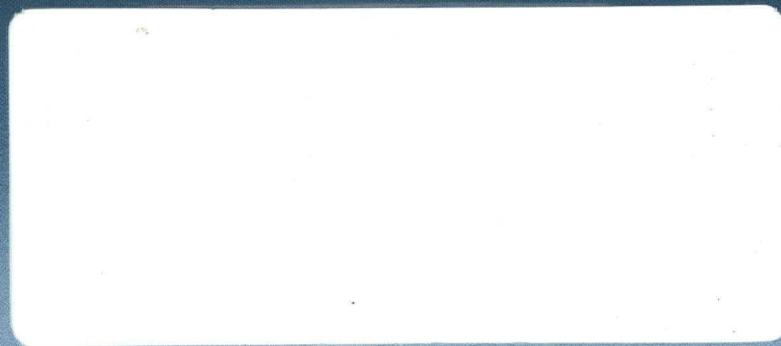




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**EUTROPHICATION OF RIVERS BY NUTRIENTS IN
TREATED KRAFT PULP MILL EFFLUENT**

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Abstract

In 1972 the start up of a 1200 ADT/day bleached kraft pulp mill in Kamloops, British Columbia resulted in a massive increase in algal standing crop in the Thompson River below Kamloops Lake. While phosphorus loading from the pulp mill was believed to be responsible for this change, the actual measured elevation in phosphorus concentration downstream of the discharge was below the detection limit ($3 \mu\text{g P}\cdot\text{L}^{-1}$) of the analytical procedure used in the joint Federal-Provincial Task Force Study of the Thompson River in 1973-75. Research at Environment Canada's Experimental Troughs Apparatus (EXTRA) located at Chase, B.C. has proven that the ambient concentration of phosphorus required to saturate the specific growth rate of attached diatom communities with a concomitant increase in algal standing crop is very low (ca. $1 \mu\text{g P}\cdot\text{L}^{-1}$). Hence, although the concentration of dissolved phosphorus in kraft mill effluent (KME) is typically below $0.5 \text{ mg P}\cdot\text{L}^{-1}$ (ca. $1 \text{ mg P}\cdot\text{L}^{-1}$ total phosphorus), even at in-river dilutions of 100-fold, the steady-state elevation of soluble phosphorus is high enough to stimulate algal production in rivers which are phosphorus-limited.

In rivers that are nitrogen-limited, nitrogen in fully treated KME can also stimulate algal production. The atomic ratio of available N and P in the McKenzie River, Oregon, is ca. 2:1 clearly indicating a nitrogen-limited system. The discharge of secondarily treated KME to this river near Springfield, Oregon has also increased algal production. Stream-side flume experiments measuring the effect of treated KME additions on algal growth have shown that the concentration of dissolved inorganic nitrogen (DIN) available for algal uptake (ammonium-N; $\sim 200 \mu\text{g N}\cdot\text{L}^{-1}$ and nitrate-N; $\sim 50 \mu\text{g N}\cdot\text{L}^{-1}$) in KME was high enough to increase specific growth rates from 0.30 to $0.56 \text{ divisions}\cdot\text{d}^{-1}$ during the summer, even with the effluent

completely diluted in the river (0.5% v/v). At other seasons of the year, KME did not stimulate algal growth at concentrations occurring in the river.

In an experiment with phosphorus added in pulses to river algal communities, the attached diatoms were able to rapidly uptake the phosphorus spikes and continue growing rapidly. Algae exposed to a P concentration of $60 \mu\text{g P}\cdot\text{L}^{-1}$ for only one min each hour grew as fast as those algae exposed to a continuous enrichment concentration of $1 \mu\text{g P}\cdot\text{L}^{-1}$. This showed the importance of maintaining stable discharge characteristics in controlling eutrophication of rivers.

Key Words: Eutrophication; phosphorus-limitation; nitrogen-limitation; pulp mill pollution; rivers; primary production; periphyton.

Introduction

Nutrients in kraft pulp mill effluent (KME) have resulted in major changes in the level of algal productivity in some rivers in western North America. However, with the exception of a few isolated cases, eutrophication has not been a major consideration for the management of pulp mill wastes. Other concerns such as biological oxygen demand (BOD) and acute toxicity to aquatic biota have been, justifiably, much higher priority. The introduction of biological oxidation systems in the 1960's for treatment of pulp mill waste has largely addressed environmental problems associated with these two concerns. Aerobic stabilization basins (ASB) reduce BOD by ca. 90% (Servizi 1989) and acute toxicity of resin acids to fishes to negligible levels (Servizi and Gordon 1986; Servizi 1989). However, the nitrogen (N) and

phosphorus (P) content of KME receiving ASB treatment is unabated and may actually be exacerbated by the practice of ASB fertilization. Partly as a result of the diminution of high BOD loading, the impact of nutrients from mills practising advanced treatment has become more evident. More recently, concerns about organochlorines, in particular dioxans and furans, have dominated the environmental agenda of the pulp and paper industry (Sodergren 1989; Eysenbach et al. 1990; Earle and Reeve 1990). The advent of chlorine-dioxide substitution with the attendant decline in organochlorine production (Berry et al. 1989) and the probable complete elimination of chlorine bleaching in future mills means that the nutrient issue will soon become the last unaddressed environmental issue in the industry.

This paper reviews current knowledge about the quantitative relationship between primary nutrients (nitrogen and phosphorus) in pulp mill effluent and the growth rate and biomass accumulation of attached algae in rocky-bottom rivers. This information was gained primarily during two series of small-scale, flowing-trough enrichment experiments. One set of trials with orthophosphorus additions was run on algal communities in the phosphorus-limited Thompson River, British Columbia. Another set of experiments using direct additions of bio-treated KME was run on the nitrogen-limited McKenzie River, Oregon. Data from these earlier small-scale experiments are here put into the context of potential whole-river impacts by considering in situ dilution effects and the influence of seasonally varying light and temperature. The comparison of results obtained from these two different rivers facilitates a discussion of similarities and differences in the response of N and P-limited algal communities to nutrients in KME.

Because both pulping and treatment processes which determine nutrient concentrations in mill effluent may vary with time, an experiment was run to determine how algal growth rates responded to a non-steady (pulsing) nutrient environment. This information may be more relevant to interpreting in situ effects of enrichment than data from the earlier "steady-state" trials.

The compilation of information provided here, including the description and illustration of some of the practical aspects of the operation of small-scale flumes for determining the potential impacts of KME in river environments, is intended to provide guidance in the conduct and interpretation of data from these kinds of experiments which are now being requested under the Canadian Federal Environmental Assessment and Review Process (EARP) guidelines by Boards reviewing proposals for new or expanded pulp mill operations (CELGAR Expansion Review Panel Final Report 1991).

Background

Thompson River: The Thompson River is located in south-central British Columbia (Fig. 1). Flow in the Thompson River is unregulated so discharge every year varies an order of magnitude from 250 to 2400 m³·s⁻¹ and the mean annual flow is about 800 m³·s⁻¹ (Fig. 2). Because flow in the Thompson is largely driven by snow pack and glacier melt, the peak freshet occurs in spring and early summer (May-June) and the lowest flows are in the late winter (January-March). The two primary tributaries that form the Thompson, the North and South Thompson Rivers, drain watersheds that are forested and relatively thinly populated. The river-lake complexes that make up the Thompson River system are for the most part pristine, nutrient-poor waters with very low phosphorus.

Beginning in May 1972 an expanded bleached kraft pulp mill located in the City of Kamloops near the confluence of the North and South Thompson Rivers began operation and the discharge of effluent into the river. At about the same time the biological character of the Thompson River downstream changed and public complaints of degraded water quality were common (Federal-Provincial Task Force Report 1976). Besides discoloration of the water during periods of low flow and reports of odour and fish tainting problems, the most obvious change in the river was the massive accumulation of benthic algae. As photographic evidence

documents (Plate 1A, B), the magnitude of the change in the river was startling. The increased biological productivity was surprising because the magnitude of dilution in the Thompson River was expected to ameliorate the effects of pollutants such as nutrients. Under all but the most extreme low flow episodes, KME is diluted more than 100-fold and during most of the year KME concentrations at complete mix range between 0.8 to 0.1% v/v (Fig. 3).

During 1973-75 a Federal-Provincial Task Force study of the Thompson River system in the vicinity of Kamloops identified phosphorus in effluent from the pulp mill and the City municipal sewage treatment plant as responsible for the increased algal production in the river (Federal-Provincial Task Force Report 1976). This judgement was based on the concentrations and ratios of N and P in the rivers upstream of the discharges (Federal-Provincial Task Force Report 1976). Background soluble reactive phosphorus concentrations in the Thompson River which were below detection by earlier methods are now known to be around 1-2 $\mu\text{g P-L}^{-1}$ (Bothwell 1985) and nitrate-nitrogen levels range between 120 and 60 $\mu\text{g P-L}^{-1}$ (Fig. 4). These levels coupled with an N:P molar ratio of 230 (Fig. 4) denote the Thompson River as a classic P-limited system. However, in spite of this evidence and the calculation that P-loadings increase the level of phosphorus in the river, the concentrations actually measured in 1973-74 in the lower Thompson River were not significantly higher than those in the North and South Thompson Rivers (Federal-Provincial Task Force Report 1976). In all other documented cases where eutrophication of natural waters has been caused by phosphorus, increases in phosphorus have been measurable and significant (Hooper 1969).

McKenzie River: The McKenzie River is a tributary of the Willamettee River in central Oregon (Fig. 1). Like the Thompson, the McKenzie drainage basin is completely forested and the river would be characterized as pristine. Nevertheless, the McKenzie River differs from the Thompson River in several ecologically important respects. Perhaps the most fundamental difference between the two rivers, relevant to how they respond to eutrophication pressure, is the nutrient water chemistry. Because of the influence of volcanic geologic parent materials in

the drainage basin, phosphorus levels in the McKenzie River are naturally very high (ie. $\sim 30 \mu\text{g P}\cdot\text{L}^{-1}$) while nitrate is low ($\sim 10 \mu\text{g N}\cdot\text{L}^{-1}$) (Fig. 4). With dissolved available N:P molar ratios around 2 (Fig. 4) the McKenzie River would be clearly judged as nitrogen-limited.

Hydrologically, the McKenzie and Thompson Rivers also differ. The McKenzie with a mean annual discharge of about $180 \text{ m}^3\cdot\text{s}^{-1}$ is an order of magnitude smaller than the Thompson (Fig. 2). Because of the milder climate and geographic location, flows are predominately driven by winter rains. Peak flows occur in December-February while low flow, and hence the lowest point-source dilution (ie. highest nutrients), occur in summer (Fig. 2). The coincidence of higher nutrient levels with warmer temperatures and higher sunlight should, potentially, make the McKenzie more susceptible to the effects of eutrophication.

The hydrograph of the McKenzie further deviates from the Thompson because of flow regulation. Amplitudes in the annual hydrograph are dampened out and there is usually only a 5-fold difference between peak and lowest flows (Fig. 2). Substantial groundwater input directly into the McKenzie River channel near Springfield, Oregon also tends to even out flows seasonally.

In spite of these major differences between the McKenzie and Thompson Rivers, the benthic algal response to the discharge of kraft pulp mill effluent (KME) appeared similar to that documented in British Columbia (Plate 1B). In both instances, large increases in the standing crop of benthic diatoms occurred during periods of low flow.

Materials and methods

Experimental flume facilities located on the banks of the McKenzie and South Thompson Rivers were used to define the relationship between nutrients in KME and the specific growth rates of attached algal communities in the rivers. Many of the design features

of the experimental apparatus used at these two sites were similar. At both locations water continuously pumped from the river to a constant head tank was gravity-fed through 20-L mixing chambers to a series of Plexiglas flumes. The mixing chambers assured thorough mixing of the pollutant (KME/nutrient) with the river water prior to overflow into the flumes. Water flow to each trough was 47-50 L·min⁻¹. This resulted in a water depth and velocity in the troughs of approximately 1 cm and 50 cm·s⁻¹, respectively. Sheets of open-cell styrofoam-DB (Customfoam Crafts, El Monte, CA) lined the bottom of the troughs and served as a substratum for microbial colonization. The troughs were covered with either plate glass or UV-opaque Plexiglas to prevent natural UV-B radiation from affecting algal growth in the very shallow water troughs.

McKenzie River apparatus: The flume apparatus on the bank of the McKenzie River near Springfield, Oregon was used to directly test the effect of bio-treated KME on the growth rates of natural river algal communities (Plate 2). In addition to the river water supply system at this site, fresh secondarily treated KME was pumped continuously into another head tank and dispensed by gravity feed through PVC ball valves into five of the six mixing chambers (Plate 2C). (In later experiments peristaltic pumps were used to dispense the KME) Each mixture of KME in river water overflowed from the mixing chamber into one of six Plexiglas flumes (Plate 2D). The KME concentrations tested were 0.5, 1.0, 2.0, 5.0 and 25% v/v. The sixth trough, the control, received no KME.

