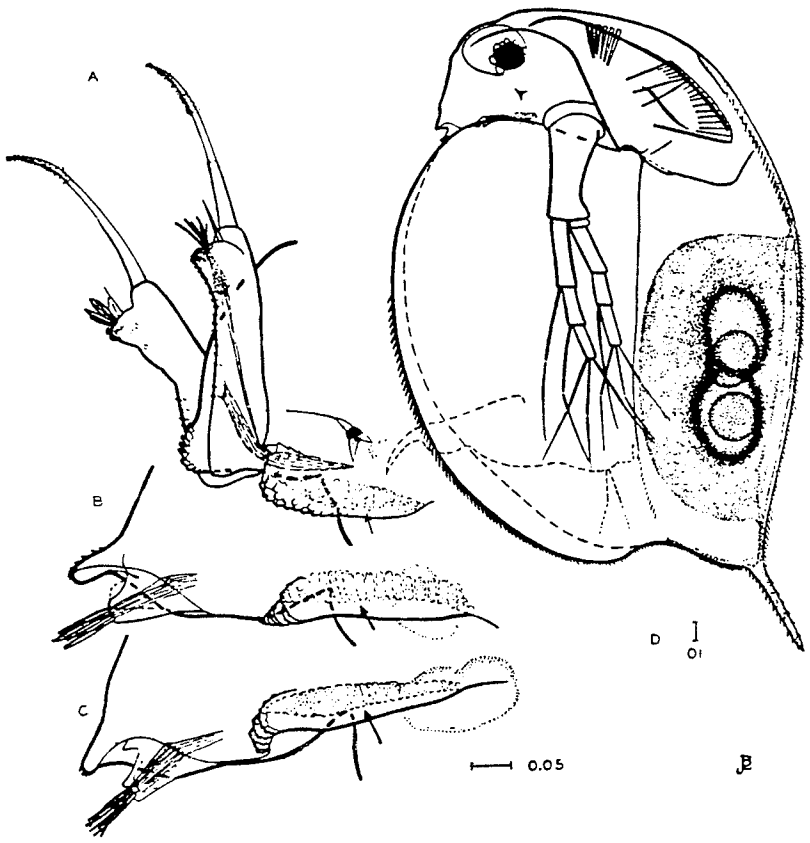

Frontispiece: Daphnia magna Straus

A. Rostrum and antennule of male from Kern County, California; B. Rostrum of A, alternate view; C. Rostrum of D; D. Ephippial female from Pahn's Lake, Saskatchewan; (after Straus in J.L. Brooks, The Systematics of North American Daphnia, Memoirs of the Connecticut Academy of Arts and Sciences, Volume 13, November, 1957, Yale University Press).

THE UNIVERSITY OF MANITOBA

A COMPARATIVE ASSESSMENT OF THE RESPONSE OF AN AQUATIC ENVIRONMENT TO THE INTRODUCTION OF LIME-AND ALUM-TREATED SECONDARY DOMESTIC SEWAGE EFFLUENTS: USING THE *Daphnia magna* AS THE INDICATOR ORGANISM.

by

Mark Ian Aimey

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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DEPARTMENT OF CIVIL ENGINEERING

WINNIPEG MANITOBA
JANUARY 1981

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MARK IAN JOHN CARLOS AIMEY

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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THE UNIVERSITY OF MANITOBA

DEPARTMENT OF CIVIL ENGINEERING

ABSTRACT

A COMPARATIVE ASSESSMENT OF THE RESPONSE OF AN AQUATIC ENVIRONMENT TO THE INTRODUCTION OF LIME-AND ALUM-TREATED SECONDARY DOMESTIC SEWAGE EFFLUENTS, USING THE Daphnia magna AS THE INDICATOR ORGANISM.

BY

MARK IAN AIMEY

ADVISORS: DR A.B. SPARLING, DR. S. LAWRENCE

JANUARY, 1981

WINNIPEG, MANITOBA

In a bench scale procedure, secondary domestic sewage effluent, from the Winnipeg South End Sewage Treatment Plant, was treated using lime and alum respectively, at the University of Manitoba Sanitary Engineering Laboratories to produce tertiary effluent. After treatment the total phosphate residuals were .05 mg/1 for the lime and .03 mg/1 for the alum effluent. The lime effluent was recarbonated to a pH of 6.0 using carbon dioxide. The exploratory study found that neither effluent was acutely toxic. A chronic toxicity study, using a replicate series of concentrations; 0, 12.5, 25, 50 and 100% of each tertiary effluent was indicated. The control, 0%, a reconstituted water, was also used for effluent dilutions. The bioassay was conducted in an environmental chamber operated at 20°C. Each parent aquarium was stocked with 50 Daphnia magna neonates from the Freshwater Institute. Feeding, removal of progeny,

counting and environmental monitoring was, on the average, done every two days. Progeny were kept in larger aquaria using an identical replicate series. The organisms in each aquarium were fed equal amounts of "Cerofood".

Although aluminum toxicity was suspected, the alum effluents appeared , in general, less toxic to the daphnids than the lime effluents. The Litchfield - Wilcoxon method showed that the lime effluents were more potent than the alum effluents in terms of mortality. Lime effluents were 1.4 - 34.6 times as potent as the alum effluents. Sulphate enrichment of the 100% alum effluents caused an immediate algal bloom, which was sustained until day 32 of the bioassay. The organisms in the alum effluents required no significant acclimation period before progeny production. Those in the lime effluents, especially the 100% concentration required a 12-day acclimation period, during which time up to 80% mortality was recorded in the parent aquaria. Mortality was mainly caused by pH fluctuations and mineral deficiency. The surviving parent Daphnia in the 100% lime effluents recovered in productivity and surpassed all other aquaria in terms of numbers of progeny generated. It could not be determined whether this response was a reaction to stress or fertilization. The alum effluents had a high sulphate and magnesium content and contained one-fourth (1/4) of the alkalinity and one-third (1/3) of the dissolved inorganic carbon as in the lime effluents. No algal bloom was noted for the control which had a sulphate content of 17.8 mg/l, a total phosphate content of 2.5 mg/l, dissolved inorganic carbon, 0.8 mg/l, and alkalinity 12 mg/l as $CaCO_3$. The control was nutrient limited.

Ephippial production was an inconclusive indicator of stress. In terms of progeny generation and total population, the 100% effluents and the 25% lime outperformed all others; but produced very diminutive opalescent organisms. Mean life expectancies determined for organisms below the 30% effluent concentration level were; 15-22 days for the control and 12.5 - 21 days for the tertiary effluents. The best performers overall were the organisms in the 12.5% lime, the 12.5% alum and the 50% alum effluents.

1. INTRODUCTION: ENVIRONMENTAL CONCERNS, SPIRIT OF THE AGE

Given expanding human populations, wastes increasing in volume and complexity and fixed territorial boundaries within which terrestrial life, as we know it, must remain, it is small wonder that concern for the environment has surged into prominence during the past two decades. Modern industrial complexes generate a variety of wastes that require unique and often sophisticated waste treatment and disposal systems. Adequate treatment of such wastes, to render them nuisance free and easily assimilable once discharged into the environment was formerly not considered to be very important in the economic scheme of industrial planning. Natural landscapes once thought to have infinite capacity to absorb wastes allowing them to disappear, as it were, began to choke visibly, deforming and killing sensitive life forms.

In North America the environmentalist movement sprouted and became popularised. Countries where industrialization is almost non-existent are being polluted to a degree; but in this case low grade wastes are generated at a level and rate which renders them easily assimilable into the immediate environment. The waste products of advanced manufacturing and refining processes present the most resistance to natural assimilative forces.

The concern with energy and pollution may be the preoccupation of our age. Ultimately all pollution involves the aquatic environment. Water is the universal solvent and once polluted, contributes to the spread of pollution through all areas of contact. Clearly the problem has become global in scope. The effects of pollution once well established are often irreversible. When these effects can be mitigated the costs may be prohibitive.

1.1. Overview and Hypothesis

With increasing popular and scientific awareness about the need for protecting the environment from pollution, present practices of secondary domestic and industrial effluent discharge into receiving streams are being reviewed. Tertiary treatment of sewage is being proposed to solve the problem of excess nutrient discharge. Phosphorous, as phosphates, has been singled out as the limiting nutrient responsible for eutrophication in fresh water. With the most practicable sanitary engineering technology available (chemical precipitation methods) the phosphate nutirents are the most amenable to removal during the tertiary treatment process. Two chemicals commonly applied in effecting the required phosphate removals are lime and alum.

Chemical reactions work via addition or substitution mechanisms. The lime, as well as the alum tertiary treatment process will impart new chemical characteristics to the treated effluent. These new characteristics may indeed promote other nuisances in the environment; shifting the problem and rendering ineffectual the intended pollution abatement.

These new effluents could possibly disrupt lower food chain patterns and in the case of very delicate ecosystems, may cause more harm than the original secondary effluents.

1.2 Research Objectives

This thesis explored the validity of this stated hypothesis by a systematic evaluation of the effects of secondary effluents treated with lime or alum on an aquatic organism. To compare the influences of the lime and alum treatments on the environment, Daphnia magna, a very important second trophic level organism, was used in a bioassay study. An attempt was made to gauge the impact of food web disturbance, directly attributable to the introduction of new chemical characteristics parent to the lime and alum processes.

1.3 Scope of the Study

The study was a bench scale determination of the effects of tertiary effluents on freshwater biota using Daphnia magna as the bioassay organism. This research was limited to the detection of those undesirable changes in the Daphnia population which could be ascribed to the presence of the tertiary effluents. No attempt was made to isolate, by more detailed testing, the causative chemical agents. An attempt was made to determine effluent discharge criteria.

2. EUROPHICATION CAUSES AND EFFECTS

"Literally, eutrophication means well nourished; whereas, in fact it means over-nourished in terms of certain nutritive components" (1). The introduction of excess nutrients in the aquatic environment stimulates and promotes the proliferation of certain flora and fauna. Nutrients having this stimulus potential are: organic carbon, inorganic nitrogen, phosphorous and a few minerals. The bloom emanates from "the biosystem that can grow at the most rapid rate" (1). In most instances blooms are aesthetically objectionable.

Algal growth is stimulated by sunlight and the presence of inorganic matter. Sunlight is the primary energy source. Lakes are classified as to the density of vegetation they will support. Table 1 outlines the distinction between eutrophic and oligotrophic lakes. Eutrophic conditions maintain low species diversity with large numbers of the few species. The condition is produced by an excess food or energy source, including photosynthesis. Oligotrophic conditions feature high species diversity with lower numbers representing each species, a condition caused by low nutrients or the presence of toxic elements.

The polluting stimulus triggers a biosystem shift, the resultant anomalous condition constituting an ecological change. "All habitats in nature have a typical plant and animal micro- and macropopulation. The relationship of this biopopulation to its environment is described as

ecology" (2). A fundamental operant concept in ecological functioning has been described by Liebig's Law of Limiting Factor, which states that the rate of growth in activity of some processes in an organism is controlled by some limiting environmental factor, in the absence of which

TABLE 1. Plankton of Oligotrophic and Eutrophic Lakes (1).

	Oligotrophic	Eutrophic
Quantity	Poor	Rich
Variety	Many species	Few species
Distribution	To great depths	Thin trophogenic layer
Diurinal migration	Extensive	Limited
Water-blooms	Very rare	Frequent
Characteristic algal groups and genera	Chlorophyceae (Desmids if Ca^{++} deficient) Staurastrum Or Diatomaceae Tabellaria Clycotella Chrysophyceae Dinobryon	Cyanophyceae Anabaena Aphanizomenon Microcystis and Diatomaceae Melosira Fragilaria Stephanodiscus Asterionella

another factor becomes limiting according to the dynamics of that particular ecosystem" (1). By extension, this law holds good for inhibition or toxic factors. In the study of limiting factors it must be remembered that "increasing the suitability of one limited factor in a combination, soon makes another factor the limiting one whether it be solar energy, density of algae, nutrients or temperature" (1).

2.1 The Role of Phosphorous in Eutrophication

Phosphorous is indispensable to terrestrial life. It is, compared to many vital elements required for cellular respiration and metabolism, incorporated in living matter in relatively small amounts. Nonetheless, complex biological processes are governed by these minute amounts.

Elemental phosphorous is toxic to living organisms. Its role in the life processes of plant and animal organisms, is performed in coalescence with other elements to form essential compounds and ions, known generally as phosphates. As demonstrated in Figure 1, the element phosphorous is continuously cycled. In algal communities on the average, the phosphorous: nitrogen: carbon relationships are 1P:7N:40C in every 100 units of dry weight matter, or for every 500 material units considering wet weight (3). If one of these three elements is growth limiting and the other elements are present in excess of physiological needs, phosphorous will be able to generate 500 times its weight in living tissue, nitrogen 71 times and carbon 12 times its weight. Further to this, Vallentyne (3) argues that, in the absence of sufficient supplies of carbon and nitrogen, plants can draw these across the water boundary from the atmospheric pool. The phosphorous and nitrogen content of raw sewage attributable to anthropogenic sources has been estimated as 100 times the amounts measured in natural waters unaffected by cultural eutrophication. Their introduction into natural waters has an amplifying effect on aquatic growth. Demand - supply ratios have been calculated for elements in aquatic communities. The higher the demand-supply ratio the greater the growth-limiting capability." Demand - supply ratios for theoretical

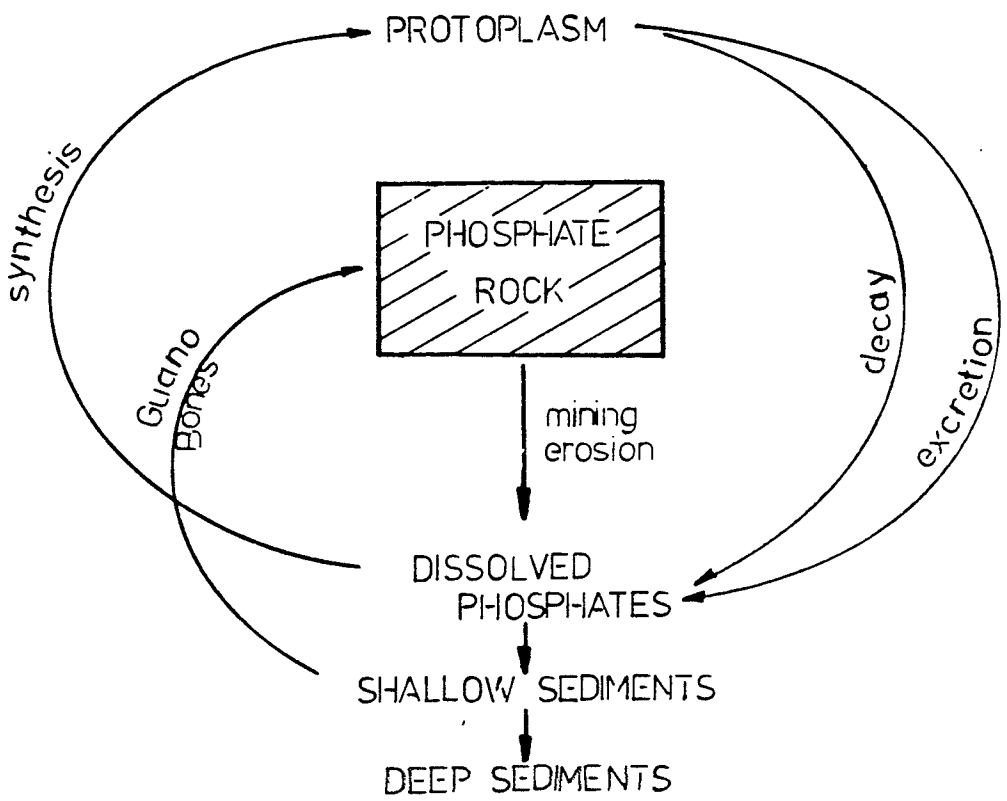

FIGURE 1. The Phosphorous Cycle (2).

world-average situations in late winter (prior to the burst of algal growth) and midsummer (at the height of maximum plant abundance)" (3) are as shown in Table 2.

2.1.1. Cultural Versus Natural Eutrophication

The complex sequence of changes in the aquatic environment, brought about by increased levels of phosphorous compounds, is a greatly amplified process. The increase in aquatic plants causes increased rates of photosynthesis, plant proliferation and stimulation of increased productivity at all trophic levels, up to and including the fishes. In lakes of sufficient depth, dissolved oxygen levels in bottom waters, may be greatly reduced in the span of a few months. Similar successional changes in natural eutrophication systems take place over millenia (3), the difference being that while cultural eutrophication is reversible, natural eutrophication is not. Fair et al. (4) state that "any stratifying lake with > 0.3 ppm inorganic nitrogen and 0.1 ppm inorganic phosphate at the time of the spring overturn can be expected to produce nuisance blooms of algae". Some species of algae are so sensitive to phosphates that they will proliferate in the presence of phosphates at levels as low as 0.001 ppm. It has also been determined that the concentration of phosphorous in bottom waters should be less than 0.08 ppm if these waters are to remain aerobic (4).

2.2 Forms of Phosphorous in Nature

The most naturally occurring phosphorous and phosphate ions are in the form of phosphorous-oxygen complexes. For convenience they have been

TABLE 2. Element Growth-Limiting Comparison (3). Ratios calculated by estimating the chemical composition of an average community of fresh water plants and then dividing by the mean chemical composition of the river waters of the world.

ELEMENT	DEMAND: SUPPLY		
	LATE WINTER		MID SUMMER
Phosphorous	80,000	up to	800,000
Nitrogen	30,000	up to	300,000
Carbon	5,000	up to	6,000
Iron, Silicon	generally low but variable		
All other elements	less than 1,000		

classified as organic and inorganic. Organic phosphorous is incorporated into the organic mass which makes up living tissues and is not readily available to organisms as a metabolite. Inorganic phosphorous is subdivided into meta- and orthophosphates. Polyphosphates are metaphosphates in polymer form. Phosphates in this form are eventually hydrolysed to orthophosphates in temperature and pH dependent reactions. Except in the presence of compounds of calcium, iron, aluminum and a few others, polyphosphates form water-soluble compounds, that, subsequent to degradation by microorganisms are directly assimilable by plants. The phosphorous, nitrogen, and potassium incorporated into plant matter is taken away from agricultrual land in the form of the crop which is harvested. Fertilization of the soil is required to replace these losses.

Orthophosphates generally occur in these forms; H_3PO_4, $H_2PO_4^-$, $HPO_4^{=}$, $PO_4^{\equiv}$. The following recations occur in natural phosphate degradation processes (5).

$$PO_4^{3-} + NH_3 + CO_2 \xrightarrow{\text{sunlight}} \text{green plants}$$

$$\text{Organic phosphates} \xrightarrow[\text{decomposition}]{\text{bacterial}} PO_4^{3-}$$

$$\text{Polyphosphates} \xrightarrow[\text{in water}]{\text{hydrolysis}} PO_4^{3-}$$

2.3 Sources of Phosphorous Compounds

Phosphates are added to domestic water supplies and to boiler waters for corrosion control. In some water treatment systems phosphates are added to eliminate the need for recarbonation after softening (6). Phosphates are also additives in some domestic detergents. As recently as

1969, phosphates in detergents formed 30 to 70% of the total mass (3). They acted as water softeners and maintained high alkalinity conditions which enhanced soap emulsification. Efficient sudsing activity promoted dirt removal. Problems arose. Since the environmental alarm has been sounded, new generation detergents using alternative building agents some of which may be nitrogen based have been marketed and have found wide acceptance (3). The total phosphate load in sanitary sewage, is therefore made up of contributions from; the chemical additives in domestic water, laundering and other household activities, human body wastes, and industrial process activities.

The ratio of nitrogen : phosphorous in domestic waste water has been reported by Fair et al. (4) as (20 to 50): (1 to 13). Specifically the ratio in urine is 14:1 and fecal matter less than 4:1. Phosphorous contributions from agricultural runoff is 0.05ppm. Algae and other aquatic plants on death, contribute their stored nitrogen and phosphorous to benthic sediments. Phosphorous in benthic sediments remains stored until immediately after the spring turn-over when they stimulate the proliferation in flora popularly known as an algal bloom.

2.4 Nitrogen

The nitrogen cycle is shown in Figure 2. The nitrogen in the atmosphere acts as a continuous buffering system (2). All the forms of nitrogen as shown are biochemically interconvertible.

2.4.1 The Significance of Nitrogen Nutrients in Eutrophication

Aerobic processes have the most direct effect on eutrophication

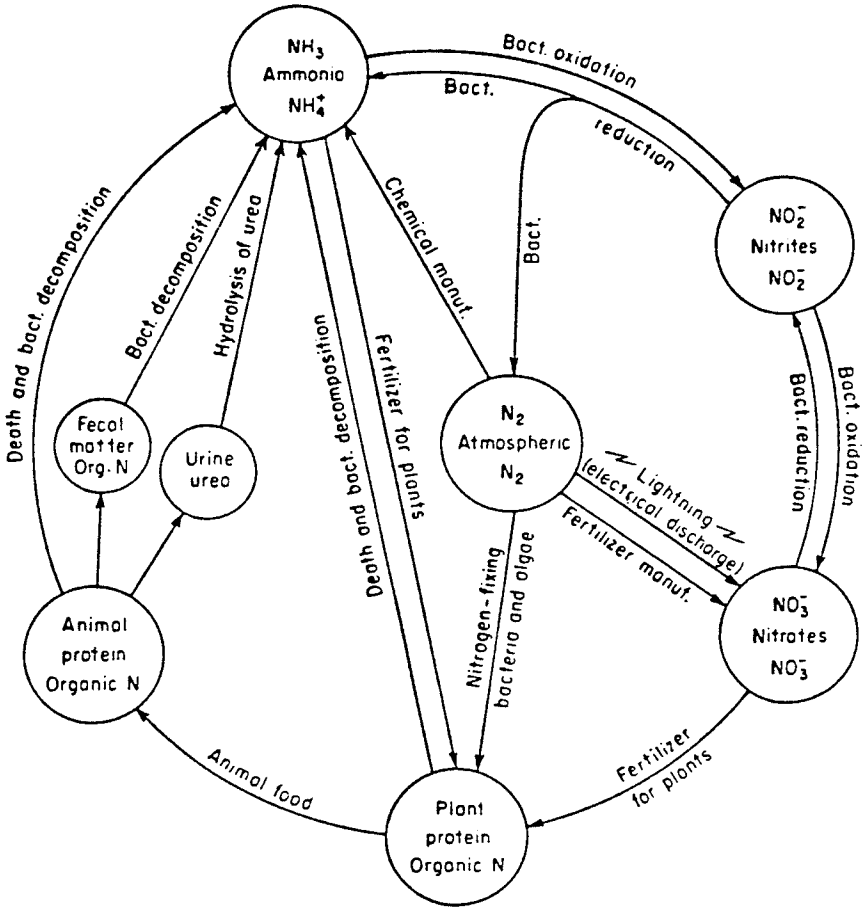

FIGURE 2. The Nitrogen Cycle (6).

processes. Next to phosphorous, nitrogen is the most growth-limiting element in stream ecology. When all other elements are present in excess of physiological needs, nitrogen is second to phosphorous in terms of its potential to stimulate the growth of tissue (3). Fair et al. (4) report the limiting nitrogen concentration for algal blooms to be 0.3 ppm at the time of the spring overturn. As a eutrophication control method, Vallentyne (3) reports it injudicious to attempt removal of nitrogen from secondary effluents by tertiary treatment processes subsequent to prior phosphate removal. This contention has been widely supported (7) because of the cost and physical difficulty of effecting nitrate removal. There is adequate opportunity for resupply to the environment in the form of nitrogen in agricultural runoff and fixation from the atmosphere by some plants.

2.5 The Role of Carbon and Other Contributors to Eutrophication

Arguments have been proposed to counter the proposition that "Phosphate is the sole controlling element in lake eutrophication"(1). In particular, Kuentzel (8) points out the anomalous demand-supply situation concerning ambient concentrations of free CO_2 and the CO_2 requirements of the algal bloom phenomenon. "Free CO_2 in most waters lies between 0.4 - 1.0 mg/litre. In some lakes an algal bloom during a single day would require a CO_2 concentration of 110 mg/litre". The alkalinity of the lake in question, Lake Sebasticook in Maine, being limited to 40 mg/litre, it would appear that as for photosynthetic requirements CO_2 would be limiting (9). Lange (10) has demonstrated the significant "effect of carbohydrates on the symbiotic growth of planktonic algae with

aerobic bacteria, even in the presence of phosphate concentrations less than 0.01 ppm". This seems to support the hypothesis that bacterial action on organic aquatic detritus liberates enough CO_2 for utilization by algae coincident with the peak photo-synthetic demand phase of the algal bloom. Figure 3 illustrates the bacteria-algae symbiotic cycle. Another source of carbon available for aquatic growth is from dissolved carbonate and bicarbonate salts. Carbon in these forms is made available during periods of elevated pH according to the following (11):

$$2\ HCO_3^- + CO_3^= \rightleftharpoons H_2O + CO_2$$

$$CO_3^= + H_2O \rightleftharpoons 2OH^- + CO_2$$

Zajic (1) argues that "cognizance should also be taken of the fact that limits for any growth-factor vital for growth will limit reproduction and that a level limiting one member may not be limiting for an other". Other nutrients required for algal growth, but which may not be necessarily limiting are:

1. Macronutrients - hydrogen, potassium, oxygen, silicon;
2. Micronutrients - boron, calcium, chloride, cobalt, copper, iron, magnesium, manganese, molybdenum, sodium, vanadium, and zinc (11).

Concentrations of these nutrients required to support algal growth under laboratory conditions are relatively small, as demonstrated in Table 3. Metcalf and Eddy (12) report that "Wastewater contains all the nutrients required for proper cell growth"; although a high industrial waste component can render the total wastewater nutrient-deficient. Table 4 lists some nutrients required by most biological systems.

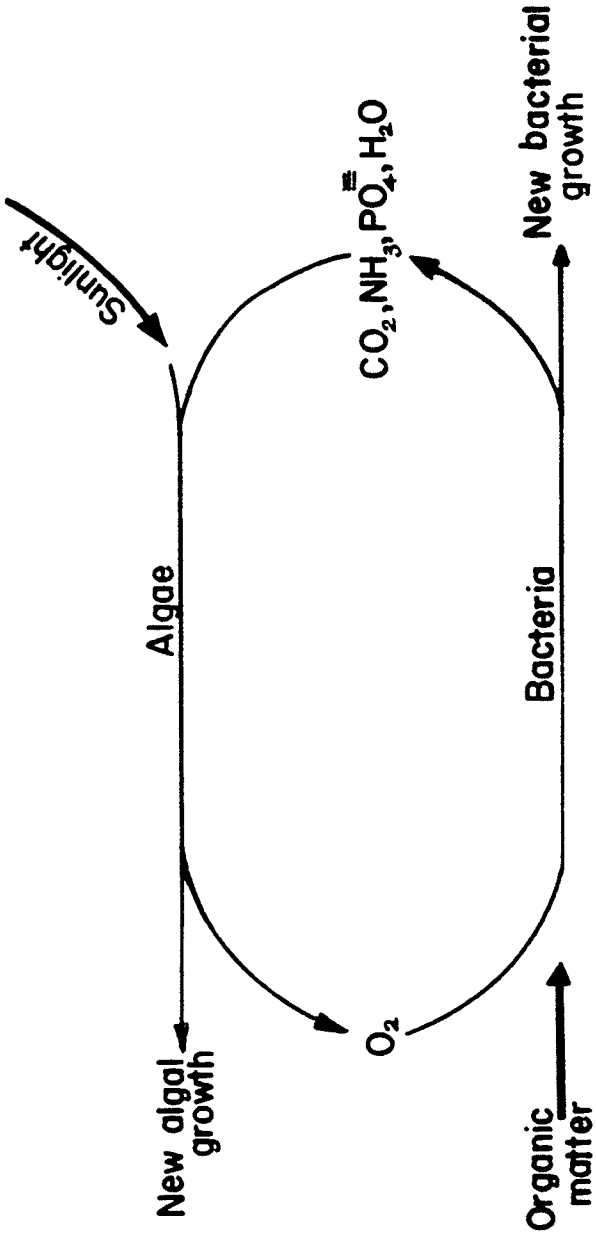

Figure 3. Algal - Bacterial Symbiosm (adapted). (5).

TABLE 3. Composition of ASM-1 algal media* (11)

Salt	Micromoles
Na_2NO_3	2,000
$MgSO_4$	200
$MgCl_2$	200
$CaCl_2$	200
K_2HPO_4	100
Na_2HPO_4	100
$FeCl_3$	4
H_3BO_3	40
$MnCl_2$	7
$ZnCl_2$	3.2
$CoCl_2$	0.08
$CuCl_2$	0.008
Na_2 E.D.T.A.	20

* Plus 1 ml/l Trace Metal Solution

TABLE 4. Inorganic Ions Necessary for Most Organisms (12)

Substantial Quantities	Trace Quantities
Na^+ (except for plants)	Fe^{++}
K^+	Cu^{++}
Ca^{++}	Mn^{++}
Mg^{++}	Zn^{++}
PO_4^{3-}	B^{3+} required by plants, certain protists
Cl^-	Mo^+ required by plants, certain protists and animals
$SO_4^=$	V^{++} required by certain protists and animals
HCO_3^-	required by certain
	Co^{++} animals, protists and plants
	I^-, $Se^=$ required by certain animals only

3. TERTIARY TREATMENT

Tertiary treatment of sewage is "an advanced waste treatment process" (5) following the conventional primary and secondary waste treatment stages. Tertiary treatment is essentially an attempt at water reclamation; "renovation of the waste water by improving the quality to such an extent that it may be directly reused" (5). This practice is becoming more important in water management to meet the demands of growing populations and expanding industry; with the degree of purification dependent on the intended use. Advanced treatment processes, beyond the secondary stage, are required to remove any refractory contaminants which have resisted removal by conventional methods.

