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A series of log storage experiments were conducted to determine whether leachates derived from water storage of logs are acutely toxic to fish. Log segments approximately 18 inches long and 16 inches in diameter were stored in tanks and held submerged for a period of 7 days. The holding water containing leached materials was made toxic with mercury to retard biological decomposition of the leached substances. Mercury was selectively removed from leachate samples by chelation prior to biochemical oxygen demand (BOD) and bioassay testing.

Trout and salmon fry were subjected to the leachate water in short term acute bioassay tests. Results are reported as a median tolerance limit, (TLm), i. e., the concentration of leachate at which 50 percent of the test fish died for any given exposure time. Leachates were also tested for BOD_5, BOD k-rate, chemical oxygen demand (COD), wood sugar and Pearl Benson Index (PBI).

Test results show that leachates from Douglas fir stored in fresh water exert a slight acute toxicity to fish. A TLm_{96} of 20 percent leachate by volume, for a 50 year old Douglas fir log, was the most toxic leachate observed. Leachates from ponderosa pine, hemlock and an older fir log stored under identical conditions produced no measurable acute toxicity. Leachates contained a significant quantity of BOD and PBI exerting substances. The highest BOD_5, (1.36 g/ft^2 of submerged surface area) was exerted by leachate from a ponderosa pine log segment stored with bark removed. The highest PBI valve (12.5 g/ft^2), was observed for leachate from a young Douglas fir log segment. BOD:COD ratios and BOD k-rate ranged widely for the various leachates, but were relatively low which indicated a significant fraction of non-biodegradable substances. Hoffbuhr (9) also observed a high non-biodegradable fraction in samples taken from log storage ponds. Wood sugars were found to account for a large part of the degradable portion of leachates. Leachate from ponderosa pine log with bark intact exerted a high BOD and also contained the highest concentration of wood sugar observed, 0.84 g/ft^2.

BOD and Toxicity of Log Leachates

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BOD AND TOXICITY OF LOG LEACHATES

INTRODUCTION

Timber from forests of the Pacific Northwest represents a major economic product of the region. Wood is required for construction, production of pulp and paper, and for other wood related industries such as clothing fibre, plastics, and wood-based chemicals. Sawmills and pulpmills store a large inventory of logs to maintain production during periods of the year when logging is not possible. Frequently, logs damaged by fire or wind must be logged rapidly and in great number in order to salvage the value of the timber.

Flotation in water is a widely used method for storage of logs. Logs are stored in ponds, lakes, rivers, and estuaries. Degrading factors such as end splitting and insect damage are reduced due to the high moisture content of the logs in water storage.

Unfortunately, there are undesirable features associated with water storage of logs. Pollution of holding water by materials leached from logs while in storage could be detrimental to fish and other aquatic life. In particular, salmon and trout are susceptible to minor changes in their environment caused by many types of pollutants. The loss of salmon as a prominent fish specie would result in a tremendous loss to the sport and commercial fishery.

The purpose of the research reported herein was to evaluate the acute toxicity of log leachates to fish. Secondary objectives were the determination of biochemical oxygen demand (BOD), BOD decay rate, and the wood sugar content of leachate samples. The research should not be construed to be conclusive for all possible storage conditions, species of timber or types of fish.

LITERATURE SURVEY

Water soluble materials enter holding water during storage of logs. In a dissolved state, leached extractives from logs may contribute a toxicity to fish and other aquatic organisms. Although the literature is limited on this subject, some reported investigations have dealt with toxicity to fish from wood, bark extractives and waste from wood processing industries.

Toxicity Measurement

The physical apparatus and techniques used for measuring acute toxicity have been described by Warner (24), Doudoroff (5), and by Standard Methods (1). The static test for acute toxicity is applicable in most situations. The acute test is a short term measurement of lethal effect as compared to a long term observation of the test organism's reaction to an imposed environment. Results of the test are expressed as a median tolerance limit (TLm), i. e., the concentration of test solution which produces a 50 percent test organism kill over the test period. Procedures for determing TLm are described in Standards Methods, (ibid).

Some research work has been reported which describes toxicity resulting from wood and wood bark extractives. However, the majority of research has been centered around industrial effluents from

wood processing industries. Numerous research reports have described the toxicity of effluents from the Kraft Process (22, 25). Stewart and Cornich (20) studied the gross toxicity of wood and bark extractives. They tested for toxic effect of extractives on lobsters in an attempt to discover which species of wood were unsuitable as crating material for live storage. Douglas fir and several other wood species were found to be non-toxic whereas Western red cedar was toxic.

Wood and Wood Bark Extractive Studies

Tabata (21) used aqueous extracts from hemlock bark in toxicity studies on fish and other aquatic life from the bays of Japan. Aqueous extracts of hemlock bark were toxic to fish when the chemical oxygen demand of the sample was above 2 ppm.

Henriksen and Samdel (8) studied water extracts of bark. They used mostly Norweigen spruce obtained from a barking machine dump. After the bark was stored in water for 65 hours, the COD of the storage water measured 42, 200 mg/l per kg of bark. Seventy-two hour toxicity tests were conducted using salmon as the test organisms. Results indicated no toxicity effect when the COD of the test water was below 89 mg/l.

Sproul and Sharpe (19) used both hardwood and softwood bark under wet leaching conditions to study characteristics of wood bark

extractives. Under wet leaching conditions the bark was stored totally immersed in water and then "leached" by a shake and rinse technique. With softwood bark, results indicated a biochemical oxygen demand, (BOD), of 780 mg/l or 7.8 lbs per ton of bark for a six day storage period. Softwood bark had a COD of 2100 mg/l of 21 lbs per ton of bark. After 69 days of wet storage followed by "leaching", BOD and COD values were measured at 15 lbs per ton of bark and 27 lbs per ton of bark, respectively. Hardwood bark under wet conditions yielded 7.8 lbs of BOD per ton and 21 lbs of COD per ton after six days storage. BOD and COD were also measured after 69 days using hardwood bark extractives. Values of 33 lbs per ton of bark as BOD and 62 lbs per ton of bark as COD were recorded.

Graham and Schaumburg (7) recently reported a study dealing with leaching of logs in water storage. They used log segments of ponderosa pine and Douglas fir held totally immersed in water. Among the parameters determined from the storage water were COD and Pearl-Benson Index (PBI). Results for ponderosa pine logs in fresh water indicated a COD of 3.9 g/ft^2 of submerged surface area and a PBI of 15 g/ft^2 after 40 days of storage. Douglas fir yielded a COD of 3.0 g/ft^2 and a PBI of 4 g/ft^2 after 37 days of storage. The results cited herein were for logs with bark intact and stored with ends sealed to prevent leaching from the ends.

The chemical composition of the bark from ponderosa and sugar pine was studied by Kurth, Hubbard and Humphrey (12). They determined a high reducing sugar content as characteristic of the ponderosa pine. Reducing sugar content ranged between three and six percent, (based on oven dry bark weight), whereas Douglas fir bark contained one-tenth that quantity.

McHugh, Miller and Olsen (14) reported reducing sugar levels in water from log ponds in the state of Oregon. Reducing sugar content ranged between 2.0 and 10.5 ppm for the ponds tested.

Leachate Preservation

Soluble substances liberated from logs during water storage must be allowed to accumulate to obtain a measure of toxicity. Biodegradation of the substances must be held to a minimum during the storage period to provide accurate and reproducible conditions for toxicity determinations. Heavy metal poisoning of the water storage environment with mercuric ion has been proven to be effective in microorganism control. Graham (7) demonstrated that a 2 ppm concentration of mercuric ion adequately arrests biological activity for a storage period of seven days.

Researchers have described the toxic effect of mercuric and other heavy metal ions. Malacea (13) found mercuric ion to be destructive to the gills of fish. The gill destruction led to asphyxia. Water

Quality Criteria of California (3) described a study in which trout were killed in 24 hours at mercury concentrations of 9.2 to 37 ppm, using mercuric chloride as a mercury source.

Poisoning storage water with mercury interferes with subsequent biological determinations unless the metal ions are removed before biological testing. Mercuric ions used to preserve the log storage water in this research were removed from collected samples before biological testing with a chelating agent. The chelating resin and the theory of chelation are described later in this text.

Reaction Rate Studies of Biochemical Oxygen Demand

Raabe (16) conducted studies on the degradation of lignin and wood sugars using river water downstream from a Kraft mill effluent. He observed a fast initial degradation and a second slower degradation using BOD tests as the study parameter. The initial degradation followed a decay rate of 0.455 per day and was attributed to the oxidation of simple wood sugars. The second phase degradation followed a decay rate of 0.027 per day, and was attributed to the biodegradable portions of lignin-like compounds present. Thus a BOD curve was developed which had two characteristic slopes.

BOD determinations using pure carbohydrates were performed by Varma and Hall (23). They described a phenomenon similar to that presented by Raabe (16). Using 500 ppm solutions of various

monosaccharides they determined BOD and decay rate. Rates ranged between 0.215 per day and 0.084 per day for the sugars studied. An unusual BOD curve was observed for the simple carbohydrates. An initial hump, attributed to absorption of oxygen, was followed by a plateau on the curve. The plateau usually occurred after 24 hours of incubation and was attributed to a decline in the rate of oxygen use following the biodegradation of the simple sugars.

CHELATION AND ION EXCHANGE

Chelation results when a metal ion is coordinately bonded to another molecule in solution to form a complex. The binding or chelate molecule donates two electrons to form a coordinate bond with a metal ion. A complex between the chelate molecule and metal ion is the result of the coordinate bond. Molecules containing atoms of nitrogen, oxygen, and sulfur often serve as chelate molecules because they can readily donate two electrons for a covalent bond (4).

Hemoglobin is one example of a naturally occurring chelate. In blood, the iron of hemoglobin is held to four nitrogen atoms of one heme group and to two other nitrogen atoms of another heme group. The coordinate bonds between iron and nitrogen form the complex which is hemoglobin.

Brey (ibid.) describes the formation of a chelate complex with an instability constant. The constant is a number describing the equilibrium dissociation or mass balance between the complex and its components. The stability constant is also used and is merely the reciprocal of the instability constant. A qualitative example of the instability or stability concept is observed when a silver chloride precipitate is dissolved by the addition of ammonia. The ammonia and silver combine, as the precipitate dissolves. The complex is in equilibrium with both dissociation and formation occurring. The

stability constant provides a number to describe the relative tendency to form the complex.

Ion exchange occurs in solutions containing ions of different elements. Large molecules have ions bound to the lattice sites of the molecular structure. Ions compete for bonding sites according to the strength of the bond formed between the free ion and the bonding lattice site. Equilibrium is established between the ions in solution and the lattice sites of the molecule. Lattice sites serve as exchange sites when ions of stronger bonding affinity for the lattice position are added to a solution. Bound ions become free ions when a different ion can form a stronger bond at the lattice site.