South Thompson River apparatus: The Experimental Troughs Apparatus (EXTRA) located on the South Thompson River near the outlet of Little Shuswap Lake in British Columbia (Plate 3) was used in experiments to determine the relationship between orthophosphate concentration and growth rates. In these trials stock solutions of K₂HPO₄ were metered into 20-L mixing chambers with high precision FMI piston pumps (RH type, Fluid Metering Inc., NY.). Seven of the twelve Plexiglas troughs at EXTRA were used in the experiments reported here. In addition to a control, with no additional phosphorus, there were six levels of constant P-enrichment; 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 µg P·L⁻¹ as PO₄³⁻.

Phosphate concentrations in the troughs were computed from the known dilutions of the standard enrichment solutions.

Growth rate experiments: All experiments began with the placement of fresh styrofoam sheets on the bottoms of the troughs and the commencement of river water flow and nutrient/KME addition. Measurements of Chl *a* density were made at frequent intervals during the period of exponential increase of algal biomass by removing quadruplicate cores (ca. 5.0 cm²) of the styrofoam with a corkborer from all the troughs (Plate 3C,D). Using this experimental trough design and sampling procedure, Chl *a* measurements on individual cores have a coefficient of variation of ca. 11% (Bothwell 1983). The replicate cores from each trough on each sampling date were extracted in 90% acetone using a Polytron grinder. The extracts were combined for a single Chl *a* determination, either fluorometric (Holm-Hansen et al. 1965) or spectrophotometric (Lorenzen 1967). Growth rates were computed by a least-squares fit of the time-course chlorophyll accrual data to the equation:

$$y = a.e^{kt}$$

where *y* is the Chl *a* density (mg·m⁻²) at day, *t*; "a" is the initial Chl *a* density, and *k* is the specific net growth rate. Values of *k*, divided by 0.693, give specific growth rate (μ) expressed as div.d⁻¹. To limit the potential influence of algal settlement (immigration) rate on the calculated growth rates (Bothwell 1983; Bothwell and Jasper 1983) Chl *a* data from the first 5 days were usually omitted. Macroinvertebrate grazers were removed from the troughs with needlenose forceps each sampling date to limit the influence of grazing pressure. Experiments usually lasted 2-3 weeks and were terminated when the rate of biomass increase began to decline.

For each experiment conducted at EXTRA the maximum growth rate attained with phosphorus enrichment (μ_{max}) was determined by a least-squares fit to an Eadie-Hofstee

transformation of the growth rate vs. P-concentration data (Dowd and Riggs 1965). Relative specific growth rates ($\mu:\mu_{\max}$) were computed for the control and all levels of phosphorus enrichment in each experiment following the recommendation of Goldman (1980). This normalization factors out the effects of physical factors (temperature and light) controlling growth so that the effects of nutrients alone can be examined (Goldman 1980).

For the three KME experiments on the McKenzie River, $\mu:\mu_{\max}$ for each of the treatments was determined by normalizing the observed μ to μ_{\max} obtained from the empirical regression between temperature and maximum specific growth rate developed from all the experimental observations at EXTRA and on the McKenzie River (Fig. 5). The estimation of μ_{\max} for each KME treatment was necessary because KME increased temperature as well as nutrients in the troughs and both effect growth rate.

Physical and chemical parameters: Water temperatures in the troughs in all experiments were measured each sampling day with an immersion thermometer. Water samples taken from the ends of the troughs were filtered immediately, preserved with mercuric chloride and analyzed for soluble reactive phosphorus (SRP), nitrate+nitrite-nitrogen and ammonium-nitrogen by the methods of Strickland and Parsons (1972). Grab samples of the KME were taken for nutrient analyses at 5-10 day intervals throughout the duration of the McKenzie River study. During the fall trial triplicate samples were taken every day. Nutrient analyses on KME were done using the same methods for water samples although samples were diluted to minimize interferences from color in the effluent.

Nutrient pulsing experiment: During one experiment at EXTRA, phosphorus was added in discrete pulses to determine the effect on growth in a non-steady state nutrient environment. The experiment was designed to test whether the growth rates of attached river algae were responding to the concentration of phosphorus in the water or to the hourly flux of phosphorus to which the cells were exposed flowing through the flumes. The FMI pumps were

controlled by a ChronTrol timer programmed to turn on each pump for the prescribed period each hour of the day, around the clock. Different combinations of orthophosphorus concentration and duration of addition were chosen such that the same hourly flux ($3000 \mu\text{g P}\cdot\text{h}^{-1}$) was obtained in each of five troughs. The five combinations tested were: $1 \mu\text{g P}\cdot\text{L}^{-1}$ added continuously; $2 \mu\text{g P}\cdot\text{L}^{-1}$ added for 30 min each hour; $4 \mu\text{g P}\cdot\text{L}^{-1}$ added for 15 min each hour; $12 \mu\text{g P}\cdot\text{L}^{-1}$ added for 5 min each hour and $60 \mu\text{g P}\cdot\text{L}^{-1}$ added for only one min each hour. With the water discharge to each trough set at $50 \text{ L}\cdot\text{min}^{-1}$, the hourly flux of phosphorus passing through the five flumes was the same, i.e. $3 \text{ mg P}\cdot\text{h}^{-1}$. The trial commenced with the start of water flow and nutrient addition and was terminated when biomass accumulation rate began to slow. Growth rates were determined from the kinetics of accrual as described in the preceding section.

Results

Thompson River experiments: Phosphorus enrichment experiments at EXTRA have consistently shown that the amount of orthophosphorus which had to be added to the river water to saturate the specific growth rates (μ) of attached diatom communities was extremely low, ca. $0.5 \mu\text{g P}\cdot\text{L}^{-1}$ (Fig. 6). With ambient levels of true orthophosphorus in the South Thompson River (estimated by radiobioassays at between 0.1 and $0.5 \mu\text{g P}\cdot\text{L}^{-1}$; Bothwell 1988), the total (ambient plus added) concentration of orthophosphorus which saturates μ is just under $1 \mu\text{g P}\cdot\text{L}^{-1}$. The maximum growth rate achieved when nutrients were no longer limiting (μ_{max}) is predominately determined by temperature (Fig. 6) (Bothwell 1988). When μ in P-enrichment experiments was normalized to μ_{max} , the relative specific growth rates ($\mu:\mu_{\text{max}}$) at different times of the year (i.e. different temperatures) as a function of external phosphorus concentration were similar (Fig. 7). $\mu:\mu_{\text{max}}$ approached unity at P-levels of less than $1 \mu\text{g P}\cdot\text{L}^{-1}$ (Bothwell 1988).

While very low P-levels saturate μ at the cellular level, the amount of accrued biomass continues to increase with increasing concentrations of phosphorus. In fact, saturation of areal algal biomass requires phosphorus concentrations 2 to 3 orders of magnitude greater than those shown in Figures 6 and 7 (Bothwell 1989). Notwithstanding the importance of this finding, the magnitude of areal biomass response to low levels of P-enrichment is still striking (Plate 4) and P-concentrations as low as $1 \mu\text{g P}\cdot\text{L}^{-1}$ can potentially cause dramatic increases in the level of productivity of rivers. Photographic evidence (Plate 4) illustrates that the massive increase in algal biomass in the lower Thompson River (Plate 1B) in the mid-1970's could have been caused by phosphorus even in the absence of measurable elevation in P-concentration in the water. It is also testimony to the fact that major increases in algal productivity in some rivers might be possible even when P-loadings are small.

McKenzie River experiments: KME also contains dissolved inorganic and organic nitrogen compounds. Of these, the inorganic forms nitrate and ammonium, are most readily available for algal uptake (Reynolds 1984). Although nitrate and ammonium are not usually present in high levels in KME, they can cause elevated algal growth in rivers. Concentrations of nitrate-N in KME discharged into the McKenzie River showed high temporal variance but generally ranged between 30 and $50 \mu\text{g N}\cdot\text{L}^{-1}$ (Fig. 8). Occasionally values up to $100 \mu\text{g N}\cdot\text{L}^{-1}$ were recorded (Fig. 8). Ammonium-N showed even greater temporal variation. Levels were usually around $200 \mu\text{g N}\cdot\text{L}^{-1}$ but peak values of up to $2 \text{mg N}\cdot\text{L}^{-1}$ occurred every few weeks (Fig. 8). The average concentration of nitrate+ammonium concentration during the experiments reported here was conservatively estimated at ca. $250 \mu\text{g N}\cdot\text{L}^{-1}$. Using this value as the "normal" concentration of dissolved inorganic nitrogen (DIN) in this particular KME, the DIN enrichments corresponding to the various KME dilutions were: 0.5% = $1.25 \mu\text{g N}\cdot\text{L}^{-1}$; 1%= $2.5 \mu\text{g N}\cdot\text{L}^{-1}$; 2%= $5.0 \mu\text{g N}\cdot\text{L}^{-1}$; 5%= $12.5 \mu\text{g N}\cdot\text{L}^{-1}$ and 25%= $62.5 \mu\text{g N}\cdot\text{L}^{-1}$.

KME also increased temperature in the troughs. While thermal influence was minimal at low concentrations, i.e. $\Delta < 0.5$ °C up to 2% KME, increases were substantial at 5% and 25% KME, i.e. $\Delta 1.5$ °C and $\Delta 6.0$ °C, respectively (Table 1).

The combined impacts of elevated temperature and available nitrogen from KME are reflected in μ of the algal communities. During summer, KME additions of only 0.5% almost doubled μ from $0.30 \text{ div}\cdot\text{d}^{-1}$ in the control to $0.56 \text{ div}\cdot\text{d}^{-1}$ (Table 1). Because temperature was barely altered by the low level KME treatment, this effect was certainly the result of nutrient (nitrogen) stimulation. During the spring and fall, KME additions up to 2% had a negligible effect on algal growth, but at 5 and 25% KME significantly increased μ (Table 1).

The response of $\mu:\mu_{\text{max}}$ to the KME treatments reflects the impact of nutrient additions alone (Donaghay et al. 1978; Goldman 1980). The fact that $\mu:\mu_{\text{max}}$ as well as μ nearly doubled with 0.5% KME during the summer trial (Table 1) strengthens the conclusion that nutrient (nitrogen) stimulation was responsible for the increase. Conversely, the fact that $\mu:\mu_{\text{max}}$ was unaffected by low KME levels during the spring and fall, indicates that nutrient additions from KME were having little impact on algal growth at those times of the year. A plot of $\mu:\mu_{\text{max}}$ versus percent KME addition during the summer trial shows a classic Monod-type response to the concentration of a limiting nutrient with growth saturation being approached (i.e. $\mu_{\text{max}} \sim 0.9$) at about 1% v/v KME (Fig. 9). This corresponds to a DIN addition of ca. $2.5 \mu\text{g N}\cdot\text{L}^{-1}$.

Phosphorus pulsing experiments: When hourly P-flux was constant, the specific growth rates of algal communities were the same (ca. $0.25 \text{ div}\cdot\text{d}^{-1}$) across a spectrum of phosphorus pulse heights ranging up to $60 \mu\text{g P}\cdot\text{L}^{-1}$ (Fig. 10). This observation corroborates the findings of other workers that P-limited algal cells have the ability to uptake P very rapidly (Blum 1966; Fuhs et al. 1972; Rhee 1973) and can obtain sufficient phosphorus during short exposures to high phosphate to maintain rapid growth rates (Suttle et al. 1988). Since P-uptake rate increases with P-concentration, faster uptake rates at increasingly higher concentrations

counterbalance the shorter duration of exposure. The net effect is that algal growth rates remain constant and appear to be set by hourly P-flux (Fig. 10).

Discussion

The P-enrichment experiments at EXTRA provided incontrovertible evidence that increasing orthophosphate concentrations on the order of $1 \mu\text{g P-L}^{-1}$ in a highly P-limited river would substantially increase algal growth rates and that this could result in higher algal biomass. The increase in P-concentration in the Thompson River resulting from the discharge of pulp mill effluent can be calculated from the measured loading of soluble-P in the effluent and the dilution in the river at complete mix. This computation indicates that phosphorus is increased by more than $1 \mu\text{g P-L}^{-1}$ at all times of the year except May and June and July (Fig. 11). However, this simple dilution calculation overestimates P-elevation during most of the year in the Thompson River because hydrodynamic mixing processes in Kamloops Lake greatly increase dilution beyond that shown in Figure 3. Mixing in the lake is especially important during the summer, fall and late spring (Carmack et al. 1979; St. John et al. 1976). Consequently, it is primarily during limnological winter (December- March) when P-concentration is actually increased ca. $2 \mu\text{g P-L}^{-1}$ that experiments indicate phosphorus could be responsible for the unsightly amounts of algal biomass accumulation that occurred in the river in the mid-1970's (Bothwell et al. 1989, Bothwell et al. in press).