3.1 Tertiary Treatment Methods

Coagulants used for chemical coagulation-precipitation methods of phosphorous removal are lime, alum ferric chloride, ferric sulfate, and sodium aluminate. To enhance removals, flocculation aids; poly-electrolytes and activated silica, are sometimes used. For dephosphatizing sewage, the chemicals alum, $Al_2 (SO_4)_3 \cdot 14 (H_2O)$ and lime, (CaO) have been the most widely used.

Coagulation techniques effect removal of particulate matter by reduction of the zeta potential forces and the promotion of flocculation, which induces gravity sedimentation. The coagulation method is therefore

the most effective for elimination of refractory particles, which exhibit colloid behaviour. Figure 4 illustrates the electrostatic forces associated with colloid particles. For effective coagulation, the coagulant selected must be opposite in charge to the most prevalent refractory components of the wastes. Characteristic colloid particles in sewage are negatively charged.

Strict chemical precipitation techniques effect phosphate removals by pH elevation and formation of insoluble phosphates which agglomerate and settle out by gravity. Lime in adequate dosages produces this effect.

3.1.1. Alum Tertiary Treatment

Alum coagulation-precipitation processes can be summarized as follows (5):

PO_4^{3-} + multivalent metal ions $\xrightarrow{\text{excess coagulant}}$ insoluble precipitates.

The alum reaction is temperature-sensitive (minimum required ambient temperatures 4°C), and because of the amphoteric quality of the hydroxides generated, the reaction is pH sensitive. The most complete precipitation is obtained between the 4.5-7.0 pH range (13).

Process reactions for alum are shown below (14):

$$Al_2(SO_4)_3 + H_2O \longrightarrow 2Al^{3+} + 3SO_4^{=} + H^+ + OH^-$$

Several hydroxides and ions are formed in combination with the Al^{3+}ion, of which, Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$ are well known examples. A reaction that depresses the pH is:

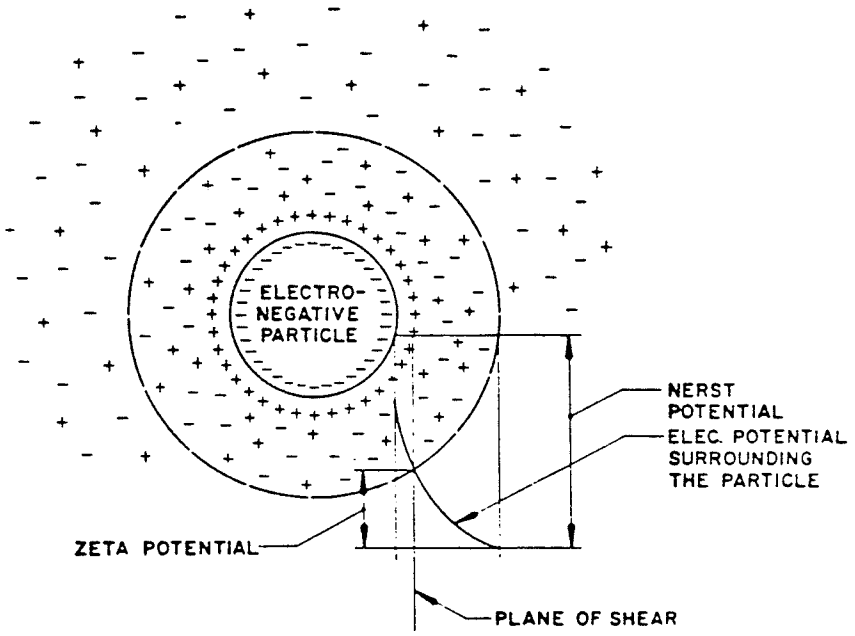

FIGURE 4. Illustrative sketch of colloidal particle and attendant electrostatic charges acquired by ionization of surface sites or adsorption of ions from solution. Note that the zeta potential is the charge at the interface between the solution and the volume of the solution held or captivated by the particle. (13).

$$Al_2(SO_4)_3 + 6H_2O \rightarrow 2Al(OH)_x + H_2SO_4 \downarrow pH$$

If there is a deficiency of natural alkalinity in the sewage to be treated it must be supplemented. The alum reaction generates six molecules of carbon dioxide $6(CO_2)$. From stoichometric computation it can be shown that 1 mg/l alum requires 0.5 mg/l alkalinity while generating 0.44 mg/l CO_2. The process reaction is:

$$Al_2(SO_4)_3 + 6H_2O + 3Ca(HCO_3)_2 \rightarrow 2Al(OH)_3 + 3CaSO_4 + 6CO_2$$

The amphoteric properties are shown below (13):

$$Al(OH)_3 + 3H^+ \longrightarrow Al^{3+} + 3H_2O$$

$$Al(OH)_3 + OH^- \longrightarrow AlO_2 + 2H_2O.$$

In theory, the alum dosage recommended for a desired phosphorous residual of less than 1 mg/l is in the range 1.6 to 2.6 moles of alum per mole of influent phosphorous. This is approximately equivalent to 300 mg/l alum required to effect 88% removal of phosphates. Actual dosages depend on the phosphorous concentration and the hardness of the wastewater. Figures 5 and 6 relate phosphorous removals to alum dosages. Aluminium phosphates are precipitated in coalescence with the gelatinous aluminum hydroxide floc.

3.1.2. Lime Tertiary Treatment

Elevation of the pH is a natural characteristic of the dephosphatizing of effluents by lime treatment. The lime reacts with the free carbon dioxide or the carbonic acid and "with the carbonic acid of the bicarbonates (half bound carbonic acid)". The calcium carbonate formed produces the coagulant effect (12). Insoluble calcium phosphate complexes formed in the elevated pH environment are entrapped in the

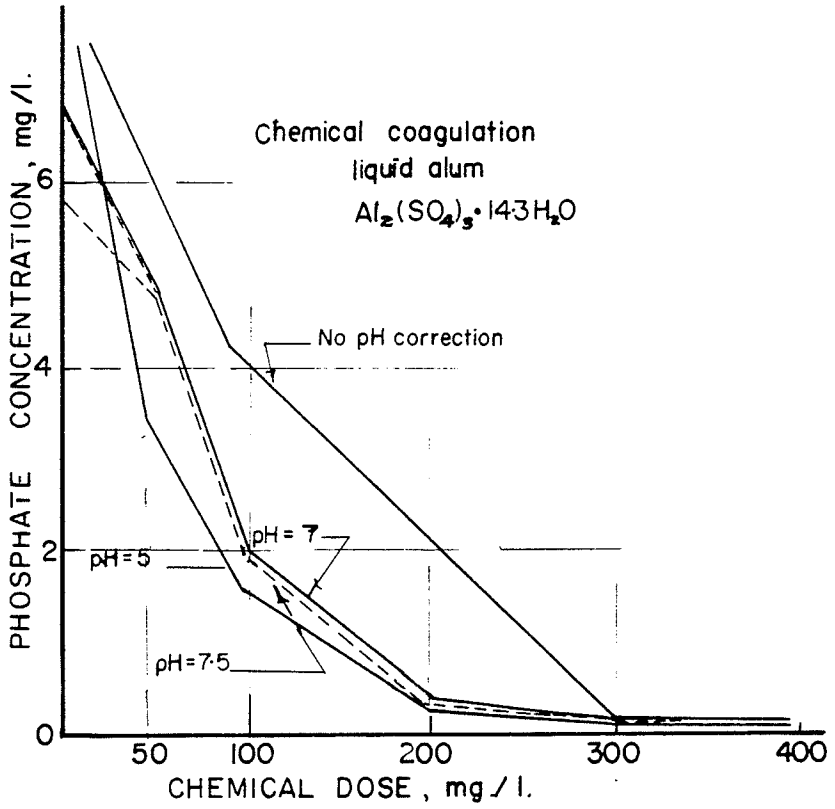

FIGURE 5. Residual phosphorous concentration as a function of alum dosage (12).

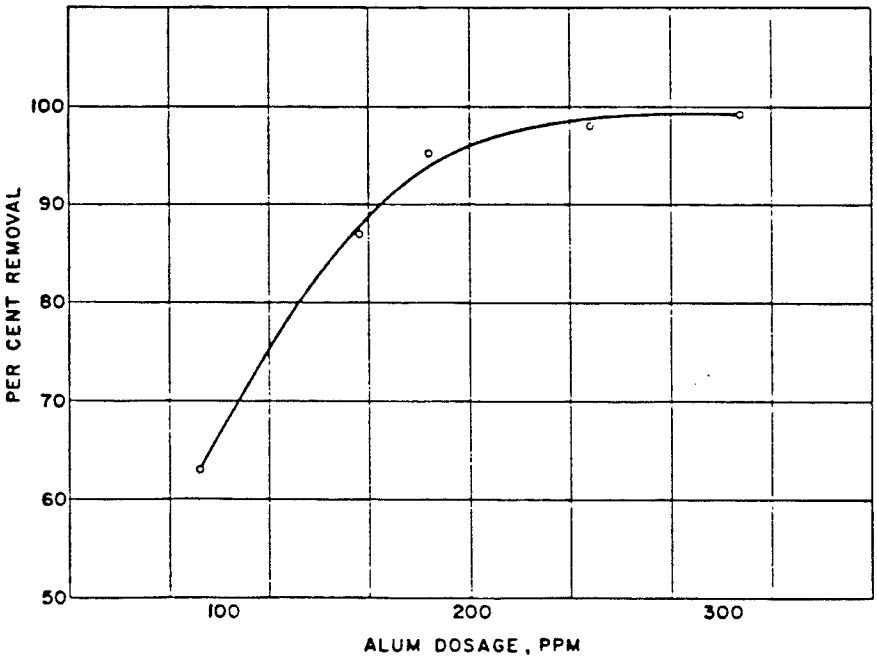

FIGURE 6. Removal of phosphates by alum treatment of effluent from secondary treatment of domestic sewage. Initial soluble phosphate concentration = 4.6 ppm (13).

settling floc. Adequate alkalinity must be present to neutralize any acidity in the effluent before precipitation can take place (12).

By comparison, alum coagulation generates six moles of carbon dioxide per mole of alum whereas lime coagulation consumes 1 mole of available carbon dioxide for every two moles of lime. When free carbon dioxide is present, for every available mole of alkalinity, two moles of lime are required to produce a neutral effluent. A lime dosage of "about 400 mg/l as CaO is necessary for maximum phosphate precipitation" (5). Recarbonation with carbon dioxide is required to reduce the pH of the effluent from values in excess of 11 to a more acceptable average value of 7.5. Figures 7 and 8 are graphs of required lime dosages. "The complexity of competing reactions, alkalinity effects, pH, trace elements and ligands found in waste water," preclude prediction on required chemical dosages of lime and alum (12). Stoichiometry is used as a bench mark for establishing jar testing dosages.

3.1.3. Nitrogen Removal

Tertiary treatment processes for nitrogen removal include air stripping of ammonia, selective ion exchange, chlorination techniques, biological removals and a few experimental techniques not at this time fully practicable.

3.2. Extent of its Practice and Limits to Applicability

Tertiary treatment is to be recommended for situations where a quality effluent relatively nutrient free is required. It may be mandated in order to protect riparian rights. It may be necessary as in the case of the Lake Tahoe basin to protect the oligotrophic nature of a water body.

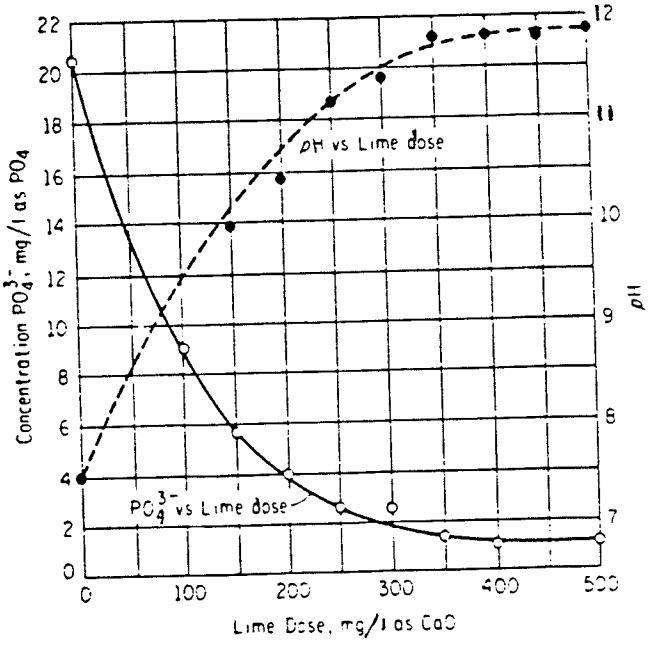

FIGURE 7. Phosphate concentration, pH, and lime dose (5).

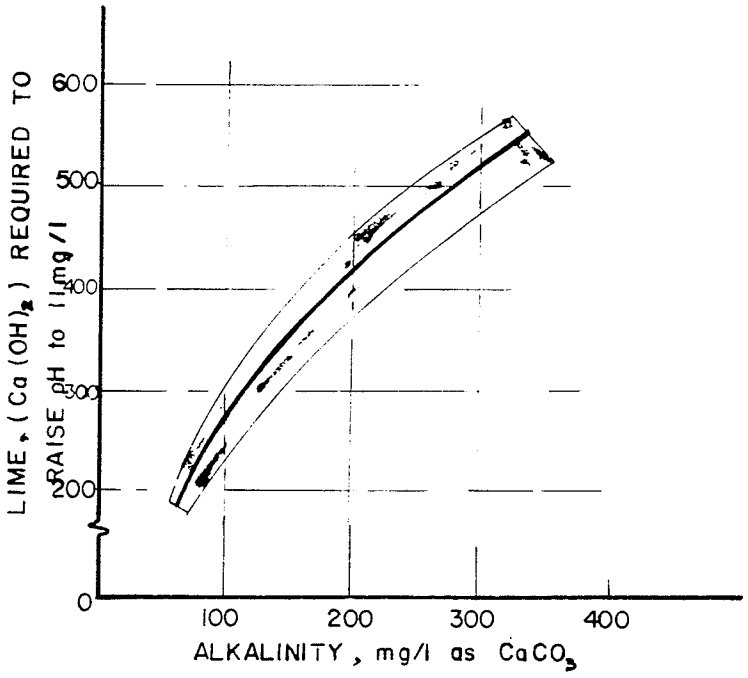

FIGURE 8. Lime dosage required to raise the pH to 11 as a function of raw waste water alkalinity (12).

4. THE AQUATIC ENVIRONMENT

The influence of industrial sewage upon a receiving water body can be considerable. Toxic compounds in certain industrial wastes interfere with biological treatment processes resulting in shock loadings and short circuiting of the treatment process. The resulting effluent, when discharged, can have adverse effects on aquatic biota. Metallic waste discharges are normally harmful. Heavy metals, in particular cadmium, mercury, lead, zinc, cobalt and molybdenum are cause for concern because of their toxic effects. Subtle deficiencies or excesses of trace metals "may lead to suboptimal health" (15) and may be eventually fatal to the organism. The full relationships and mechanisms are not completely known (15).

"Every element becomes toxic if it is concentrated above a certain level" (16). Heavy metals are toxic because of their activity in organisms (2):

(a) They replace sulfhydryl groups and therefore act as enzyme inhibitors;

(b) They damage membranes and enzymes associated with membranes and cellular integrity. They interfere with transport mechanisms;

(c) They cause protein denaturation and interfere with macro-molecule functioning;

(d) They are not degraded organically or chemically hence their activity

remains unabated;

(e) They produce synergistic effects;

(f) They are persistent;

(g) They are susceptible to biological magnification.

In the absence of masking by chelators, copper is poisonous to some primary organisms when present in surface water at concentrations of 1 µg/l. Manganese is poisonous to blue-green algae at a level of 200 µg/l, cobalt at 2µg/l and zinc at 200 µg/l. Pesticide residues and their degradation products are limiting to productivity by virtue of their toxic effects in the environment (16).

4.1 Indices of Environmental Quality

In the natural unpolluted state, an aquatic body is well oxygenated and supports many aerobic species ranging from the microscopic to the macroscopic. The population numbers are kept in check by factors such as the availability of oxygen, typical food, water quality, temperature, pH and the size of the range. The introduction of domestic sewage creates an equilibrium shift. The oxygen saturation potential of the waterbody may be lowered. The available organic detritus favours microscopic life forms, the metabolic and respiratory demands of which further deplete the oxygen reserve. The pollution load causes slight temperature increases during insolation. This increases metabolic activity, which causes a decrease in the oxygen holding capacity of the water. Such waters are turbid and interfere with light penetration so that growth of algae is inhibited. In addition, algae present in the euphotic zone are transported downwards by the soil burden out of the zone of illumination.

Submerged plant life and algae discharge oxygen during the photosynthetic process. During peak periods of insolation waters may become supersaturated with oxygen. During the nocturnal phase, due to excess respiratory demands of algae, a significant oxygen depletion may be observed.

Excess nutrients in the environment cause disturbances in population equilibria, favouring fewer species with larger numbers of each species. Anaerobic conditions develop when the pollution load swamps the capacity of the system to maintain the required minimum oxygen content. Adaptive and facultative species can survive. Only when the detrimental pollution load is discontinued will the natural processes of dilution, aeration and assimilation of wastes within the system slowly restore the pre-pollution balance.

4.2. The Aquatic Food Web and Ecological Balance

In the aquatic ecosystem, a community of organisms is kept healthy and well-graded when a normal level of prey-predator dynamism exists. According to Gause's Competitive Exclusion Principle; "no two species can occupy the same ecological niche for a prolonged period with success" (2). Competition itself is a "negative interaction where both populations suffer a decrease in number" (17). The interaction involves competition for sustenance, habitat and nesting sites. Consequently one population either finds alternative accomodation or becomes extinct. "In less extreme cases the two populations can seek accommodation in the same niche by adaptation of their behaviour patterns and surviving in a modified way in lesser numbers. There is overlapping of some ecological

niches. A niche is more specifically a term describing the modus operandi of the organisms; their food gathering habits, their use of it, their reproduction behaviour and needs, their systems of defence..." (17).

In the aquatic food web the primary consumers, holozoic protozoa and the microphages depend on the primary producers as their food source. "In addition to their direct effect on prey populations, consumers play a significant role in nutrient cycling and therefore in setting the patterns and levels of abundance of all types of aquatic organisms" (18). The aquatic food web is schematically presented in Figure 9. Disruption of one of the primary or other links in the food web triggers repercussions via energy transfer relationships up the food pyramid. Severe ecological imbalance can result.

4.2.1. Toxicity Factors in Communities

Amongst organisms living in close community, refuge or concealment is not the only mechanism of predation avoidance. Some organisms repel, discourage or poison their predators. Blue-green algae are known to release toxins during algal blooms. This eliminates some of the competition for space in the environment and may be a population regulatory mechanism.

Species of Cyanophyceae when ingested are poisonous to some animals. Chlorella for instance, produces a bactericide which is suspected of slowing the food-filtering ability of Daphnia (19). Another source reports experimental evidence that "senescent Chlorella cells contain a substance which inhibits growth and reproduction of Daphnia magna feeding

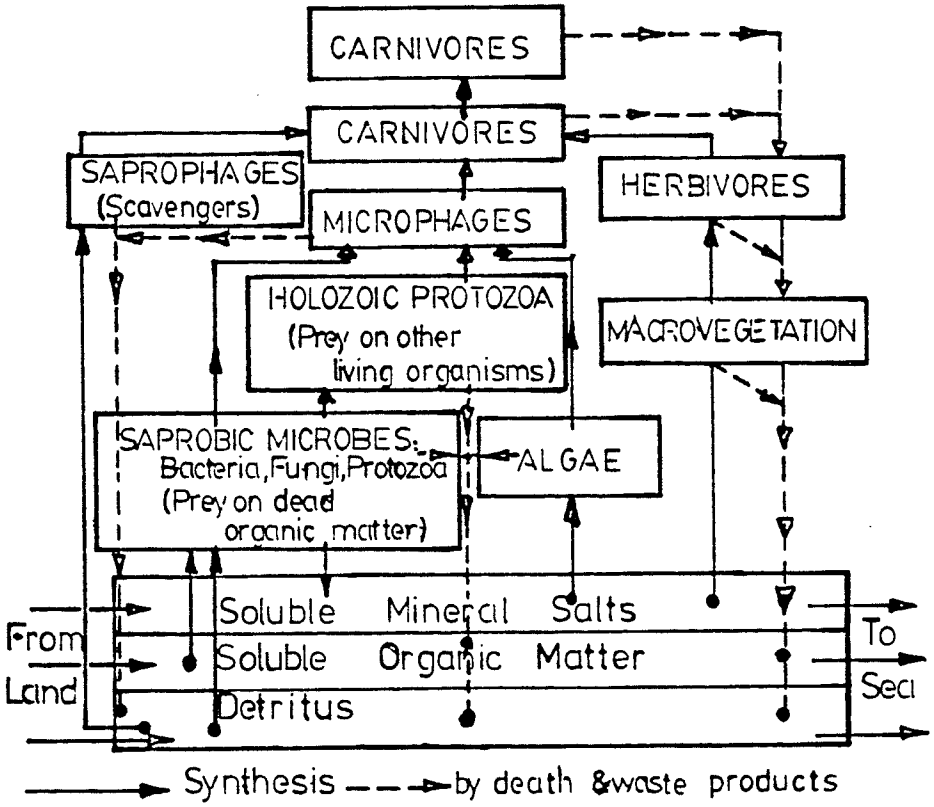

FIGURE 9. Theoretical food cycle classification in a stream bed community (after Klein, 1962). Note: the sun is the common energy source for autotrophs and all life (1).

upon them, while rapidly growing Chlorella exhibit no such antagonistic effect" (20).

4.3. Chemical Equilibria in the Aquatic Environment

Toxicity, derived from metallic wastes, vegetable and other sources is but one of the physico-chemical qualities that promote instability and equilibrium shifts in the aquatic environment.

Detritus stimulates the proliferation of saprophytic bacteria, which discharge as a by-product of cellular respiration, CO_2 in amounts sufficient to trigger and maintain the algal bloom phenomenon. Oxygen is made available to the aquatic environment via algal discharges during photosynthesis. Wolschlag et al. (21) noted that the amount of free CO_2 in solution may have some effect in stimulating algal growth. In exposed areas of Lake Mendota, increased growth of algae was regularly noted subsequent to wind storms. The storm winds induced full turbulence in these waters and maximized CO_2 uptake. Adjacent leeward parts of the lake demonstrated no comparable bloom. A contributing factor to this bloom was the increased nutrient availability due to the turbulence. These workers reported that soluble phosphates when added to a river containing 0.23 to 1.09 mg/l of phosphates produced only a delayed increase in algal growth whereas secondary effluent when added produced an increased growth response. Other factors being equal, they concluded that the organic matter and bacteria provided the stimuli for algal growth. The soluble P when added in isolation had to depend on CO_2 generated by slower physical-chemical processes. Explosive logarithmic growth of bacteria, under favourable conditions, could deliver the large

amounts of CO_2 required to produce algal blooms. The requirements are approximately 2g CO_2 per gram algae produced.

Intensity of algal photosynthesis is indicated by pH and alkalinity relationships and by dissolved oxygen concentrations. The pH is the most sensitive measure. In oxidation ponds, carbon dioxide removal by photosynthesis results in elevated pH values, "with peaks of 10 being reached under intense light conditions of summer days"(20). Lapses in photosynthetic activity are reflected in decreased pH values. Respiration, by higher organisms like Daphnia, contributes to this decrease by restoring CO_2 concentrations. Photosynthesis affects alkalinity, principally by "the incorporation of ammonia nitrogen into algal protein and the precipitation of carbonates and phosphates at raised pH", which processes consume alkalinity at nearly stoichiometric ratios (20). Hence, whereas the loss of CO_2 may not contribute directly to alkalinity increase; alkalinity tends to decrease with pH rise.

"Alkalinity in natural waters is primarily due to salts of weak acids, although weak or strong bases may also contribute" (6). Bicarbonates are the major source. They originate when carbonic acid reacts with basic materials in the soil. Humic acid forms salts that add to the alkalinity of natural waters. "In polluted anaerobic waters, salts of weak acids such as acetic, propionic and hydrosulfuric may be produced and would also contribute to alkalinity"(6). Algae can remove CO_2 to levels below the equilibrium concentration with air. With pH increases, the alkalinity forms; carbonate, bicarbonate and hydroxide change, resulting in CO_2 extraction for algal growth from bicarbonates and carbonates in accordance with the equilibrium expression (10):

$$2HCO_3^- \rightleftharpoons CO_3^{2-} + H_2O + CO_2$$

$$CO_3^{2-} + H_2O \rightleftharpoons 2OH^- + CO_2$$

Carbon dioxide removal by algae causes an alkalinity shift from bicarbonate to carbonate to hydroxide. The limiting pH range beyond which there is no extraction of carbon dioxide from water is 10 to 11.

Ammonia losses in streams are linked to the pH alkalinity relationships by the following pH dependent relationship; an equilibrium between the hydroxyl and gaseous forms:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

"Alkaline conditions favour the gaseous form NH_3, while neutral or acid pH, conditions favour the residence of the ammonium ion. In this way small shallow streams under very active algal or other photosynthesis can lose large quantities of ammonia nitrogren to the air as the pH rises" (22). This equilibrium affects the stressing or crowding of organisms. Under laboratory conditions concentrations of metabolic waste products like "CO_2 should not become too high and the organisms should not be stressed by crowding" (23), since this affects the concentrations of gaseous ammonia in the water. Standard Methods (23) reports that the concentrations of un-ionized ammonia in test aquaria should not exceed 20 µg/l. Table 5 relates temperature and pH to the percentage of un-ionized ammonia in distilled water. In the absence of consumers, dense populations of primary producers generate ammonia nitrogen. Nitrogen in its form of gaseous ammonia inhibits bacterial proliferation and primary production. When consumers are present "added nutrients are made available at higher rates, and in increased amounts" (18) as evidenced by several growth periods and a higher maximum standing crop.

4.4 Possible Effects of Tertiary Effluents on the Aquatic Biota

"The attempt to establish chemical criteria, in terms of toxicity to aquatic organisms is difficult and, indeed, may prove to be impossible. The great host of potentially toxic compounds, the vast numbers of species of organisms, the innumerable interaction effects among compounds, and the wide range effects produced by variations in temperature, dissolved solids, pH, and other physical and chemical factors produce permutations which may exceed the capability of adequate testing"(1). An important consideration is the buffering capacity of the receiving water.

Tertiary effluents may be potential pollutants because of the new characteristics imparted by treatment chemicals, especially when in quantities in excess of the dampening capacity of the receiving system. This buffering is a function of the chemical composition of both effluent and receptor body and the volume ratio of effluent to receptor reservoir.

Lime discharges cause sudden changes in pH. Aluminum hydroxides and phosphates may affect stream biota by changing the ionic characteristics of the environment. To-date "the effects of combining chemical coagulation with biological metabolism have not been evaluated in detail" (5). Dr. R. Gallop of the University of Manitoba has implicated, by hypothesis, aluminum hydroxides as the most likely pollution concern of the future (24). These wastes are often discharged into the aquatic environment, subsequent to alum coagulation clarification processes in water treatment and dephosphatizing waste processes.

Although only limited work has been done in this area to-date, some workers have documented the observation of mysterious toxic influences when tertiary effluents have been discharged into receiving streams.

TABLE 5. Percentage of Ammonia Un-Ionized in Distilled Water. (23)

Temperature °C	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
5	0.01	0.04	0.11	0.40	1.1	3.6	10	27	54
10	0.02	0.06	0.18	0.57	1.8	5.4	15	36	64
15	0.03	0.08	0.26	0.83	2.6	7.7	21	45	72
20	0.04	0.12	0.37	1.2	3.7	11	28	55	80
25	0.05	0.17	0.51	1.7	5.1	14	35	63	84
30	0.07	0.23	0.70	2.3	7.0	19	43	70	88

Paul D. Smith (25) observed that 10% tertiary effluent from an alum coagulation-filtration (CF) process "severely altered the capacity of the secondary sewage to stimulate planktonic primary productivity", and gave rise to an 85% negative difference in photosynthetic activity, when compared with 10% secondary effluent. When compared with the control basin which contained lake water with no sewage additions, the 10% tertiary effluent showed a 59% negative difference in photosynthetic activity. He suspected that factors other than nutrient removals were responsible for this anomaly. His suspicion was that a normal constituent of the tertiary effluent "became disruptive at the 10% level of enrichment, or a transient material which entered the tertiary treatment system" could be the responsible agents. In a subsequent experiment these results were reproduced. Smith (25) noted that although the test concentrations were five (5) to ten (10) times greater than actual full scale lake loading, the observations did give reasonable cause for consideration of anomalous toxic effects from treated domestic wastewater effluents. The reliability of these assessments are questionable since in studies conducted by others no such toxic effects were demonstrated.

5. THE TEST ORGANISM, CLASSIFICATION, PHYSIOLOGY, LIFE-CYCLE

Daphnia magna (Arthropoda: Crustacea) or "water fleas" are common, cosmopolitan, small aquatic cladocerans, about 1 - 3 mm in length, found in ponds, lakes and relatively sheltered freshwater bodies (26). Daphnia are primary consumers which inhabit shallow ponds, pools and ditches. They are microphages which can select from a variety of food sources. They are predominantly herbivores and also consume detritus and bacteria (27). For a full taxonomic classification the reader is referred to Brooks (28).