Calcium ion removal in the water softening process is an example of ion exchange. A resin with sodium ion at the lattice sites is a common exchange media. Calcium ions replace sodium ions on the resin structure and are then removed from the solution with the resin molecule.

METHODS AND MATERIALS

Special Equipment and Supplies

Several items of equipment were required in this research study. Two 50-liter plexiglas tanks were constructed and used previously by Graham (7) in another log storage study. Two small ring clamps were secured to the inside bottom of each tank. The clamps held log segments completely submerged when the tanks were filled with water. The water was agitated by two magnetic stirrers installed beneath the storage tanks to impart motion to teflon coated stirring bars in the tank.

A Beckman Instruments Inc. DU spectrophotometer was required for colorimetric determination of the Pearl-Benson Index. A respirometer BOD device supplied by the Hach Chemical Co., was used to obtain data required to determine biochemical oxygen demand decay rate.

Chelex 100 [1] was selected as the mercury chelating agent in this research study. Chelex 100 is an analytical grade resin and has unusually high chelating preference for heavy metal cations, especially mercury and copper. Chelex 100 in sodium form has sodium ions bound covalently to oxygen atoms on the chelate molecule. Metal ions

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[1] Chelex 100 was obtained from Bio-Rad Laboratories, Richmond, California.

exerting a stronger preference for sharing of oxygen electrons, replace the sodium ions. Data supplied with Chelex 100 indicate that the mercuric ion possesses an affinity for the resin 1.06×10^3 times greater than that of the sodium. Cu^{++}, by comparison, has an affinity of 1.26×10^2 and Zn^{++} an affinity of 1.0, equal to that of sodium ion.

Douglas fir, ponderosa pine and hemlock wood species were selected for evaluation in water storage. Douglas fir and hemlock logs were obtained from a pulp mill in western Oregon. Freshly felled logs of both species were acquired with back intact. The two Douglas fir logs differed in age, one 50 years old, whereas the other fir log was approximately 120 years old. The hemlock log was from a tree approximately 50 years old. Ponderosa pine was cut from a 70 year old tree felled in central Oregon.

Paraffin wax was used as an end sealant. This procedure was described by Graham and Schaumburg (7). The end sealant eliminated end effects from the short log segments. They found significant differences between logs stored without sealing the ends and those logs stored with ends sealed.

Water for log storage and bioassay testing was taken from Oak Creek, a small stream flowing through Corvallis, Oregon and the Oregon State University campus. Oak Creek was used as a water source since the Oregon State University Water Pollution and Fisheries Laboratory has its bioassay test fish stock reared in Oak Creek

water. Bioassays were conducted at the Oak Creek laboratory. Oak Creek water was also used for log storage to eliminate differences in quality between bioassay and storage water.

Bioassay Test Organisms

Two types of fish were acquired for use as test organisms in toxicity determination. Chinook salmon, Oncorhynchus tshawytscha (Walbaum), approximately three months of age, were used until the supply was exhausted. The salmon were obtained in May, 1969 from the Department of Fisheries and Wildlife Hatchery, Netarts, Oregon. Kamloops rainbow trout, Salmo gairdneri (Richardson), also three months of age, were purchased from Trout Lodge Springs Hatchery, Soap Lake, Washington. The trout were obtained in June, 1969 and were used during the remainder of the study. All test fish were acclimated to Oak Creek water.

Analytical Methods

Pearl-Benson Index (PBI)

The concentration of tannin, lignin and other phenolic compounds leached from log segments while in water storage was determined by the standardized Pearl-Benson, or Nitroso method (2). PBI is a colorimetric determination of the quantity of lignin and other

phenolic compounds present in a sample. The PBI test is used to evaluate spent sulfite liquor (SSL) concentration in waste streams from pulp mills. A DU spectrophotometer was used to measure the color developed in the test. A standard SSL in a range of known concentrations was used to standardize test results. The standard was calcium SSL, (Orzan^{+}), made from a ten percent concentration of SSL.

Chemical Oxygen Demand (COD)

COD analyses were made using the Jeris "rapid" COD technique (11). The "standard" COD method (1) was applied to one series of samples to obtain a correlation between the rapid and standard procedures. Data which shows the standard method to be about 96 percent of the Jeris method, is presented in Table 9. Using log pond water, Hoffbuhr (9) also correlated the Jeris rapid method with the standard method. He found the rapid method to yield COD values between 96 percent and 100 percent of the values obtained using the standard method.

Biochemical Oxygen Demand (BOD)

Five day BOD analyses were made using 300 milliliter BOD bottles and applying the dilution technique described in Standard Methods (1). Seed for the BOD tests was obtained from a bench scale activated sludge unit in which the microorganisms were acclimated

to effluent from a fibreboard plant. The unit was batch fed daily to accomplish the acclimation of seed.

BOD Reaction Rate (k-rate)

Oxygen uptake data used in k-rate determination was obtained using the Hach manometric BOD apparatus. Interval oxygen uptake readings correspond to the BOD exerted. The determination of k-rate was accomplished by matching curves obtained from experimental data to standard curves obtained using a mechanical plotter in connection with the Oregon State University computer. To obtain a standard curve for matching with an experimental curve, 5-day BOD values from experimental data and an assumed series of k-rates were supplied as input to the computer program. A series of mechanically plotted curves was obtained. The closest fit between standard and experimental plots determined k-rate.

Reducing Sugar Content

Reducing sugar content was determined using techniques described by Somogyi (18) and the procedure presented by Hodge and Hofreiter (10). The titrimetric method applying the 1945 alkaline copper reducing agent was used.

Bioassay Technique for Toxicity Determination

Bioassays were conducted at the Oak Creek Laboratory. The test tanks were located in a constant temperature room. Test fish were acclimated to Oak Creek water at 14°C.

Each bioassay test unit consisted of a 2-gallon circular cardboard container fitted with a plastic liner. Six liters of water were placed into the container and a stream of air was bubbled slowly through the water for 24 hours before the test fish were introduced. A total of 10 fish were used in every unit and the number of dead fish counted visually at 24 hour intervals for a period of 96 hours. The water was not mechanically stirred and the fish were not fed during the 96 hour test period. No fish was used more than once and surviving fish were kept separated from the remaining unexposed test fish.

EXPERIMENTAL

Introduction

The goals of this research study were to determine acute toxicity and BOD associated with leachates derived from water storage of logs. To ascertain leachate toxicity, material leached from logs had to be preserved against biological decay until tested for toxicity. Preservation of the leachate material was accomplished by poisoning the log holding water with the heavy metal mercury. Toxicity tests using fish as test organisms and BOD tests using microorganisms, were conducted for samples of log storage water. Removal of the mercury ions was necessary before biological testing could be undertaken.

Tests were conducted on synthetic biodegradable samples to study the effectiveness of mercury as a preservative. After preservation, samples were subjected to a chelating process for mercury removal. Techniques for mercury preservation and removal by chelation were developed by Schaumburg (17). The necessity for mercury removal prompted development of a technique and standard procedure to be followed in these studies. Several mercury removal experiments were conducted to develop the standard procedure.

Determination of leachate toxicity to fish was undertaken following the development of a procedure for handling water samples obtained from the log storage system. Biochemical oxygen demand,

chemical oxygen demand, BOD k-rate, wood sugar content and PBI were determined to characterize the leachate samples.

Log segments used during this research were indigenous to the Pacific Northwest. The selection of wood species included the two major commercial softwoods of the region and a hardwood commonly logged in the Northwest.

Procedure

Sample Preservation

Two tests were conducted to illustrate sample protection against biodegradation. A synthetic sample of known biochemical oxygen demand was prepared. The sample contained equal parts, by weight, of glucose and glutamic acid. The standard bottle BOD technique (1) was performed to check the BOD of the synthetic sample.

A three-liter sample was prepared for each test. The sample was seeded with 15 milliliters of primary sewage from the Corvallis, Oregon sewage treatment plant. A volume of seeded dilution water was also prepared according to Standard Methods, ibid. BOD tests were conducted on the synthetic sample to determine its initial strength. The remainder of the three liter sample was divided into three parts of equal volume. One sample was incubated at 20°C for 24 hours. One sample was refrigerated at 4°C for 24 hours. The

remaining sample was incubated at 20°C for 24 hours after being poisoned with 5 mg/l Hg^{++} in the form of $HgCl_2$. BOD tests were performed on all samples following storage. The sample containing mercury ions was treated with 5 g/l Chelex 100 (200 mesh) for removal of mercury before biological testing. The resin and sample were mixed for ten minutes using a magnetic mixer, and then held quiescent for 20 minutes to permit settlement of the insoluble Chelex resin- Hg^{++} complex. Supernatant was decanted and used in the BOD test.

The second test program was conducted following the general procedure outlined above. Mercury ion concentration producing the preservative effect was reduced to 2 mg/l after personal communication with Schaumburg (17). After 24 hour storage, each of the three samples were filtered through a millipore filter apparatus. Standard BOD tests were then conducted on the filtered samples.

Acute Toxicity of Mercury

The acute toxicity of mercury was determined for the trout test fish. A mercuric chloride solution was pipeted into a series of bioassay test units. The quantity of mercuric chloride solution added to each unit was controlled to obtain a precise dilution series in the test units. Eleven units were arranged with concentrations ranging from zero to 2 mg/l Hg^{++} in the form of $HgCl_2$. Several dilutions

were made to ensure accuracy in obtaining low concentrations of mercuric ion in the test units.

Mercury Removal

Mercury removal experiments were patterned after the techniques used in the sample preservation tests. Initial mercury removal was attempted using Chelex 100 in 200 mesh size. Subsequent tests were carried out with Chelex 100 in 50 mesh size because of improved settling characteristics inherent with the larger particle size.

In every mercury removal experiment a 20 liter sample of Oak Creek water was made toxic by the addition of 2 mg/l Hg^{++}. After each sample was prepared, it was allowed to stand for seven days in a laboratory carboy. The storage period duplicated the storage time of log segments to be studied later in the research.

In the initial experiment, 5 g/l of 200 mesh Chelex 100 were added to the 20 liter sample. The sample and resin were stireed 20 minutes by an air driven rotary stirrer to obtain contact of the resin and Hg^{++} ions. The chelated sample was left standing for 24 hours, after which time the supernatant was siphoned into another carboy. Six bioassay test units were then arranged. These included a unit with Oak Creek water only, and a unit of Oak Creek water poisoned with 2 mg/l Hg^{++}. The chelated sample was tested together with

dilutions of 25, 50 and 75% by volume. All sample dilutions were made with Oak Creek water. Test organisms used for the 96-hour bioassay tests were chinook salmon fry adapted to Oak Creek water.

The poisoned sample used for the second mercury removal experiment, was chelated and allowed to settle as in the first experiment. In this experiment, however, supernatant was filtered through a glass mat following settling in an attempt to remove colloidal sized particles of resin from the decanted supernatant. Bioassays were conducted using the sample and a blank containing Oak Creek water made toxic with 2 mg/l Hg^{++}.