Direct enrichment with KME also increased algal growth rates on the McKenzie River during summer. Even 0.5% KME caused algal growth rates to double. Because this concentration conservatively reflects the actual dilution in the river at complete mix (Fig. 3) an increase of this magnitude indicates that KME has a strong effect on the algal productivity in the McKenzie River during the summer. However, the computed increase in DIN

concentration of 1-2 $\mu\text{g}\cdot\text{L}^{-1}$ in situ in summer (Fig. 11) is lower than levels which saturate algal growth (Fig. 9). Hence, in this instance, further increases in DIN loading would likely result in even greater algal production and biomass.

Direct enrichment experiments with KME, such as those conducted at the McKenzie River site, have three major advantages over those conducted at EXTRA where one nutrient was added in isolation. First, they measure algal responses to the same mix of nutrient compounds actually being added to the environment. This is important because the effect of single nutrient addition is frequently not the same as when two or more are added simultaneously. For example, in lakes (Schindler et al. 1971; Suttle and Harrison 1988; Dodds et al. 1989) and rivers (Stockner and Shortreed 1978; Bothwell unpubl. data) additions of N and P together may have greater effect than either added alone. Second, trials with actual effluent additions measure the impact of interactions with other parameters in the effluent, such as temperature. Temperature is of particular importance because it substantially influences all physiological processes and KME is nearly always warmer than ambient receiving environments. For this reason using relative specific growth rates to factor out physical effects is important.

While the $\mu:\mu_{\text{max}}$ concept has been in the scientific literature for more than a decade it is not widely used in environmental assessments even though in those instances where it has been employed it has proved to be invaluable in identifying varying degrees of nutrient limitation between sites with different temperature regimes (Bothwell 1985; Biggs 1990). In the Thompson River, the relative growth rates of algae responded to P-additions the same year round (Fig. 7), but the absolute effect on growth rate was greatest during the warmer months of the year (Fig. 8). This indicates that while the low levels of P added to the Thompson River during winter can stimulate algal production, the situation would likely be worse if the annual hydrograph of the Thompson were like that of the McKenzie (Fig. 2) with lowest flows during the warmer summer months.

A third aspect of realism embodied in the direct, in-line (fresh) KME addition trials, is the effect of varying nutrient levels on algal growth. The daily sampling program during the fall trial showed that the erratic fluctuations in nutrient levels from bioponds (see Fig. 8 for ammonia and nitrate data) were real and not the result of sampling error (Fig. 12). These order of magnitude nutrient spikes could be several days in duration and may have resulted from process upsets in the mill, the discharge of industrial cleansers, or from the intentional addition of nutrients to stimulate biopond oxidation. While the role of nutrient pulses in controlling algal composition, size distribution and species competitiveness has been thoroughly examined in laboratory experiments (Turpin and Harrison 1979, 1980; Robinson and Sandgren 1983; Scavia et al. 1984; Sommer 1984, 1985; Suttle et al. 1987) the effect of pulses on the growth of attached algae in rivers has not been previously demonstrated. The P-pulsing experiment at EXTRA provided evidence for the importance of time-averaged nutrient flux on algal growth in rivers. Although the timing and magnitude of the nutrient spikes coming from pulp mill bioponds are much different than the P-pulses examined here, the overall effect would be similar. Algae have the ability to uptake and store phosphorus in excess of immediate needs to facilitate growth during times of nutrient deficiency. Hence, slugs of nutrients discharged into rivers, even for only a few hours, could be sufficient to increase algal growth for many days. For this reason, maintenance of temporal stability of effluent characteristics should be a consideration in the control of eutrophication effects of pulp mill effluent.

The most important observation in comparing how nitrogen-limited rivers respond to KME enrichment relative to phosphorus-limited systems is that during spring and fall, KME had no impact on algal growth in the McKenzie River, in spite of in situ N:P ratios suggesting a strongly N-limited community. Light levels during the spring ($818 \text{ J}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$) and fall ($648 \text{ J}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$) experiments were half those during the summer trial ($1915 \text{ J}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$). Light has been shown in algal culture studies to be important in the uptake and assimilation of nitrate-

nitrogen (Bongers 1956; Hattori 1962; Grant and Turner 1969; Eppley et al. 1971) and ammonium-nitrogen (MacIsaac and Dugdale 1972). Nitrate enrichment studies in lotic systems have also shown that light is an important parameter determining nitrate uptake by periphyton communities where increasing shading was found to progressively decrease the uptake of nitrate-N (Triska et al. 1983). While some studies have shown the uptake of phosphorus can also be stimulated by light (Nalewajko and Lean 1980), the effect is only seen at high concentrations of phosphorus (Healey 1973). Hence, the eutrophication of N-limited rivers is probably more strongly dependent on the seasonality of light availability than P-limited rivers.

In summary, the nitrogen and phosphorus content of KME which has been secondarily treated in extended aeration lagoons can cause algal blooms in receiving rivers. In some cases the increases in primary production have been large enough to alter both the appearance of the river bottom as well as the river's trophic status. However, the role of pulp mill effluent in the eutrophication of rivers is not widely appreciated. In part, this is because of the common misconception that concentrations of nutrients must be greatly elevated to have a measurable impact on algal production. Enrichment experiments conducted in stream side, outdoor flumes have clearly demonstrated that an elevation in orthophosphorus of as low as $0.2 \mu\text{g}\cdot\text{L}^{-1}$ can produce a substantial increase in algal growth rate in P-limited algal communities. An increase in dissolved inorganic nitrogen (DIN) of $2.5 \mu\text{g}\cdot\text{L}^{-1}$ can produce a similar response in N-limited systems. While, elevated concentrations of orthophosphate and DIN of around $1 \mu\text{g}\cdot\text{L}^{-1}$ and $10 \mu\text{g}\cdot\text{L}^{-1}$, respectively, are sufficient to completely saturate growth rates of thinner periphyton communities, higher nutrient concentrations will still produce thicker algal mats (Bothwell 1989).

Experiments with phosphorus added in pulses demonstrated that the growth rates of algae actually respond to the time averaged flux of a nutrient, not simply the steady-state concentration. This illustrates the need to minimize disturbances in mill operations and treatment processes which might result in slugs of nutrients being discharged to rivers. The importance of maintaining stable discharge characteristics in controlling eutrophication of rivers, would also be a primary consideration in controlling potential toxicity as well.

Acknowledgements

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References

- BERRY, R.M., B.I. FLEMING, R.H. VOSS, C.E. LUTHIE and P.E. WRIST. 1989. Toward preventing the formation of dioxan during chemical pulp bleaching. *Pulp and Paper Canada* 90:T279-T289.
- BIGGS, B. J. F. 1990. Use of relative specific growth rates of periphytic diatoms to assess enrichment of a stream. *New Zealand J. Mar. Freshwater Res.* 24:9-18.
- BLUM, J.J. 1966. Phosphate uptake by phosphate-starved *Euglena*. *J. Gen. Physiol.* 49: 1125-1137.
- BONGERS, L.H.J. 1956. Aspects of nitrogen assimilation by cultures of green algae (*Chlorella vulgaris*, strain A and *Scenedesmus*). Mededel. Landbouwhogeschool Wageningen 56:1-52.
- BOTHWELL, M.L. and J.G. STOCKNER. 1980. Influence of secondarily treated kraft mill effluent on the accumulation rate of attached algae in experimental continuous-flow troughs. *Can. J. Fish. Aquat. Sci.* 37:248-254.
- BOTHWELL, M.L. 1983. All-weather troughs for periphyton studies. *Water Res.* 17:1735-1741.
- BOTHWELL, M.L. and S. JASPER. 1983. A light and dark trough methodology for measuring rates of lotic periphyton settlement and net growth: An evaluation through intersite comparison. p.253-265. In R.G. Wetzel [ed.], *Periphyton of freshwater ecosystems*. Junk.

- BOTHWELL, M.L. 1985. Phosphorus limitation of lotic periphyton growth rates: An intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). *Limnol. Oceanogr.* 30:527-542.
- BOTHWELL, M.L. 1988. Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. *Can. J. Fish. Aquat. Sci.* 45:261-270.
- BOTHWELL, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Can. J. Fish. Aquat. Sci.* 46:1293-1301.
- BOTHWELL, M. L., S. JASPER and R. J. DALEY. 1989. Phosphorus control of algal production and biomass in the Thompson River, British Columbia. IWD Scientific Series No 165. 9 pp.
- BOTHWELL, M. L., G. DERKSEN, R. N. NORDIN and J. M. CULP. 1991. Nutrient and grazer control of algal biomass in the Thompson River, British Columbia: A case history of water quality management. Proceedings of the Rawson Academy Conference on Aquatic Ecosystems in Semi-Arid Regions. Saskatoon Sask. (in press).
- CARMACK, E. C., C. B. GRAY, C. H. PHARO and R. J. DALEY. 1979. Importance of lake-river interaction on seasonal patterns in the general circulation of Kamloops Lake, British Columbia. *Limnol. Oceanogr.* 24:634-644.

- CELGAR EXPANSION REVIEW PANEL. 1991. Final report submitted under the Federal Environmental Assessment and Review Process and British Columbia Major Project Review Process. 98pp.
- DONAGHAY, P.L., J.M. DEMANCHE and L.F. SMALL. 1978. On predicting phytoplankton growth rates from carbon:nitrogen ratios. *Limnol. Oceanogr.* 23:359-362.
- DODDS, W. K., K. R. JOHNSON and J. C. PRISCU. 1989. Simultaneous nitrogen and phosphorus deficiency in natural phytoplankton assemblages: Theory, empirical evidence, and implications for lake management. *Lake and Reservoir Management* 1: 21-26.
- DOWD, J. E. and D. S. RIGGS. 1965. A comparison of estimates of Michaelis-Menton kinetic constants from various linear transformations. *J. Biol. Chem.* 240:863-869.
- EARLE, P. F. and D. W. REEVE. 1990. Chlorinated organic matter in bleached chemical pulp production. Part 6. Chlorinated compounds in effluent. *Tappi.* Jan 1990. pp179-184.
- ENVIRONMENT CANADA. 1979. Analytical methods manual, v.1. Inland Waters Directorate, Water Quality Branch, Ottawa.
- EYSENBACH, E. J., L.W. NEAL and J.W. OWENS. 1990. Pulping effects in the aquatic environment. *Tappi.* Aug 1990. pp.104-106.
- FEDERAL-PROVINCIAL THOMPSON RIVER TASK FORCE. 1976. Sources and effects of algal growth, colour, foaming and fish tainting in the Thompson River system. Summary Report.

- FUHS, G.W., S.D. DEMMERLE, E. CANELLI and M. CHEN. 1972. Characterization of phosphorus limited algae. In: Likens, G.E. (ed.): *The Limiting Nutrient Concept*, Limnol. Oceanogr. Special Proceedings, 113-33.
- GOLDMAN, J.C. 1980. Physiological processes, nutrient availability, and the concept of relative growth rate in marine phytoplankton ecology. In: Falkowski, P.G. [ed.]: *Primary productivity in the sea*. Brookhaven Symposia in Biology. Plenum Press. pp. 179-194.
- GRANT, B. R. and I. M. TURNER. 1969. Light stimulated nitrate and nitrite assimilation in several species of algae. *Comp. Biochem. Physiol.* 29:995-1004.
- HATTORI, A. 1962. Light- induced reduction of nitrate, nitrite and hydroxylamine in a blue-green algae, *Anabaena cylindrica*. *Pl. Cell Physiol. Tokyo* 3:355-369.
- HEALEY, F.P. 1973. Inorganic nutrient uptake and deficiency in algae. *CRC Crit. Rev. Microbiol.* 3: 69-113.
- HOLM-HANSEN, O., C.J. LORENZEN, R.W. HOLMES and J.D.H. STRICKLAND. 1965. Fluorometric determination of chlorophyll. *J. Cons. Perm. Int. Explor. Mer.* 30:3-15.
- HOOPER, F. F. 1969. Eutrophication indices and their relation to other indices of ecosystem change. In *Eutrophication: causatives, consequences, correctives*. National Academy of Sciences. Washington D.C. pp. 225-235.
- LORENZEN, C.J. 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnol. Oceanogr.* 12: 343-346.