As branchiopods; they are equipped with a pair of thoracic legs each with five branches covered with cilia which enable Daphnia to strain particulate suspended matter from the water in which they are enveloped. These legs generate a water current which directs the sifted particles in a stream towards the buccal cavity, where they are macerated by powerful mandibular structures prior to ingestion (26). Digestion occurs along the digestive tract. Undigested debris and other metabolites are expelled through the anus. Daphnia magna have a single multifaceted eye located in the centre of the head, a translucent body, and a myogenic heart located in the upper dorsal area. Locomotion is via their branchiopoda which propel them in a jerky mode. They are weak swimmers and are restricted to the quiescent waters of ponds and lakes. At birth they measure 0.7 - 1.0 mm in length and may grow to 2.3 - 6.0

mm. Typical dry weight is 0.25 - 0.45 mg (27).

Under suitable environmental conditions the reproductive mode is by diploid parthenogenesis. Parthenogenic females can produce female offspring which are genetically similar. Under adverse environmental conditions where crowding occurs, either through inadequate space or indirectly through a reduction in the available food supply, the organisms become stressed and parthenogenic females develop eggs which can hatch into males. Males and females mate and produce sexual eggs encased in a specialized protective pouch known as the ephippium. In this enclosure the eggs are well protected, sufficient to withstand adverse environmental conditions of freezing and dessication. On exposure to habitable conditions they hatch in limited numbers into parthenogenetic females.

Lake density maxima for non-crowding of Daphnia have been reported as 1-2 organisms ml^{-1}. Optimum conditions for laboratory reared organisms are quoted as one organism 20 ml^{-1} (29).

The sequence of parthenogenic cloning is: the detachment of eggs from the ovary and arrival to the brood chamber via the fallopian tubes followed by hatching of live young in the brood chamber and subsequent discharge into the surrounding waters as fully developed neonates.

These are called first instar larvae. With each brood clutch, the parent daphnid sheds her carapace or body shell. More eggs descend into the brood chamber and the process is repeated. Body proportions enlarge while the new carapace hardens. By the sixth or seventh instar the fledgling young is ready to reproduce. Initial clutch sizes are small and may be limited to two off-spring. Clutch sizes of mature animals can

number as many as 60 (27). The life span of Daphnia magna at 25° C is two months (30).

5.1 Utility of Daphia magna as a Bioassy Test Organism

As a test animal for bioassy studies Daphnia magna presents many advantages. Daphnids are small, attaining a maximum size of 6 mm (27); permitting the rearing of a great many in a small space. The short life span and reproductive cycles permit the study of effects over several generations in a short time. "Daphnids are easy to culture, requiring only water containing bacteria or their equivalent, for food. They can be grown individually in small bottles or in mass culture in large aquaria. They mature early giving birth to young within their first week of life. After the first brood they spawn new broods every two or three days throughout the remainder of their lives. An average of twenty or more young may be produced in each brood. Each female who lives to a ripe old age can bear four hundred or more off-spring" (30). If produced parthenogenically, all the young from any one female are genetically like the mother. Reproduction can be limited to parthenogenesis if the proper conditions are maintained (30).

Stock cultures of daphnids are relatively easily available. Animals, during their early larval stages, are easily separable into age groups, so that for a particular study animals of virtually the same age can be secured. Many workers have found that the susceptibility of Daphnia to toxic substances is a function of the age of the organism; the period of greatest sensitivity occuring during the first instar, with a

gradual decrease noted with an increase in age. To secure closely reproducible results, the use of animals of the same age has been recommended (30).

Daphnids are representatives of a class of animals that serve as food for many fish; particularly during the fry and fingerling stage. "Fishes do not remain long in waters where their food supply has been destroyed, even though the causative destructive agents do no direct harm to the fish. For these reasons daphnids should prove satisfactory for testing waters for toxic materials" (30).

The past history of the animals is important in determining the results of experiments. "Well fed animals grow more rapidly, reproduce in greater numbers and have a higher heart beat frequency than those reared on limited food. The stage in the life history is signficant since heart beat frequency, oxygen consumption, and resistance to toxic materials vary with the age of the animal. The time within the instar is not be ignored since a new layer of chitin forms during the latter part of each instar and this may modify rates of permeability of various substances" (31). Moreover, Anderson reports that daphnids may be more susceptible during ecdysis than at other times (32). Reactions may also vary depending on the time within the instar. Males are more susceptible to poisons than females, they also have a generally higher metabolic rate than females. To assure reproducible results, "experimental animals must be reared under adequately controlled conditions and selected with respect to age, time within the instar, sex and clone"(31).

5.2 Responses of Daphnia magna to Ecological Events

Daphnia magna are periodic inhabitants of waste water stabilization ponds which have been described as a "hypereutrophic environment of extremes" (33). Daphnia are extremely sensitive to changes in their environment. Table 6 gives extreme tolerance and positive increase ranges for some of these parameters. Some of the conditions that are inimical to daphnids are lampooned in the cartoon presentation; Figure 10. There are three conditions with which Daphnia are unable to cope: (a) zero dissolved oxygen; (b) toxic soluble sulphides; and, (c) ammonia toxicity due to pH and temperature increases. For simplicity "some lines of interaction have been omitted" (26) from Figure 10. A description of the responses of this organism to changes effected in environmental quality by some of the most common forces of change, follows.

5.2.1 Algal Toxins

The dashed line in Figure 10 is an addition to the original cartoon, inserted to include the role played by phytoplanktonic chemical releases which have an inimical effect on zooplankters. Hardy's theory of animal exclusion (Hardy and Gunther (35), Hardy (36)) states that dense populations of phytoplankton produce" conditions which are unfavourable for the zooplankton, and that the latter consequently avoid or are excluded from these high concentrations of algae" (37). Ryther (37) has produced evidence in support of Hardy's theory, which states that; "dense populations of phytoplankton, in both marine and freshwater habitats, may create conditions which are inimical or actually lethal to a great

TABLE 6. Extreme Tolerances and Positive Population Increase Ranges for Daphnia magna. (34).

Factor	Extreme Tolerances	Positive Increase
Temperature ^{o}C	1.5 - 27	12 - 20
pH	6 - 9.4	7 - 8.5
Dissolved Oxygen, mg/l	0.2 - 24	0.5 - 10
H_2S, mg/l	0 - 3	0 - 0.4
Ammonia, mg/l	0 - 32	0 - 17
Nitrite, mg/l	0 - 20	0 - 8
Bacteria/ccm	$1.5x10^{2}$-$1x10^{8}$	$1x10^{4}$-$1x10^{5}$

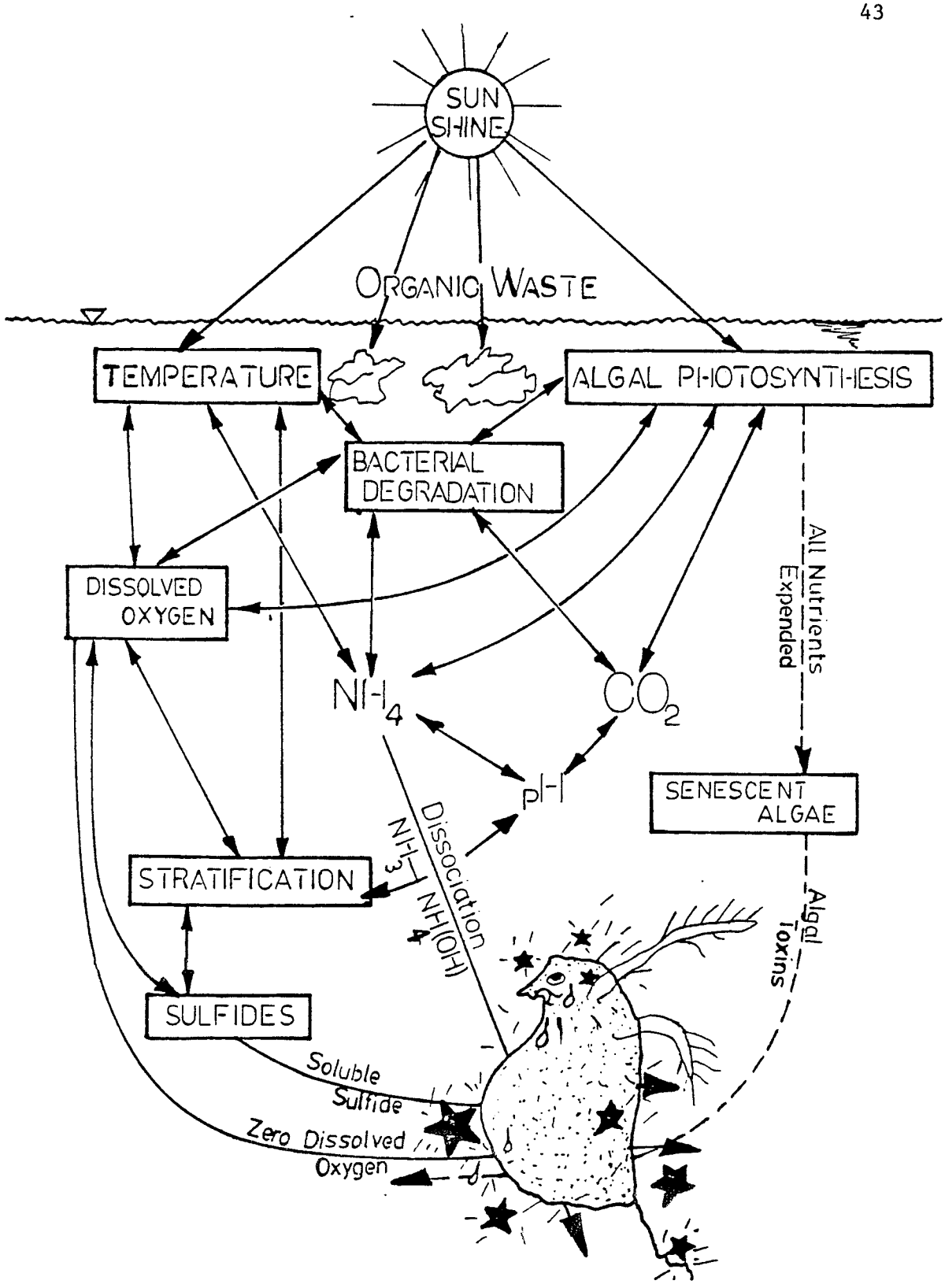

FIGURE 10. Demise of the Daphnia (adapted) (26).

variety of aquatic organisms".

Studies were conducted using Daphnia magna and three species of planktonic algae; the Chlorophyta, Chlorella vulgaris and Scenedesmus quadricauda and the diatom, Navicula pelliculosa. These feeding experiments with Daphnia demonstrated that "within a limited range, the concentration of algae in the water may have a pronounced effect upon the filtering rate of that animal" (37). Moreover, the age of the phytoplankton was the most significant factor affecting the feeding behaviour and health of the cladoceran. Conclusions to Ryther's study of relevance to this dissertation are listed below: (37)

(a) "The filtering rate of Daphnia is inhibited by substances produced by the three species of algae tested. The inhibitory product of Chlorella appears to be identical to the...antibiotic, chlorellin. The inhibition is mediated partially by substances which diffuse from the cells and accumulate in the water. A much more pronounced effect appears to be produced by the release of the inhibitory products from ingested cells within the animals.

(b) The minimum inhibition is produced by actively growing algae from cultures which are in their log phase of growth. The maximum effect is produced by senescent, non-dividing algae.

(c) No sign of feeding could be detected in Daphnia which had been exposed to suspensions of senescent, non-dividing Chlorella for period of 12 hours prior to the feeding experiments.

(d) Within the size range represented by Chlorella ($3.6\mu^3$) and

Scenedesmus (25.5 μ^3) Daphnia shows no selectivity in its feeding, removing cells of both species at the same rate from mixed populations of these algae.

(e) Daphnia cultured upon actively growing Chlorella cells grew rapidly and, upon reaching maturity, maintained a high rate of reproduction. Daphnia cultured on senescent, non-dividing Chlorella grew very slowly, failed to reproduce, and died after 11-13 days".

Ryther's study has demonstrated the ecological occurrence of the inhibition of zooplankton filter feeding populations by phytoplankton. In particular, the study has implicated senescent phytoplankton with the release of "exocrine" metabolites which are inimical to Daphnia. In a more recent study Porter (38) reports growth enhancement of the alga Sphaerocystis schroeteri directly due to grazing by Daphnia magna. Cells of the alga which may remain undamaged during passage through the gut of the Daphnia, are stimulated by the nutrients absorbed from the Daphnia.

5.2.2 Light and Temperature

In wastewater stabilization ponds Daphnia show sensitivity to the photoperiod which "indirectly regulates the animal population pulse." Optimum, production may be restricted to the interval of minimum photo period; averaging around 500 minutes per day. During which period the temperature averages 22° C. The optimum temperature range is 12 - 20°C. High temperatures are inimical to Daphnia especially through complications caused by pH - ammonia balance.

5.2.3 pH

The hydrogen ion concentration is determined by the carbon dioxide-carbonate-bicarbonate-ammonia equilibrium, bacterial metabolism and algal photosynthetic processes discussed in Chapter 5. Despite the effectiveness of the system buffering capacity the pH generally tends to an increase with an increase in photoperiod. Subject to conditions of ammonia equilibrium and water temperature, the optimum pH range for Daphnia magna according to Scheithauer (34) in Table 6 is from 7 to 8.5. Lower temperatures and pH values in this range tend to favour the non-toxic ammonium ion. The un-ionized forms, NH_3 and NH_4OH are inimical to Cladocera. Figure 11 shows the pH - ammonia effect on Daphnia magna. The highest population densities for Daphnia magna are supported below a pH of 7.8 and below 17 mg/l ammonia concentration. Ambient temperatures have not been shown for these relationships.

5.2.4 Soluble Sulphides

Sulphide formation is the work of sulphate reducing bacteria under anaerobic conditions. Elevated water temperatures and increasing anaerobiosis favour the proliferation of sulphate reducing bacteria. Lack of mixing can promote layering which further enhances this condition. "Once the sulphate reducing bacteria population has attained a high level, sulphide production is likely to increase in ponds having an abundance of sulphates" (26). The rate of decomposition of sulphates is a function of the sulphate levels present, the numbers of bacteria and the water temperature. Free nitrates restrict soluble sulphide production. The degree of toxicity of soluble sulphides to aquatic life

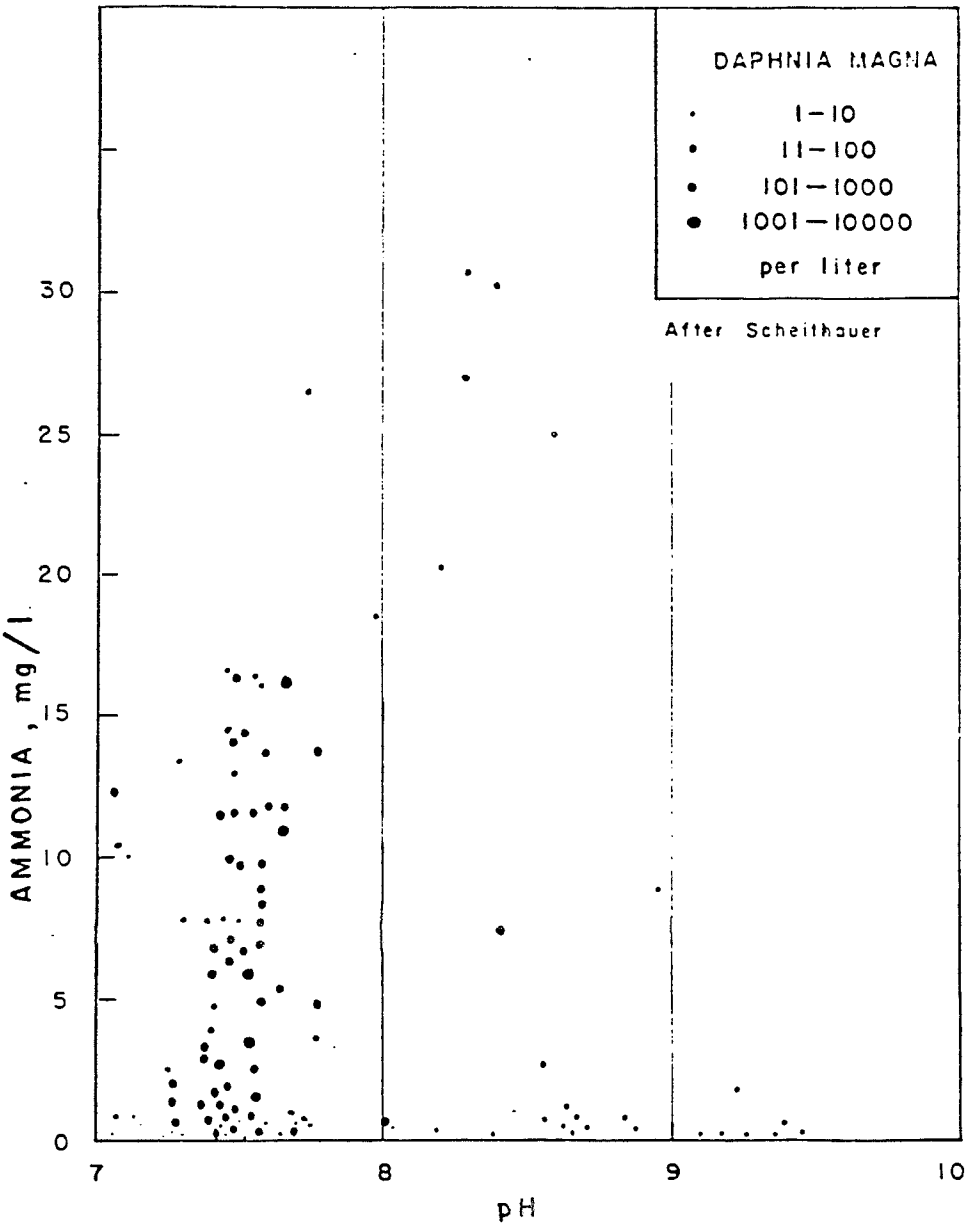

FIGURE 11. Effects of ammonia vs pH on Daphnia (34).

increases with pH rise. The growth of Daphnia is optimal between 0 - 0.4 mg/l, Table 6, although in some instances populations can survive up to 3.0 mg/l soluble sulphides (34).

5.2.5 Loading

Reported organic loadings on ponds supporting Daphnia populations were from 30 to 161 pounds of BOD_5 per acre per day, when the oxygen supply required to promote bacterial decomposition of accumulated sludge was adequate. The Daphnia population maintains and equilibrium with the available food supply. Food scarcity results in the appearance of males and sexual females and the curtailment of parthenogenic production (26).

5.2.6 Dissolved Oxygen

Daphnia may thrive at a DO range from 0.6 mg/l - saturation and can survive short periods when no measureable dissolved oxygen is present in the water. At these times through movements near the air-water surface adequate oxygen for respiration can be entrained through surface turbulence. The movements of a Daphnia swarm are co-ordinated to effect such a distribution (26). In times of low oxygen concentration Daphnia develop haemogloblin pigment which assists in efficient oxygen transport to tissues (39).

Supersaturation by photosynthesis may be harmful to Daphnia: Oxygen bubbles collecting below the carapace may float organisms to the surface where dessication and death occur. The same effect may be created by the liberation of oxygen by algae in the gut of the organisms (26). Figure 12 relates Daphnia production to available dissolved

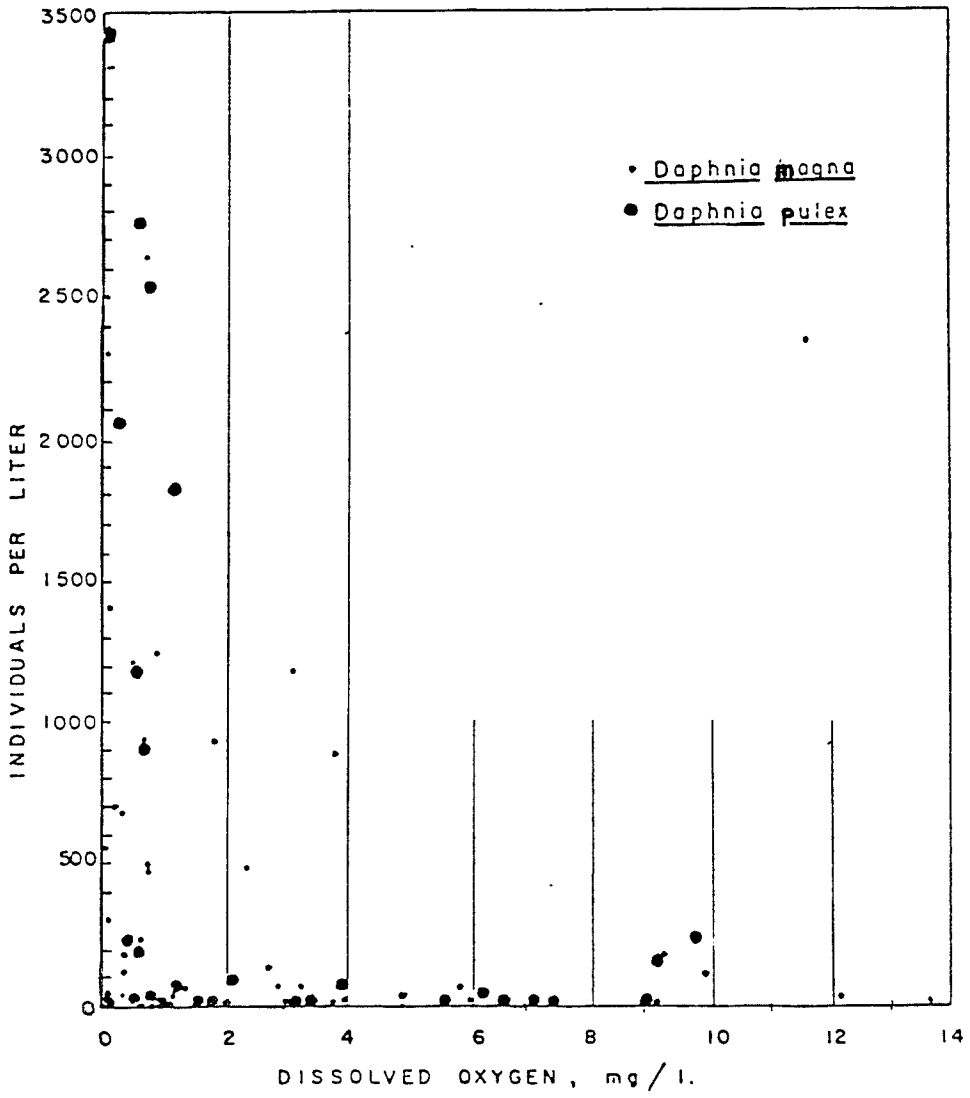

FIGURE 12. Dissolved oxygen and Daphnia production (26).

oxygen.

5.2.7 Mineral Content

"The sodium to potassium ratio when less than 10:1, could be toxic" (26). The chloride radical should be the dominant anion and chloride content should always exceed the nitrate levels. Nitrate toxicity was found to be variable but decreased with chloride increase (26). Nitrate levels should be as "low as consistent to algae growth(26)". Daphnia populate waters over the hardness range from 27 - 660 mg/l as $CaCO_3$. They can survive in waters containing 2490 mg/l dissolved solids. The lowest sodium - potassium ratio found to support Daphnia was 4.7:1. The maximum nitrate content, of waters supporting healthy Daphnia, was 82 mg/l(26). Daphnia species show wide variability with respect to response to environmental features and to changes in them.

5.2.8 Predation

Fish are the main predators of Daphnia populations. Copepods eat Daphnia young (29). Migratory water fowl and shore birds also use Daphnia as food. Predation by migratory birds assists in the dissemination of Daphnia over a widespread range by transporting ephippia in fecal material and on their feathers to other bodies of water. Predaceous insects, notably the diving beetle and Chaborus, the phantom midge (29); can cause significant impact on small Daphnia populations. A balanced predation on Daphnia populations may be beneficial due to the conversion of organic matter to a higher order and a stimulation of Daphnia reproduction (26).

5.2.9 Sensitivity of the Organism to Anthropogenic Pollutants

All substances which form emulsions or simple solutions with water can be harmful to aquatic life. Particularly "all salts are toxic when they are present in concentrations high enough to exert an unfavourable osmotic pressure"(32). Salts of the sodium and potassium genre have special importance to pollution studies because of the special role they play in altering the dynamics of cellular transport mechanisms. Pollutants contribute to chemical poisoning of and physical discomfort to the organism by sufficiently altering the ecology of their environment. Salts, causing acid or basic conditions in solution are poisonous to Daphnia. Examples are the salts sodium bisulfate or bisulfite, when of sufficient molarity to depress the pH to below 6; calcium oxide and the salts sodium carbonate and sodium hydroxide when they elevate the pH above 9.1. Precipitates may exert a mechanical effect by obstructing the straining mechanism of the Daphnia (32).

Daphnia are most susceptible to toxic substances during their "first instar and become less so as to their age increases" (30). Adult organisms are more susceptible during ecdysis than at other times. The salts; sodium acetate, sodium bromide, sodium chloride, sodium formate, and sodium nitrate, "have approximately the same threshold concentrations in terms of molarity and are probably toxic only when their concentrations are high enough to exert an unfavourable osmotic effect" (32), when added to certain lake waters. Figure 13 is a graphical presentation of threshold concentrations for the substances shown. The threshold concentration is that level at which the "immobilization curve parallels the ordinate" (30). A few readily ionized chemicals, having a common

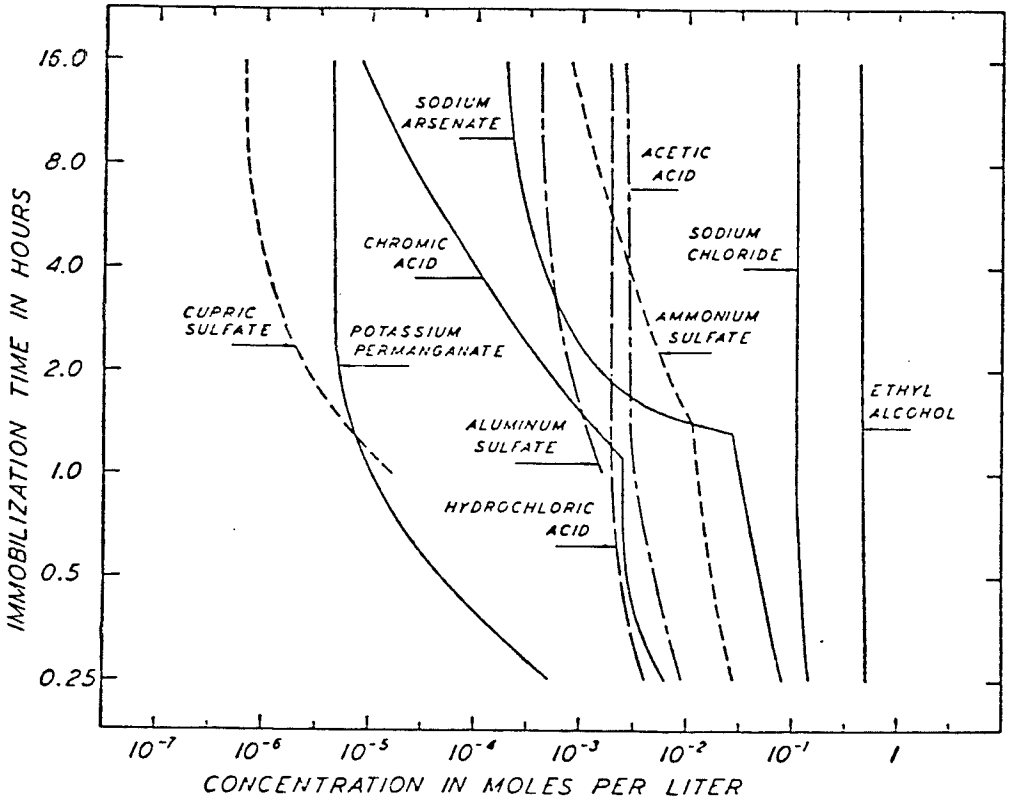

FIGURE 13. Relation of immobilization time to concentration for Daphnia magna (30).

cationic radical, are toxic at threshold concentrations directly dependent on the solution concentration of the common cation. Aluminum ammonium sulfate and aluminum potassium sulfate, are in this category. Hydrochloric, nitric and sulfuric acids exhibit threshold toxicity concentrations that vary directly with their hydrogen equivalents. The toxicity of these acids is due to pH depression. Acetic, citric and lactic acids can be metabolised by living organisms. This effectively reduces their availability for acid activity. Toxic solutions of the acids after an experimental run, show higher pH values than would test solutions of equivalent concentrations of the non-biochemically important acids. Alcohol toxicity is generally governed by Traube's rule, which states that, "the concentration, in terms of molarity, required to narcotize decreases approximately in negative powers of three as the number of carbon atoms in the chain increases" (30).

Many distilled waters are reported to be toxic to daphnids. "Water distilled from ordinary glass was non-toxic but that from hard glass was toxic. Single-distilled waters from each of several different metal stills immobilized first instar young and older daphnids in two hours. Older daphnids survived a week or longer in double-distilled water, the final distillation from Pyrex glass;...first instar young were immobilized within three hours" (30).

A study was conducted by Biesinger et al. (40) on the chronic effects of several metals on the survival, growth reproduction and metabolism of Daphnia magna. In the study, toxicities were evaluated on the basis of a 48-hr 50% lethal concentration (LC 50), a 3-week LC 50, and 16 and 50% reproductive impairment over a two week period. Chronic effects

on growth were evaluated by assessment of weight and protein changes. Metabolic effects were studied by monitoring glutamic oxalacetic transaminase (GOT) activity. The following observations were made:

"For acute and chronic tests median lethal concentrations were higher with than without food added. Many of the metals tested were from one to two orders of magnitude more toxic on a chronic basis than in 48 hr. tests." Reproductive impairment was found to be a more sensitive measure of toxicity than survival. Although reproduction was usually stimulated by small metal additions, the study found that the 16% level of reproductive impairment was the basal level at which impairment could be generally detected. The results of this study are presented in Table 7.