The test procedure was altered for the third mercury removal experiment to show the effect of a reduced dosage of chelating resin on both mercury removal and fish survival. Two 20 liter samples were used. One sample was made toxic with 2 mg/l Hg^{++}, as previously described, whereas the other sample contained unpoisoned Oak Creek water. Both samples were handled identically. One gram per liter of Chelex 100 in 200 mesh size was added to each sample. Both samples were mixed for 20 minutes and allowed to settle for 24 hours. Bioassay tests were conducted using chinook salmon as test organisms. As described above, a blank of Oak Creek water and a toxic control (2 mg/l Hg^{++}) were also bioassay tested.

The fourth experiment was similar to the third except the chelating resin was changed to 50 mesh size and mixing time was increased

to 30 minutes. The larger particle size of the chelating resin reduced the required settling time to one hour. Bioassay tests were conducted for the samples using chinook salmon as test organisms. A bioassay was also conducted on a blank test unit of unpoisoned Oak Creek water.

The final experiment was designed to determine the quantity of chelating agent required for effective mercury removal and to determine whether or not the required dosage was toxic to fish. Three 20 liter samples were prepared with Oak Creek water. Two of the samples contained 2 mg/l Hg^{++}. The other sample was an unpoisoned control containing no mercury. All samples were subjected to the same mixing and settling procedure as described above. Three grams per liter Chelex 100 was added to one toxic sample and 2 g/l to the other. The sample without mercury was dosed at 3 g/l. Bioassay tests were conducted as in previous experiments with chinook salmon as test organisms.

Log Storage, Bioassay and Leachate Testing

Log storage studies were conducted after mercury preservation and mercury removal techniques were established. Five leaching periods were evaluated by holding logs submerged in water for a period of time. After the holding period, water samples containing leachates were analyzed for toxicity to fish, BOD, BOD k-rate, COD, reducing sugar and PBI. All leaching periods were conducted in a

constant temperature room thermostatically controlled at 20°C ± 1°.

A standard procedure was followed for preparation of all logs before water storage. Two segments, approximately 18 inches (46 cm) long, were cut from the interior part of a larger log with a chain saw. One of the segments was stripped of its bark by hand or by a chisel. Each segment was measured for length and cross sectional dimensions. The log segment with bark left intact was prepared for the study by coating the ends with a layer of paraffin wax, as described by Graham, et al (7). A quantity of wax was melted in a pan and then ladeled onto the flat end surface of the log segment. A smooth layer of paraffin was achieved by distributing the molten material over the surface with a thin piece of cardboard.

Each log segment was stored in a plexiglas tank. A hook was driven into the ends of both logs to attach the log to the bottom of the storage tank. Following placement of the logs into the tanks a magnetic stirring bar, $1\frac{1}{4}$-inch long, was placed in each tank and the tanks were moved to the constant temperature room where storage was conducted. Each tank was placed on a wood support frame fitted with a magnetic stirrer.

Water obtained from Oak Creek was poured slowly into the storage tanks until each log segment was completely immersed. The logs being less dense than water, bouyed to the extension limit of the hooks. A distance of two inches (5 cm) above the tank bottom was normal.

The volume of water added to each tank was recorded and the magnetic mixers were started. A volume of mercuric chloride stock solution was pipeted into each tank to achieve a Hg^{++} concentration of 2 mg/l. Plastic covers were placed over the tanks to reduce evaporation losses. Daily observation of the apparatus was performed to insure proper functioning of the mixers and to make sure the logs stayed submerged.

The physical dimensions and approximate age of log sections, the volume of Oak Creek water used to submerge each log segment and the type of fish used as bioassay test organisms are presented in Table 1.

A storage period of seven days was selected. Graham (7) has shown that a significant portion of the leachate is released to the holding water in the first seven days of storage.

Chemical oxygen demand (COD) was determined for the Oak Creek water used as a storage medium. Water was obtained from Oak Creek at one week intervals to determine variability of the COD.

Leachate samples were collected after seven days of log storage. Approximately 26 liters of leachate were collected from each tank from a sample port near the bottom of the tank. Of the total sample volume, 20 liters were used for bioassay study and four liters were used in BOD, BOD k-rate and COD testing. The remaining 2 liters of sample was put into two separate plastic containers and

Table 1. Physical characteristics of logs and log storage conditions.

	Approx. Age (years)	Length (in.)	Length (cm.)	Diameter (in.)	Diameter (cm.)	Storage Water Volume (liters)	Bioassay Test Fish
Douglas Fir							
with bark	50	18	46.0	16	41.0	105	Salmon
without bark	50	19	48.3	15.5	39.4	100	Salmon
Douglas Fir							
with bark	50	18	46.0	16.5	41.9	100	Salmon
without bark	50	17.5	44.5	15.5	39.4	100	Salmon
Hemlock							
with bark	50	17.5	44.5	14.5	36.9	100	Salmon
without bark	50	18.5	47.3	15	38.1	100	Salmon
Douglas Fir							
with bark	120	16.5	41.9	16.5	41.9	100	Trout
without bark	120	16.5	41.9	15.5	39.4	100	Trout
Ponderosa Pine							
with bark	70	17	43.2	14	35.6	100	Trout
without bark	70	18	46.0	12	30.5	82	Trout

frozen for later analysis of reducing sugar and PBI. Twenty-four liters of leachate sample were chelated for mercury removal with 3 g/l Chelex 100 in 50 mesh size. Twenty liters of the chelated sample were used for BOD and k-rate determination. Samples were transported to the Oak Creek Laboratory following chelation for bioassay testing. BOD was determined for the chelated sample and the Hach manometric BOD device was used for obtaining oxygen uptake data required for determination of k-rate. Directions furnished with the device were followed closely. Nutrients and seed required in the procedure were identical to those used in BOD determination. Seed organisms were obtained from the bench scale activated sludge unit operated throughout the study period.

Chemical oxygen demand and PBI were determined on unchelated samples by methods described previously.

Reducing sugar content in each leachate sample was determined using the Somogyi method (18). Two preliminary tests were conducted on glucose solutions of known concentrations to check reliability of reagents required for the test. Frozen samples were thawed, mixed thoroughly and tested for simple reducing sugar content.

Results and Discussion

Sample Preservation

Preservation of samples against biodegradation with a heavy metal poison and subsequent removal of the poison was the object of procedures developed from experiments conducted on synthetic biodegradable samples. Results of the two preservation tests follow.

The synthetic glucose and glutamic acid sample was found to exert a BOD_5 of 210 mg/l. The portion of sample poisoned with 2 mg/l mercury and the treated with Chelex 100 following incubation, exerted a BOD_5 of 130 mg/l. The sample which was incubated at 20°C for 24 hours but not poisoned exerted a BOD_5 of 170 mg/l. It was expected that the poisoned but chelated sample would exert a BOD approximately equal to original value. Furthermore, the sample incubated at 20°C had more BOD remaining than the refrigerated sample. This was opposite from what was expected since organisms which were incubated should have been biologically more active and therefore would have exerted a greater demand for oxygen. Biodegradation during incubation would occur at a faster rate than during refrigeration. The initial procedure was therefore not considered to be valid. After 24 hours of storage all samples were not in the same biological state at the start of BOD testing. Seed organisms incubated during storage were in a more active state than were organisms which

were refrigerated during storage. For an initial period of time during the BOD test, organisms which were incubated would exert a greater demand for oxygen.

The revised procedure included millipore filtration of samples to remove microorganisms and reseeding before BOD tests were started. Table 2 represents the BOD_5 results of the revised tests. The chelated sample was found to exert a BOD_5 of 213 mg/l. The presence of mercury in the sample during storage obviously prevented biodegradation of substrate. Subsequent Hg^{++} removal was also near complete. BOD_5 of the sample incubated for 24 hours at 20°C was less than the refrigerated sample, 216 mg/l versus 226 mg/l, which indicated the millipore filtering technique eliminated most of the biological differences which had existed prior to BOD testing.

The preservation experiments showed that a biodegradable sample could be preserved against decomposition by poisoning the sample with heavy metal ions. Furthermore, the toxic effect of the poison could be removed to enable biological determination of the oxygen demand present in the sample. The positive results of the experiments allowed the log storage environment to be poisoned for preservation of biodegradable portions of materials leached from logs. Subsequent mercury removal allowed biological testing to be conducted on samples which had not been altered by biological activity.

Table 2. BOD_5 results (mg/l) of preservation tests conducted on synthetic samples.

Synthetic Sample*	Synthetic Sample Incubated and Chelated	Synthetic Sample Incubated	Synthetic Sample Refrigerated
213	204	234	234
213	234	210	222
207	198	204	222
207	216		
Ave. (210)	Ave. (213)	Ave. (216)	Ave. (226)

* Glucose 150 mg/l
Glutamic Acid 150 mg/l

Acute Toxicity of Mercury

The mercuric ion was found to be toxic to test fish. Therefore mercury had to be removed before successful bioassay testing could be undertaken. The acute toxicity bioassay test provided an indication of the effectiveness required of Chelex 100 for removal of mercuric ion.

Results of the mercury toxicity study are shown in Table 3. Under the conditions imposed, the test fish lost tolerance for mercury ions at a concentration between 0.25 and 0.50 mg/l Hg^{++}. Test data are plotted in Figure 1. The TLm_{96} was 0.35 mg/l. As previously described, the bioassay test units were made with plastic liners. Some mercury ions could have adsorbed onto the plastic during the 24 hour period of acclimation prior to the time fish were introduced. Therefore, TLm data is only valid for the imposed conditions. If mercury ions were adsorbed, the tolerance level stated would be too high. However, the level is sufficiently low to demonstrate the toxic effect of mercury in the acute or short term test.

Mercury Removal

Five attempts at mercury removal with chelating resin were made before an acceptable procedure was established whereby the test organisms could survive during the test period without signs of

Table 3. The effect of mercury concentration and exposure time on mortality of rainbow trout fry.