- MACISAAC, J.J. and R.C. DUGDALE. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.* 19:209-232.
- NALEWAJKO, C. and D.R.S. LEAN. 1980. Phosphorus. In: Morris, I. [ed.] *The physiological ecology of phytoplankton.* Univ. Cal. Press. pp. 235-258.
- RHEE, G. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp.. *J. Phycol.* 9: 495-506.
- SCAVIA, D., G.L. FAHNENSTIEL, J.A. DAVIS and R.G. KREIS. 1984. Small-scale nutrient patchiness: some consequences and a new encounter mechanism. *Limnol. Oceanogr.* 29:785-793.
- SERVIZI, J. 1989. Protecting Fraser River salmon from waste waters: An assessment. *Can. Special Publ. Fish. Aquat. Sci.* 105:136-153.
- SERVIZI, J. and R. W. GORDON. 1986. Detoxification of TMP and CTMP effluent alternating in a pilot scale aerated lagoon. *Pulp and Paper Canada* 87:T404-T409.
- SODERGREN, A. 1989. Biological effects of bleached pulp mill effluent. National Swedish Environmental Protection Bd. Report 3558.
- SOMMER, U. 1984. The paradox of the plankton: Fluctuations of phosphorus availability maintain diversity of phytoplankton in flow-trough cultures. *Limnol. Oceanogr.* 29:633-636.

- SOMMER, U. 1985. Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton. *Limnol. Oceanogr.* 30:335-346.
- STOCKNER, J.G. and K.R.S. SHORTREED. 1978. Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. *J. Fish. Res. Board Can.* 35:28-34.
- ST. JOHN, B. E., E. C. CARMACK, R. J. DALEY, C. B. GRAY and C. H. PHARO. 1976. The limnology of Kamloops Lake, British Columbia. *Can. Centre Inland Waters, Pacific Yukon Region. West Vancouver, B.C.* 167 p.
- STRICKLAND, J.D.H. and T.R. PARSONS. 1972. A practical handbook of seawater analysis. *Fish. Res. Board Can. Bull.* 167:1-311.
- SUTTLE, C.A., J.G. STOCKNER, and P.J. HARRISON. 1987. Effects of nutrient pulses on community structure and cell size of a freshwater phytoplankton assemblage in culture. *Can. J. Fish. Aquat. Sci.* 44:1768 - 1774.
- SUTTLE, C.A., J.G. STOCKNER, K.S. SHORTREED and P.J. HARRISON. 1988. Time-courses of size-fractionated phosphate uptake: are larger cells better competitors for pulses of phosphate than smaller cells? *Oecol.* 74: 571-576.
- TRISKA, F. J., V. C. KENNEDY and R. J. AVANZINO. 1983. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. In: Fontaine, T.D. and Bartell, S.M. [eds]. *Dynamics of lotic ecosystems.* Ann Arbor Science Publ. Ann Arbor, MI. pp. 129-159.

TURPIN, D.H. and P.J. HARRISON. 1979. Limiting nutrient patchiness and its role in phytoplankton ecology. *J. Exp. Mar. Biol. Ecol.* 39: 151-166.

TURPIN, D.H. and P.J. HARRISON. 1980. Cell size manipulation in natural marine, planktonic, diatom communities. *Can. J. Fish. Aquat. Sci.* 37:1193-1195.

Figure legends

Figure 1. Map showing the general locations of the basins of the Thompson River in south central British Columbia and the McKenzie River in central Oregon. Both rivers are impacted by effluent from kraft pulp mills.

Figure 2. Annual hydrographs of the Thompson and McKenzie Rivers. Data for the Thompson River are the mean daily values downstream of Kamloops between 1915-1987. The curve for the McKenzie River is interpolated from mean monthly values during the 1970's at Springfield, Oregon. This figure illustrates the major differences in the annual hydrographs of the two rivers.

Figure 3. Percent (v/v) KME in river water at complete mix in the Thompson and McKenzie Rivers. These data are computed using the seasonal discharge data in Figure 2 and assumed constant KME discharge rates of $1.7 \text{ m}^3\cdot\text{s}^{-1}$ for the Thompson River and $0.44 \text{ m}^3\cdot\text{s}^{-1}$ for the McKenzie River.

Figure 4. Concentrations of soluble reactive phosphorus (SRP) and nitrate-nitrogen (NO_3) in the Thompson and McKenzie Rivers during their respective periods of low flow. Values for the Thompson are averages on each sampling date from the North and South Thompson Rivers just upstream of Kamloops. The N:P ratios are computed on a molar basis using the sum of nitrate and ammonium (not shown) concentrations for N and SRP for P. Water samples from the McKenzie River were taken at the experimental flume site near Springfield, Oregon.

Figure 5. The empirical relationship between maximum specific growth rate (μ_{max}) and water temperature during 13 experiments at EXTRA (Bothwell 1988) and 3 trials on the McKenzie River (this paper). Data are fitted with a least-squares linear regression model.

Figure 6. Attached algal community growth rates ($\text{div}\cdot\text{d}^{-1}$) as a function of added orthophosphate concentration at different times of the year (i.e. different temperatures). The results of four experiments are shown. The temperatures given are the mean values during that experiment. Error bars are ± 1 SE. Redrawn from Bothwell (1988).

Figure 7. Relative specific growth rates ($\mu:\mu_{\text{max}}$) of the attached algal communities as a function of added orthophosphate concentration at different times of the year (i.e. different temperatures). Values from five experiments are plotted. The temperatures given are the mean values during that experiment. Redrawn from Bothwell (1988).

Figure 8. Concentrations of ammonia-N and nitrate-N in KME from biopond discharge during the months of April -October. Grab samples were taken at 5-10 days intervals.

Figure 9. Relative specific growth rates ($\mu:\mu_{\text{max}}$) of the attached algal communities in the summer trial on the McKenzie River as a function of both percent KME added and the added concentration of dissolved inorganic nitrogen (DIN) in ppb ($\mu\text{g}\cdot\text{L}^{-1}$). Points are fitted with a line drawn by eye. Error bars are ± 1 SE.

Figure 10. Specific algal growth rate (μ) plotted against the log of the phosphate concentration added as a timed pulse. The hourly phosphate flux of $3000 \mu\text{g P}\cdot\text{h}^{-1}$ flowing through all troughs was the same and was set by choosing appropriate P-pulse heights and durations as outlined in the text.

Figure 11. The left hand ordinate scale shows the computed increase in concentration of soluble reactive phosphorus (SRP) in ppb ($\mu\text{g}\cdot\text{L}^{-1}$) in the Thompson River resulting from the discharge of KME at Kamloops, BC. Computations assume the river discharge curve shown in Figure 2 ; a constant KME effluent volume of $1.7 \text{ m}^3\cdot\text{s}^{-1}$; and a dissolved-P level in KME of 300

$\mu\text{g}\cdot\text{L}^{-1}$ (Bothwell et al. 1989). The right hand ordinate scale shows the computed increase in dissolved inorganic nitrogen (DIN) in ppb ($\mu\text{g}\cdot\text{L}^{-1}$) in the McKenzie River, Oregon resulting from the discharge of KME at Springfield, OR. Computations assume the river discharge curve shown in Figure 2; a constant KME effluent volume of $0.44 \text{ m}^3\cdot\text{s}^{-1}$; and a DIN content in the KME of $250 \mu\text{g}\cdot\text{L}^{-1}$.

Figure 12. Concentrations of ammonia-N and soluble reactive phosphorus (SRP) expressed as ppm ($\text{mg}\cdot\text{L}^{-1}$) measured in daily grab samples taken during the fall experiment. Samples were taken in triplicate. Error bars are $\pm 1\text{SD}$.

Plate legends

Plate 1. Benthic algal biomass in the Thompson River near Chase, BC., upstream (A) of Kamloops and the pulp mill and downstream (B) of the pulp mill discharge at Savona in early April 1975. Downstream of the pulp mill benthic diatom biomass became so great that at times it completely obscured the topography of the river bottom. The reach of river affected in this manner in the mid-1970's was 20-40 km in length. Photos by O. Langer.

Plate 2. A. Overview of the experimental trough site on the bank of the McKenzie River near Springfield, Oregon. B. Gate valve controlling flow of fresh river water to a 1200-L head tank. C. Front view showing six, 20-L tanks for continually mixing river water and KME in known dilutions. D. Oblique view showing overflow pipes leading from mixing tanks to the head of each of six Plexiglas flumes (3.6 mX19 cm) arranged in three pairs. In the upper right is the 120-L constant head tank for KME continuously pumped to the site. Horizontal distribution pipes for both river water and KME are seen above the mixing chambers. E. Oblique view showing the six, 3-m long flumes in operation with discharged water returned to the river. The flumes are covered with plate glass to reduce inhibitory effects of UV-B radiation. PVC piping was used for all plumbing.

Plate 3. EXTRA on the South Thompson River at Chase, BC. A. Twelve troughs (each 2 mX19 cm), arranged three on a platform. B. Three troughs after 21 days of periphyton accumulation. C. Closeup showing holes remaining following removal of styrofoam plugs for chlorophyll analysis. D. Styrofoam plugs removed from the corkborer for chlorophyll analysis. E. Two troughs during a trial showing holes resulting from chlorophyll sampling .

Plate 4. Algal biomass accumulation at the end of a long-term experiment at EXTRA on the South Thompson River. Photo taken after 61 days of growth comparing the control (no phosphorus added) to the response with 1 ppb (ie. $1 \mu\text{g} \cdot \text{L}^{-1}$) of phosphorus added.

TABLE 1. Results of KME enrichment experiments on the specific growth rates (μ) of attached diatom communities in the McKenzie River, Oregon during spring, summer and fall. Also shown for each trial are the mean temperatures for each treatment (KME increased temperature as well as nutrient level) and the computed relative specific growth rates ($\mu:\mu_{\max}$) where the theoretical μ_{\max} for each treatment was determined from the empirical regression of μ_{\max} against temperature in all experiments on the McKenzie and Thompson Rivers combined (Fig. 5).

PERCENT KME (v/v)	SPRING			SUMMER			FALL		
	TEMP °C	μ (div.d-1)	$\mu:\mu_{\max}$	TEMP °C	μ (div.d-1)	$\mu:\mu_{\max}$	TEMP °C	μ (div.d)	$\mu:\mu_{\max}$
CONTROL	9.0	0.24	0.53	16.5	0.30	0.44	11.0	0.23	0.45
0.5%	9.1	0.29	0.64	16.6	0.56	0.82	11.1	0.20	0.39
1.0%	9.2	0.29	0.63	16.7	0.63	0.93	11.2	0.24	0.46
2.0%	9.5	0.32	0.68	17.0	0.72	0.97	11.5	0.27	0.50
5.0%	10.2	0.42	0.86	17.7	0.76	1.05	12.2	0.62	1.13
25.0%	15.0	0.69	1.10	22.5	0.88	1.02	17.0	0.71	1.03

FIGURE 1.

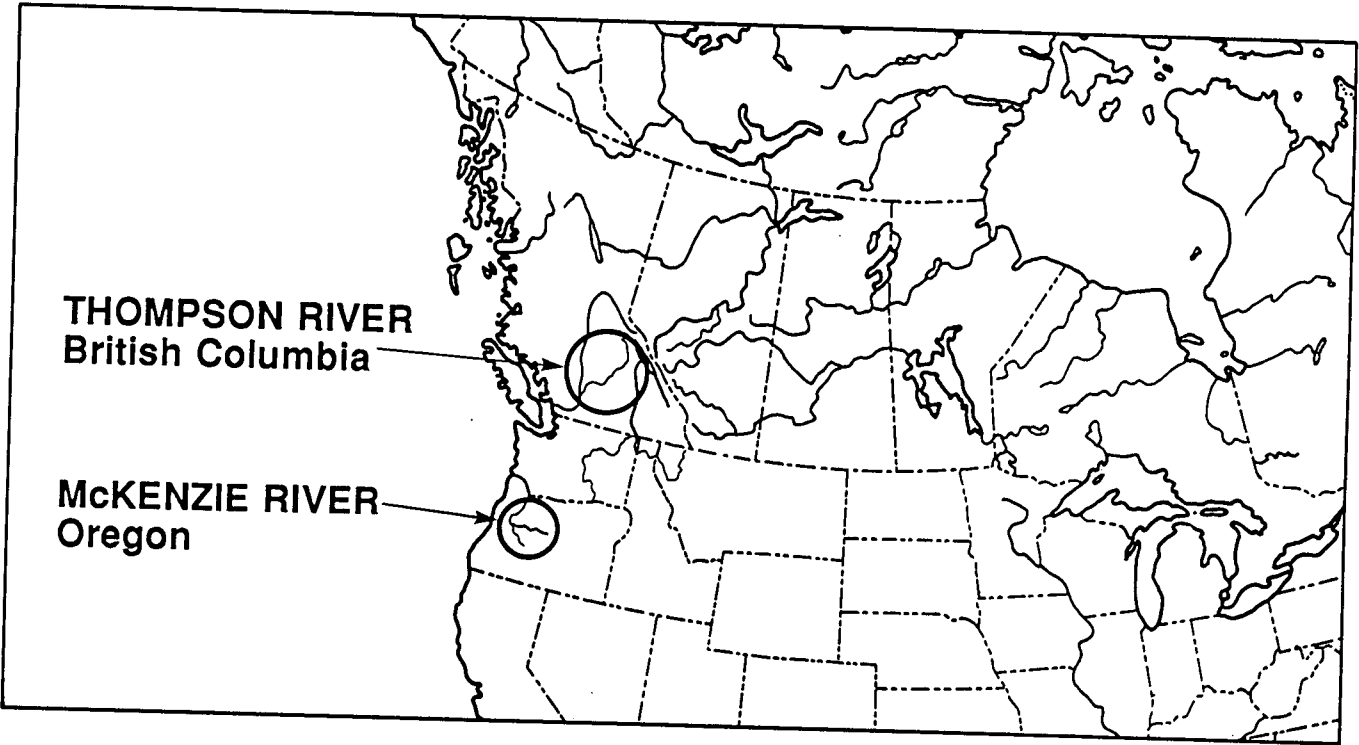


FIGURE 2.