In a study of the "Population dynamics of Daphnia galeata mendotae as modified by chronic cadmium stress", using comparable levels of cadmium, Marshall (41) concluded that the stress effects observed were similar to "those observed in" Daphnia populations in response to chronic radiation stress or removal of fixed percentages of new born". In particular, chronic "cadmium stress reduced the populations' average numbers and biomass, while it increased population variability, probability of extinction, turnover rate and the proportion of adults in the populations". These net effects were realized by "reduced average life expect ancy and increased pre-natal mortality, individual growth rates and brood size" (41).

In the biochemical assessments of Biesinger et al. (40), the animals after a three week period, with the exception of those subjected to the potassium samples, generally weighed less than the control group. The

TABLE 7. Chronic (3-week) toxicities of various metal ions (µg/liter) to Daphnia magna in Lake Superior water (40).

Metal ion	LC50	Reproductive impairment	
		50%	16%
Sodium	1,480,000 (1,180,000-1,840,000)[a]	1,020,000	680,000
	S 2.22[b]	S 1.50	
Calcium	330,000 (308,000-353,000)	220,000	116,000
	S 1.36	S 1.94	
Magnesium	190,000 (167,000-217,000)	125,000	82,000
	S 2.00	S 1.63	
Potassium	97,000 (87,000-108,000)	68,000	53,000
	S 1.42	S 1.28	
Strontium	86,000 (82,000-90,000)	60,000	42,000
	S 1.14	S 1.39	
Barium	13,500 (12,200-15,000)	8900	5800
	S 1.45	S 1.55	
Iron	5900 (5180-6730)	5200	4380
	S 2.15	S 1.18	
Manganese	5700 (5380-6040)	5200	4100
	S 1.24	S 1.29	
Arsenic	2850 (2520-3220)	1400	520
	S 1.63	S 2.66	
Tin	42,000 (23,000-76,000)	1500	350
	S 5.86	S 4.41	
Chromium	2000 (650-2600)	600	330
	S 3.70	S 1.82	
Aluminum	1400 (1080-1820)	680	320
	S 2.24	S 2.14	
Zinc	158 (146-170)	102	70
	S 1.40	S 1.48	
Gold	1050 (861-1281)	180	60
	S 1.65	S 3.00	
Nickel	130 (98-173)	95	30
	S 2.03	S 3.16	
Lead	300 (236-381)	100	30
	S 2.52	S 3.36	
Copper	44 (35-55)	35	22
	S 1.83	S 1.60	
Platinum	520 (437-619)	82	14
	S 1.51	S 6.22	
Cobalt	21 (14-31)	12	10
	S 1.62	S 1.21	
Mercury	13[c] (9-19)	6.7	3.4
	S 3.16	S 1.99	
Cadmium	5 (4.0-6.2)	0.7	0.17
	S 2.42	S 4.06	

[a]Ninety-five percent confidence limits.
[b]Slope function.
[c]Extrapolated value from three partial concentrations less than the LC50.

test animals were visibly smaller. The percentage of protein was notably greater with a few exceptions. GOT activity was stimulated by all ions with the exception of Fe, As, Cr, Al, Cu, Pt and Hg. These results are summarized in Table 8. Increases in protein and GOT activity were related to the molecular dynamics of protein-forming enzymes, which once altered, effected a general increase in protein metabolism.

In the same study (40) "certain correlations were found between the toxicity of these metals and their physicochemical characteristics". In particular, the correlation between toxicity and the solubility of metal sulfides suggested "the possibility that metals may combine *in vivo* with sulfhydryl groups on enzymes, which affects their solubility and catalytic activity" (40).

In a comparison of the 64-hr. "apparent-threshold metal concentrations for daphnids with acute values for fish, "Anderson (42) concluded that "in general *Daphnia* and related forms are more susceptible to cations than are fish". Differences in chemistry of source waters used for studies along with testing methods and exposure times render evaluations of toxicity results by various workers difficult. Complete relationships between the toxicity of various substances can not be satisfactorily worked out without complete analyses of diluent waters. Moreover, threshold response comparisons for daphnids with fishes or other organisms can only be reliable when all species being compared are "subjected to the same substances when added to identical diluents"(30).

5.3 Laboratory Methods for Culturing *Daphnia magna*

Successful methods for culturing *Daphnia magna* under laboratory

TABLE 8. Effects of 3-week exposures to metal chlorides on body weight, total protein, and GOT activity of Daphnia magna (expressed as mean percentage deviation from control means)[a] (40).

Metal ion	No. experiments	Effect concentrations		Weight/animal % Δ	Protein/animal % Δ	GOT/animal % Δ
		In M × 10^6	In μg/liter			
Sodium	3	43,500	1,000,000	−18	−7	+12
Calcium	2	10,000	400,000	−29	+18	+3
Magnesium	2	10,300	250,000	−22	+40	+65
Potassium	2	2040	79,800	+27	−5	+5
Strontium	2	1140	99,900	−24	+15	+55
Barium	1	144	19,800	−12	0	+1
Iron	1	134	7480	−77	+48	−13
Manganese	3	91.0	5000	−8	+24	+100
Arsenic	4	13.3	996	−18	−15	−18
Tin	2	25.3	3000	−23	+5	+15
Chromium	4	11.9	619	−11	−3	−4
Aluminum	2	23.0	620	−38	+3	−13
Zinc	1	2.68	175	−28	+10	+1
Nickel	2	2.13	125	−43	−9	−26
Lead	3	0.300	62	−12	+8	+15
Copper	3	0.628	40	−26	−5	−10
Platinum	2	0.318	62	−12	−13	−20
Cobalt	1	0.423	24	−15	+12	+45
Mercury	1	0.050	10	−5	−5	−19
Cadmium	1	0.0089	1	−7	+6	+15

[a]Precision (range) = ±10% (weight); 8% (protein); ±5% (GOT).

conditions have been reported in the literature. These presentations are still in the experimental stage. Generally, laboratory methods attempt to simulate, using distilled waters and reagent grade chemicals, conditions under which the organisms can thrive. Laboratory methods attempt to sustain an environment in which the proliferation of Daphnia magna through diploid parthenogenesis would be favoured. When natural source waters are unavailable, dechlorinated tap waters have been used. Waters used for culture media should be of a constant quality, by definition; a water in which the fluctuation in monthly ranges relevant to "hardness, alkalinity, conductivity, total organic carbon or chemical oxygen demand, and salinity, are less than 10% of the respective averages and if the range of pH is less than 0.4 unit" (23). Double-glass-distilled tap waters; deionized to remove unacceptable contaminants imparted by the distillation process, have been reconstituted with satisfactory result. Reconstituted nutrient media are usually similar to preparations used to sustain healthy algal growths. An example of such is the NC-2 media used in the culture of Daphnia magna by the Freshwater Institute of Canada (43). Table 9 is a list of the stock solutions for a modified reconstituted medium. Sodium bicarbonate has been added to this list, in an amount equivalent to the concentration suggested for algal stock nutrient solutions in Standard Methods (23).

The stock culture of daphnids must be reared in aquaria made of non toxic materials. Materials from which toxic substances can be leached must not be used. Aquaria should be suitably covered to reduce evaporation. Environmental conditions; pH, temperature, oxygen and illumin-

TABLE 9. Modified reconstituted medium for growing *Daphnia magna*. (43).

KCl	50 g/l
$NaNO_3$	50 g/l
KH_2PO_4	6 g/l
K_2HPO_4	6 g/l
$MgSO_4.7\ H_2O$	40 g/l
$CaCl_2.6\ H_2O$	21.9 g/l
or	
$CaCl_2.2\ H_2O$	14.7 g/l
$NaHCO_3$	15 g/l
$Na_2SiO_3.9\ H_2O$	20 g/l

Preparation

1. Before adding calcium solution add 1 ml of each stock solution per litre of deionized distilled water.
2. Bring the mixture to a pH of 6.65 using 2N HCl.
3. Add 7 ml calcium stock solution per litre of reconstituted water. To avoid precipitation of the $CaCL_2$ the pH must be below 7.0.
4. For axenic cultures, sterilize the solution in an autoclave. The final solution pH should be in the range of 6 - 7.

ation favourable to the organism must be maintained. Daphnia magna are sensitive and delicate organisms and must be handled with consumate care.

Food used to sustain stock cultures of Daphnia include bacteria, algae, infusoria, and a wide variety of preparations which generate organic detritus. These include yeast preparations, "soil extracts and organic materials such as cotton seed meal, herring meal, powdered dried grass, and enriched trout fry granules" (23). Manure-soil media have been widely used. Aliquots are withdrawn and introduced into the culture aquaria as feeding demand dictates.

A successful culture method, in which the conditions for parthenogenic reproduction were maintained over a one year period, has been outlined in the literature by Ryther (37). "Eggs or young were always plentiful in mature animals and no males or females with ephippia were ever observed". The animals were cultured in cement aquaria, with surface dimensions 1680 x 610 mm, with water to a depth of 50 mm; maintained at this level by constantly dripping tap water. "The Daphnia lived in association with a population of snails, Lymnaea palustris, which was maintained by feeding regularly with lettuce (37). The Daphnia fed upon the fecal matter from the snails and the bacterial fauna generated by the organic detritus.

6. MATERIALS AND METHODS

Secondary sewage effluent was drawn from the North Clarifier of The City of Winnipeg South End Water Pollution Control Facility, on March 10, 1978 at 4:45 p.m. The effluent was refrigerated at 1°C until required for processing into tertiary effluent. Bioassay procedures extended from April 7, 1978 to June 12, 1978.

The experimental testing programme was arranged and conducted according to the following schedule:

1) Preliminary analysis of secondary sewage effluent;
2) Tertiary treatment of secondary effluent using two chemical methods; lime and alum, to produce separate batches of tertiary effluent of the same low residual phosphate concentration;
3) Preparation of the dilution water, bioassay apparatus and the effluent concentration series for both systems;
4) The exploratory bioassay study;
5) The main bioassay study with attendent monitoring and testing;
6) Post experimental testing and data collection.

6.1 Miscellaneous Analytical Methods

Extensive effluent testing and monitoring was required. A complete differential chemical analysis of the secondary sewage effluent, tertiary effluent and the reconstituted dilution water was required, before

beginning the actual bioassay. Post experimental differential chemical analysis was conducted on the effluent remaining in the aquaria on termination of the experiment. The analytical testing schedule included the following determinations, from which an appropriate selection was made, based on utility and the experimental objectives:

(a) Total Organic Carbon (TOC):

(b) Total Alkalinity;

(c) Dissolved Oxygen (DO):

(d) pH;

(e) Conductivity;

(f) Total Hardness and Calcium Hardness;

(g) Total Ammonia Nitrogen, NH_3-N;

(h) Free Carbon Dioxide (CO_2);

(i) Total Dissolved Solids;

(j) Turbidity.

Some analytical work requiring specialized analytical equipment was conducted by laboratory staff, at The Freshwater Institute (FWI), Winnipeg, Manitoba. The other analyses, and all bioassay studies were conducted at the Sanitary Engineering Laboratories at the University of Manitoba. These analyses included:

1) Turbidity analysis, using a Hellige Turbidimeter;
2) Dissolved Oxygen (DO) analysis using a YSI Model 54 Oxygen-meter with a 0 to 20 mg/l range;
3) pH Analysis using a Type PHM206, Radiometer Copenhagen meter;
4) Conductivity analysis using a Type CDM2e meter;

5) Total Organic Carbon (TOC) determinations were conducted at both laboratories. The analyses at the Sanitary Engineering Lab were conducted using a Beckman Model 915A, TOC Analyser, with a Beckman Model 865 Infrared Analyser.

6) Total and Calcium Hardness and Alkalinity did not require any specialized apparatus. The procedures used were as found in Standard Methods for the Examination of Water and Wastewater (23), hereinafter called Standard Methods. Bicarbonate and CO_2 Alkalinity, as well as free CO_2 content was found by nomograph methods in the same text. Magnesium Hardness by the difference between Total and Calcium Hardness.

7) Total Dissolved Solids in mg/l was found by the estimation method using: Conductivity μmhos/cm x an empirical factor ranging from 0.55 to 0.9. For these analyses 0.6 was used.

8) Total Amonia Nitrogen, NH_3-N analyses were done using Kjeldahl Apparatus and the distillation method as per Standard Methods. Figure 14 shows the standard curve developed for this experimental series. Total un-ionized ammonia content of samples was then computed from Table 5.

9) The success of the jar testing procedures was ascertained by constant monitoring of the supernatant for phosphorous residues. Phipps and Bird jar testing apparatus with six (6) 2000 ml pyrex beakers was used. Phosphate content was determined spectrophotometrically using the Bausch and Lomb

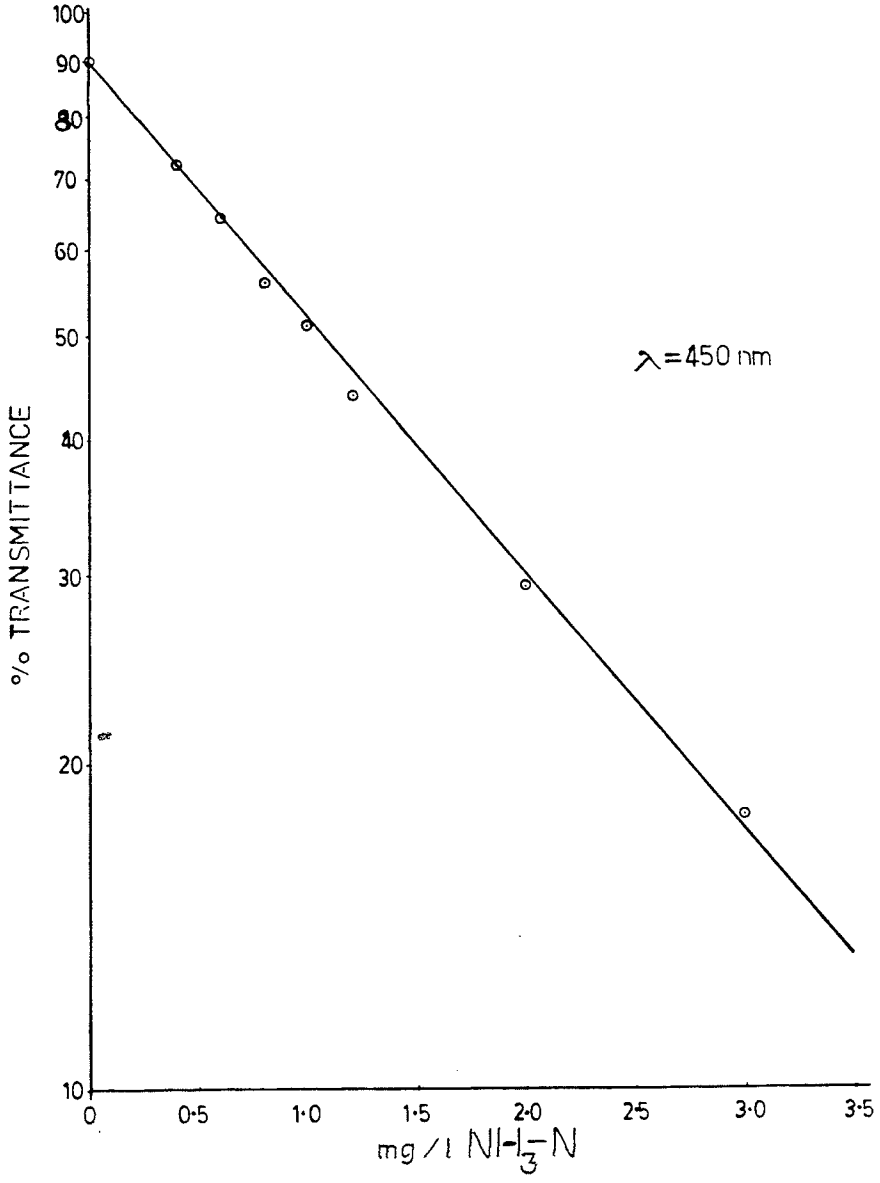

FIGURE 14. Standard curve for Ammonia Nitrogen as N. May 9, 1978 for λ = 450 nm. Best fit by eye.

Spectronic 20 apparatus.

Samples requiring heavy metal analysis were acidified with 0.5% nitric acid per 100 ml sample prior to analysis at the FWI.

During the bioassay phase of experimentation, sample volumes for eventual analysis were obtained from each aquarium on two occasions; 80 ml and 100 ml respectively. The volumes removed were replaced after temperature equalization, using identical stock concentrations from the containers in storage.

6.1.1 Analysis of Secondary Sewage Effluent

For comparative assessments, a complete differential chemical analysis of the secondary sewage effluent was obtained. Analysis of the secondary effluent preliminary to tertiary treatment was important for a complete documentation of the effluent profile.

6.2 Tertiary Treatment Using Lime and Alum

Trial jar tests were conducted using both lime and alum to reduce the phosphate content to a concentration of 0.1 mg/l. This represented a 98.4% reduction from the 6.1 mg/l total phosphate concentration in the secondary effluent. Total phosphate determinations for monitoring the effectiveness of the jar testing trials, were conducted, using the Stannous Chloride Method with Preliminary Digestion as per Standard Methods. An American Sterilizer Company Model 608A autoclave operating at 200°F for a period of 30 minutes was used to effect complete hydrolysis of the sample to orthophosphates. Figure 15 shows the calibration curve developed for this analysis. As reported in the literature, the

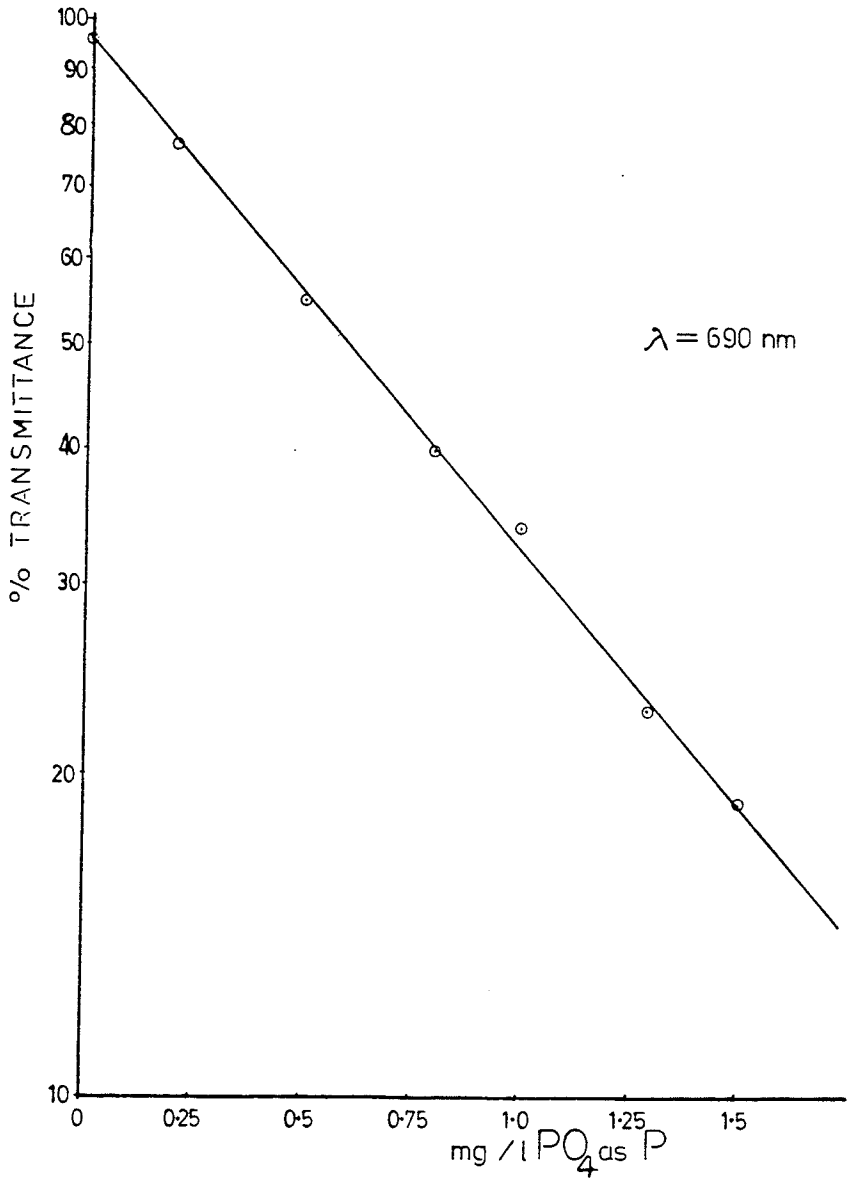

FIGURE 15. Standard curve for total Phosphates as P. March 14, 1978 for λ = 690 nm. Best fit by eye.

removal efficiencies by both lime and alum methods were pH dependent. For effective removals using lime treatment, pH levels in excess of 10.4 were required. For effective alum removals the critical pH range observed was from 5.5 to 7.0. The process manipulation found to be the most successful in obtaining the desired phosphate residuals was the Split-Treatment Method using 400 mg/l slaked lime and for the alum batch 450 mg/l dissolved aluminum sulphates. Low removals were also obtained using 300 mg/l alum. However, 450 mg/l concentration was chosen to keep both dosages numerically similar to facilitate comparison. The Split-Treatment technique devised was as follows:

1) Rapid Mix secondary effluent with full required dosage of chemical for 5 minutes at 100 rpm; using only one-half of the liquid volume to be treated.
2) Slow Mix at 40 rpm for 5 minutes. Effective removals for the lime treatment required the minimum pH of the mix at 11.5
3) The liquid volume was brought up to the 100 percent mark by adding secondary effluent, and paddled in a slow mix mode for 40 minutes.
4) The treated volumes were allowed to settle for at least 1 hour, whereupon the supernatant was carefully poured off into thoroughly cleansed plastic containers originally containing a dilute solution of sodium hypoclorite.
5) The only modification used for the alum system was a total mix time of 45 instead of 50 minutes.

Figures 16 and 17 show the experimental jar testing curves for the dephosphatization of secondary sewage effluent for the lime and alum

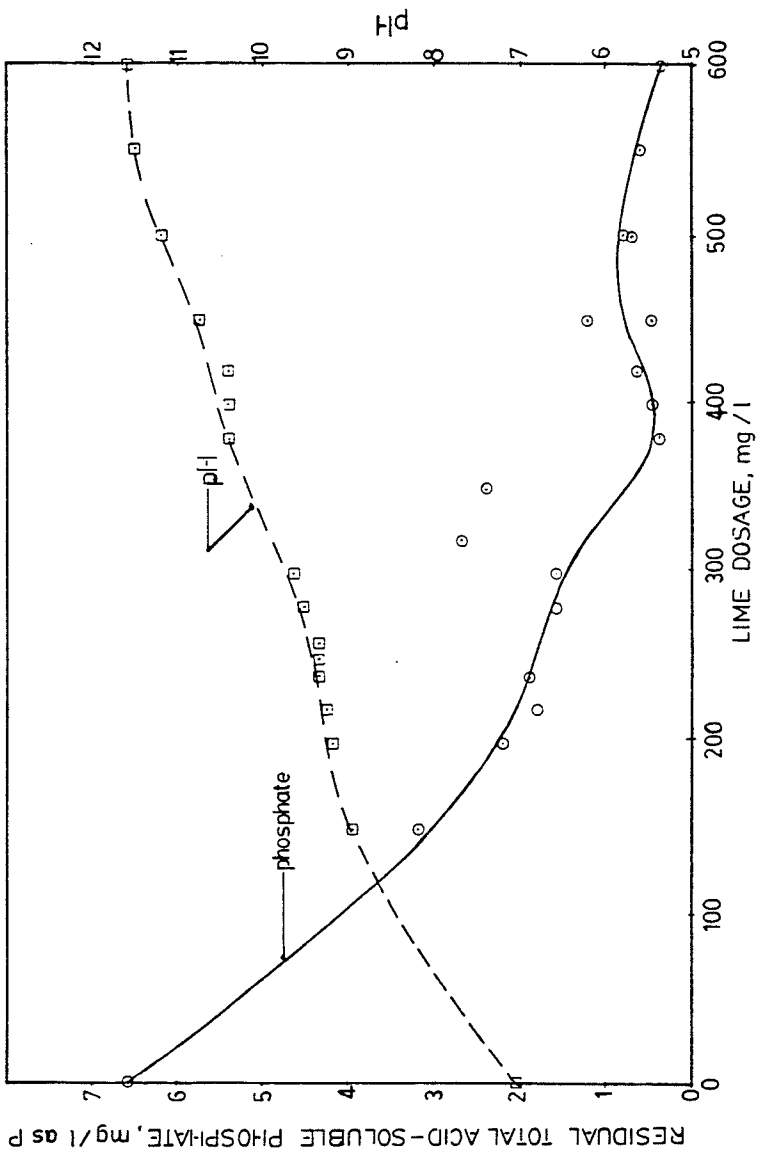

FIGURE 16. Jar test functions for Lime dephosphatization of Secondary Sewage Effluent, at 22°C. Note: dashed line is pH curve.

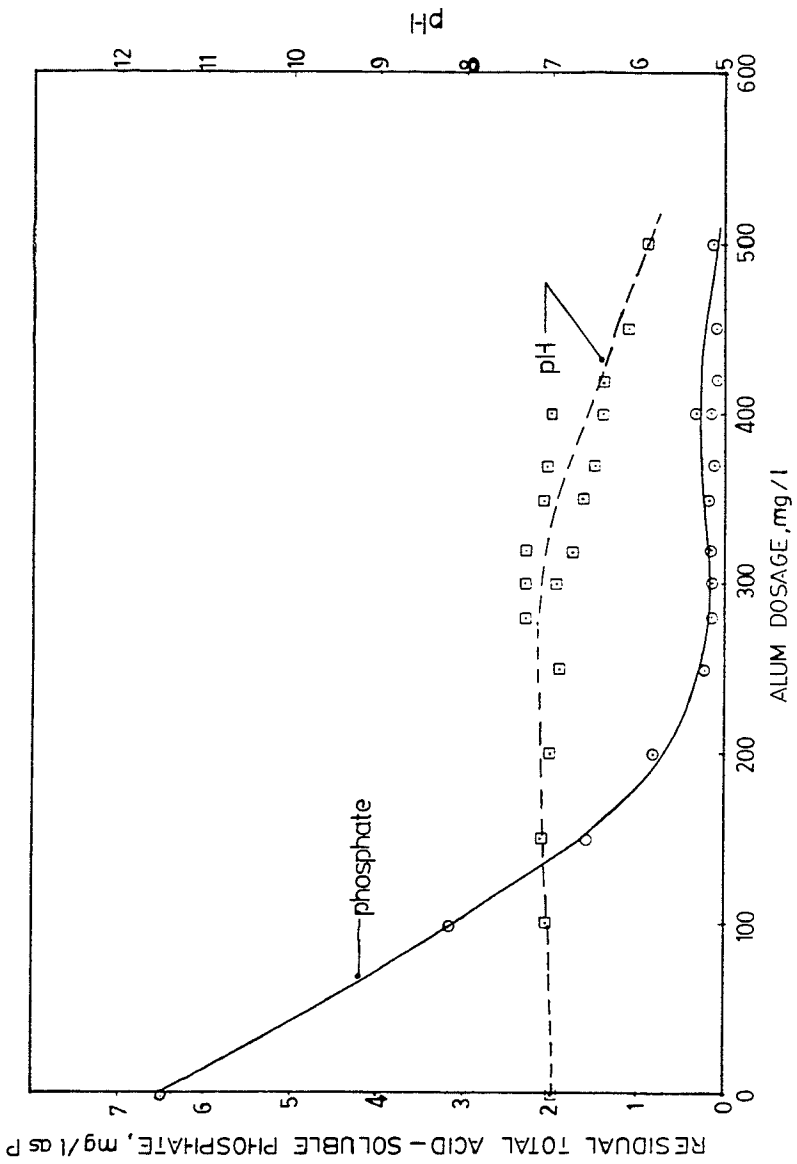

FIGURE 17. Jar test functions for Alum dephosphatization of Secondary Sewage Effluent, at 22°C. Note: dashed line is pH curve.

systems respectively.

To complete the process the lime treated effluent had to be recarbonated by bubbling carbon dioxide through the effluent. This reduced the pH from levels in excess of 11.0 to a value of 6.0, on par with the pH level of the alum treated effluent. The alum and lime effluent, after storage, appeared to stabilize at pH values of 6.3 and 6.4 respectively.

The chemical profile of the tertiary effluent was ascertained for the same parameters used in the secondary effluent evaluation.

6.3. Dilution Water Bioassay Apparatus and *Daphnia magna* Stock

Double glass-distilled deionized water was used in the preparation of the reconstituted medium or reconstituted dilution water as is illustrated in the procedure of Table 9. A supply of this distilled water was obtained in quantities sufficient for the complete bioassay from the FWI. The media was sterilized in an American Company Model 608A autoclave before use in the preparation of the dilution volumes.

The bioassay apparatus was set up within an Econaire Environmental Chamber. Two sizes of aquaria were used in the bioassay runs. A 1000 ml size, wide-mouthed, soft glass jar, was used for the parent aquaria. A 3000 ml series of wide-mouthed, soft glass containers, "battery jars", were used for the progeny aquaria. The bioassay test series was conducted in replicate for each tertiary effluent series, with a common replicate control; the reconstituted dilution water. Figures 18 and 19 illustrate the apparatus used in the arrangement of the replicate series for both parent and progeny aquaria. To minimize evaporation "Tygon" tubing was attached to the rims of the aquaria, over which transparent

FIGURE 18. Replicate Series for parent aquaria.
Photo Courtesy Elvin Hewitt.