Mercury Ion Concentration	Exposure Time 24 hrs.	48 hrs.	74 hrs.	96 hrs.
2 ppm	10	10	10	10
1 ppm	8	10	10	10
500 ppb	1	1	10	10
250 ppb	0	0	0	0
100 ppb	0	0	0	0
50 ppb	0	0	0	0
25 ppb	0	0	0	0
10 ppb	0	0	0	0
5 ppb	0	0	0	0
2 ppb	0	0	0	0
0 ppb	0	0	0	0

ppm - parts per million

ppb - parts per billion

Table values represent number of dead fish out of a total of 10

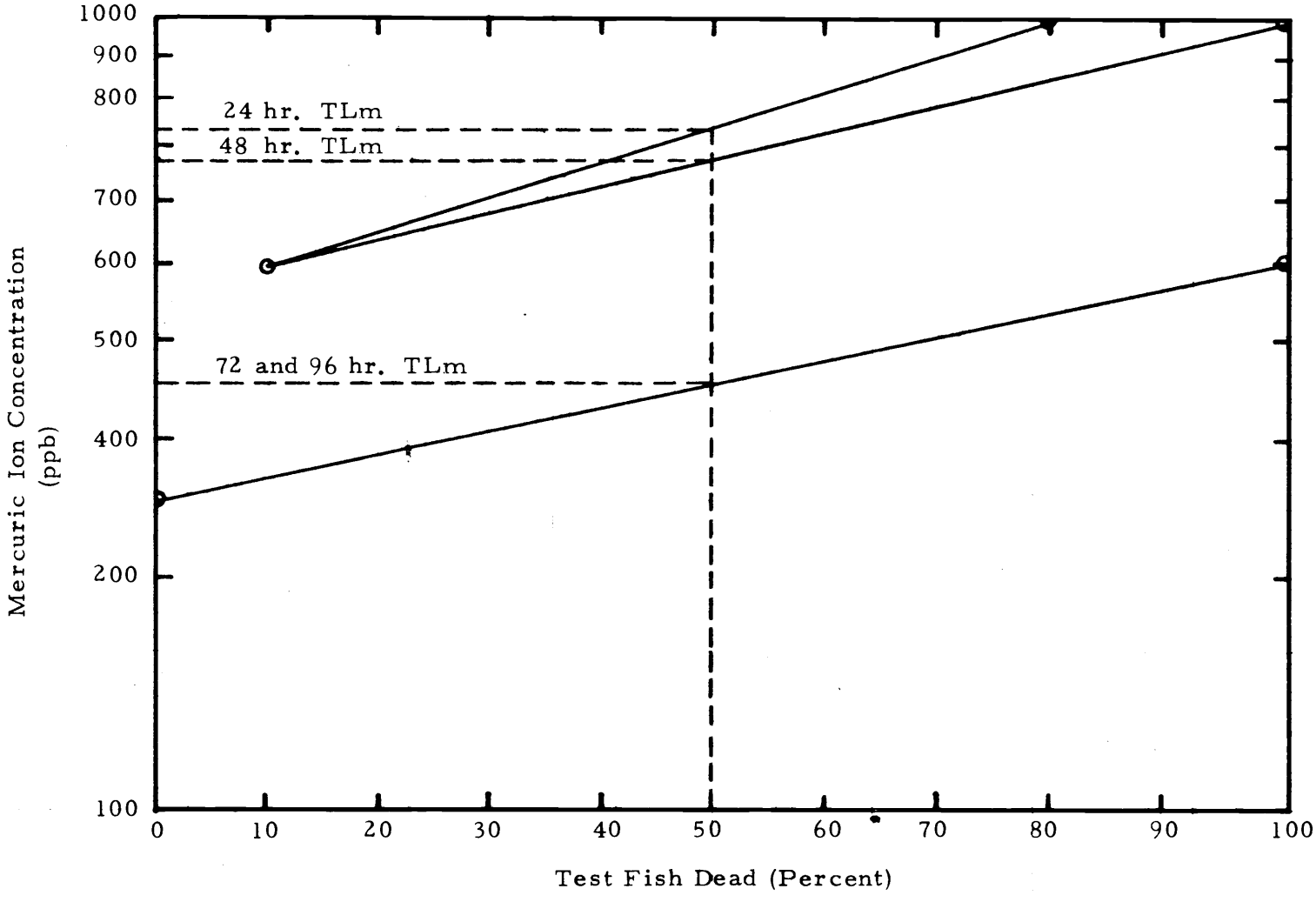

Figure 1. Results of bioassay tests measuring acute toxicity of mercury.

distress. All samples had previously contained a lethal concentration of mercury ions. Results of the mercury removal experiments are summarized in Table 4.

All test fish died in the initial mercury removal experiment involving 5 g/l 200 mesh Chelex 100 when the sample was used full strength. At 75% of full strength, none of the test fish died in the 96-hour exposure period. A problem other than poor mercury removal was suggested by the results obtained. The sample remained turbid because of the 200 mesh chelating resin, even though a 24 hour settling period was allowed following stirring. The fish used as test organisms were small, therefore their gill capacity was small. It is hypothesized that colloidal sized particles remaining in the sample could have caused the fish to suffocate as a result of clogged gills.

The sample in the second mercury removal experiment was filtered through a glass mat after settling in an attempt to eliminate colloidal and suspended resin. The chelated sample was used without dilution and the test fish died within 48 hours. Colloidal particles of resin were observed to persist in the sample.

The quantity of chelating resin was reduced in an attempt to refine the mercury removal procedure. One g/l 200 mesh Chelex 100 was used to reduce the quantity of suspended resin. Results obtained were satisfactory. No fish died during a 96-hour exposure to the undiluted chelated sample. A settling period of 24 hours following

Table 4. Bioassay test results for mercury removal experiments.

Trial No. 1	BLANK	CONTROL	Chelex 5 g/l 200 Mesh No Dilution	Chelex 5 g/l 200 Mesh 25% Dilution	Chelex 5 g/l 200 Mesh 50% Dilution	Chelex 5 g/l 200 Mesh 75% Dilution
	None Dead 96 hrs.	All Dead 24 hrs.	All Dead 48 hrs.	None Dead 96 hrs.	None Dead 96 hrs.	None Dead 96 hrs.
Trial No. 2	BLANK	CONTROL	Chelex 5 g/l 200 Mesh Filtered			
	None Dead 96 hrs.	All Dead 24 hrs.	All Dead 48 hrs.			
Trial No. 3	BLANK	CONTROL	Chelex 1 g/l 200 Mesh	Chelex 1 g/l 200 Mesh No Mercury		
	None Dead 96 hrs.	All Dead 24 hrs.	None Dead 96 hrs.	None Dead 96 hrs.		
Trial No. 4	BLANK		Chelex 1 g/l 50 Mesh	Chelex 1 g/l 50 Mesh No Mercury		
	None Dead 96 hrs.		All Dead 24 hrs.	None Dead 96 hrs.		
Trial No. 5	BLANK		Chelex 3 g/l 50 Mesh	Chelex 2 g/l 50 Mesh	Chelex 3 g/l 50 Mesh No Mercury	
	One Dead 72 hrs.		None Dead 96 hrs.	None Dead 96 hrs.	None Dead 96 hrs.	

mercury removal was still necessary to achieve adequate settling. During the 24 hour settling period, considerable biodegradation could have occurred without mercury present as a preservative.

It was desirable to decrease the settling time necessary to obtain a clear supernatant. Increasing the particle size of the chelating resin reduced the time required for settling to one hour. Chelex 100 (50 mesh) settled rapidly but when used in the same concentration as 200 mesh, mercury removal was not effective. Results of the fourth experiment gave an indication that the quantity of chelating resin had to be increased when the 50 mesh size was used. All fish died within 24 hours when 1 g/l Chelex 100 was used.

The final refinement in procedure was designed to determine the quantity of 50 mesh Chelex 100 required to achieve effective mercury removal. Results in Table 4 show that the chelating resin produced satisfactory removal when used in 2 g/l and 3 g/l concentrations. Furthermore, samples, treated with 3 g/l chelating resin, but containing no mercury, caused no harm to the test fish. This indicated that the 3 g/l was not inherently harmful when allowed to settle a minimum of one hour after mixing.

Results of the mercury removal procedures were used to develop a standard technique for use in all subsequent mercury removal applications. With mercury concentrations less than or equal to 2 mg/l Hg^{++} in aqueous samples, the procedure was as follows:

1. 20-liter samples were obtained in laboratory carboys
2. 60 grams Chelex 100 (50 mesh size) was added to each sample.
3. Sample was mixed for 30 minutes with an air driven rotary stirrer.
4. Samples were settled for one hour.
5. Supernatant was siphoned into another carboy for bioassay tests and BOD analysis.

Log Storage, Leachate and Bioassay Testing

Logs in ponds or rafts are usually 15 feet or longer and have a considerable portion of bark remaining intact. The logs usually have a total circumferencial surface area which is considerably larger than the surface area of the ends. Some of the logs used in this study were stored in water with ends sealed and bark intact, thereby simulating a longer, floating log. Logs stored without bark and without sealed ends were intended to provide samples containing high concentrations of leached material. The object was to simulate conditions which would produce the most toxic sample possible. Bark was stripped to expose the cambium layer of some log segments. The cambium layer, the most active portion of the log cross section, carries moisture and nutrients throughout the tree.

Extracts from the Douglas fir log with bark intact displayed slight toxicity to salmon test fish. Fish kill was not sufficient to calculate a median tolerance limit (TLm), i. e., less than 50% died at full strength. However, a small kill was observed. A 60 percent solution of the sample, by volume, resulted in a 10 percent fish kill after a 72-hour exposure. Parameters determined in the laboratory which characterize the leachate are presented in Table 5. Leachate from the log without bark was more toxic. TLm data is plotted in Figure 2. TLm_{48} was 93 percent by volume and remained at that level during the full 96-hour exposure.

Toxicity of leachate from logs with and without bark was only slightly different. The similarity between the two samples was further supported by BOD_5 and COD tests. Douglas fir with bark had a BOD_5 to COD ratio of 0.28 whereas Douglas fir without bark had a ratio of 0.29. No relationship between BOD, COD and toxicity is inferred. The quantities are reported only as a means of characterizing leachate strength.

Leachates from the 50 year old Douglas fir log used in the second period exhibited a greater toxicity to fish. Bioassay results presented in Figure 3 show a TLm_{96} value of 20 percent by volume for the log with bark. Leachate from the log without bark had Tlm_{96} of 24 percent, as shown in Figure 4.

Table 5. Results of tests conducted to characterize log leachates. Storage period no. 1.