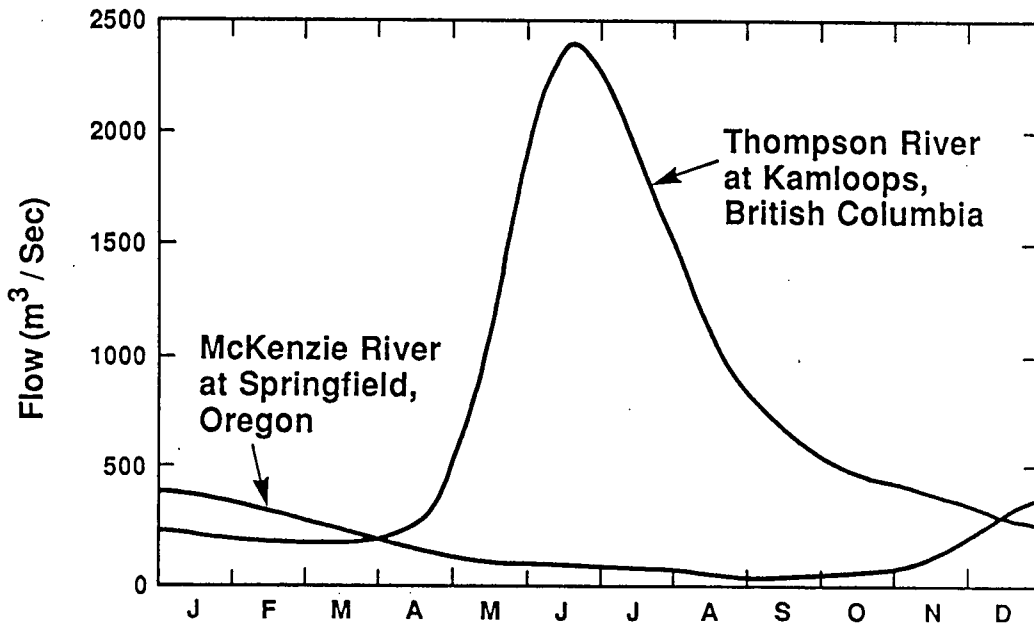
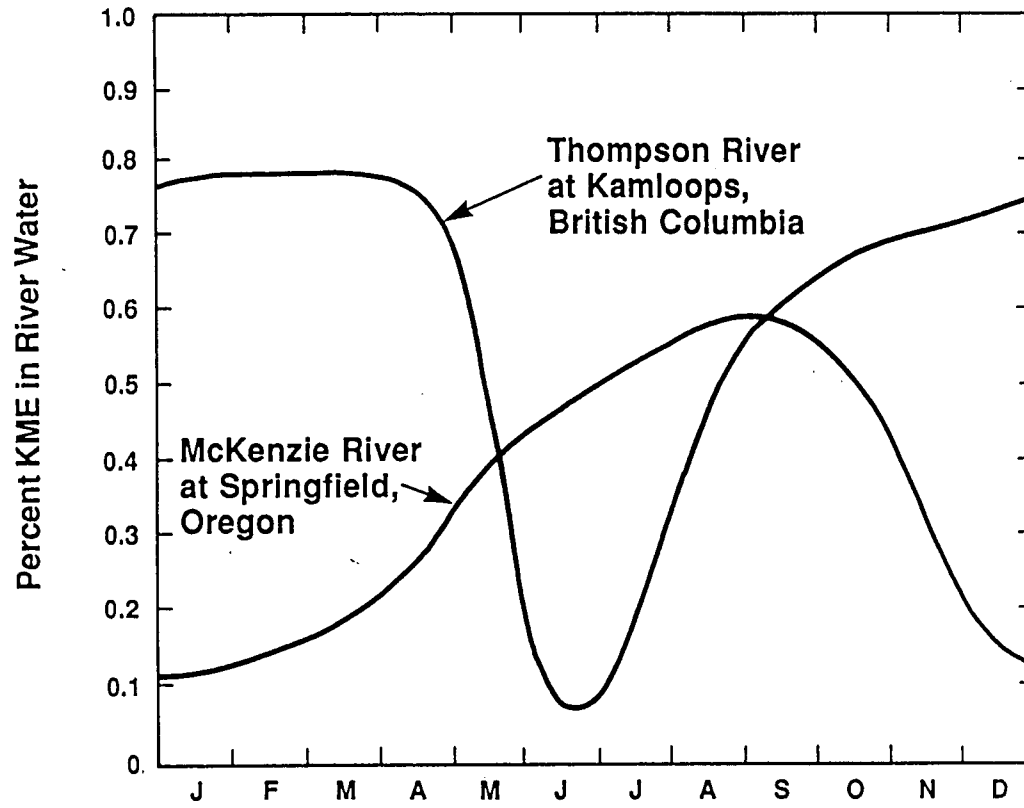


FIGURE 3.



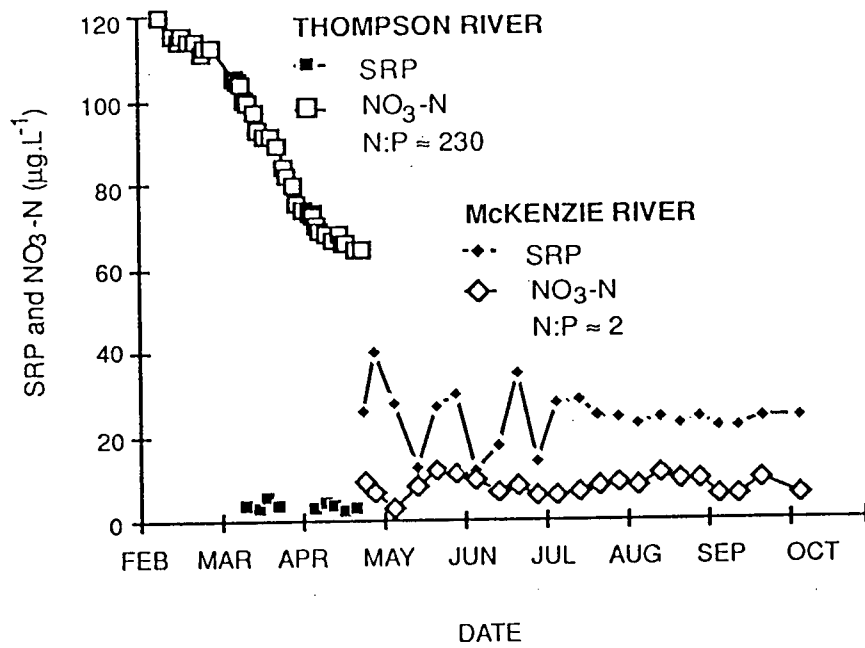


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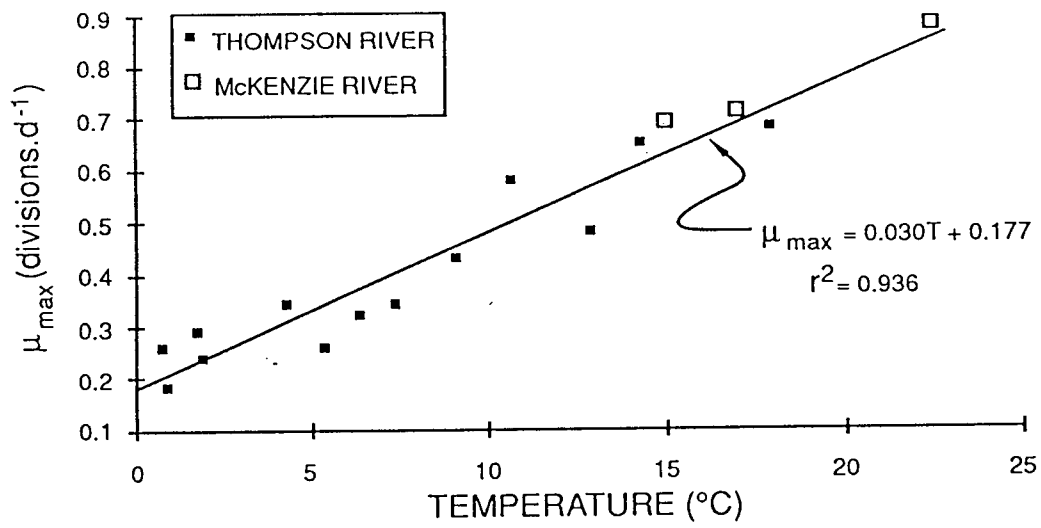


FIGURE 5.

FIGURE 6.