FIGURE 19. Replicate Series for progeny aquaria.
Photo Courtesy Elvin Hewitt.

acetate covers were placed as shown in Figure 18. For each system of aquaria; parent and progeny, there was a total of 18 aquaria (the control group in replicate and four serial dilutions in replicate for each effluent). Containers were numbered and randomized to eliminate experimental bias.

Miscellaneous items to be used in the bioassay study were labelled and kept in isolation, where necessary, to reduce the possibility of cross-contamination. Contact probes of analytical apparatus were cleansed after each effluent reading was taken. A series of eye droppers and filled wash-bottles in replicate corresponding to the effluent series was kept on hand. Specially constructed sieves, prepared by the FWI, were used in the transfer and counting of the organisms. The sieves were made of nylon mesh, affixed to plastic barrels by a non-toxic silicone adhesive. These materials are reputed to present no hazard to the organisms. The small gauge sieve had square mesh openings of size 0.113 mm. The larger sieve mesh size was 0.75 mm. Two banks of fluorescent lighting provided adequate lighting for the study.

The stock neonates provided by the FWI for the exploratory and main bioassay study, were estimated, by that department to be fiftieth (50) generation laboratory parthenogenic clones (29). A standard food preparation "Cerofood" shown in Table 10, was obtained from the FWI for feeding the test organisms on a regular feeding schedule. The recommended feeding schedule per adult organism was 5 µl every second day, or 0.1 ml of "Cerofood" for each 25 animals, every second day (43). Progeny generated during the experiment were held in small vials 15 mm in diameter with a minimum liquid depth of 5.7 cm. The feeding dose and

schedule was 5 µl per vial every three days.

TABLE 10. Standard food ("Cerofood") preparation (43).

Components are:

(1) Algal media

FW6

Stocks are all 100 mmol/litre	ml/litre stock
$NaNO_3$	30
K_2PHO_4	3
$MgSO_4$	4
$CaCl_2.6H_2O$	2
$NaHCO_3$	10
Distilled water	950

Sterilize by autoclave.

10 µl of each stock solution are added to 950 ml of double distilled water. To this is added a drop of 1% $FeCl_3$ solution.

(2) 10 g trout crumbles, ground fine in a blender (eg. Victor Fox TF crumbles or #6 ration, Purina).

(3) 0.5 g Cerophy^R powder (Source: Cerophyl Laboratories, Kansas City, MO, U.S.A.).

Preparation:

Mix trout crumbles and cerophyl. Add to 150 ml algal media and blend thoroughly. Strain through cheesecloth three times. Store in refrigerator in sterile dilution bottles or sterile vials. Shake before using. Must be remade monthly.

6.4 Exploratory Study and Test Concentrations

An exploratory study was conducted to ascertain the toxicity of the waste in terms of the EC 50 criterion, the effective concentration fatal

to 50% of test organisms. Replicate test concentrations of both effluents were prepared in the logarithmic scale 0.1, 1.0, 10 and 100% effluents. An experimental control of 0% effluent concentration, the reconstituted dilution water, was included. Each 600 ml volume concentration was held in the soft glass jars in the Econaire Environmental Chamber and allowed to equilibrate over a period of 3 days before insertion of the organisms. This procedure was followed in anticipation of small upward shifts particularly in the lime-treated effluent after removal from closed storage at 1°C.

Initial and final differential chemical analyses were conducted on the prepared concentrations. These analyses determined; DO, pH, Conductivity, Total Alkalinity, Total Hardness, Calcium Hardness, Magnesium Hardness, Free CO_2, Total NH_3-N, and Total Dissolved Solids content. In some cases, the quantities were estimated using the weighted mean method:

$$L_{mix} = \frac{V_A L_A + V_B L_B}{V_A + V_B}$$

where L = concentration in mg/l;

V = volume of liquid in litres

Ten (10) neonates, of maximum age 3 days, from the stock aquarium kept in the Environmental Chamber, were, at the zero hour, decanted into each concentration. Observations for mortality (criteria: immobility, cessation of heart beat or gut contraction) or moribund condition were made at the following intervals: 1.5, 3.0, 6.0, 12.0, 24.0, 48.0, and 96 hours.

No food was given during the exploratory study.

The results of this study suggested a test concentration series; 0% (reconstituted water), 12.5%, 25%, 50% and 100% effluents for both alum and lime effluent, as a means of guaging whether the effluents produced any reproductive impairment. The parent and progeny aquaria held 800 and 1500 ml of effluent respectively.

6.5 Main Bioassay Study

The main bioassy study was conducted over a period of 6 weeks from April 13 to May 25, 1978. The end of the study was predicted by a visible decline in the populations. In each of the eighteen parent aquaria, 50 neonates of maximum age 4 days, were placed and allowed to reproduce. Every two (2) days, the aquaria were monitored for DO, pH, conductivity, adult mortality, ephippial and progeny production. Records were kept and progeny were transferred to other aquaria containing the same effluent. The organisms were then fed, as per feeding schedule previously noted. To keep pace with their development and nutrient demands, the food given to the parent Daphnia was increased from 0.1 ml during the first 10 days to 0.2 for the next 7 days, to 0.25 ml for the next 6 days, to a maximum of 0.3 ml to the end of the bioassay. All aquaria were administered the same amounts of food. Figure 20 illustates the layout of some of the apparatus and aquaria in the Environmental Chamber.

The chamber was maintained at 20°C. A water-filled tray was affixed beneath the overhead fan. Water was also held in open containers below the tables to improve the ambient humidity and reduce evaporation from test aquaria. The two banks of cool-white fluorescent lights were

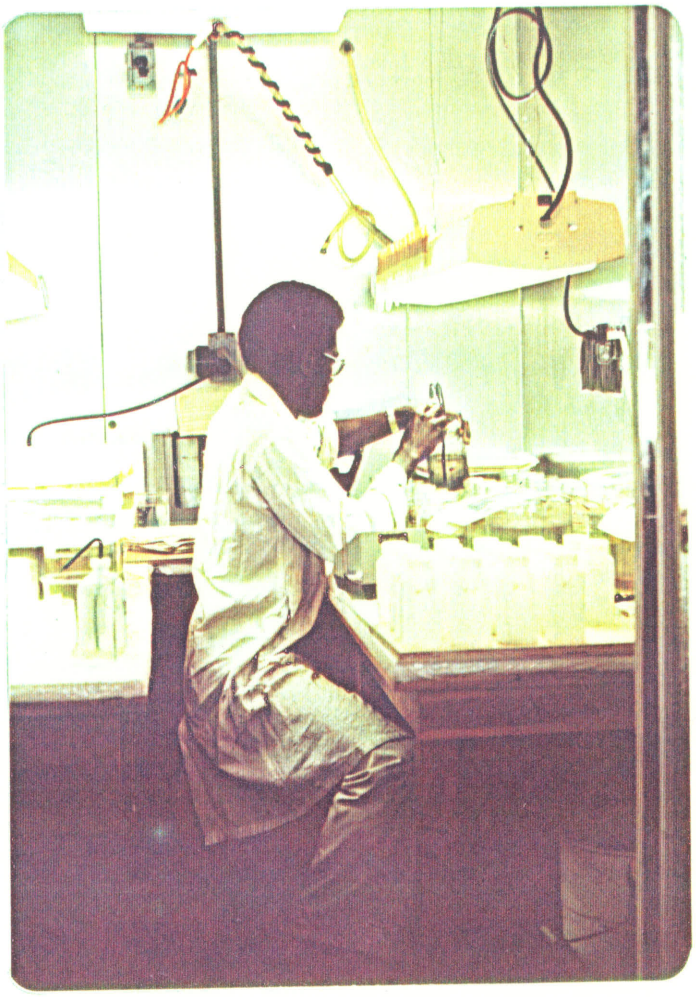

FIGURE 20 . Bioassay apparatus in Environmental Chamber.
Photo Courtesy Elvin Hewitt.

adjusted, with the aid of a light meter, to provide 200 candles of illumination at mid-water level, according to Standard Methods. Lighting was continuous for the first twelve (12) days; however, in an attempt to reduce the algal growth in some of the aquaria, a day-night cycle, 15 hrs. light; 9 hrs. dark, was introduced after day 12.

Screening and counting of adults and young was performed using the two sieves previously described.

6.6 Post experimental Testing and Data Collection

The post experimental phase could be categorized into five divisions described as follows:

1) Record of numbers of parents alive and tabulation of numbers and sizes of progeny at the particular time of counting. Progeny were categorized into adults and young based on size separation with the nylon mesh sieves. Those retained on the sieve with 0.75 x 0.75 mm square openings were classified "adults". Those passing this sieve and eventually retained on the 0.113 x 0.113 mm mesh were termed "young".

2) A random sampling of progeny aquaria for 25 organisms to be used to ascertain a size profile of animals remaining. Each of the progeny aquaria was agitated in turn to randomize the collection, simultaneously avoiding the bias inherent in the vortex. About 40 organisms were withdrawn by the single dip of a labelled beaker. These animals were subsequently removed from the liquid and fixed in a sugar formalin solution, 40 g/l sucrose in 4% formalin (44), after which they were selected at

random for measurement of head to spine lengths, under a microscope, with the aid of a special ocular, and Sedgwick-Rafter Cell. The microscope used was a Leitz Wetzlar instrument, equipped with a 2 mm long micrometer, an ocular G 12.5X, and a barrel magnification 4X. The collected data was compiled and used in statistical computations.

3) Biomass measurements were made by ascertaining "Wet" and Ash Weights of all progeny generated during the experiment. A Muffle Furnace equipped with Solid State Controller was used for drying the specimens; Thermoclyne, Model No. FA1730 with Dubuque III Solid State Controller Model No. CPS A 8720. Specimens were dried in a Boekel Dessicator, Model 1340. To determine "wet" weight specimens were dried for 12.5 hours at 60°C and cooled to room temperature in the dessicator before final measurements were taken. To determine "Ash" weight specimens were further dried for 24 hours at 500°C, and cooled in the dessicator, before the ultimate weight was determined. A sensitive scale was required. A scale on which weights could be determined to five decimal places was used; Gram - Alic Balance, Model No. B6.

4) Samples of algal growths on aquaria walls were taken to the University of Manitoba Botany Lab for identification.

5) Statistical analysis, computations and evaluations using data generated over the course of the study.

7. PRESENTATION OF EXPERIMENTAL RESULTS

The experimental results are presented in order in the following categories:

1. Exploratory-study data.
2. Effluent-analysis data.
3. Bioassay effluent-monitoring data.
4. Presentations generated from bioassay data.
5. Log-probability and statistical assessment data.
6. Appendix I.

7.1 Exploratory Study Data

Data derived from the 96-hour exploratory bioassay study is presented in table 11 and 12. This data assesses the physical-chemical quality of the test effluents and the cumulative mortality counts at the selected intervals. Figure 21 is a log-probability graphical expression of the survival percentage at hour 96.

7.2 Effluent-Analysis Data

Tables 13 through 16 present the physical-chemical quality of the batch effluents and reconstituted water and the actual test concentrations used. Table 13 gives an in-depth initial analysis of the secondary sewage, lime and alum tertiary effluents and the reconstituted water.

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numbering

	Effluent Concentration	Dissolved Oxygen mg/l Initial	Dissolved Oxygen mg/l Final	pH Initial	pH Final	Conductivity millimho Initial	Conductivity millimho Final	Alkalinity as mg/l $CaCO_3$ Initial	Alkalinity as mg/l $CaCO_3$ Final	T.Hardness as mg/l $CaCO_3$ Initial	T.Hardness as mg/l $CaCO_3$ Final	Ca^{++} Hardness as mg/l $CaCO_3$ Initial	Ca^{++} Hardness as mg/l $CaCO_3$ Final	Free CO_2* mg/l Initial	Free CO_2* mg/l Final	Total NH_3-N* mg/l Initial	Total NH_3-N* mg/l Final	Total* Dissolved Solids mg/l
Control	0%	9.2	9.6	6.5	6.6	.30	.32	6	6	94	94	74	74	3.8	3.3	1.0	6.0	192
		9.2	9.4	6.6	6.4	.30	.34							2.8	3.3	1.0	6.0	204
Lime Tertiary Effluent	0.1%	9.0	9.4	6.5	6.5	.32	.33	6	6	86	86	68	68	3.6	3.6	1.0	8.0	198
		9.2	9.6	6.5	6.5	.32	.32							3.7	3.6	1.0	8.0	192
	1.0%	9.1	9.5	6.5	6.4	.30	.32	4	4	88	88	70	70	2.4	2.3	1.0	5.3	192
		9.1	9.6	6.6	6.4	.30	.33							2.2	2.3	1.0	5.3	198
	10%	8.9	9.5	7.5	7.2	.34	.37	32	32	96	96	70	70	2.0	2.0	3.0	3.0	222
		9.1	9.0	7.5	7.4	.35	.37							2.0	2.0	3.0	3.0	222
	100%	8.8	9.5	8.0	8.2	.80	.75	285	285	162	162	106	106	5.5	5.5	20.5	3.3	450
		8.8	9.4	8.0	8.1	.81	.72							5.5	5.5	20.5	3.3	432
Alum Tertiary Effluent	0.1%	9.2	9.8	6.5	6.4	.30	.32	2	2	96	96	72	72	1.0	1.3	1.0	7.1	192
		9.2	9.8	6.5	6.5	.30	.34							1.1	1.3	1.0	7.1	204
	1.0%	9.2	9.6	6.6	6.5	.30	.32	3	3	96	96	74	74	1.4	1.4	1.0	2.0	192
		9.2	9.8	6.6	6.6	.30	.36							1.5	1.4	1.0	2.0	216
	10%	9.0	9.8	7.2	7.2	.35	.37	11	11	108	108	90	90	1.9	1.9	4.3	3.0	222
		9.0	9.8	7.2	7.2	.35	.37							1.1	1.9	4.3	3.0	222
	100%	8.9	9.5	7.7	7.8	.85	.86	68	68	148	148	234	234	2.5	2.5	34.0	3.0	516
		9.0	9.6	7.7	7.9	.85	.86							2.5	2.5	34.0	3.0	516

TABLE 11. Environmental monitoring of effluent during 96-hr bioassay exploratory study. Ten (10) daphnids were used per 600 ml aquarium in replicate series of concentration. Temperature was constant at 20°C.

* Values found by estimation using nomograph, weighted mean and empirical techniques respectively.

	Effluent Concentration	No of Tests Organisms	Mortality at Times Shown						
			1.5 hr	3.0 hr.	6 hr	12 hr	24 hr	48 hr	96 hr
Control	0%	10	0	1	2	4	5	8	9
		10	0	0	0	0	2	5	9
Lime Tertiary Effluent	0.1%	10	0	0	1	1	1	5	8
		10	0	1	1	2	2	8	9
	1.0%	10	0	0	0	0	0	7	7
		10	0	0	0	0	1	7	7
	10%	10	0	0	0	0	0	0	3
		10	0	0	0	0	0	0	2
	100%	10	0	0	0	0	0	0	2
		10	0	0	0	0	0	1	3
Alum Tertiary Effluent	0.1%	10	0	0	1	1	1	6	9
		10	0	0	0	1	4	6	7
	1.0%	10	0	1	1	1	3	6	9
		10	0	0	0	0	1	2	4
	10%	10	0	0	0	0	0	0	0
		10	0	0	0	0	0	1	2
	100%	10	0	0	0	1	1	1	2
		10	0	0	0	1	1	1	3

TABLE 12 . Exploratory bioassay study; summary of mortality as afunction of time. Ten (10) Daphnia used for each replicate concentration shown. Daphnia not fed during study.

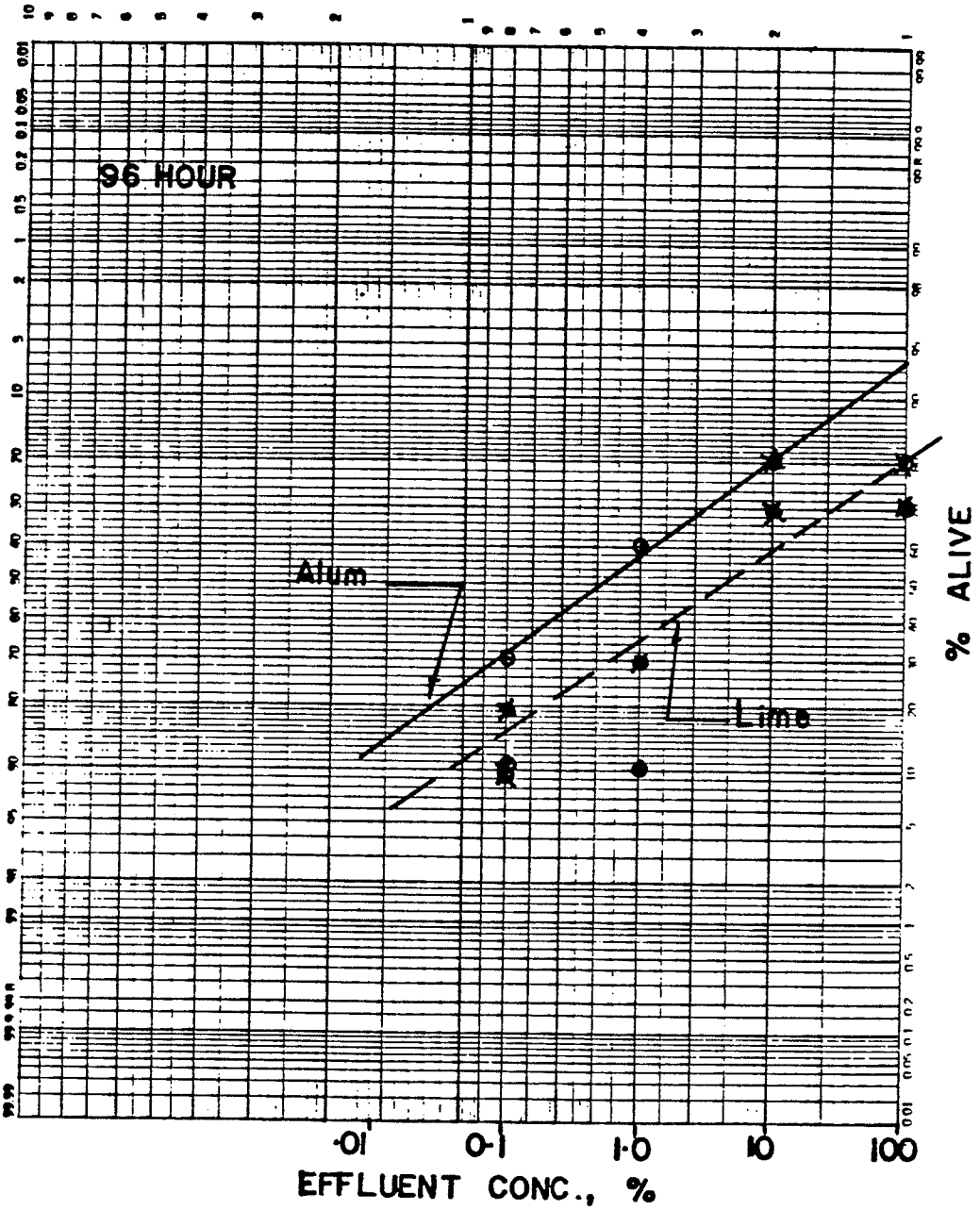

FIGURE 21. Log-probability curves for 96-hour exploratory bioassay study.

Quality Analysed	Unit	Secondary Sewage	Tertiary Alum	Tertiary Lime	Reconstituted Water
Ammonia Nitrogen NH_3-N	mg/l	32	30.6	17.5	.07
Nitrite Nitrogen NO_2-N	mg/l	2	3.0	6.0	2.0
Nitrate Nitrogen NO_3-N	mg/l	.01	.01	.02	8.2
Total Dissolved Nitrogen TDN	mg/l	39	35.8	22.3	9.8
Suspended Nitrogen	mg/l	1.1	0.1	0.2	0.02
Soluble Reactive Phosphate	mg/l	5.8	.01	.014	2.4
Total Dissolved Phosphates	mg/l	6.1	.03	.05	2.5
Suspended Phosphates	mg/l	0.4	.05	.02	.01
Dissolved Inorganic Carbon	mg/l	68.8	25.9	88.9	0.8
Dissolved Organic Carbon	mg/l	16.6	10.3	11.6	3.4
Suspended Carbon	mg/l	6.8	0.5	1.4	0.2
Chloride Cl	mg/l	71.5	70.0	72.5	61.5
Sulphates SO_4	mg/l	64.0	258	58.0	17.8
Soluble Reactive Si	mg/l	4.86	4.49	3.50	2.63
Sodium Na	mg/l	62.2	60.2	61.7	17.5
Potassium K	mg/l	13.4	13.0	13.4	4.8
Magnesium Mg	mg/l	15.2	15.0	0.8	4.2
Calcium Ca	mg/l	38.3	37.2	90.9	28.4
Manganese Mn	mg/l	0.06	2.06	0.07	0.01
Iron Fe	mg/l	0.09	0.05	0.02	0.02
pH		6.98	6.30	6.56	6.40
Total Dissolved Solids (estimated)	mg/l	498	546	510	192
Conductivity at 25°C	millimho	.83	.91	.85	.32
Total Dissolved Solids (analytical)	mg/l	410	510	460	200
Chlorophyl-a	µg/l	1.2	-	<0.1	0.1
Total Suspended Solids	mg/l	15	1	3	<1
Total Hardness as $CaCO_3$	mg/l	160	162	112	92
Calcium Hardness as $CaCO_3$	mg/l	91	105	234	74
Magnesium Hardness as $CaCO_3$	mg/l	69	56	122	18
Alkalinity as $CaCO_3$	mg/l	262	72	286	6
Bicarbonate Alkalinity as $CaCO_3$	mg/l	250	68	280	58
$CO_3^=$ Alaklinity as $CaCO_3$	mg/l	12	4	6	0.2
Free CO_2 as $CaCO_3$	mg/l	50	70	140	5
Lead Pb	µg/l	4	<1	<1	-
Copper Cu	µg/l	161	34	48	-
Nickel Ni	µg/l	103	86	22	-
Arsenic As	µg/l	1.0	<0.5	<0.5	-
Cadmium Cd	µg/l	0.2	0.4	0.2	-
Selenium Se	µg/l	<0.5	<0.5	<0.5	-
Aluminum Al	µg/l	157	236	56	-
Total CO_2 as $CaCO_3$ (estimated)	mg/l	275	132	389	10
Free CO_2 as $CaCO_3$ (estimated)	mg/l	50	70	140	5

TABLE 13. Initial Sewage Analysis, Day 1, April 13, 1978.

	Effluent Concentration	Parent Aquaria Day 1		Parent Aquaria Day 41		Progeny Aquaria Day 41		Progeny Aquaria Day 60	
		Free CO_2 as mg/l $CaCO_3$	Total CO_2 as mg/l $CaCO_3$	Free CO_2 as mg/l $CaCO_3$	Total CO_2 as mg/l $CaCO_3$	Free CO_2 as mg/l $CaCO_3$	Total CO_2 as mg/l $CaCO_3$	Free CO_2 as mg/l $CaCO_3$	Total CO_2 as mg/l $CaCO_3$
Control	0%	3.2	13.8	0.3	18.6	0	31.2	0.6	42.8
		2.8	11.6	0.0	16.3	0	24.6	5.5	56.5
Lime Tertiary Effluent	12.5%	9.0	49.5	0.0	29.0	0	37.4	0.2	45.1
		7.0	47.5	0.0	33.4	0	48.8	0.7	52.8
	25%	13.0	81.6	0.2	63.8	0.2	74.6	0.2	67.5
		12.0	82.4	0.2	60.5	0.3	66.3	0.6	80.0
	50%	36.0	169.8	0.4	110.4	0.4	89.3	3.0	118.6
		38.0	171.8	0.7	65.8	0.2	42.4	0.8	107.9
	100%	65.0	314.9	0.0	44.0	0	34.3	0.8	68.6
		65.0	314.9	0.5	81.9	0.5	66.5	0.2	48.2
Alum Tertiary Effluent	12.5%	3.5	17.6	0.0	11.4	0	8.8	0.2	12.5
		3.2	20.8	0.0	5.3	0	7.0	0.2	14.3
	25%	7.0	31.6	0.0	13.2	0	5.3	1.0	6.3
		7.0	31.6	0.2	16.9	0	8.8	1.2	10.0
	50%	9.0	46.0	0.0	7.0	0	7.0	0.5	11.1
		7.0	44.0	0.4	19.8	0	5.3	1.2	11.8
	100%	15.0	78.4	0.2	17.8	0	5.3	3.4	8.7
		15.0	78.4	20.0	21.8	0	0.0	7.0	71.8

TABLE 14. Environmental monitoring for free and total Carbon Dioxide, as mg/l $CaCO_3$, in parent and progeny aquaria, reflecting quality for dates as shown.

Quality Analysed	Units	Secondary Sewage	Tertiary Alum	Tertiary Lime	Reconstituted Water
Total Hardness as $CaCO_3$	mg/l	170	162	110	84
Calcium Hardness as $CaCO_3$	mg/l	90	104	230	52
Magnesium Hardness as $CaCO_3$	mg/l	60	58	-120	32
Total Alkalinity as $CaCO_3$	mg/l	256	74	274	12
Conductivity	millimho	.74	.75	.70	.25
Dissolved Oxygen at $14^{o}C$	mg/l	7.3	11.8	11.8	9.6 at $20^{o}C$
Turbidity	J.T.U.	2.7	1.0	1.5	.42
Total CO_2 as $CaCO_3$	mg/l	270	132	365	20
Free CO_2 as $CaCO_3$	mg/l	47	70	130	10
Total Dissolved Solids (estimated)	mg/l	444	450	420	150
Total Inorganic Carbon (dissolved)	mg/l	70	14	66	2
Total Organic Carbon (dissolved)	mg/l	26	19	23	8
Total Carbon (dissolved)	mg/l	96	33	89	10
pH		6.98	6.36	6.56	6.4

TABLE 15. Post Experimental Sewage Analysis, Day 49, May 31, 1978.

	Effluent Concentration	pH	Conductivity millimho	Total Dissolved Solids mg/l	Total Alkalinity as mg/l $CaCO_3$	HARDNESS			DISSOLVED CARBON		
						Total as mg/l $CaCO_3$	Ca^{++} as mg/l $CaCO_3$	Mg^{++} as mg/l $CaCO_3$	Inorganic Carbon mg/l	Total mg/l	Organic Carbon mg/l
Control	0%	8.2	.46	276	48	132	112	20	7.0	22	15
		7.3	.49	294	58	144	116	28	10.0	32	22
Lime Tertiary Effluent	12.5%	8.6	.52	312	52	144	120	24	7	21	14
		8.2	.52	312	60	152	120	32	9	23	14
	25%	8.9	.56	336	80	164	148	16	10	27	17
		8.4	.54	324	86	168	144	24	13	30	17
	50%	7.9	.71	426	132	216	192	24	21	46	25
		8.5	.63	378	124	200	176	24	20	44	24
	100%	8.3	.64	384	78	132	88	44	12	48	36
		8.7	.57	342	56	92	88	4	7	32	25
Alum Tertiary Effluent	12.5%	8.2	.53	318	14	152	76	76	1.0	14	13
		8.2	.48	288	16	156	104	52	2	14	12
	25%	7.3	.60	360	6	176	124	52	1	11	10
		7.2	.57	342	10	160	116	44	1	14	13
	50%	7.7	.73	438	12	192	140	52	1	14	13
		7.3	.76	456	12	196	136	60	1	14	13
	100%	6.5	.95	570	6	244	168	76	1	23	22
		4.6	.97	582	2	236	160	76	1	30	29

TABLE 16. Post Experimental Sewage Analysis of Progeny Aquaria, Day 60, June 12, 1978.

Table 14 gives the analysis of the full range of test effluents for CO_2 content as mg/l $CaCO_3$. These analyses were done on days 1, 41 and 60 as shown. Table 15 gives the post experimental analysis, for selected parameters for the secondary effluent, batch tertiary effluents and the reconstituted water. Batch effluents were kept in refrigeration throughout the course of the experiment; refrigeration at 1°C. Table 16 gives the post experimental sewage analysis of the effluents remaining in the progeny aquaria.

7.3 Bioassay Effluent - Monitoring Data

Effluent monitoring data is presented in figures 22 through 29. The assessment is for; dissolved oxygen, pH, conductivity, hardness, alkalinity, total dissolved organic and inorganic carbon, total ammonia nitrogen, and total un-ionized ammonia respectively.