	Douglas fir with bark 50 year log	Douglas fir without bark 50 year log
BOD_5	0.90 g/ft^2 (84 mg/l)	0.93 g/ft^2 (44 mg/l)
k-rate*	0.25	0.19
COD**	3.23 g/ft^2 (193 mg/l)	3.18 g/ft^2 (287 mg/l)
BOD_5:COD	0.28	0.29
Reducing sugar	0.41 g/ft^2 (24 mg/l)	0.41 g/ft^2 (37 mg/l)
PBI	7.11 g/ft^2 (426 mg/l)	4.45 g/ft^2 (402 mg/l)

* base 10 per day

** Jeris method

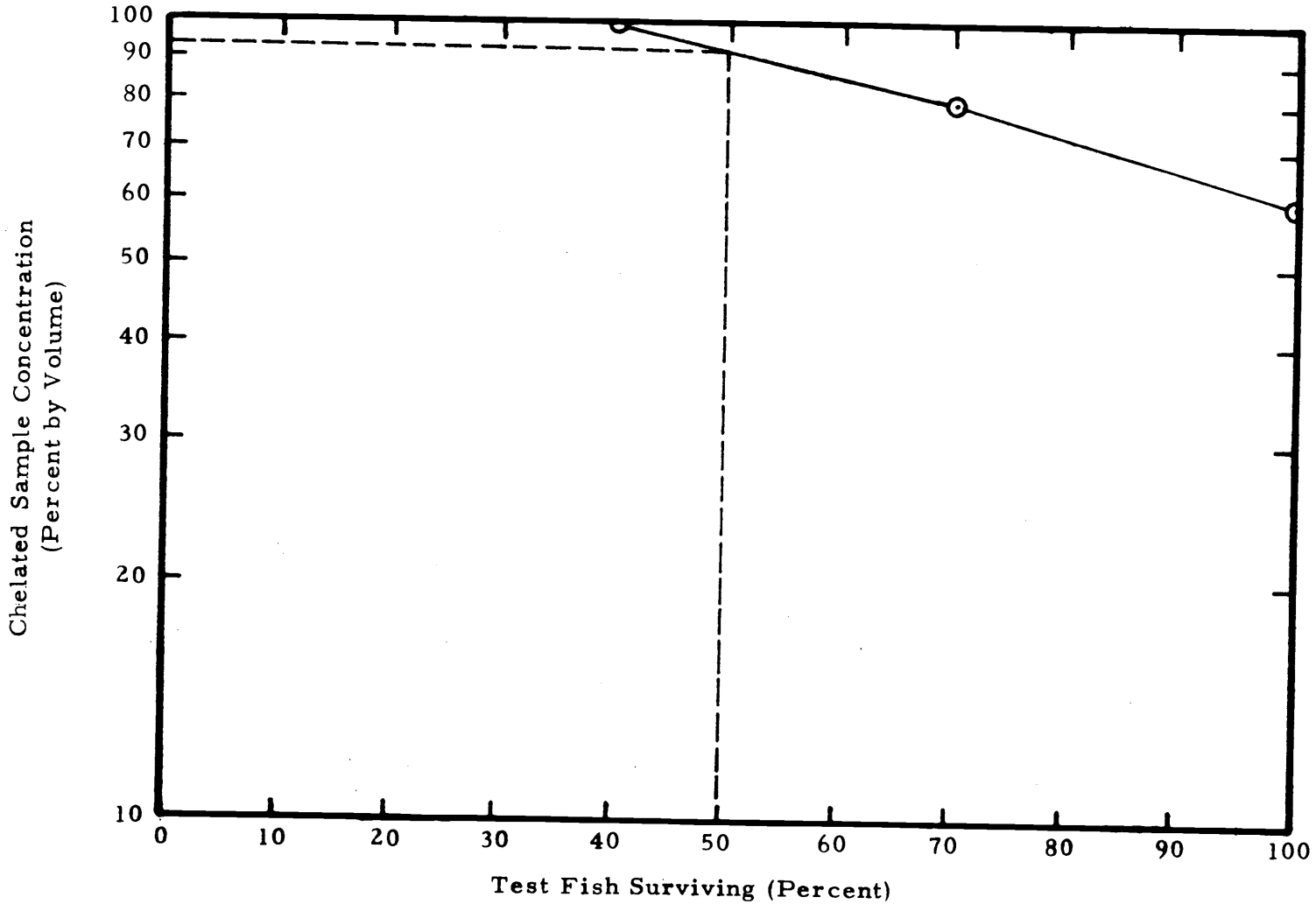

Figure 2. Bioassay test results. Leachate from Douglas fir, storage period no. 1, 50 year old log without bark.

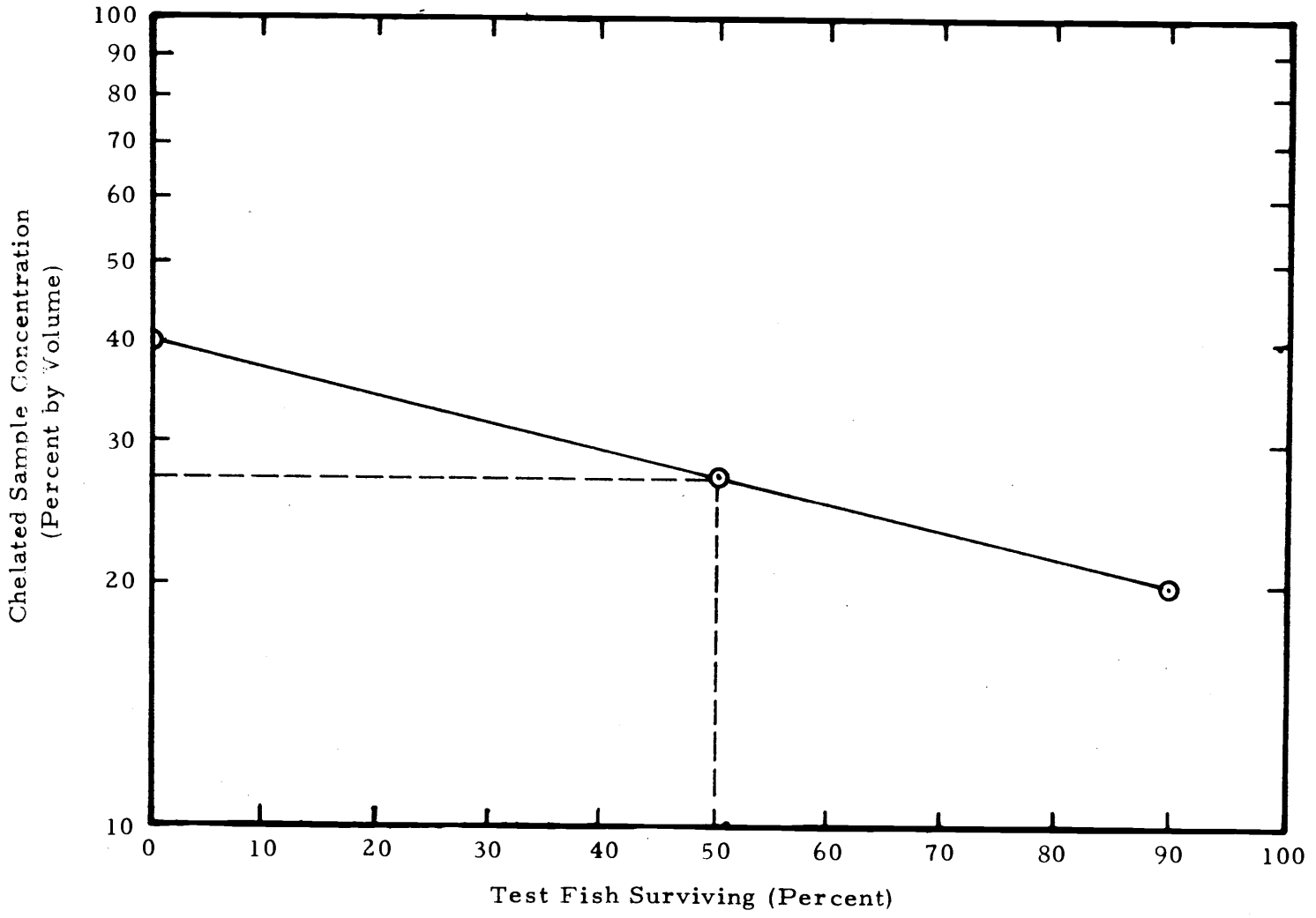

Figure 3. Bioassay test results. Leachate from Douglas fir, storage period no. 2, 50 year old log without bark.

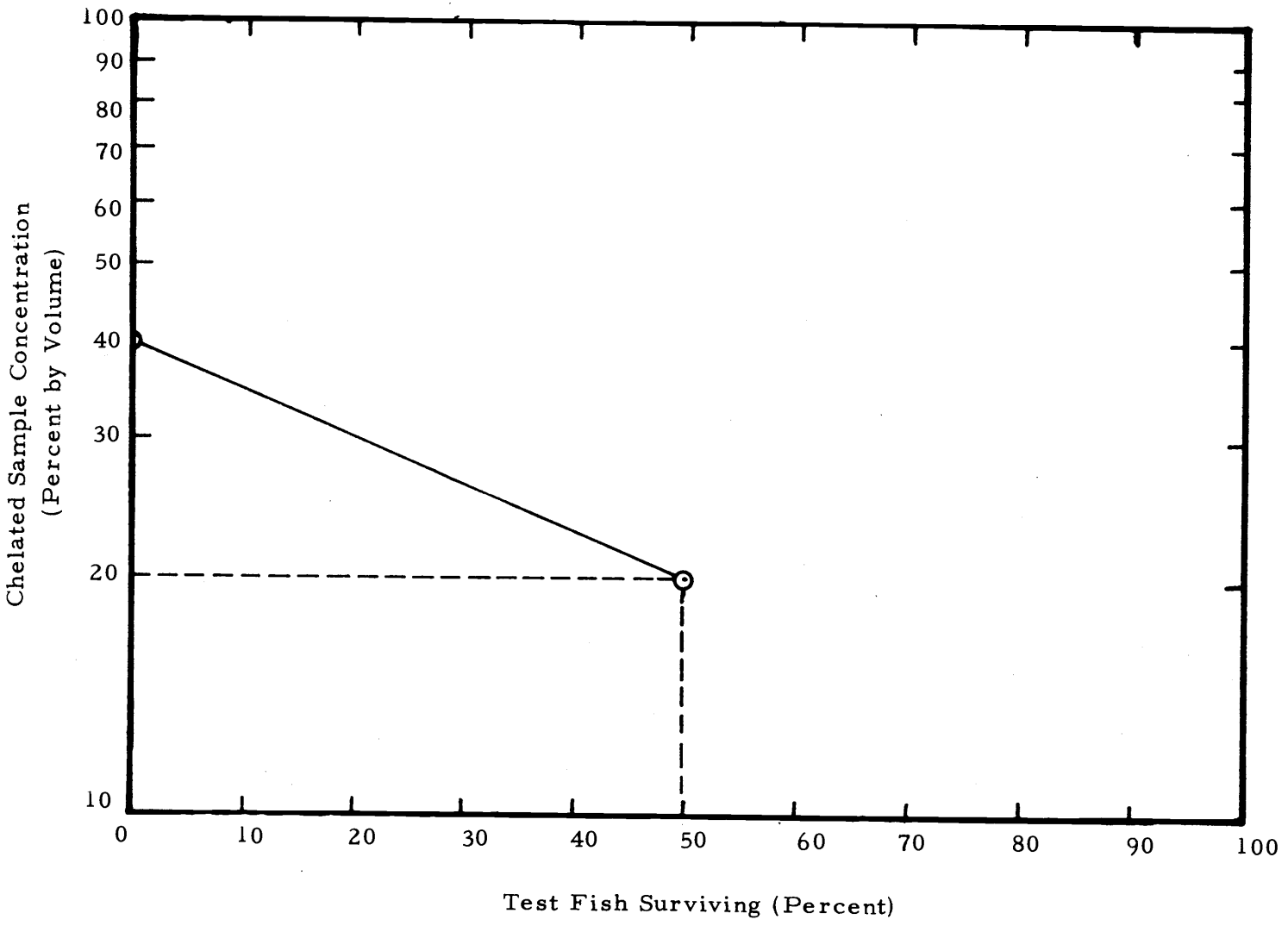

Figure 4. Bioassay test results. Leachate from Douglas fir, storage period no. 2, 50 year old log with bark

Full strength leachates from ponderosa pine, hemlock and the older Douglas fir log produced no fish kill in 96 hour exposure periods. Tables 7, 8 and 9 present data characterizing leachates of the pine, hemlock and older Douglas fir logs. Leachate from the hemlock log and the 120 year old Douglas fir log did not exert BOD or COD as large as the oxygen demand found in the first two Douglas fir trials. Leachate from the ponderosa pine without bark exerted a BOD_5 of 1.36 grams per square foot of log surface area (92 mg/l), which was higher than most of the values found for the young fir log leachates. Leachate from the pine log exerted a large COD based on log surface area, (4.24 g/ft^2, 221 mg/l). However, the young Douglas fir leachates possessed higher COD concentrations. The highest was 313 mg/l. The large COD determined for the pine leachate was an obvious result of the large sugar content (0.84 g/ft^2, 44 mg/l) contained in the leachate.