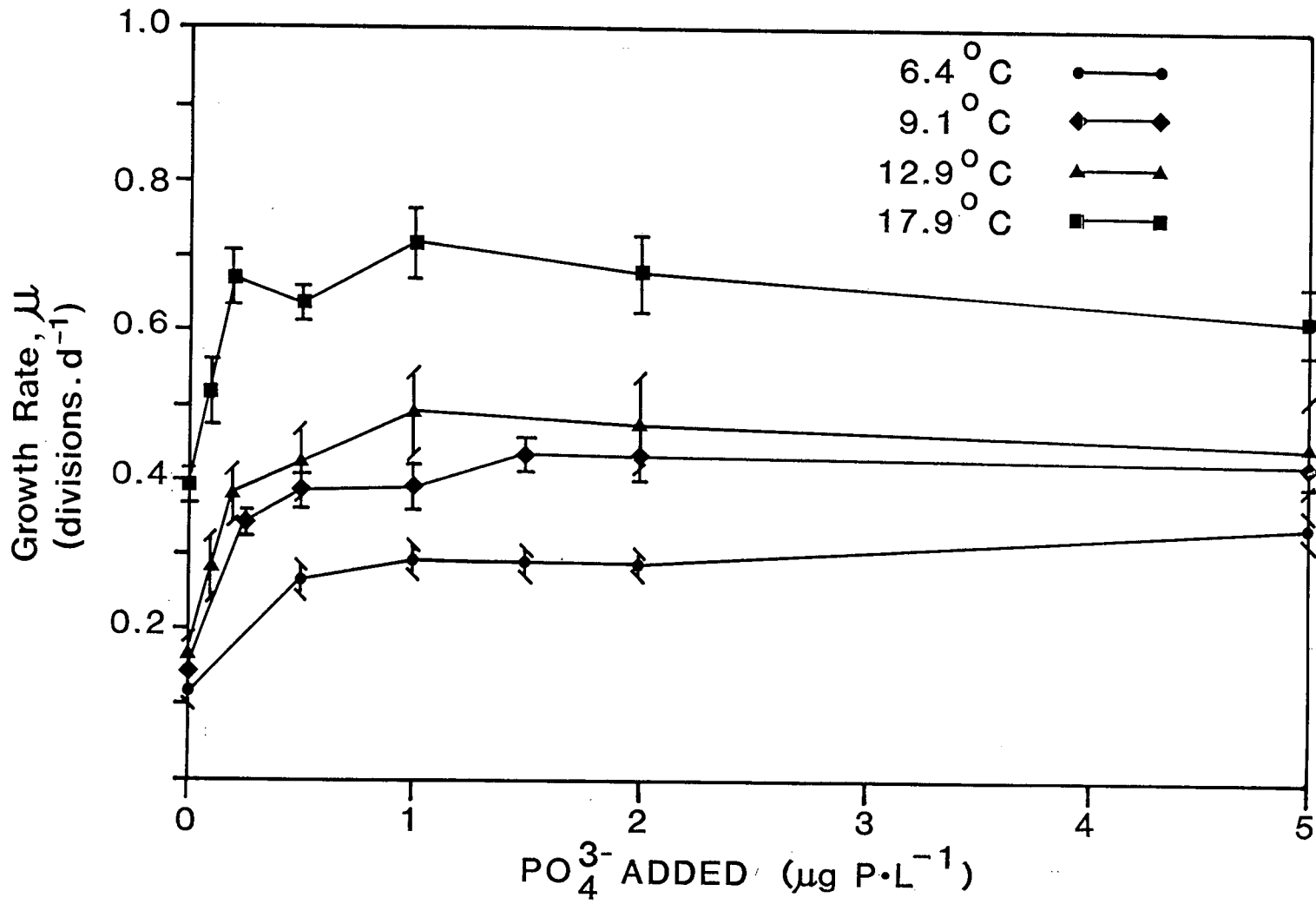
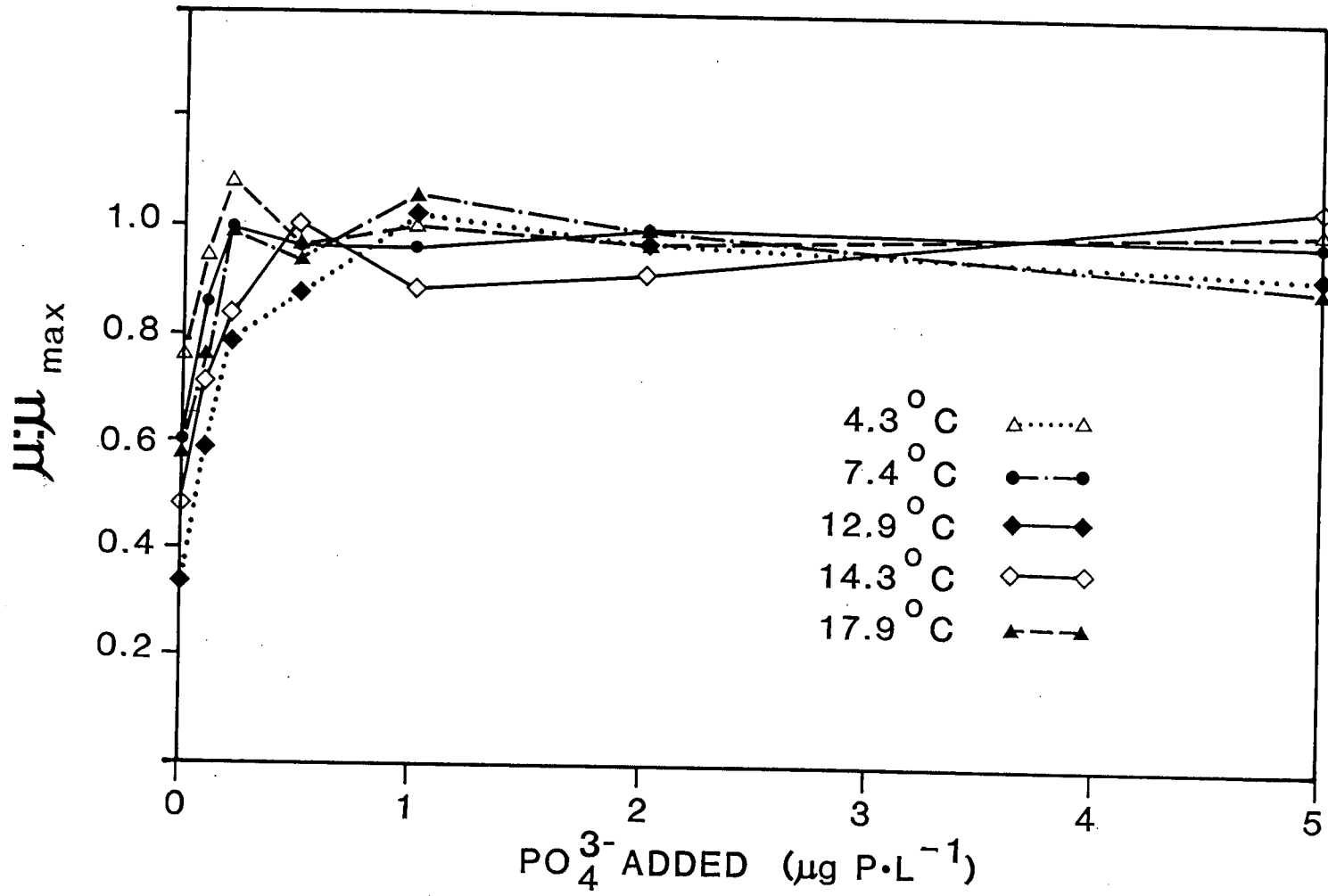


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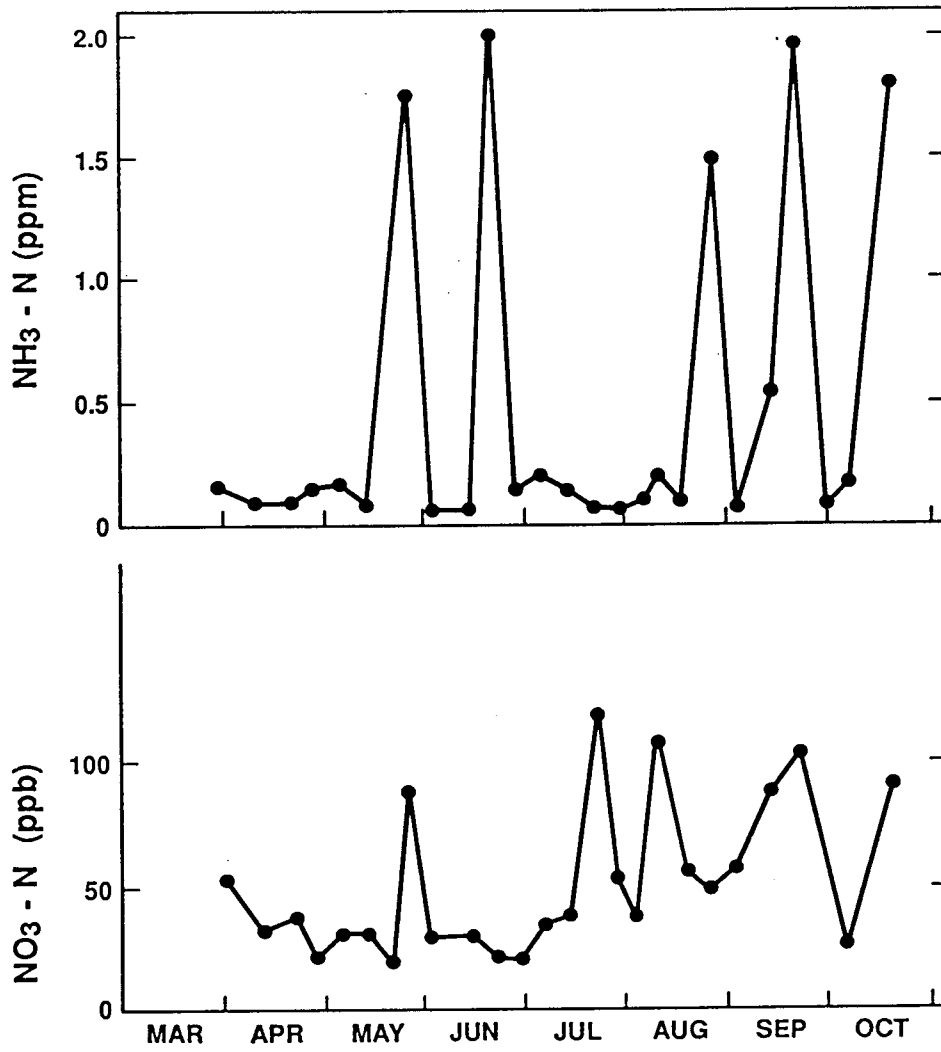
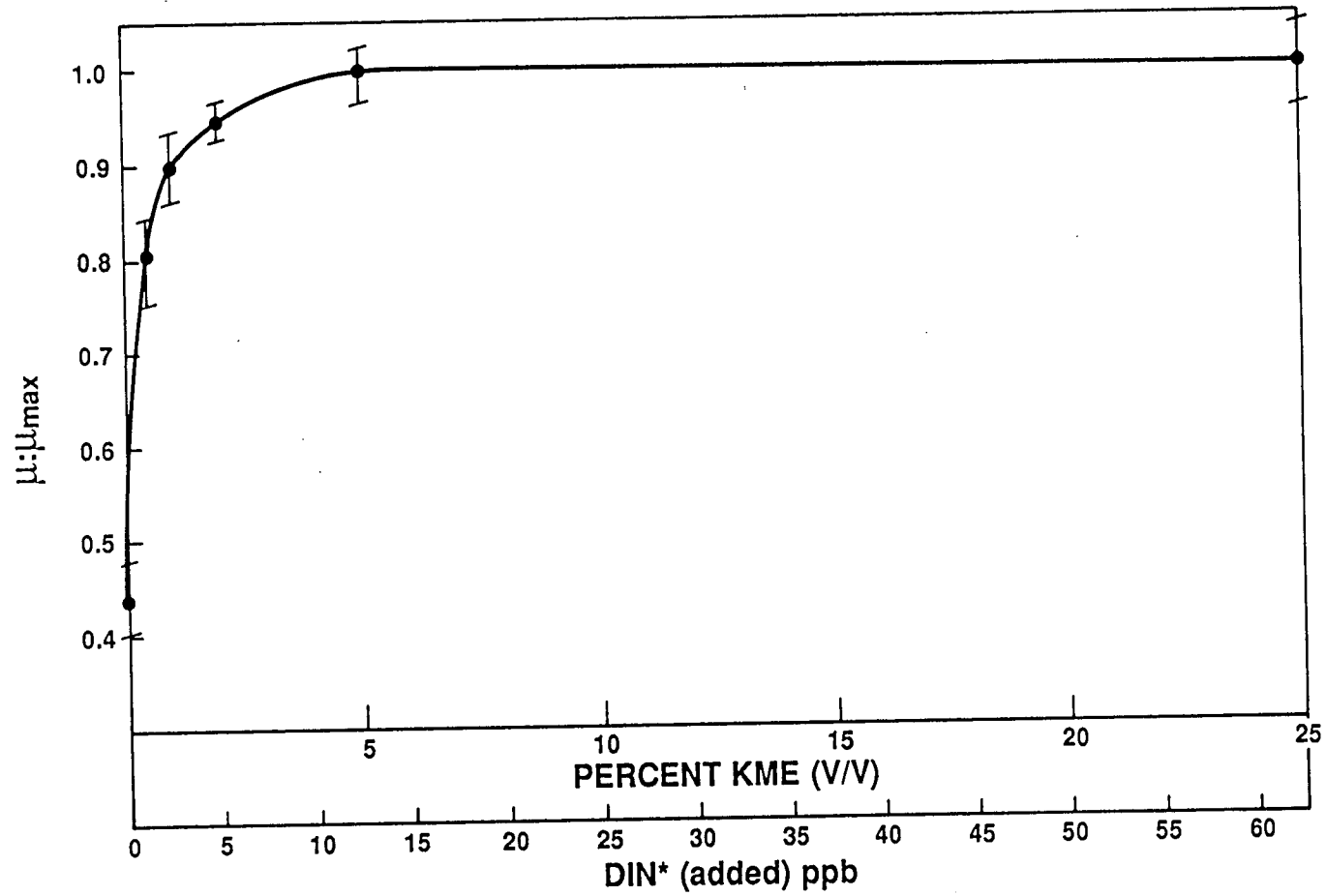


FIGURE 8.

FIGURE 9.