7.4 Presentations Generated From Bioassay Data

Figures 30 to 32 show the log-probability, mortality-concentration curves on days 12, 32 and 39 respectively. Figures 33 and 34 show ephippial production expressed as a monthly rate for shown intervals and as a cumulative time-dependent function for the test effluents respectively. Figure 35 compares the mortality in the control group with the mortality found for the tertiary effluent concentrations. Figure 36 gives the empirical relationship, for this study, between ephippial production and effluent concentrations. Figures 37 and 38 relate mean length of organisms to effluent concentration. Figures 39 and 40 show progeny production on an interval basis, characteristic of the individual

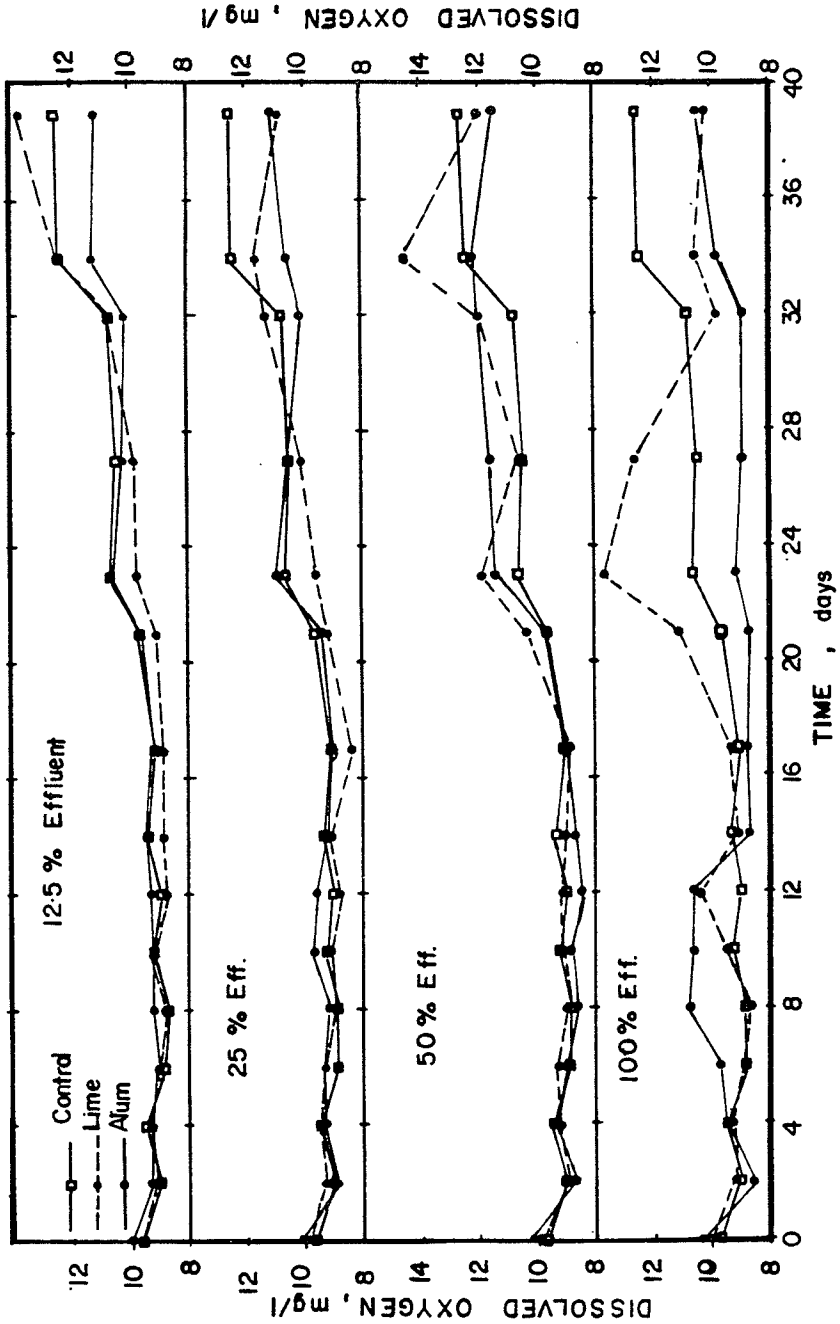

FIGURE 22. Effluent monitoring for dissolved oxygen content. Temp. = 20°C

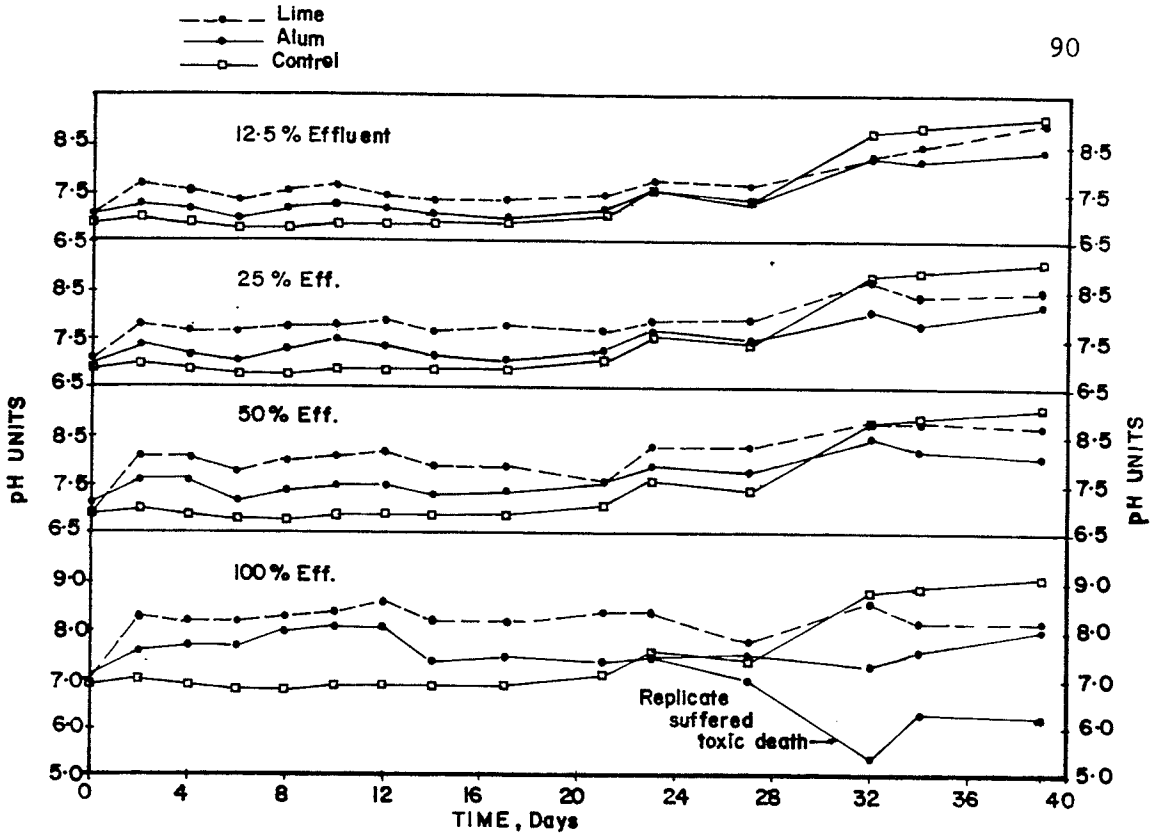

FIGURE 23. Effluent monitoring for pH.

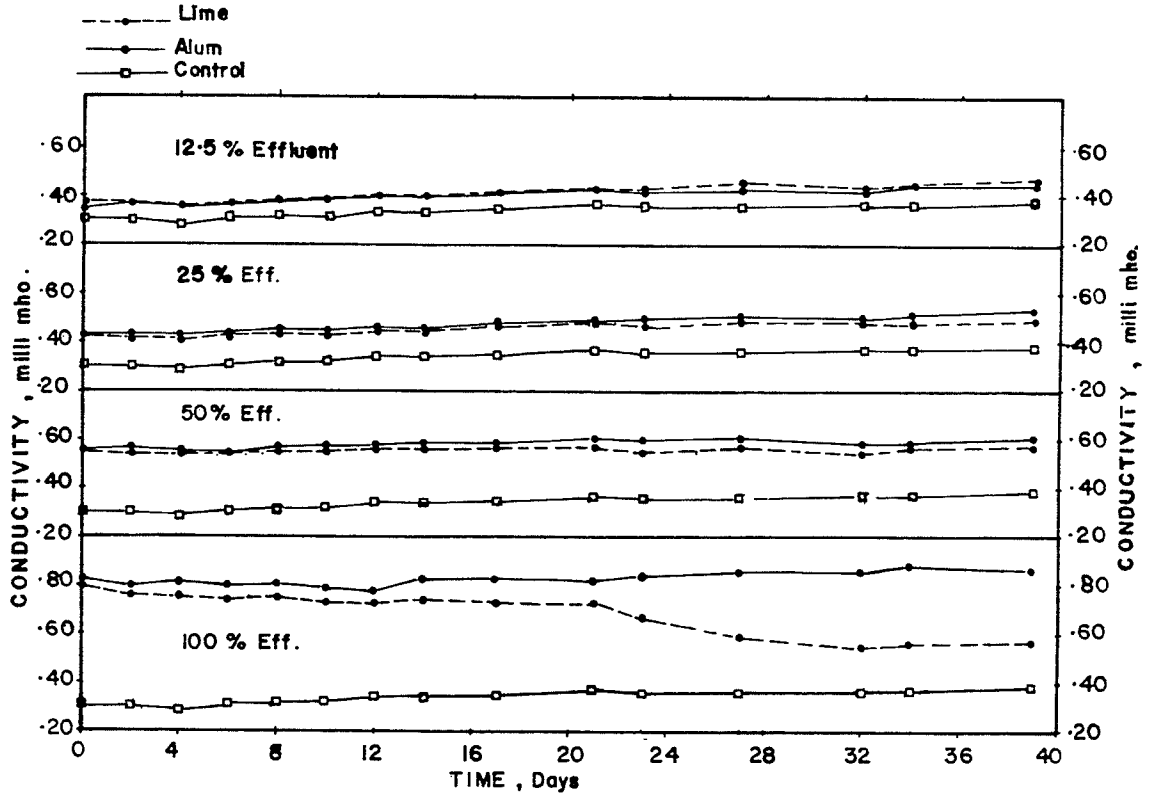

FIGURE 24. Effluent monitoring for Conductivity.

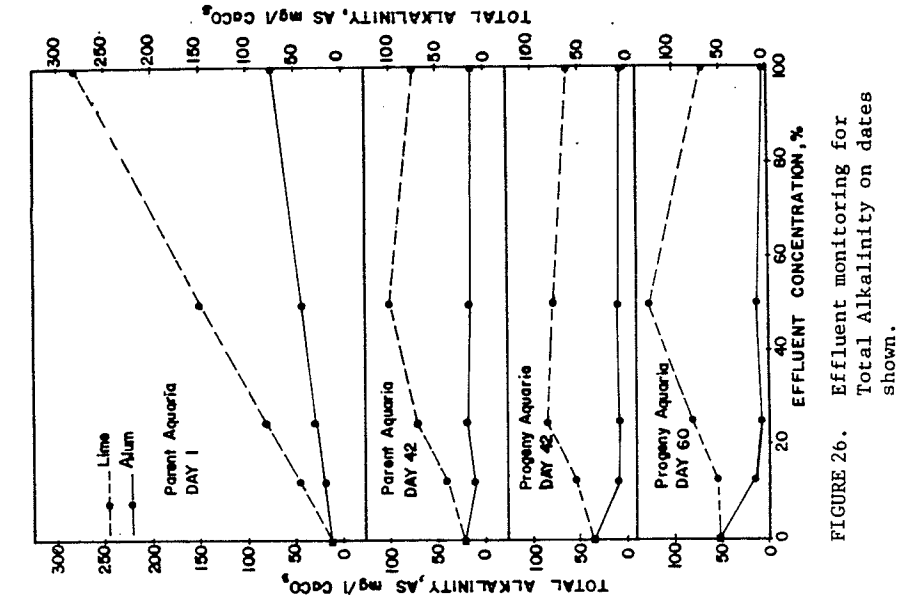

FIGURE 26. Effluent monitoring for Total Alkalinity on dates shown.

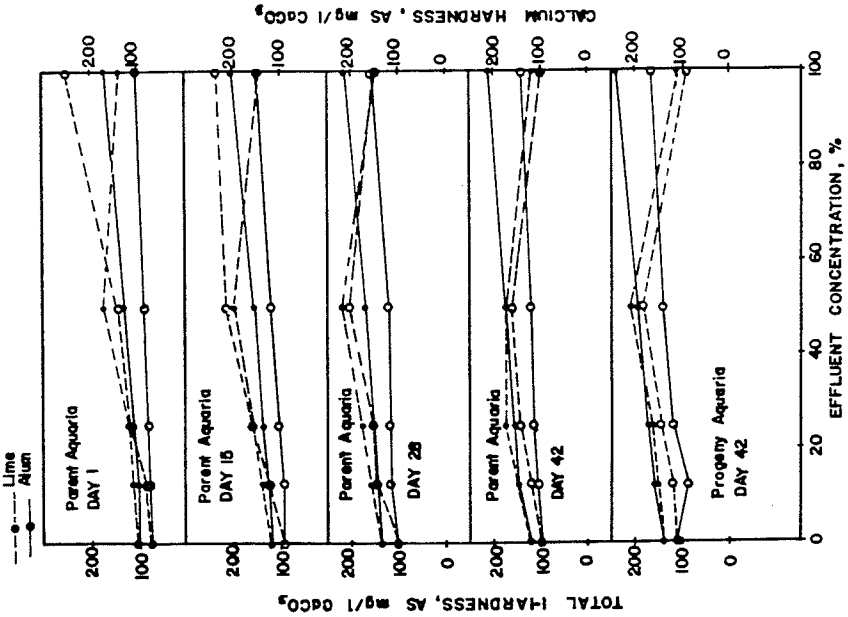

FIGURE 25. Effluent monitoring for Total Hardness and Calcium Hardness on dates shown. Open holes signify Ca^{++} Hardness curves.

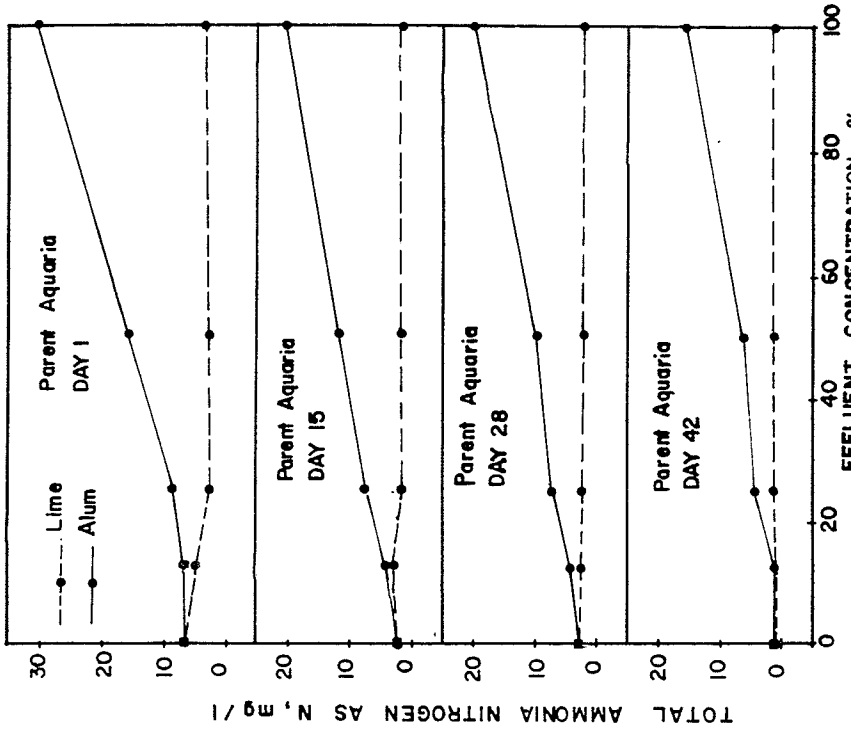

FIGURE 28. Effluent monitoring for Total Ammonia Nitrogen as N.

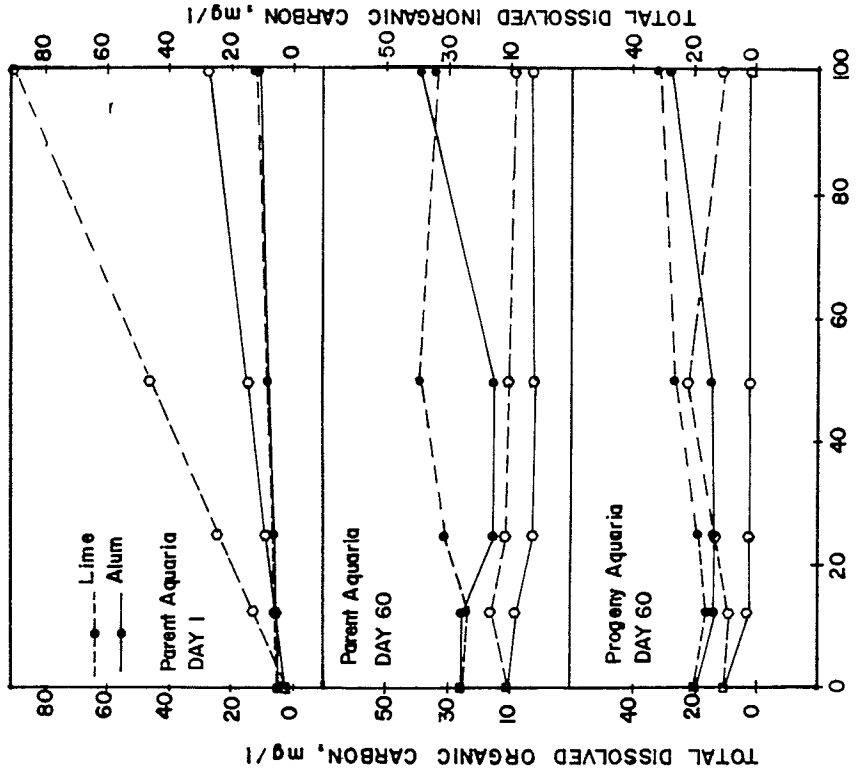

FIGURE 27. Effluent monitoring for Total Dissolved Organic Carbon and Total Dissolved Inorganic Carbon. Open holes signify Inorganic Carbon curves.

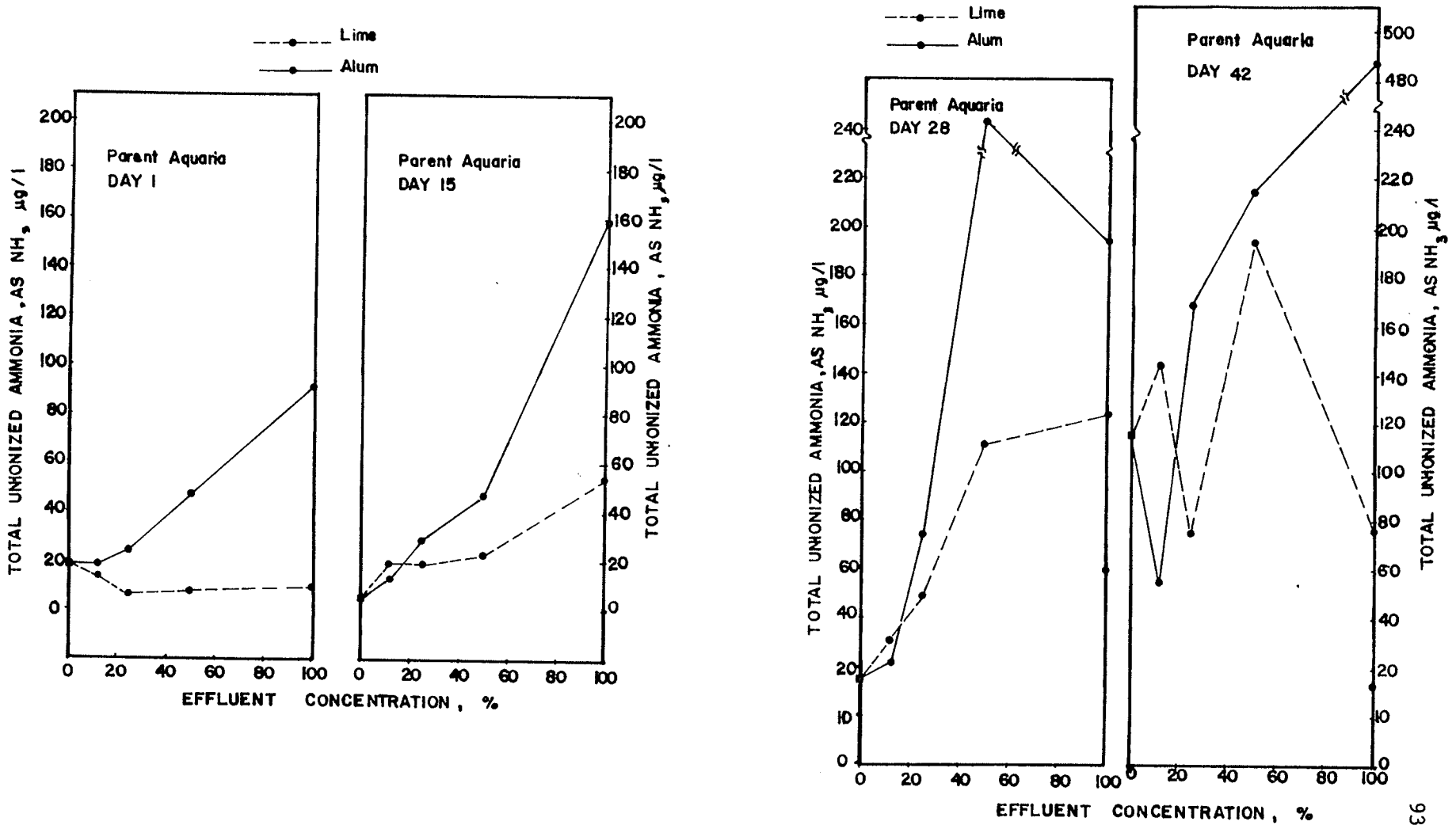

FIGURE 29. Effluent monitoring for Total Unionized Ammonia as NH_3 on dates shown. Unionized Ammonia values were derived from: Total Ammonia values shown in Figure 28, pH data given in Figure 23 and ideal values for distilled water given in Table 5.

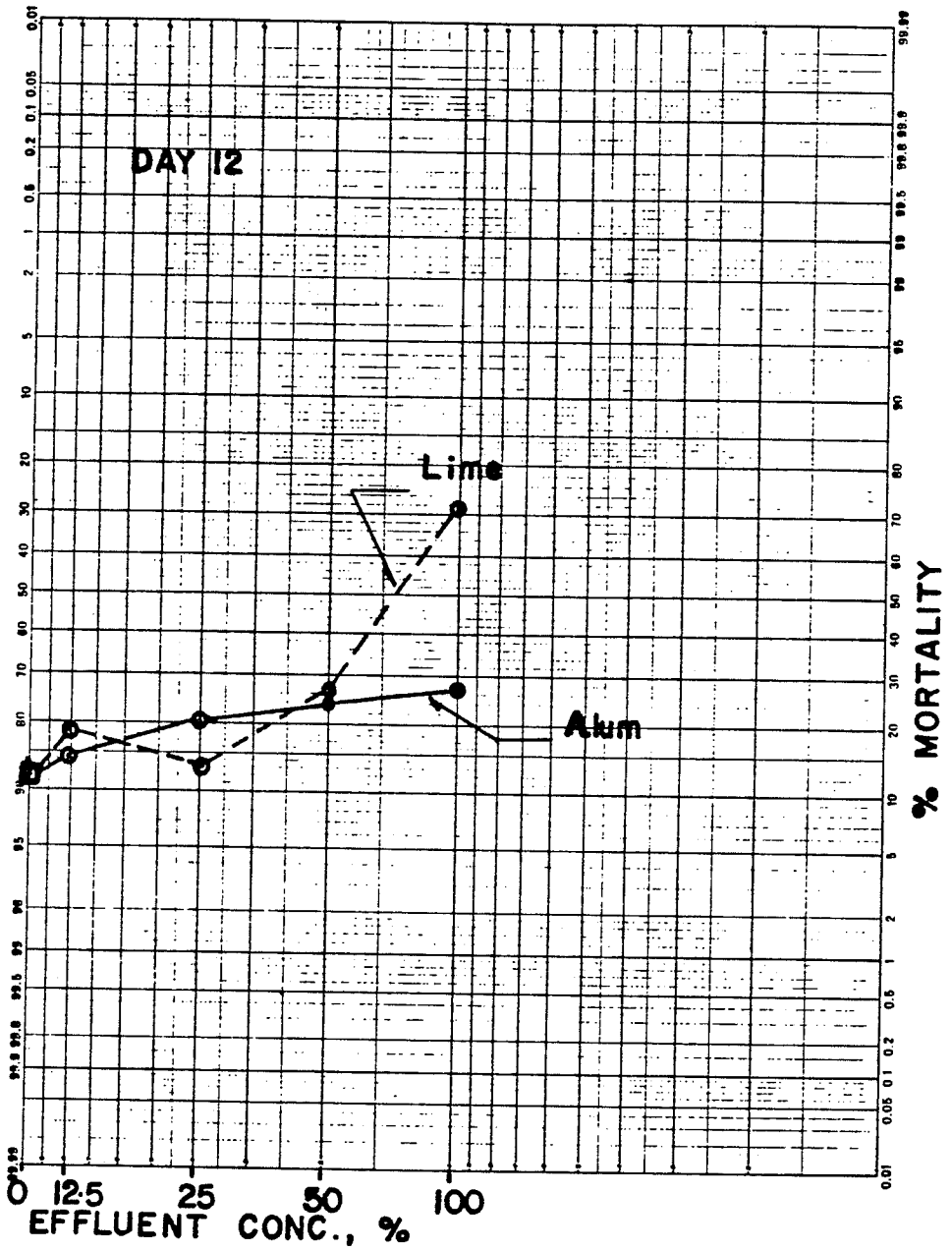

FIGURE 30. Log-probability curves for effluents on day 12. Mortality percent versus effluent concentration percent.

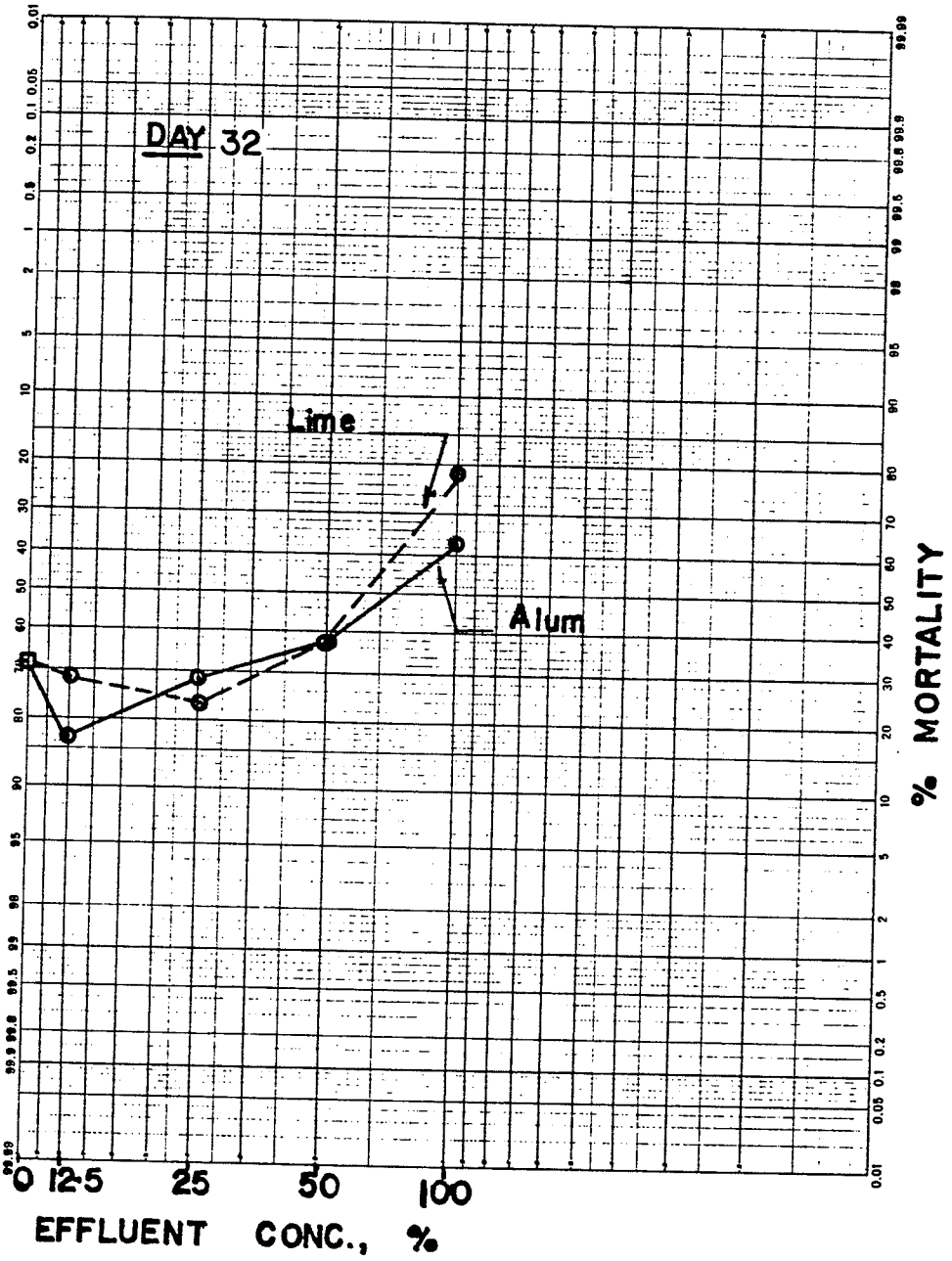

FIGURE 31. Log-probability curves for effluents on day 32. Mortality percent versus effluent concentration percent.