Segments from the same Douglas fir log were used in the first two trials to measure reproducibility and reliability of results. In both trials acute toxicity to fish was observed. Leachate from the second trial was more toxic, i.e., a TLm_{96} of 24 percent versus 93 percent. Parameters measured to characterize the leachate were more concentrated for the second trial. For some unexplainable reason BOD_5 was nearly 40 percent higher in the second storage period. The PBI, which is a measure of the concentration of phenolic,

Table 6. Results of tests conducted to characterize log leachates. Storage period no. 2.

	Douglas fir with bark 50 year log	Douglas fir without bark 50 year log
BOD_5	1.20 g/ft^2 (84 mg/l)	1.30 g/ft^2 (120 mg/l)
k-rate*	0.32	0.26
COD**	3.91 g/ft^2 (272 mg/l)	3.38 g/ft^2 (313 mg/l)
BOD_5:COD	0.31	0.38
Reducing sugar	0.66 g/ft^2 (46 mg/l)	0.50 g/ft^2 (46 mg/l)
PBI	12.5 g/ft^2 (872 mg/l)	10.8 g/ft^2 (1005 mg/l)

* base 10 per day

** Jeris method

Table 7. Results of tests conducted to characterize log leachates. Storage period no. 3.

	Hemlock with bark 50 year log	Hemlock without bark 50 year log
BOD_5	0.27 g/ft^2 (15 mg/l)	0.93 g/ft^2 (79 mg/l)
k-rate*	0.13	0.28
COD**	1.82 g/ft^2 (101 mg/l)	2.04 g/ft^2 (174 mg/l)
BOD_5:COD	0.15	0.45
Reducing sugar	0.23 g/ft^2 (13 mg/l)	0.18 g/ft^2 (15 mg/l)
PBI	2.06 g/ft^2 (114 mg/l)	1.91 g/ft^2 (163 mg/l)

* base 10 per day

** Jeris method

Table 8. Results of tests conducted to characterize log leachates. Storage period no. 4.

	Douglas fir with bark 120 year log	Douglas fir without bark 120 year log
BOD_5	0.11 g/ft^2 (6 mg/l)	0.56 g/ft^2 (42 mg/l)
k-rate*	0.17	0.30
COD**	1.0 g/ft^2 (53 mg/l)	1.89 g/ft^2 (142 mg/l)
BOD_5:COD	0.11	0.30
Reducing sugar	0.31 g/ft^2 (16 mg/l)	0.41 g/ft^2 (31 mg/l)
PBI	0.66 g/ft^2 (35 mg/l)	2.98 g/ft^2 (223 mg/l)

* base 10 per day

** Jeris method

Table 9. Results of tests conducted to characterize log leachates. Storage period no. 5.

	Ponderosa pine with bark 70 year log	Ponderosa pine without bark 70 year log
BOD_5	0.80 g/ft^2 (42 mg/l)	1.36 g/ft^2 (92 mg/l)
k-rate*	0.31	0.40
COD**	---	2.75 g/ft^2 (185 mg/l)
COD***	4.24 g/ft^2 (221 mg/l)	2.63 g/ft^2 (177 mg/l)
BOD_5:COD	0.19	0.52
Reducing sugar	0.84 g/ft^2 (44 mg/l)	0.16 g/ft^2 (11 mg/l)
PBI	7.48 g/ft^2 (416 mg/l)	0.79 g/ft^2 (53 mg/l)

* base 10 per day

** Jeris method

*** Reflux method

tannin, or lignin-like compounds, was higher than the level measured in the first trial. For logs without bark, the PBI values were 10.8 g/ft^2, (1005 mg/l), in the second test compared to 4.45 g/ft^2, (402 mg/l), in the first test. Higher PBI values correspond to samples which were the most toxic. A relationship between phenolic or lignin-like compounds in the leachates and toxicity could exist.

The color of leachate samples was observed throughout the study and a relationship between color and sample strength was noted. Leachates which were most highly colored also possessed the highest concentrations of BOD exerting substances, reducing sugars and PBI. For example, storage water for the younger Douglas fir log produced highly colored leachates and the highest PBI concentration measured in the study, 1005 mg/l. BOD_5 values were consistently high, in the range of 1.0 g/ft^2 log surface area. Ponderosa pine with bark intact also produced a highly colored leachate with an orange tint. As previously mentioned, the pine leachate produced the highest COD and sugar content observed.

Classification of leachate toxicity measured in this study is relative to the length of the log storage period used and the bioassay test conditions employed. Results of tests and observations made in this study indicate that leachates from hemlock and ponderosa pine which had BOD_5 as high as 1.36 g/ft^2 and PBI as high as 7.98 g/ft^2 are not acutely toxic to fish. Douglas fir exerted toxicity only when leachate

originated from a relatively young log. As previously described the younger fir logs produced a large PBI which could indicate a relationship between toxicity and PBI. Leachate from a log more than twice as old produced no toxic effect. Log sections without bark were more toxic than were comparable sections with bark remaining intact.

Parameters measured to characterize leachate samples have shown that a portion of the materials which leach from logs are biodegradable and could represent a significant oxygen demand on a body of water used for storage. BOD_5 values were found to be as high as 1.36 g/sq. ft. for the ponderosa pine log. BOD_5:COD values ranged from 0.1 to 0.45. These values are lower than that for many wastewaters. For example domestic raw sewage averages 0.5. This indicates that leachates from logs include some substances which are not readily biodegradable.

Some of the compounds in the leachate samples which exerted BOD were wood sugars. Leachates contained wood sugar in a range of 0.18 to 0.84 grams per square foot of submerged log. High BOD decay constants ranging from 0.17 to 0.40 were determined for the log leachates. A high constant indicates a readily biodegradable substance. The leachates, with the highest sugar content produced the highest decay rate. BOD curves for all leachate samples are presented in Figures 5, 6, 7, 8 and 9.

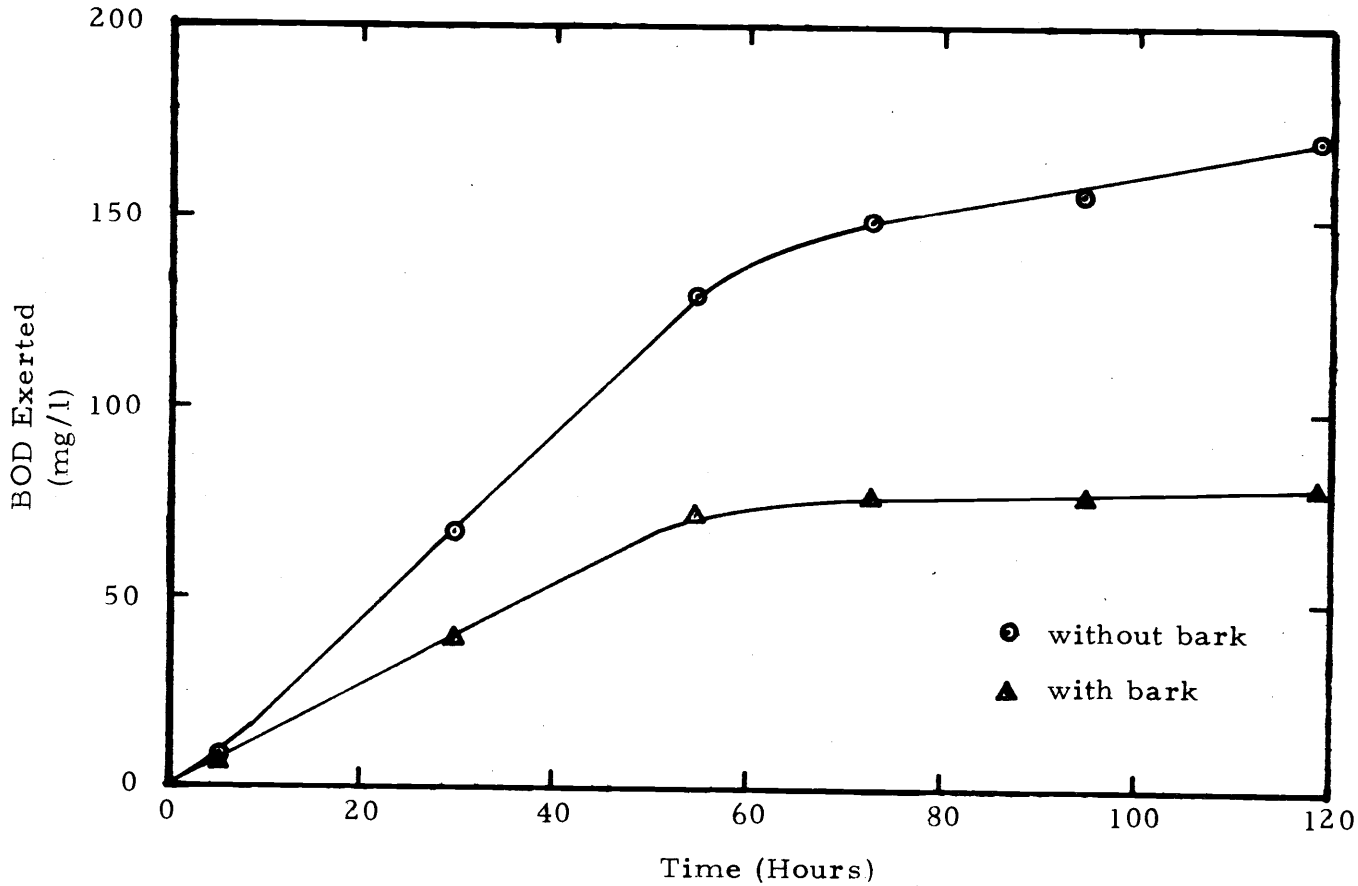

Figure 5. BOD exerted by Douglas fir leachate, storage period no. 1, 50 year old log.