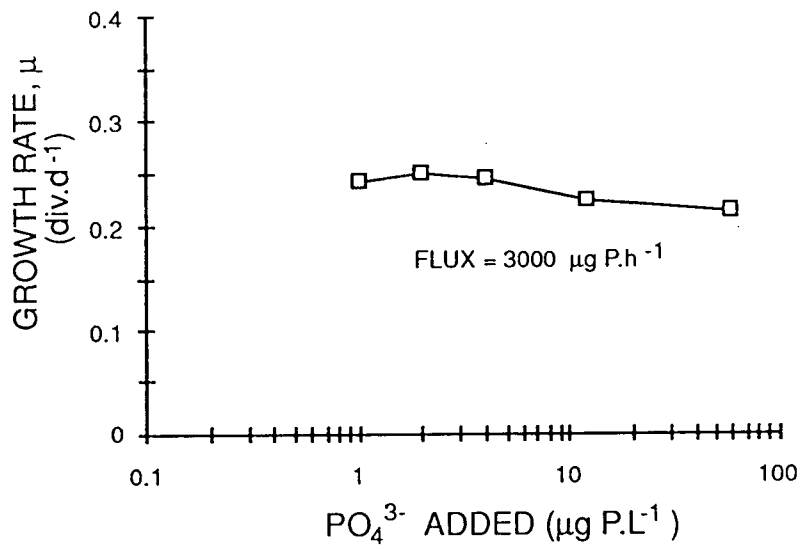
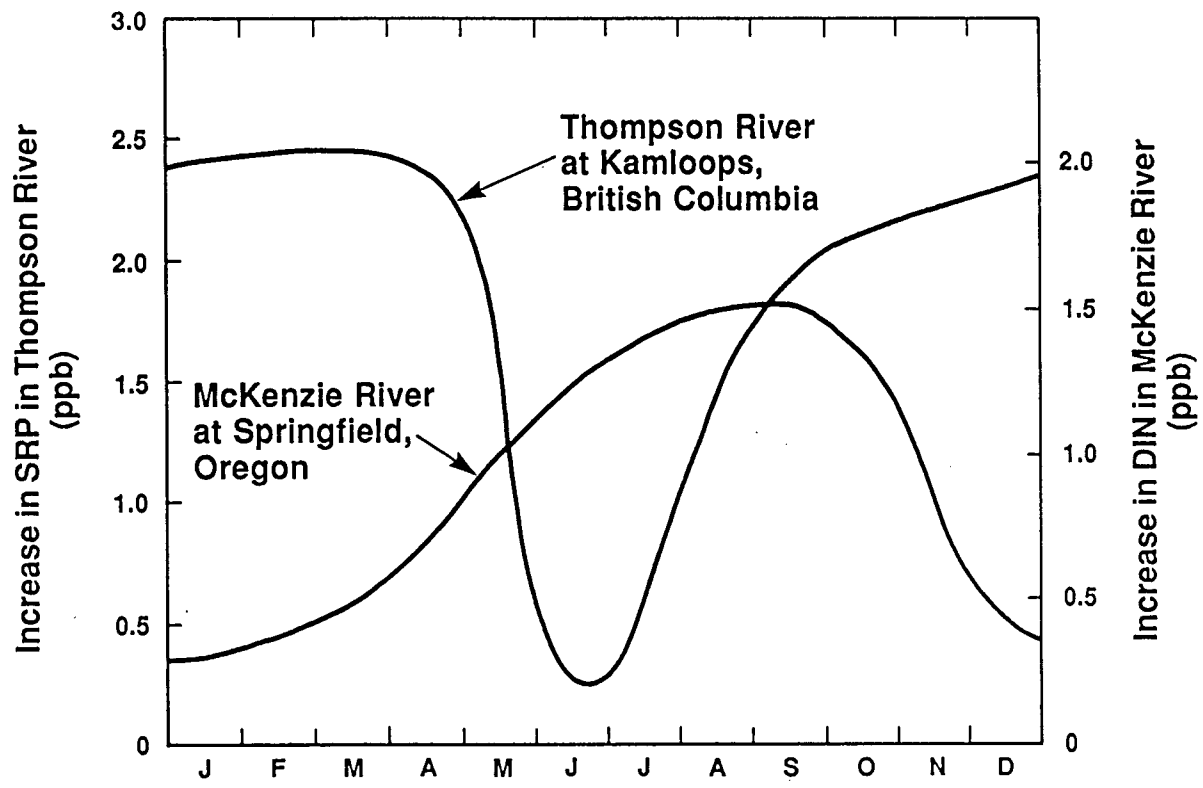


FIGURE 10.

FIGURE 11.



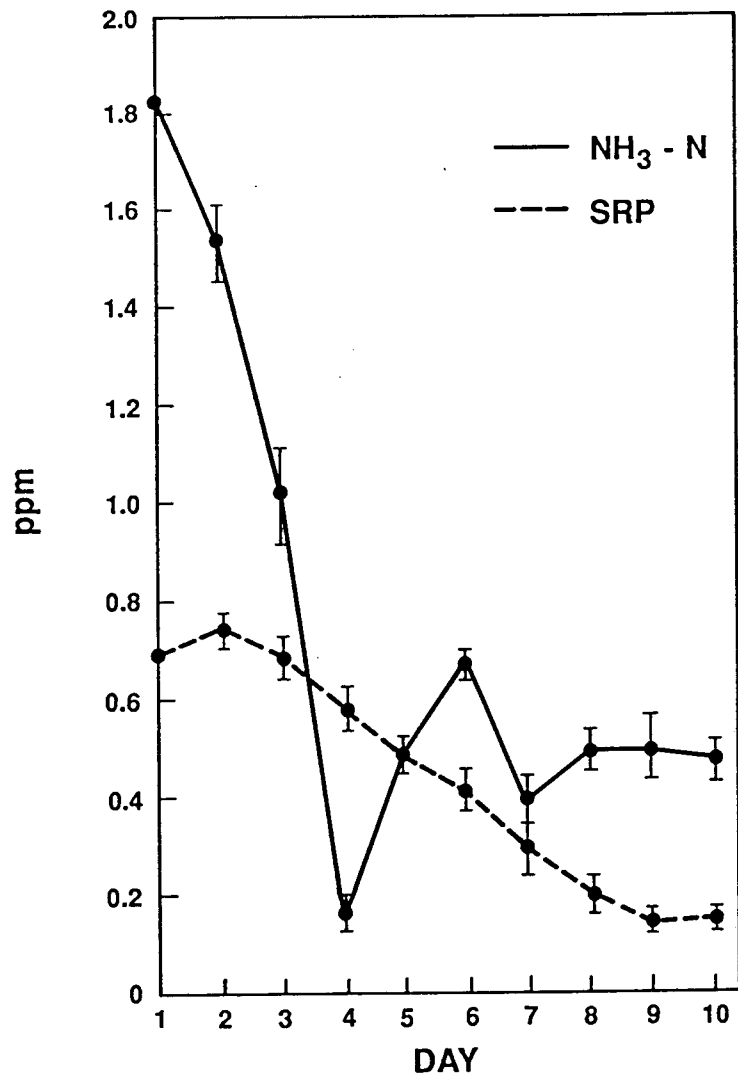


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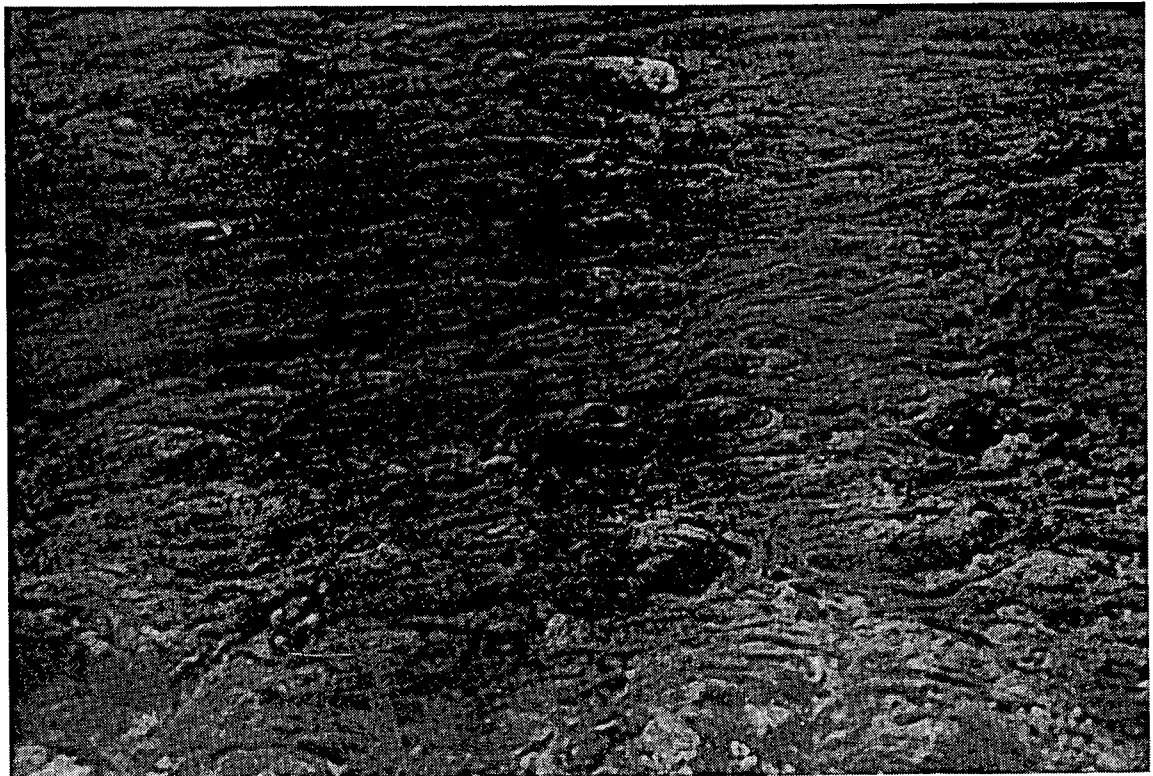


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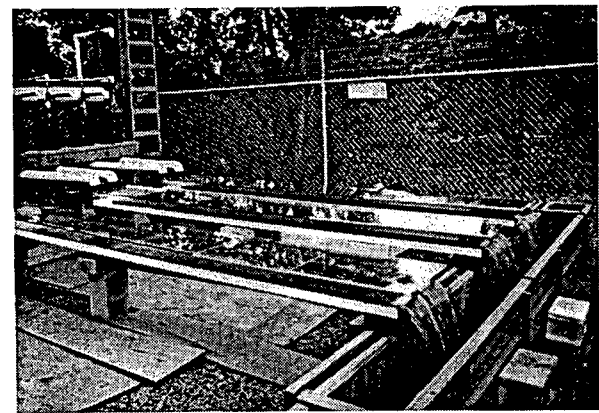
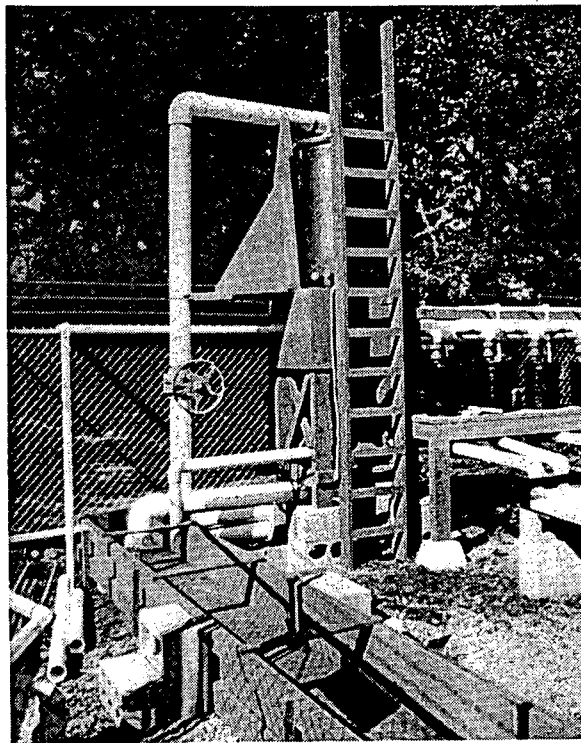
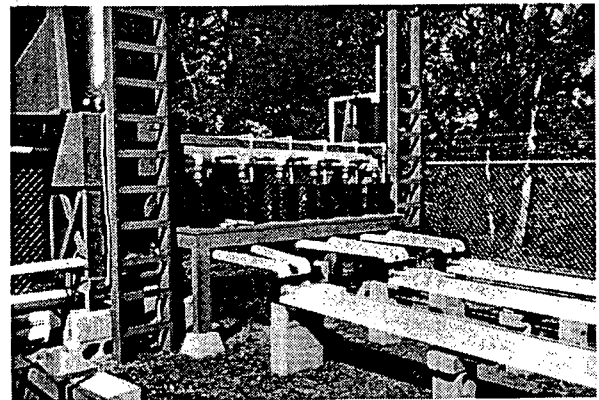
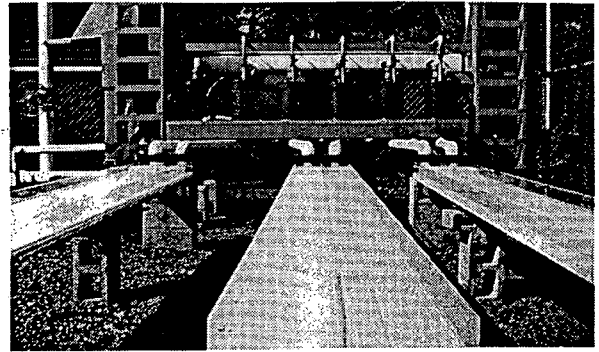
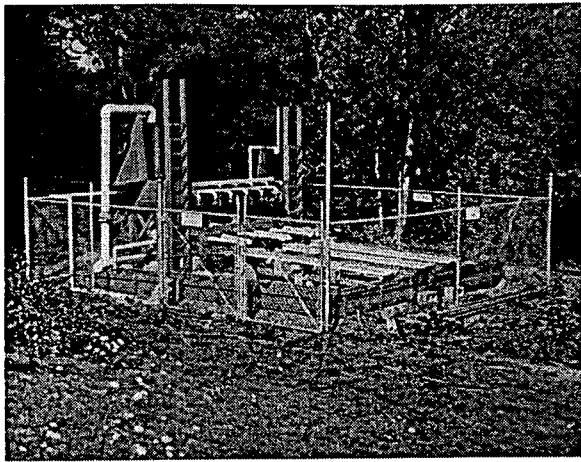