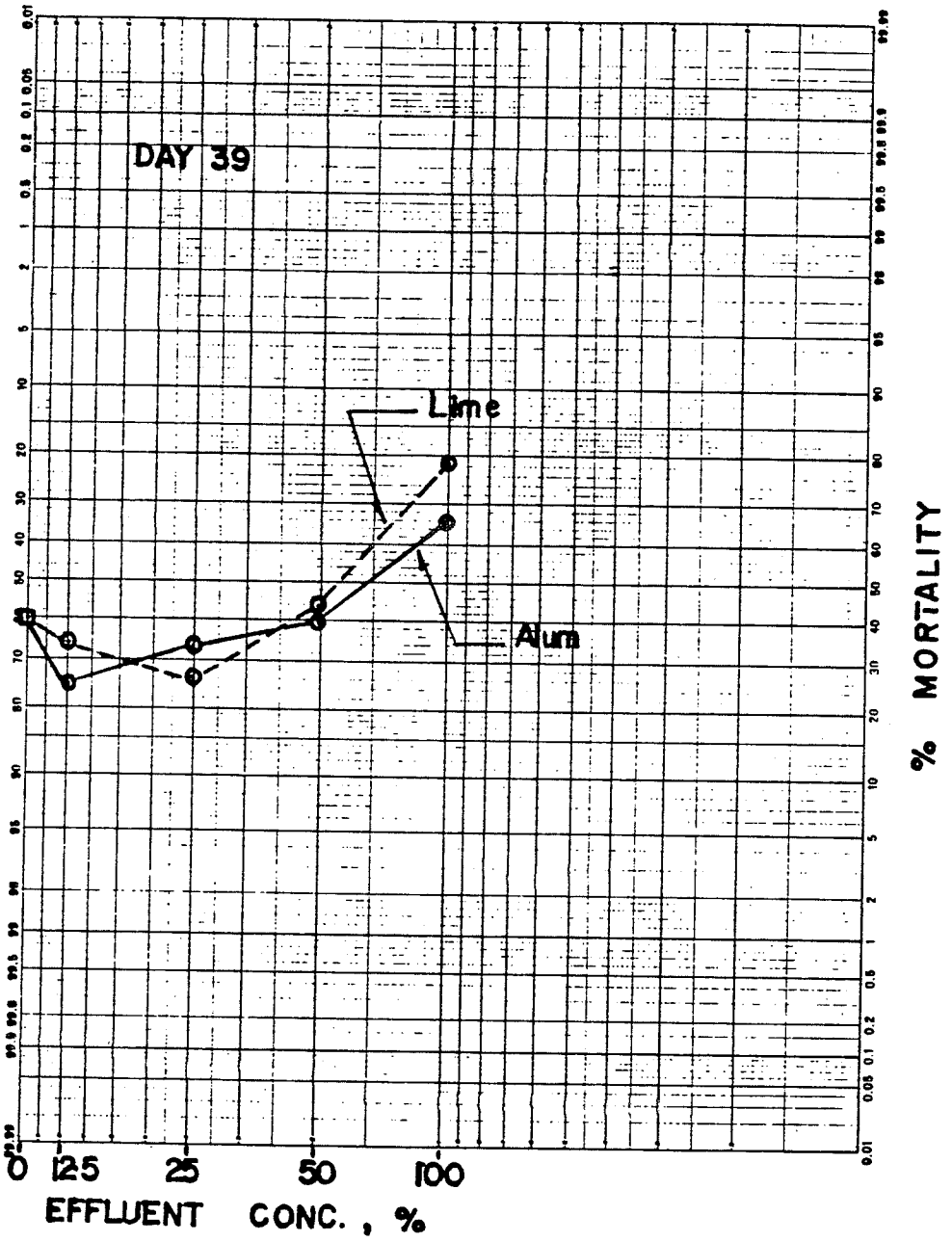

FIGURE 32. Log-probability curves for effluents on day 39. Mortality % versus effluent concentration %.

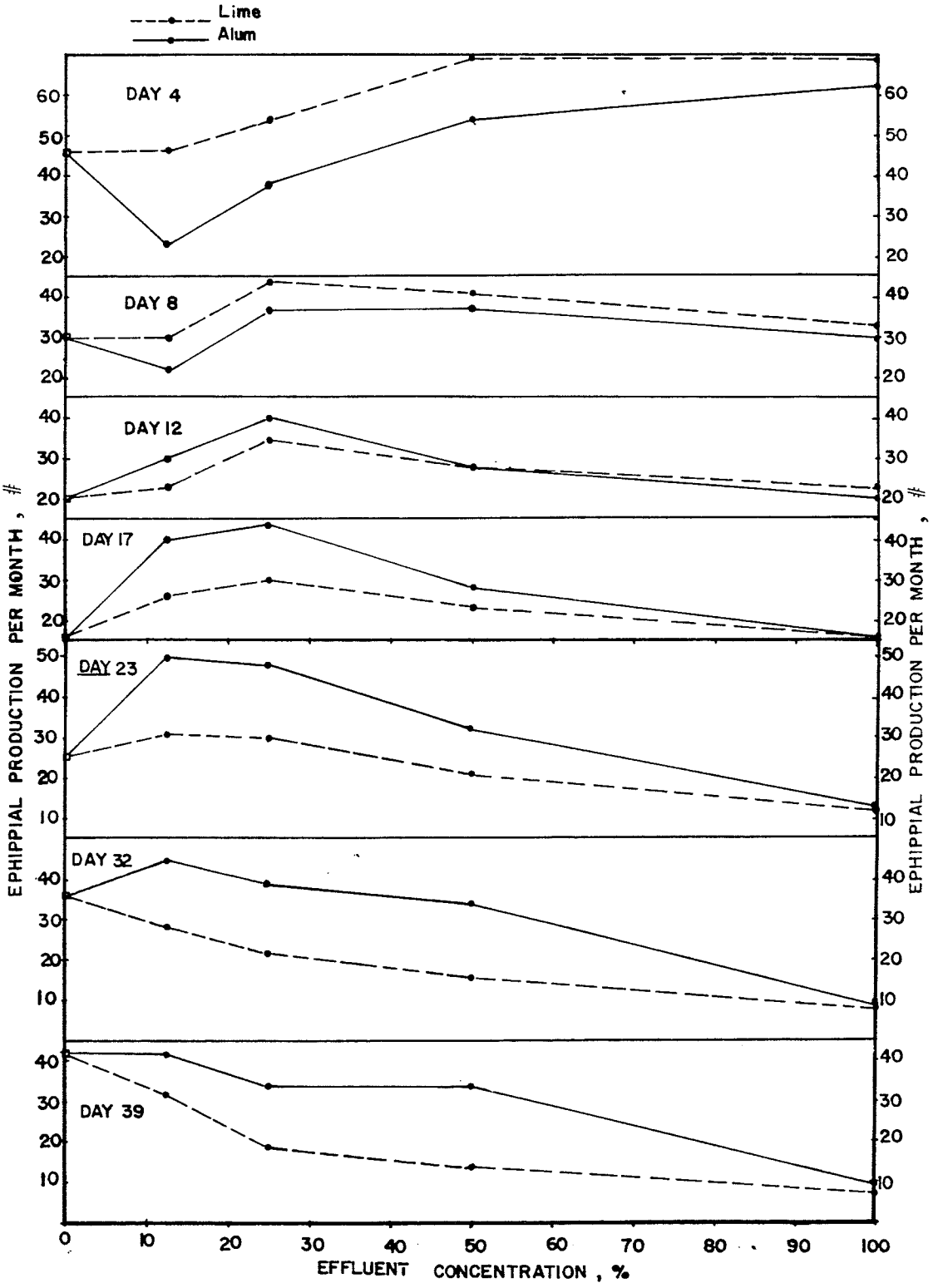

FIGURE 33. Ephippial production, during intervals shown as a monthly average versus effluent concentration, %.

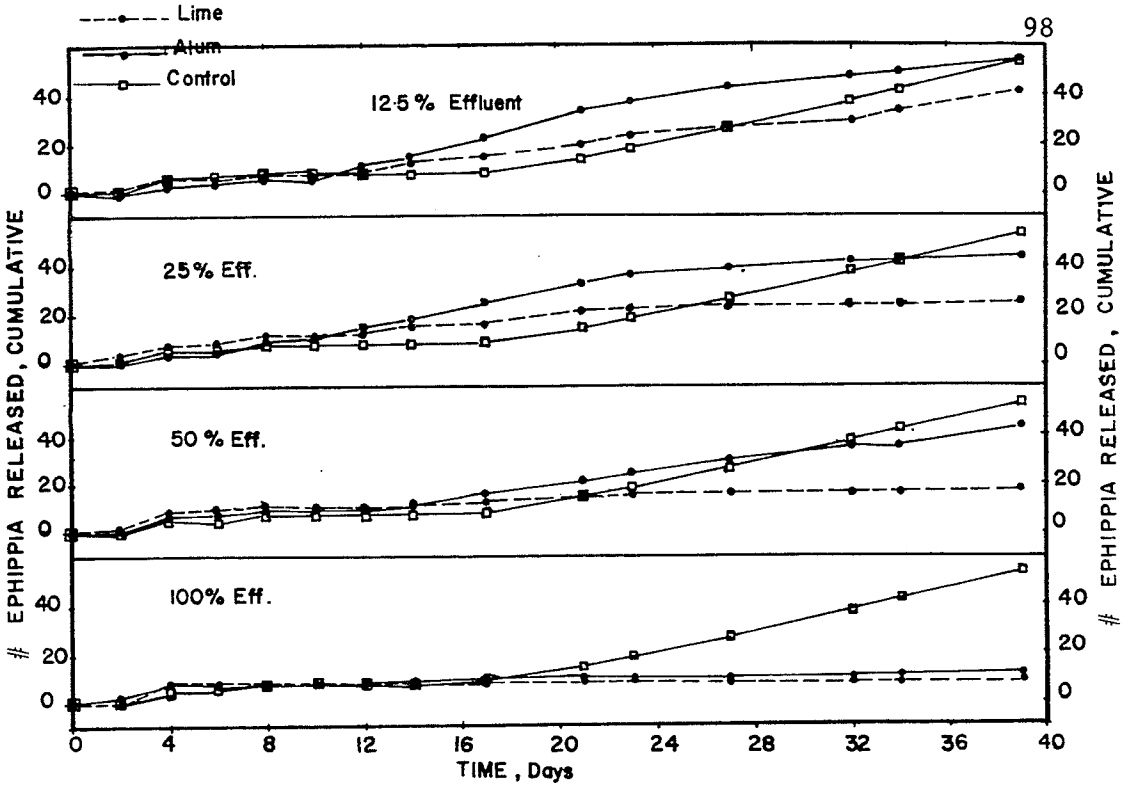

FIGURE 34. Total ephippia released for each effluent concentration, shown as a function of time , for original animals.

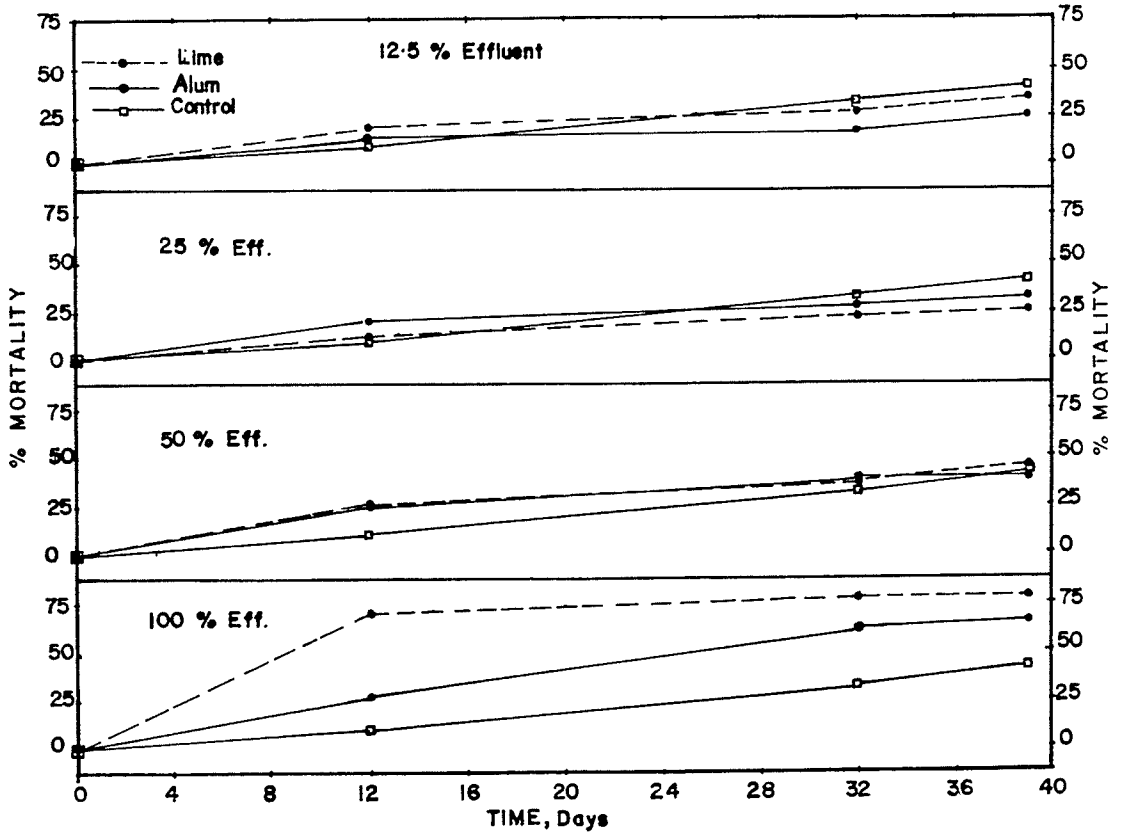

FIGURE 35. Percentage mortality for each effluent concentration shown as a function of time , for original animals.

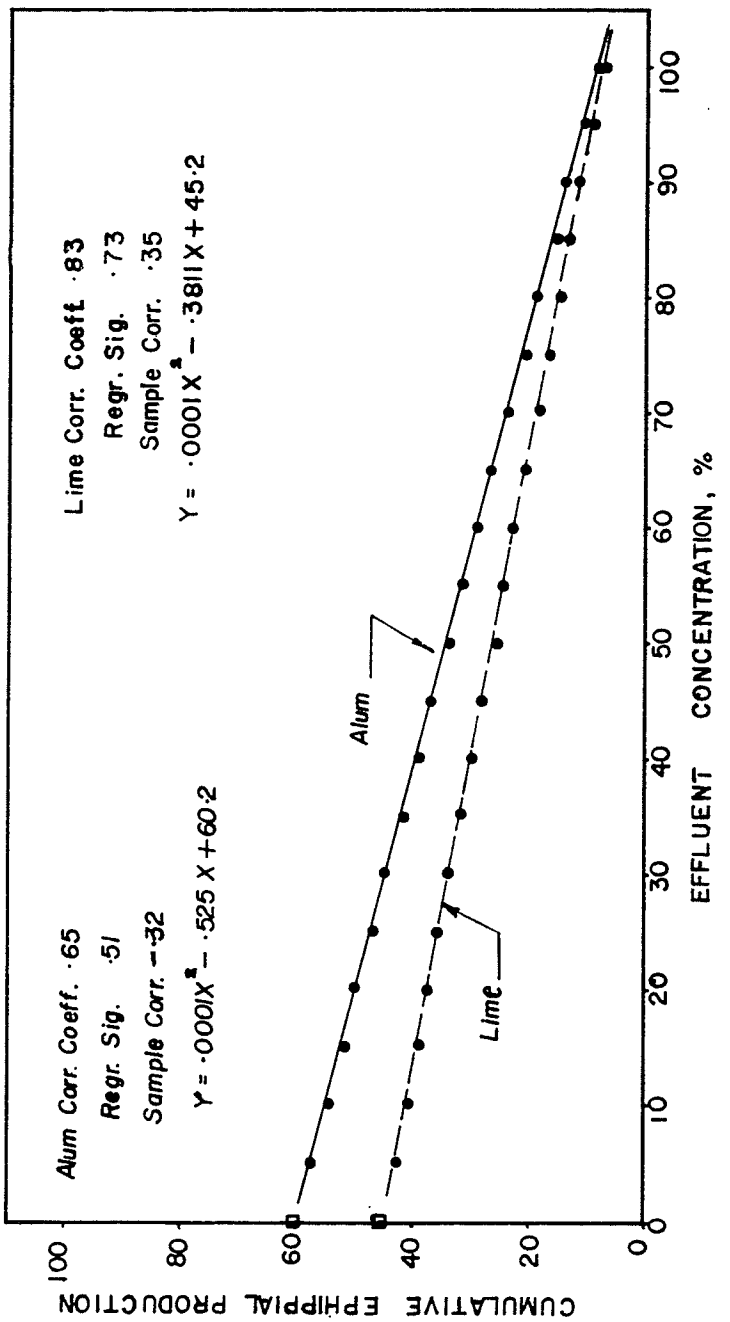

FIGURE 36. Statistical APL Software Package, Univariate Regression Curves generated from experimental data. Cumulative Ephippial production versus effluent concentration, %.

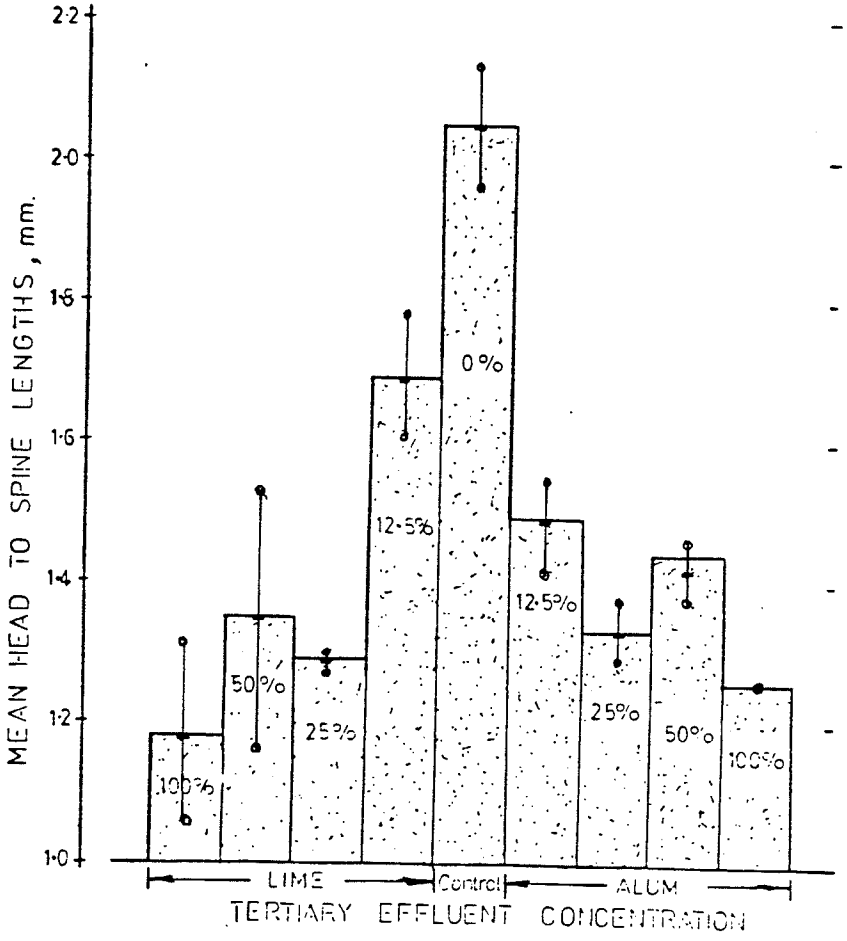

FIGURE 37. Mean head to spine lengths of 25 randomly selected daphnia from progeny aquaria. Daphnids were cultured in effluent concentration series in replicate. The statistic plotted above represents the averages of these replicates.

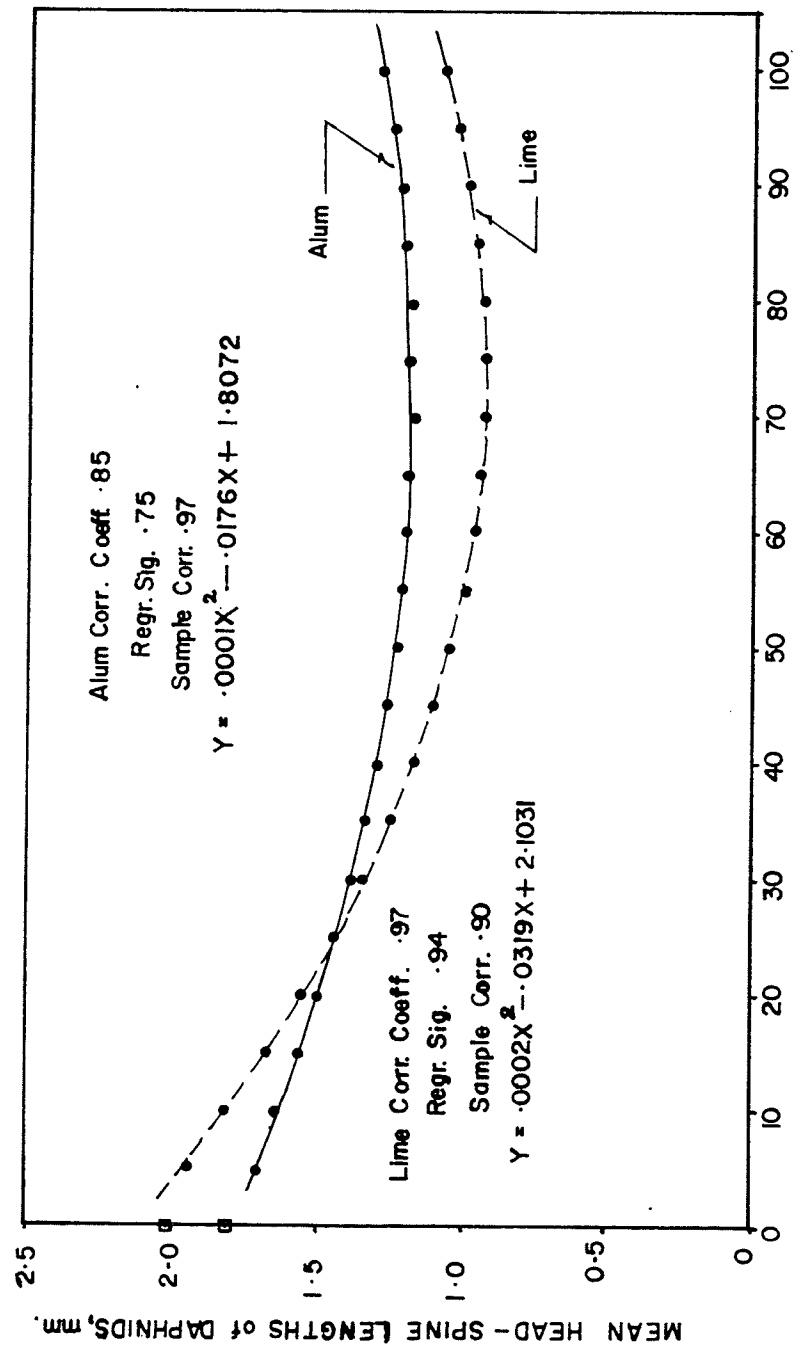

FIGURE 38. Statistical APL Software Package, Univariate Regression Curves, generated from experimental data. Mean Head to Spine lengths in mm. versus effluent concentration, %.

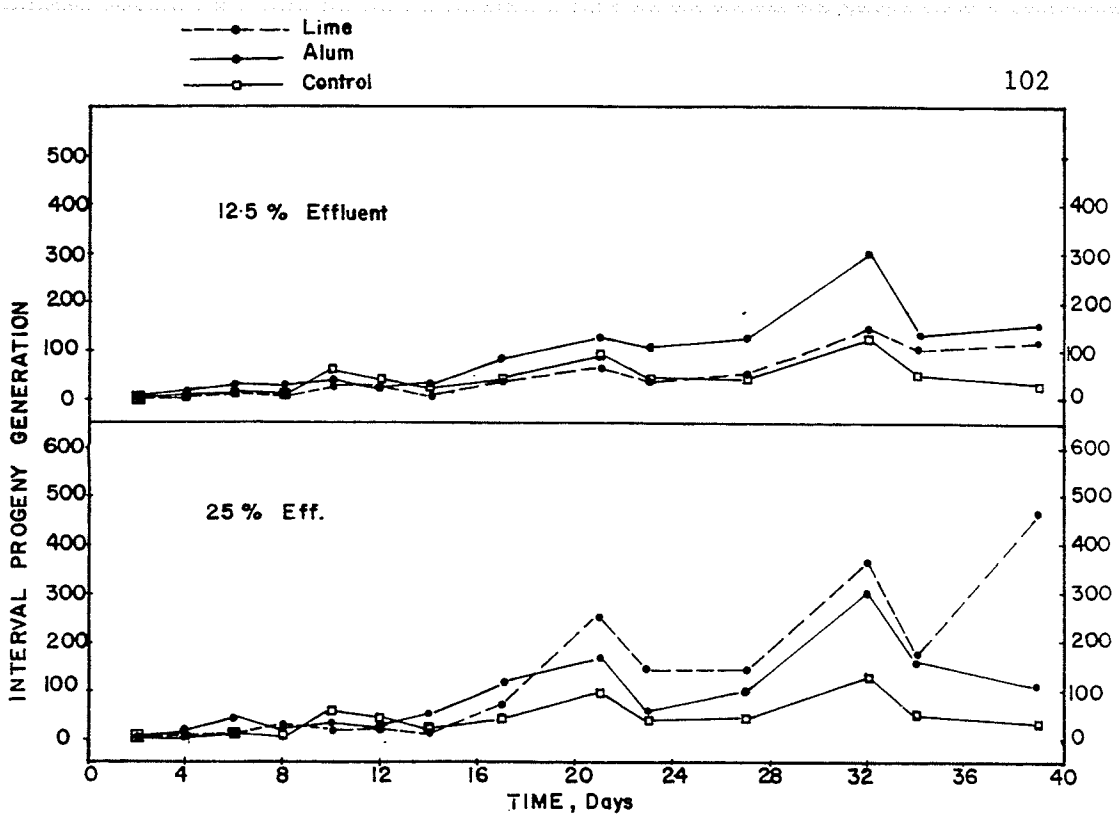

FIGURE 39. Interval progeny production for effluents shown, 12.5% and 25% respectively.

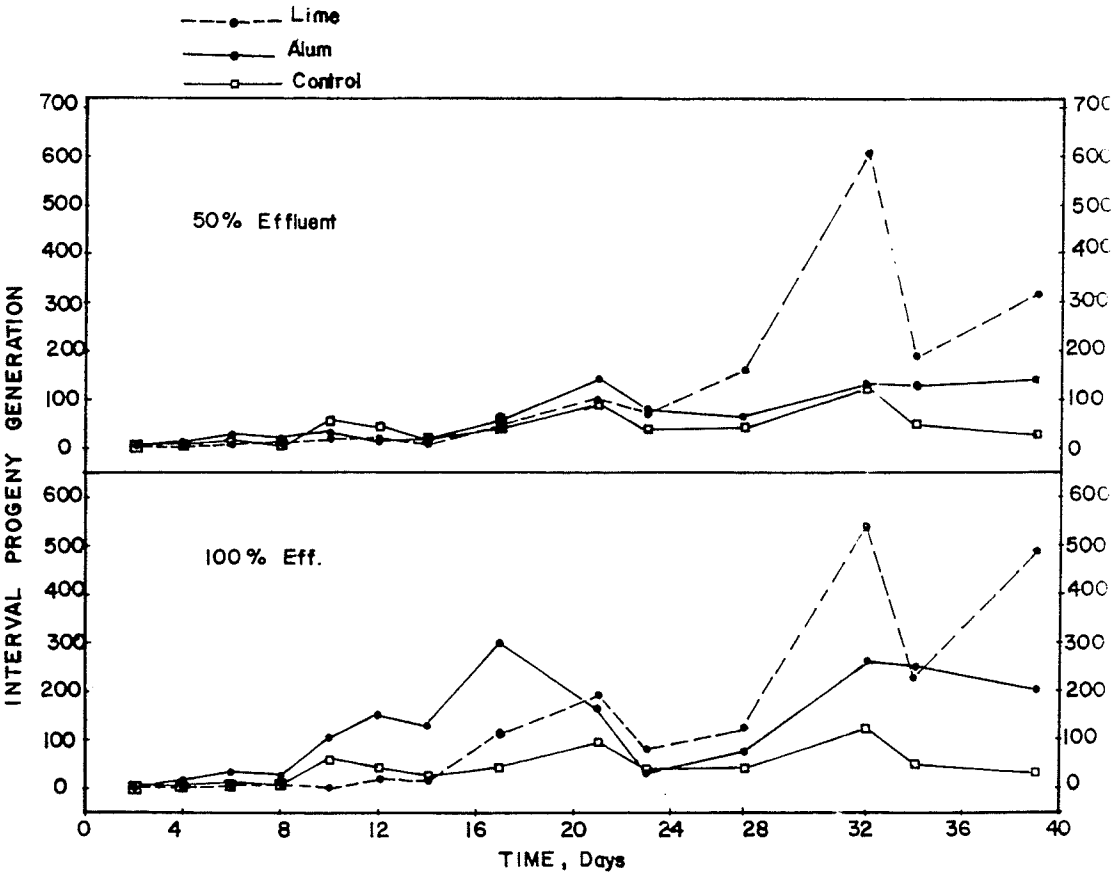

FIGURE 40. Interval progeny production for effluents shown, 50% and 100% respectively.

effluents. Figure 41 considers the cumulative progeny production computed as a daily statistic, as a function of effluent concentration. Figures 42 through 47 present results on progeny generation and final population counts, as a function of effluent concentration. Table 17 is a summary of population data expressed as birth rate, rate of increase for specific intervals, population death rate and mean life expectancy. These rates are shown related to the effluent concentrations. Figures 48 and 49 are graphical expressions of two of the tabulations in Table 17. Figure 50 expresses the computed biomass as a function of effluent concentration.

7.5 Log-Probability and Statistical Assessment Data

Figures 51 and 52 show probit curves for the effluents over the course of the experiment. Figures 53 through 55 are probit curves for Days 12, 32 and 39 respectively. Survivorship is shown as a function of effluent concentration. These probit curves were used in statistical analyses to gauge whether significant differences between the lime and alum effluent were evident. Tables 18 , 19 and 20 summarize the statistical treatments of the experimental data. These treatments are based on; mean length of organisms, progeny production, total population count, and average birth rate. Figure 56 is an illustration of the statistical confidence intervals for all effluents when compared to mean length of the control group.

