0	39.4	-1.0	-1.0	46.1	48.4	50.6	51.7	54.0
7	26.4	-1.0	33.0	-1.0	-1.0	37.4	38.5	39.6	41.8	41.8
8	34.0	-1.0	38.5	-1.0	-1.0	42.9	44.0	45.0	47.2	48.3
9	37.4	-1.0	40.7	-1.0	-1.0	45.0	47.2	49.4	51.6	52.7
13	0	-1.0	0	-1.0	-1.0	0	0	0	0	0
14	0	-1.0	0	-1.0	-1.0	0	0	0	0	0
15	2.5	-1.0	2.5	-1.0	-1.0	2.5	2.5	2.5	2.5	2.5

Table 21. Continued

Bottle Number	Day Number									
	21	22	23	24	25	26	27	28	29	30
1	63.1	64.4	65.6	-1.0	70.6	71.8	-1.0	71.8	71.8	71.8
2	172.1	174.6	174.6	-1.0	174.6	174.6	-1.0	174.6	174.6	174.6
3	120.1	123.8	125.1	-1.0	131.3	133.7	-1.0	133.7	133.7	133.7
4	51.7	54.0	56.2	-1.0	59.6	60.7	-1.0	63.0	64.1	65.2
5	84.4	87.7	88.9	-1.0	92.2	93.4	-1.0	95.6	96.7	97.9
6	56.2	59.6	61.9	-1.0	67.5	69.7	-1.0	75.4	76.5	79.9
7	42.9	44.0	45.0	-1.0	48.3	49.4	-1.0	52.7	53.8	54.9
8	50.5	52.7	52.7	-1.0	53.8	53.8	-1.0	53.8	56.0	57.1
9	56.0	57.1	59.3	-1.0	64.8	67.0	-1.0	71.4	74.7	78.0
13	0	0	0	-1.0	0	0	-1.0	0	0	0
14	0	0	0	-1.0	0	0	-1.0	0	0	0
15	2.5	2.5	2.5	-1.0	2.5	2.5	-1.0	2.5	2.5	2.5

Table 21. Continued

Bottle Number	Day Number									
	31	32	33	34	35	36	37	38	39	40
1	-1.0	71.8	-1.0	71.8	-1.0	-1.0	-1.0	74.3	-1.0	74.3
2	-1.0	174.6	-1.0	174.6	-1.0	-1.0	-1.0	177.1	-1.8	179.6
3	-1.0	133.7	-1.0	136.2	-1.0	-1.0	-1.0	136.2	-1.0	137.5
4	-1.0	68.6	-1.0	69.7	-1.0	-1.0	-1.0	69.7	-1.0	69.7
5	-1.0	100.1	-1.0	103.5	-1.0	-1.0	-1.0	103.5	-1.0	104.6
6	-1.0	79.9	-1.0	84.4	-1.0	-1.0	-1.0	86.6	-1.0	88.9
7	-1.0	58.2	-1.0	60.4	-1.0	-1.0	-1.0	62.6	-1.0	62.6
8	-1.0	59.3	-1.0	62.6	-1.0	-1.0	-1.0	65.9	-1.0	65.9
9	-1.0	84.6	-1.0	86.8	-1.0	-1.0	-1.0	87.9	-1.0	91.2
13	-1.0	0	-1.0	0	-1.0	-1.0	-1.0	0	-1.0	0
14	-1.0	0	-1.0	0	-1.0	-1.0	-1.0	0	-1.0	0
15	-1.0	2.5	-1.0	2.5	-1.0	-1.0	-1.0	2.5	-1.0	2.5

Table 21. Continued

Bottle Number	Day Number				
	41	42	43	44	45
1	-1.0	76.8	-1.0	78.0	78.0
2	-1.0	179.6	-1.0	179.6	179.6
3	-1.0	137.5	-1.0	137.5	138.7
4	-1.0	69.7	-1.0	72.0	73.1
5	-1.0	105.7	-1.0	108.0	108.0
6	-1.0	92.2	-1.0	94.5	96.7
7	-1.0	63.7	-1.0	64.8	65.9
8	-1.0	67.0	-1.0	68.1	69.2
9	-1.0	94.5	-1.0	96.7	100.0
13	-1.0	0	-1.0	0	0
14	-1.0	0	-1.0	0	0
15	-1.0	2.5	-1.0	2.5	2.5