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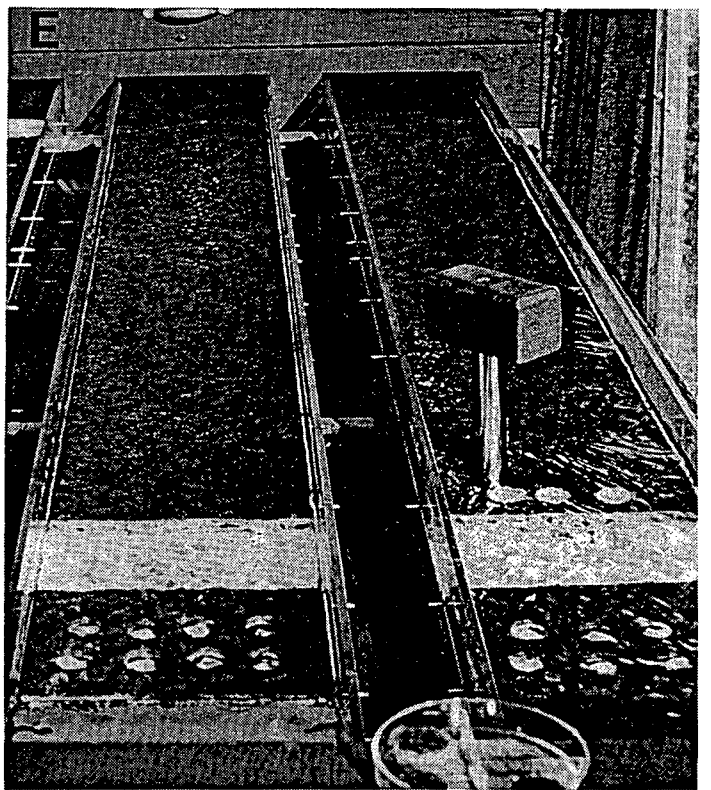
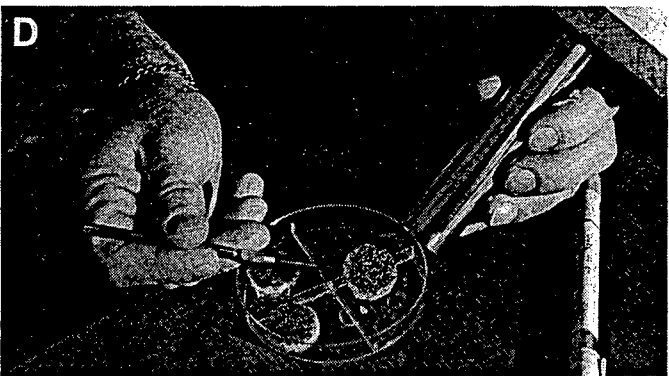
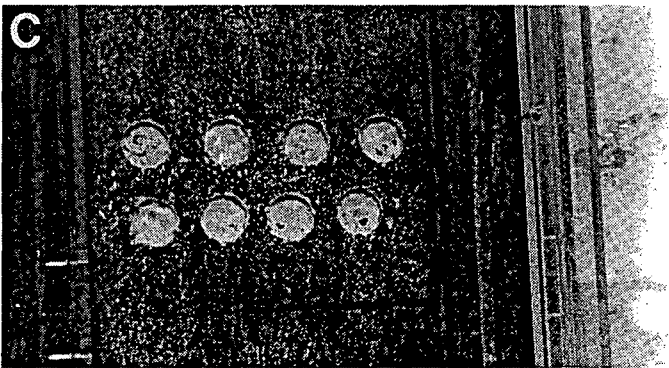
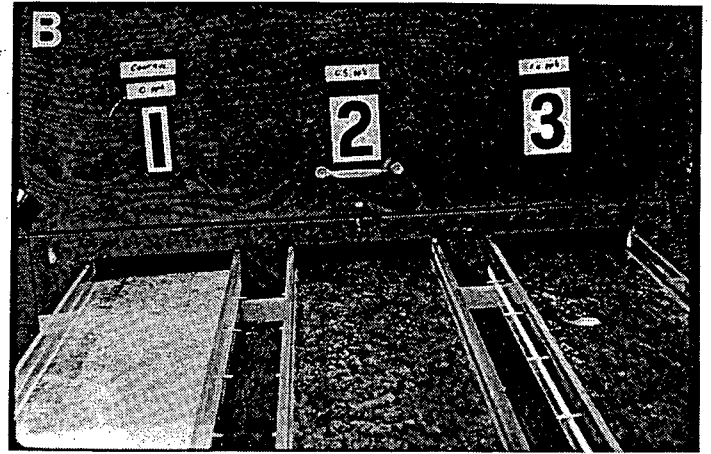
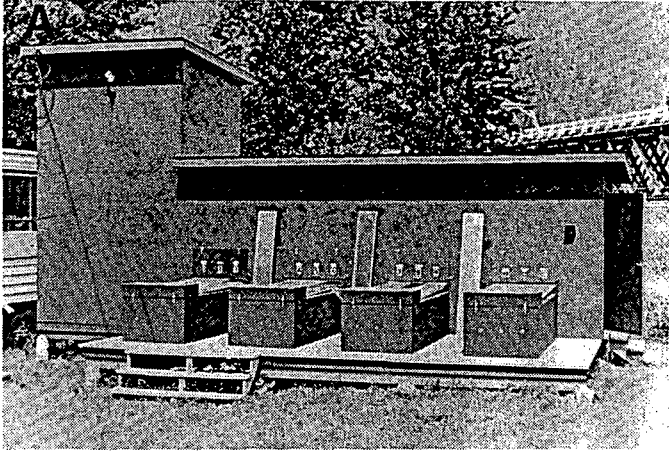


PLATE 3.

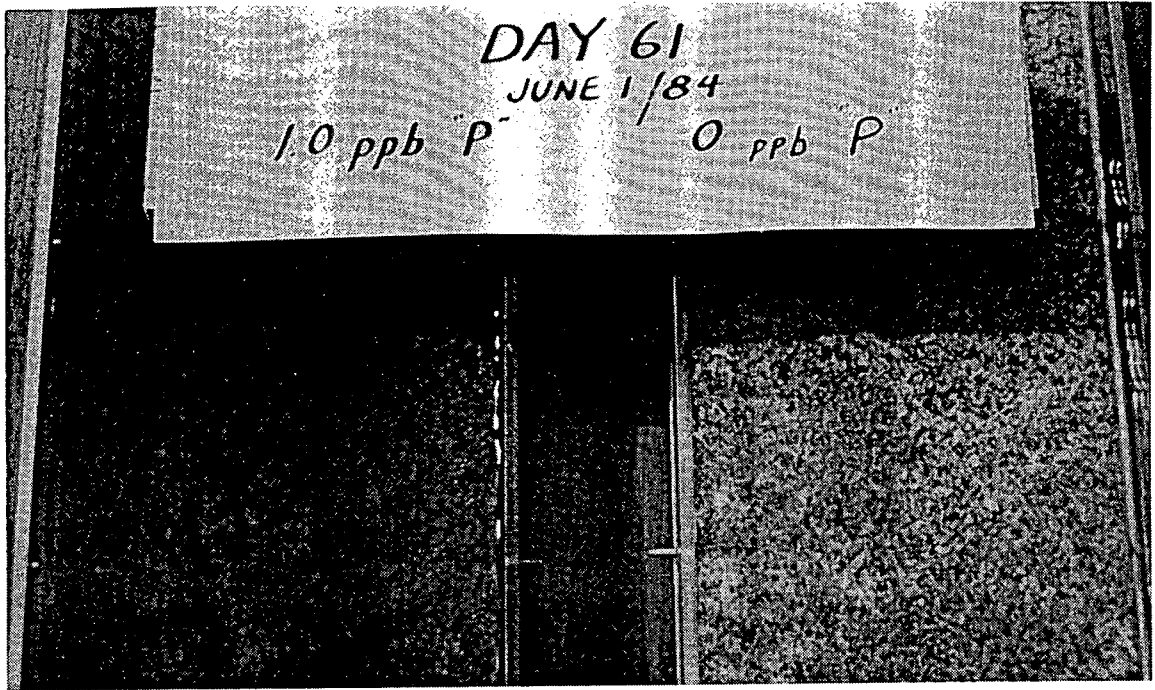
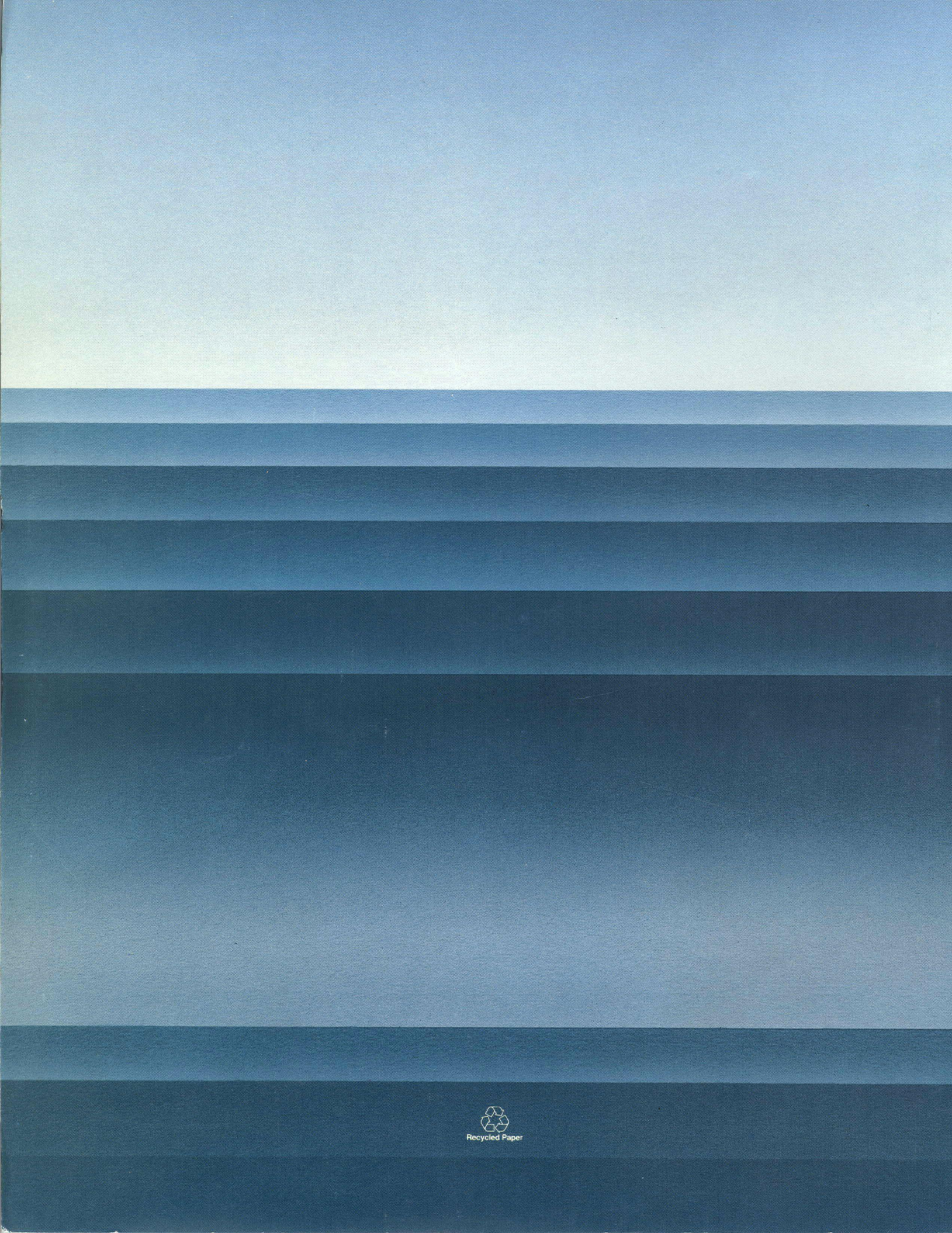


PLATE 4.



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