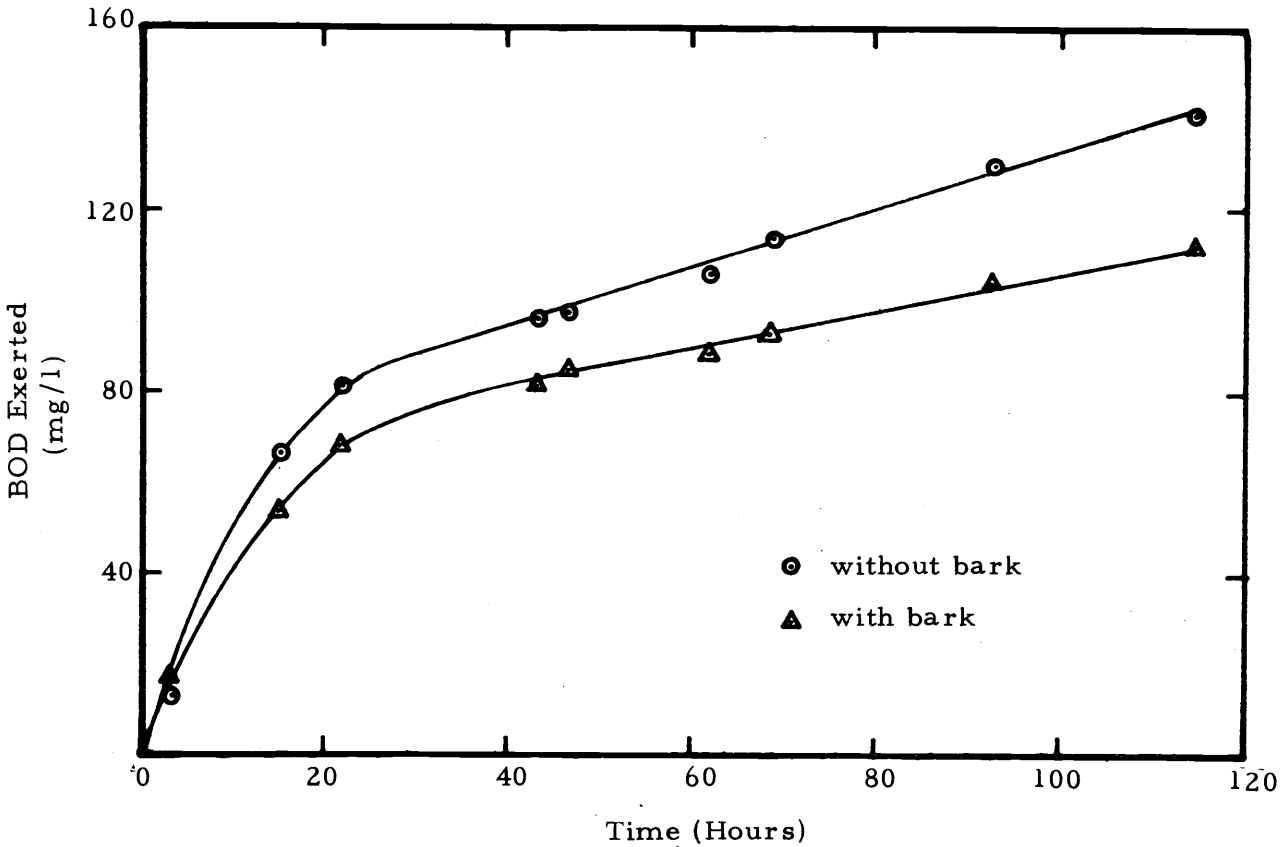

Figure 6. BOD exerted by Douglas fir leachate, storage period no. 2, 50 year old log.

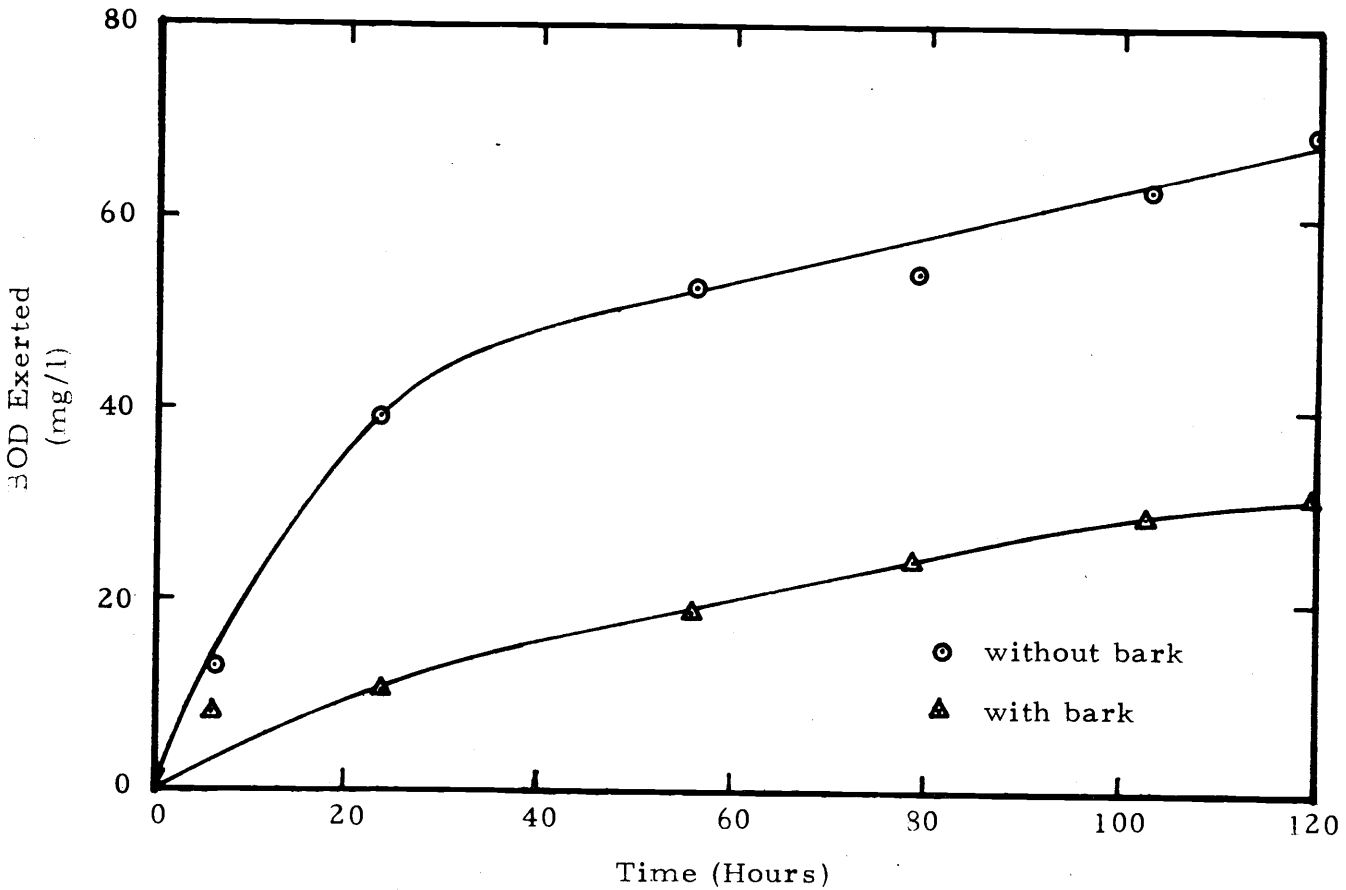

Figure 7. BOD exerted by hemlock leachate, storage period no. 3.

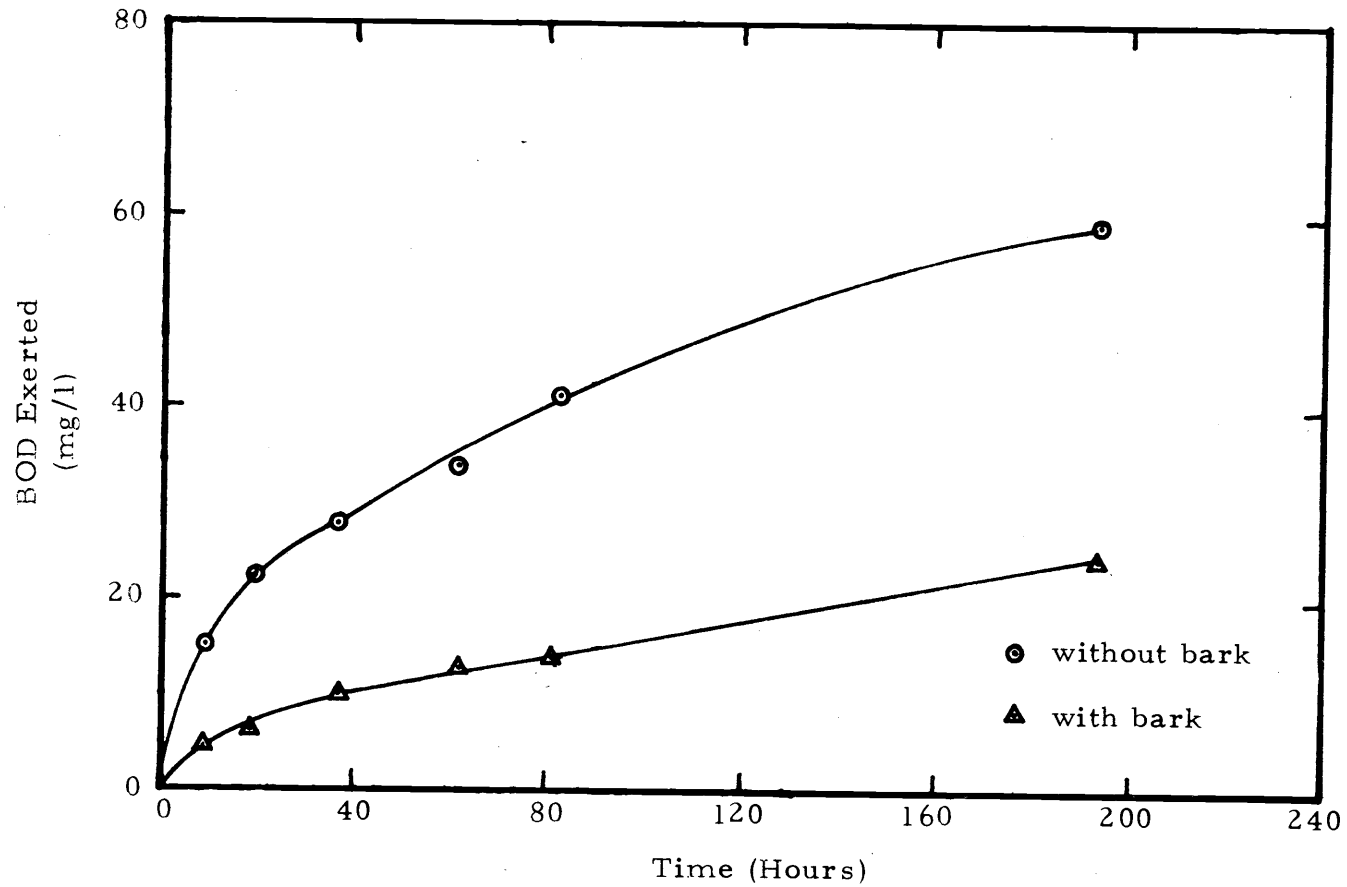

Figure 8. BOD exerted by Douglas fir leachate, storage period no. 4, 120 year old log.

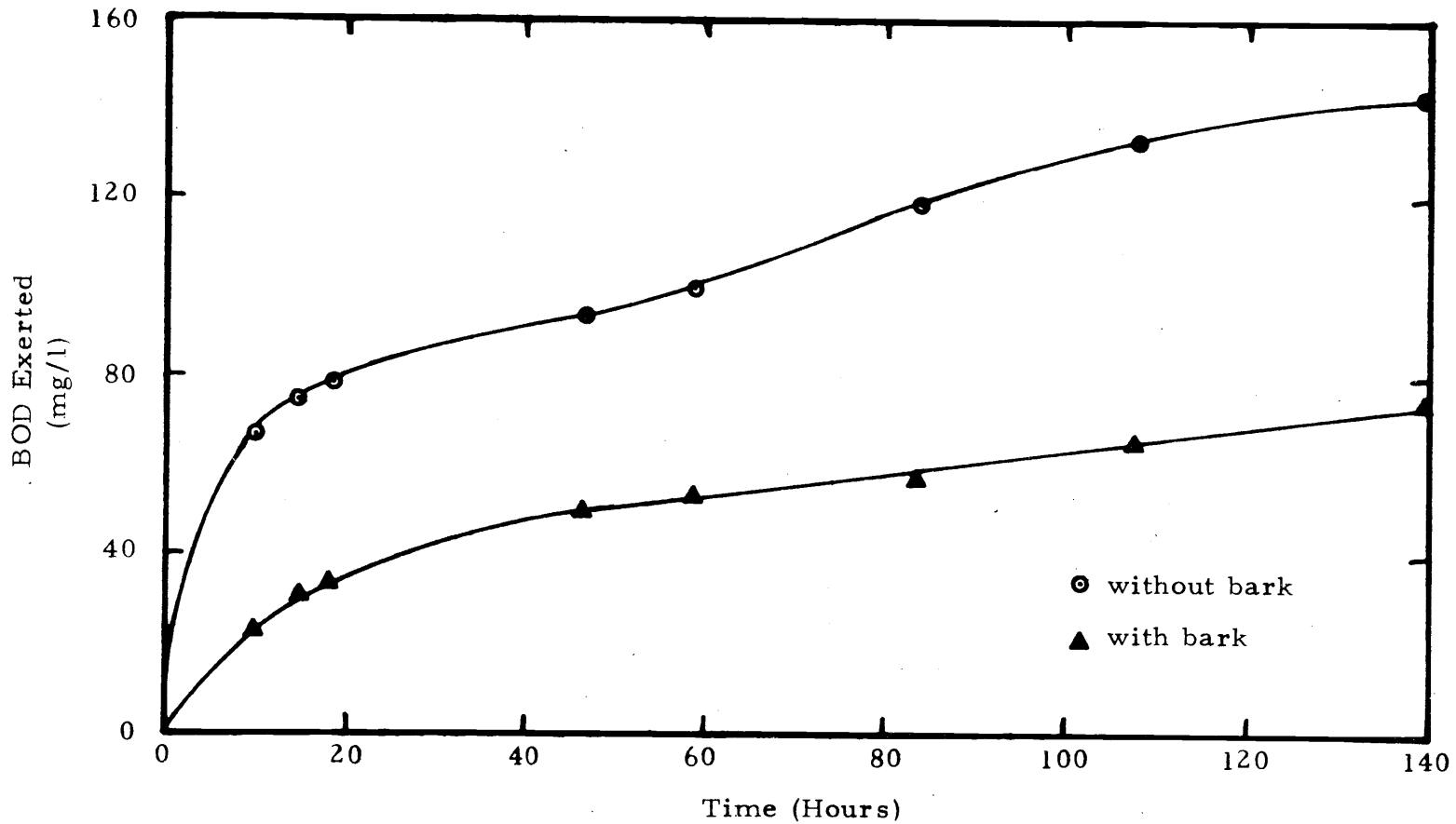

Figure 9. BOD exerted by ponderosa pine leachate, storage period no. 5.

The significane which underlies the above discussion is related to the characterization of what leachates from logs are capable of imposing on a receiving body of water. Leachates have oxygen depleting potential and a rapid decay rate, suggesting a condition whereby rather large oxygen demand could be exerted in a short period of time. Furthermore, some logs appear to possess an acute toxicity to fish which is not based on oxygen depletion. Leachates studied in this research study had low BOD_5:COD ratios indicating a relatively high quantity of non-biodegradable material present in the samples.

Williamson (26) estimated BOD and COD for a hypothetical situation involving a 50-acre raft of ponderosa pine logs stored in a river for 30 days. Using the leaching equation developed by Graham (7) and BOD:COD ratios determined in this study a COD of 700 pounds per day was calculated. The population equivalent was 2800 people based on 0.25 pounds of COD per capita per day. BOD was calculated at 320 pounds per day, which yields a population equivalent of 1900 at 0.17 pounds BOD per capita per day.

CONCLUSIONS

Based upon the studies reported herein, for log segments approximately 18 inches long, 16 inches in diameter and stored in approximately 100 liters of fresh creek water for a period of seven days, the following conclusions are drawn:

1. Leachate from Douglas fir logs can be slightly toxic to fish exposed for a 96-hour period depending on the age of the tree from which the log was cut.
2. Leachates from hemlock and ponderosa pine logs are not measurably toxic to fish exposed for a 96-hour period.
3. Mercuric ions can be chelated from water samples to eliminate the toxic effects of mercury.
4. Some of the substances leached from logs in water storage are rapidly degraded by microorganisms. Soluble wood sugars contribute a large portion of the biodegradable matter.

BIBLIOGRAPHY

1. American Public Health Association. Standard methods for the examination of water and wastewater. 12th ed. New York, 1965. 769 p.

2. Barnes, C. A. et al. A standardized Pearl-Benson, or nitroso, method recommended for estimation of spent sulfite liquor or sulfite waste liquor concentration in waters. Tappi 46:347-351. 1963.

3. Belding, D. L. Toxicity experiment with fish in reference to trade and waste pollution. Transactions of the American Fisheries Society, 57, p. 100, 1927.

4. Brey, W. S. Principles of Physical Chemistry. Appleton-Century Crofts, Inc. New York, N. Y. 1958.

5. Doudoroff, P. Biological observations and toxicity bioassay in the control of industrial waste disposal. Proceedings of the Sixth Industrial Waste Conference, Purdue University Engineering Extension Bulletin, Series no. 76, pp. 88-104.

6. Felicetta, V. F. and J. L. McCarthy. The Pearl Benson, or nitroso, method for the estimation of spent sulfite liquor concentration in water. Tappi 48:337-347. 1963.

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

8. Henrikson, A. and J. E. Samdol. Centralized log barking and water pollution. Vattenhygien 2:55-60. 1966.

9. Hoffbuhr, J. W. The character and biological treatability of log pond waters. M. S. Thesis, Oregon State University, June, 1970.

10. Hodge, J. E. and B. T. Hofreiter. Determination of reducing sugars and carbohydrates. Methods in Carbohydrate Chemistry.

11. Jeris, J. S. A rapid COD test. Water and Wastes Engineering. 4:89-91. May, 1967.

12. Kurth, E. F., J. K. Hubbard and J. D. Humphrey. Chemical composition of ponderosa and sugar pine barks. Paper presented at the Third Annual National Meeting of the Forest Products Research Society, Grand Rapids, Michigan, May 3, 1949.

13. Malacea, I. and E. Gruia. Contributions to the study of the toxic effect of heavy metals on some aquatic organisms. Institute Hydrotechnical Research and Science, Bucharest, Sect. 4, 47-49, 1964. Water Pollution Abstracts, 38, 1567, 1965.

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

15. McKee, J. E. and H. W. Wolf. Water quality criteria. State of California. State Water Quality Control Board, 2nd edition, 1963.

16. Raabe, E. W. Biochemical oxygen demand and degradation of lignin in natural waters. Journal of the Water Pollution Control Federation, Research Supplement. 40:R145-R150. 1968.

17. Schaumburg, F. D. Associate Professor of Civil Engineering, Oregon State University, Personal communication.

18. Somogyi, M. A new reagent for the determination of sugars. Journal of Biological Chemistry. 160:61-68. 1945.

19. Sproul, O. J. and C. A. Sharpe. Water quality degradation by wood bark pollutants. Water Resources Center, University of Maine, Orono, Maine, 1968.

20. Stewart, J. E. and J. W. Cornich. Lobster tolerance for tris buffer, sodium flouride and sea-water extracts of various woods. Journal of Fisheries Research Board of Canada. 1964. 21, 1549-1551.

21. Tabata, K. Systematic studies on toxic components in industrial wastes with reference to the tolerance of aquatic lines-III on acute toxic component in water-extract liquor from bark. Bulletin Tokai Region Fish Research Laboratory, 42:17-21, 1965. Water Pollution Abstracts, 37:1857. 1964.

22. Technical Bulletin, No. 51, National Council for Stream Improvement. The effects of Kraft mill waste liquors and some

of their components on certain salmonoid fishes of the Pacific Northwest. May 29, 1952.

23. Varma, N. M. and L. H. Hall. BOD of pure carbohydrates and its relation to the chemical structure. Water and Sewage Works 113:102-109. 1966.

24. Warner, R. E. Bioassays for microchemical environmental contaminants. Bulletin of the World Health Organization 36:181-207, 1967.

25. Warren, C. E. and E. N. Marvell. A study of constituents responsible for the toxicity of Kraft Pulp mill wastes to fish. U. S. Public Health Service National Institute of Health, research grants RG5045 and RG5710, progress report, June 1, 1961.

26 Williamson, K. E. Personal communication and unpublished research.

27. Wise, L. E. Ed. Wood Chemistry. Reinhold Publishing Corporation, New York, N. Y. 1944. 860p.