7.6 Appendix I

Appendix I contains miscellaneous experimental tabulations which can

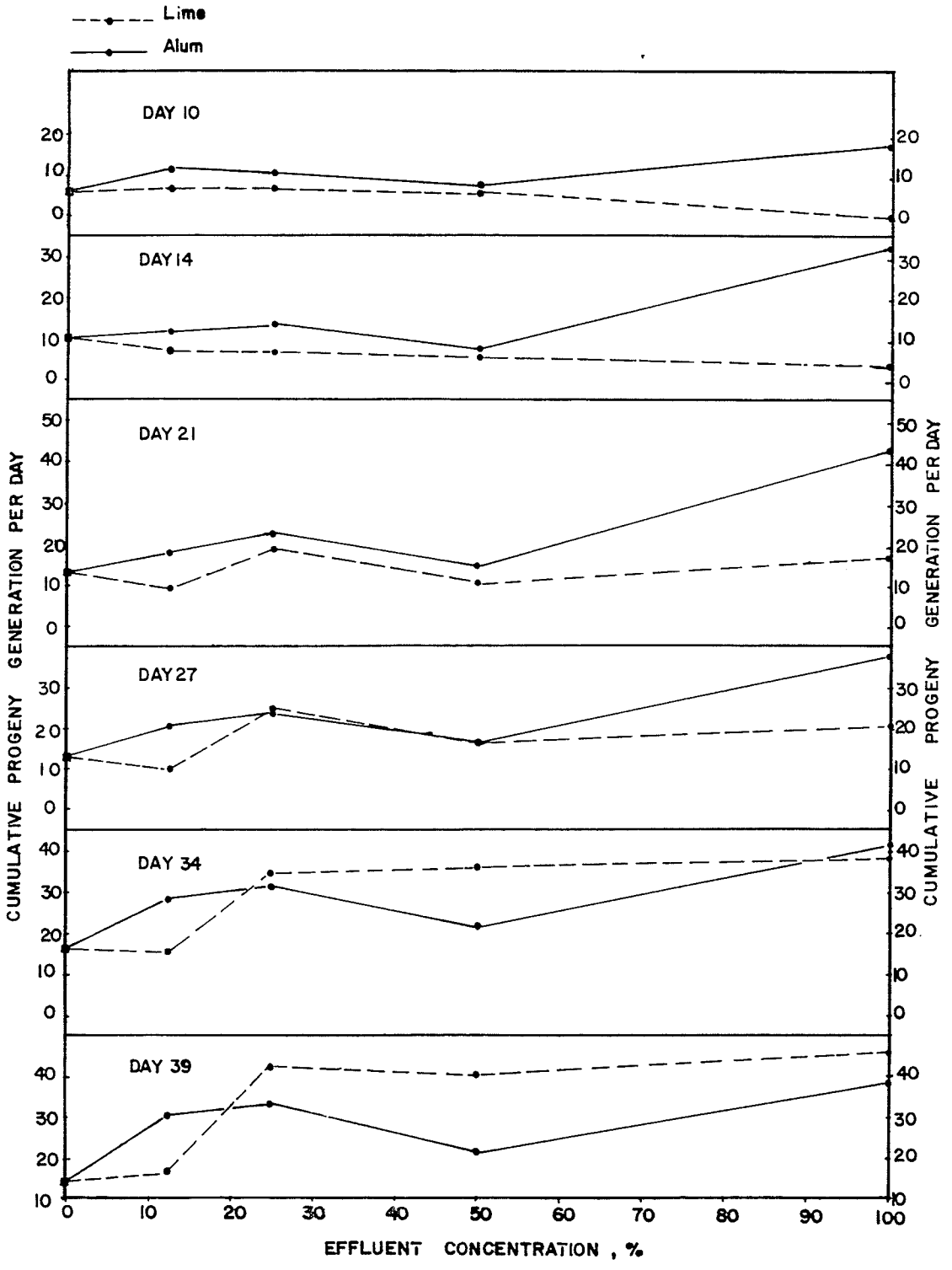

FIGURE 41. Cumulative progeny generated per day, over intervals shown, as a function of effluent concentration, %.

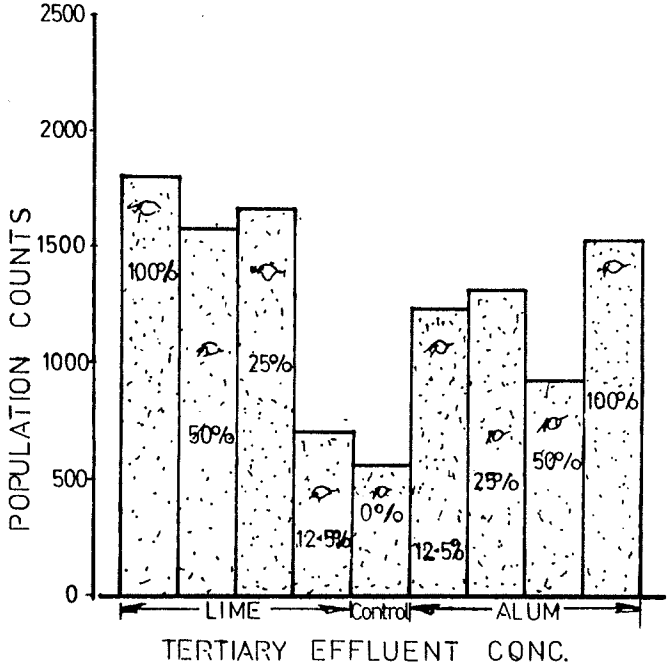

FIGURE 42. Averages of cumulative progeny counts for replicate effluent concentrations in bioassay series.

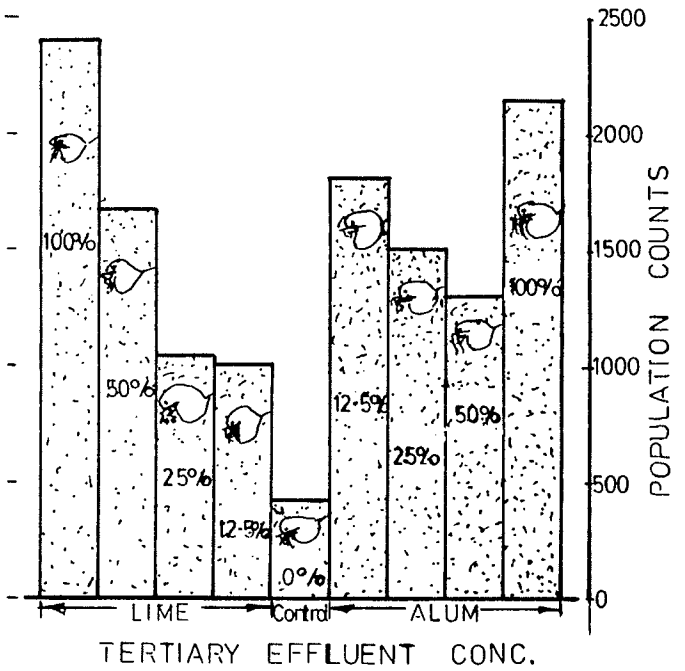

FIGURE 43. Averages of final population counts for replicate effluent concentrations in bioassay series

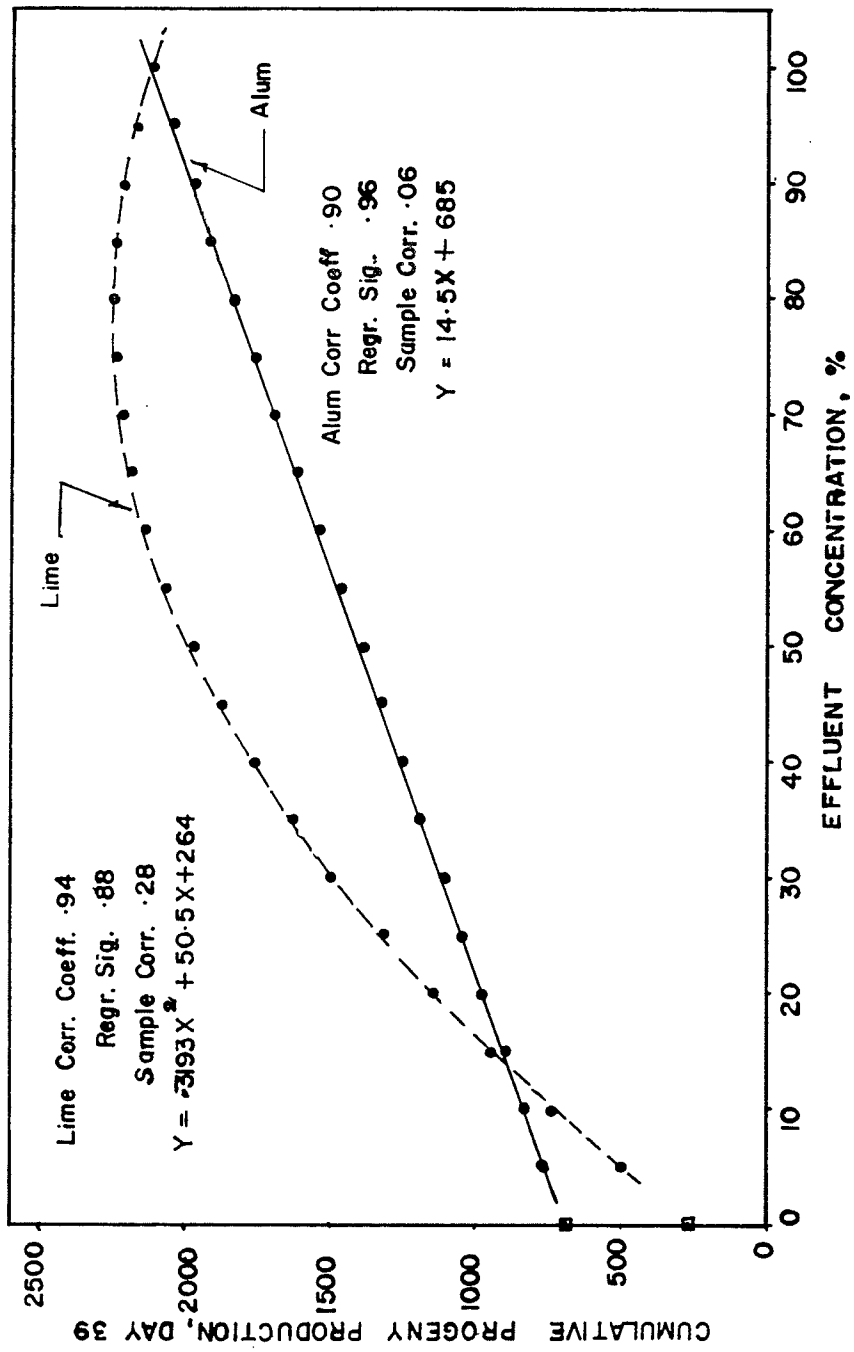

FIGURE 44. Statistical APL Software Package, Univariate Regression Curves, generated from experimental data. Cumulative progeny production by day 39 versus effluent concentration, %.

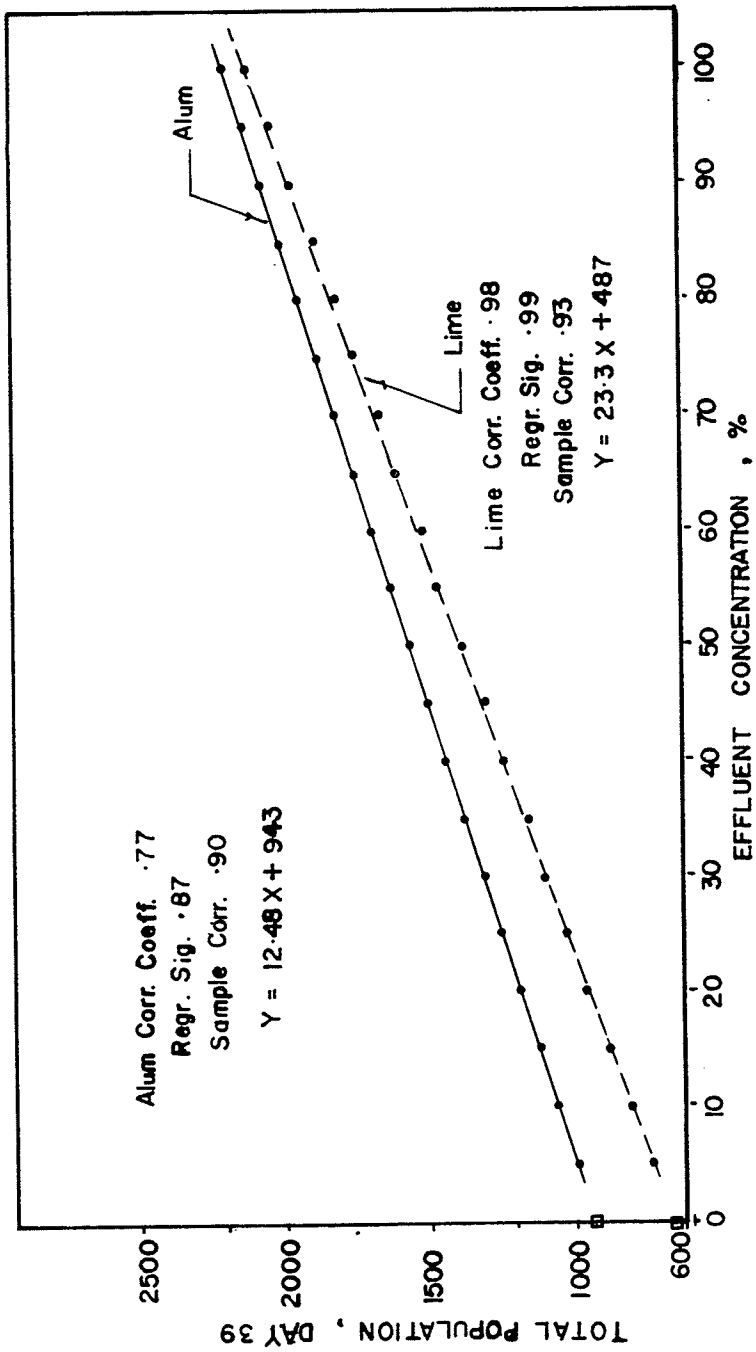

FIGURE 45. Statistical APL Software Package, Univariate Regression Curves, generated from experimental data. Total population on hand, day 39, versus effluent concentration, %.

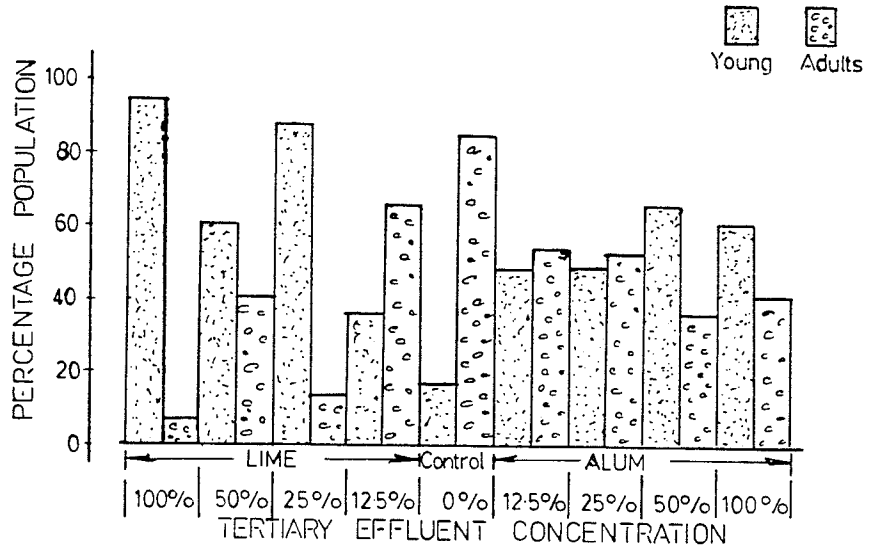

FIGURE 46. Final Population Characteristic; Adults to young statistic at end of experiment.

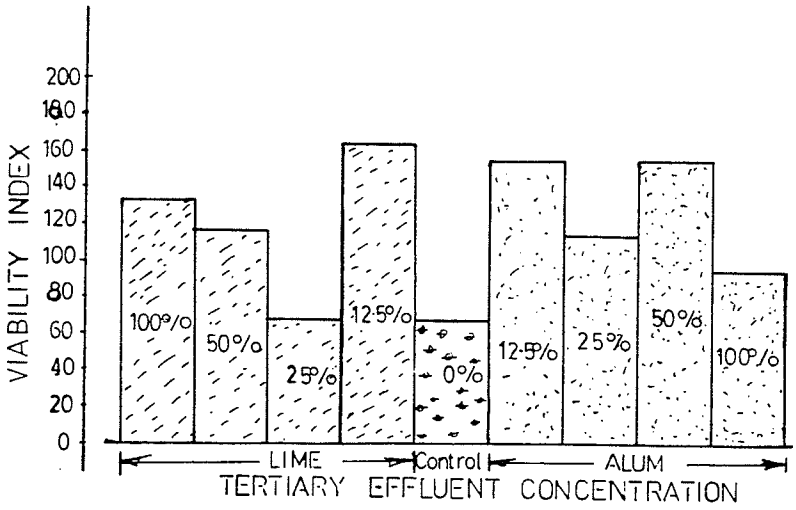

FIGURE 47. Viability differential; Viability differential = 100+ (Final population count-Total Progeny generated by parent group / Total progeny generated by parent group) X 100

	Effluent Concentration	Average per diem birth rate $\bar{b}$	Rate of increase r Days 4-12	r days 12-32	r days 32-39	Average per diem rate of increase $\bar{r}$	Population death rates average $\bar{d} = \bar{b}-\bar{r}$	Mean life expectancy days 1/d
Control	0%	.07	.35	.07	.03	.009	.061	16
		.05	.57	.08	.02	.007	.043	23
Lime Tertiary Effluent	12.5%	.08	.32	.09	.06	.004	.076	13
		.06	.27	.07	.06	0	.060	17
	25%	.10	.49	.14	.05	.003	.097	10
		.08	.31	.10	.12	.006	.074	14
	50%	.08	.54	.10	.06	.009	.071	14
		.11	.26	.15	.05	0	.110	9
	100%	.12	.39	.20	.05	-.008	.128	8
		.12	.45	.17	.09	-.004	.124	8
Alum Tertiary Effluent	12.5%	.09	.38	.09	.06	.008	.082	12
		.08	.25	.09	.03	-.006	.086	12
	25%	.09	.30	.10	.03	-.001	.091	11
		.09	.33	.09	.06	0	.090	11
	50%	.07	.30	.09	.05	-.006	.076	13
		.09	.26	.08	.06	-.001	.091	11
	100%	.13	.40	.07	.03	-.008	.138	7
		.07*	.32	.07	0	-.017	.087	11

TABLE 17. Summary of average birth rate, rates of increase, population death rates, and mean life expectancy. $b = \ln(1 + E) / D$. ; $r = (\ln N_t - \ln N_{t-7}) / \Delta t$.

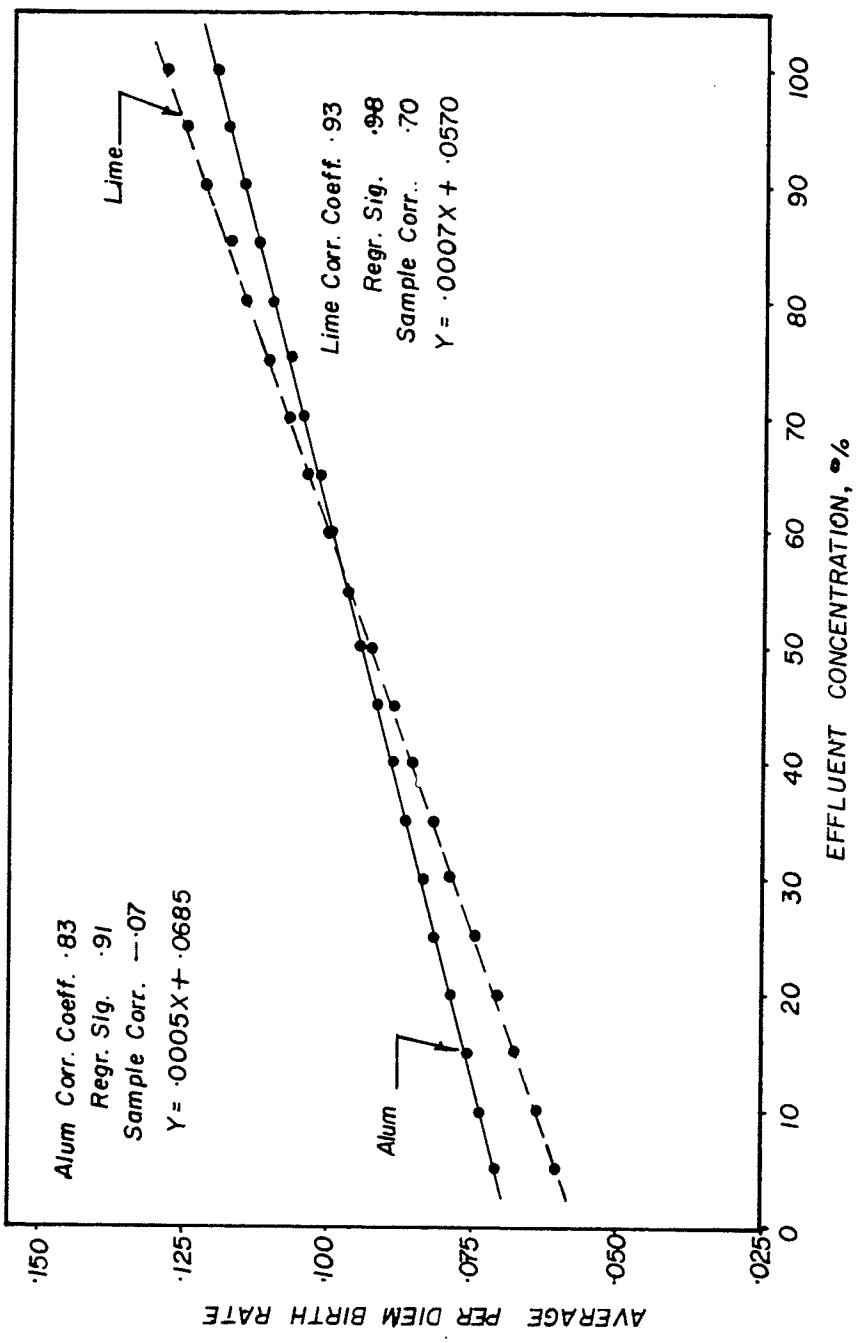

FIGURE 48. Statistical APL Software Package, Univariate Regression Curves, generated from experimental data. Average birth rate per day versus effluent concentration, %.

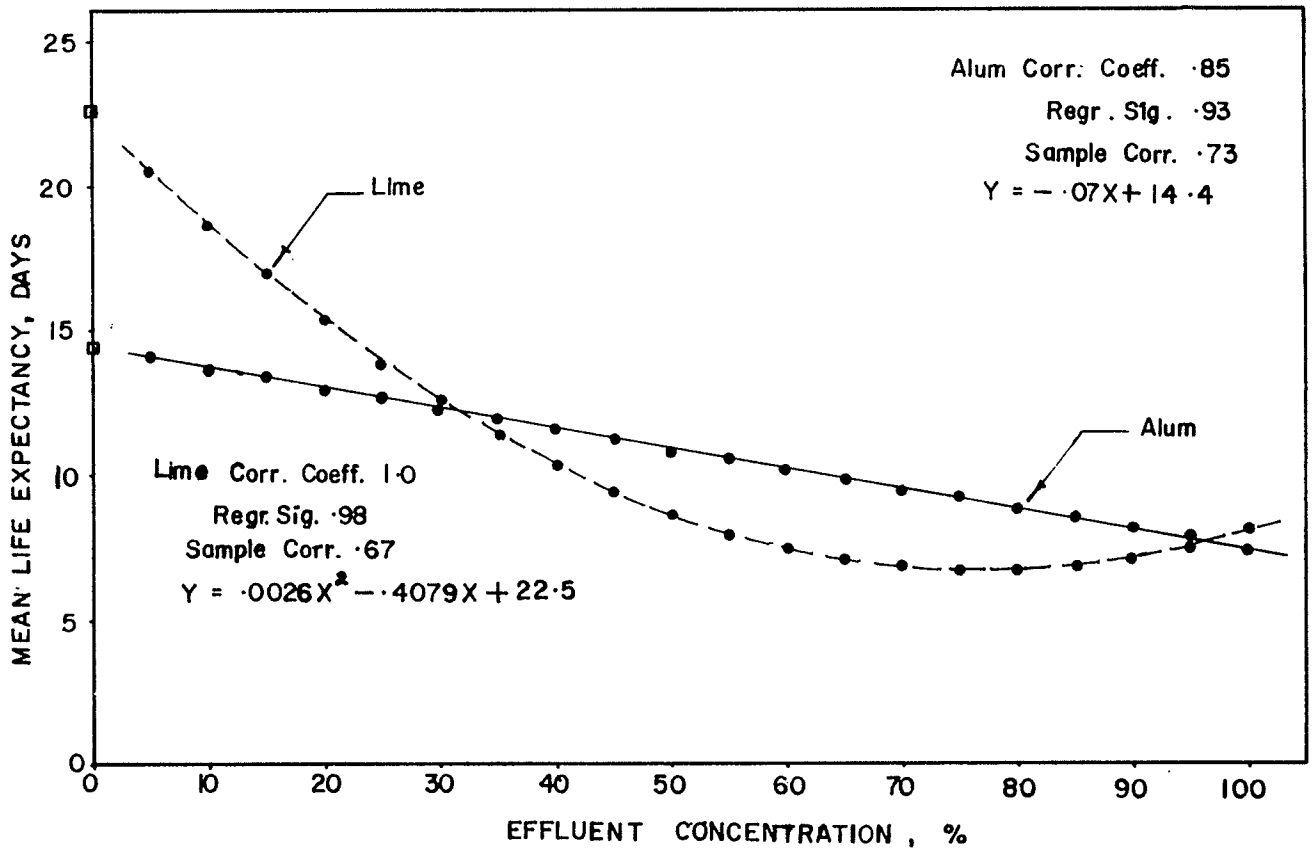

FIGURE 49. Statistical APL Software Package, Univariate Regression Curves, generated from experimental data. Mean life expectancy, days, versus effluent concentration, %.

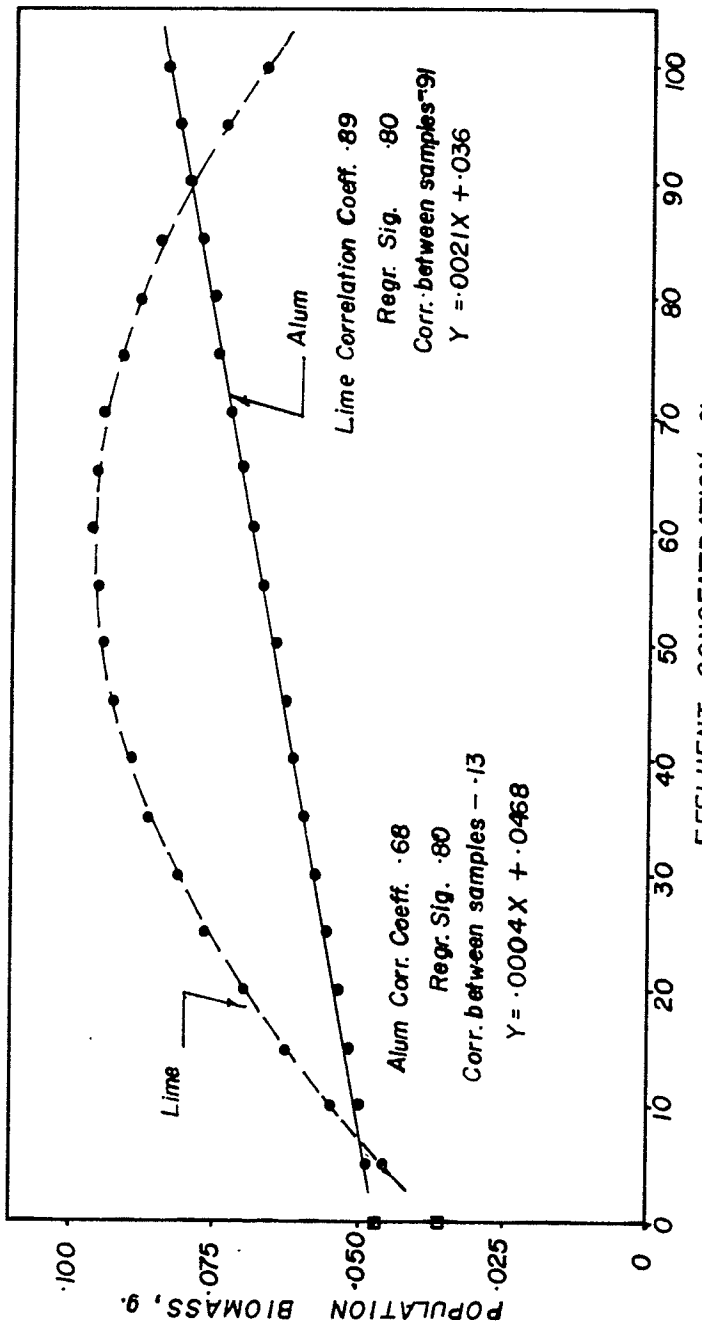

FIGURE 50. Statistical APL Software Package, Univariate Regression Curves, generated from experimental data. Population biomass, grams versus effluent concentration, %.

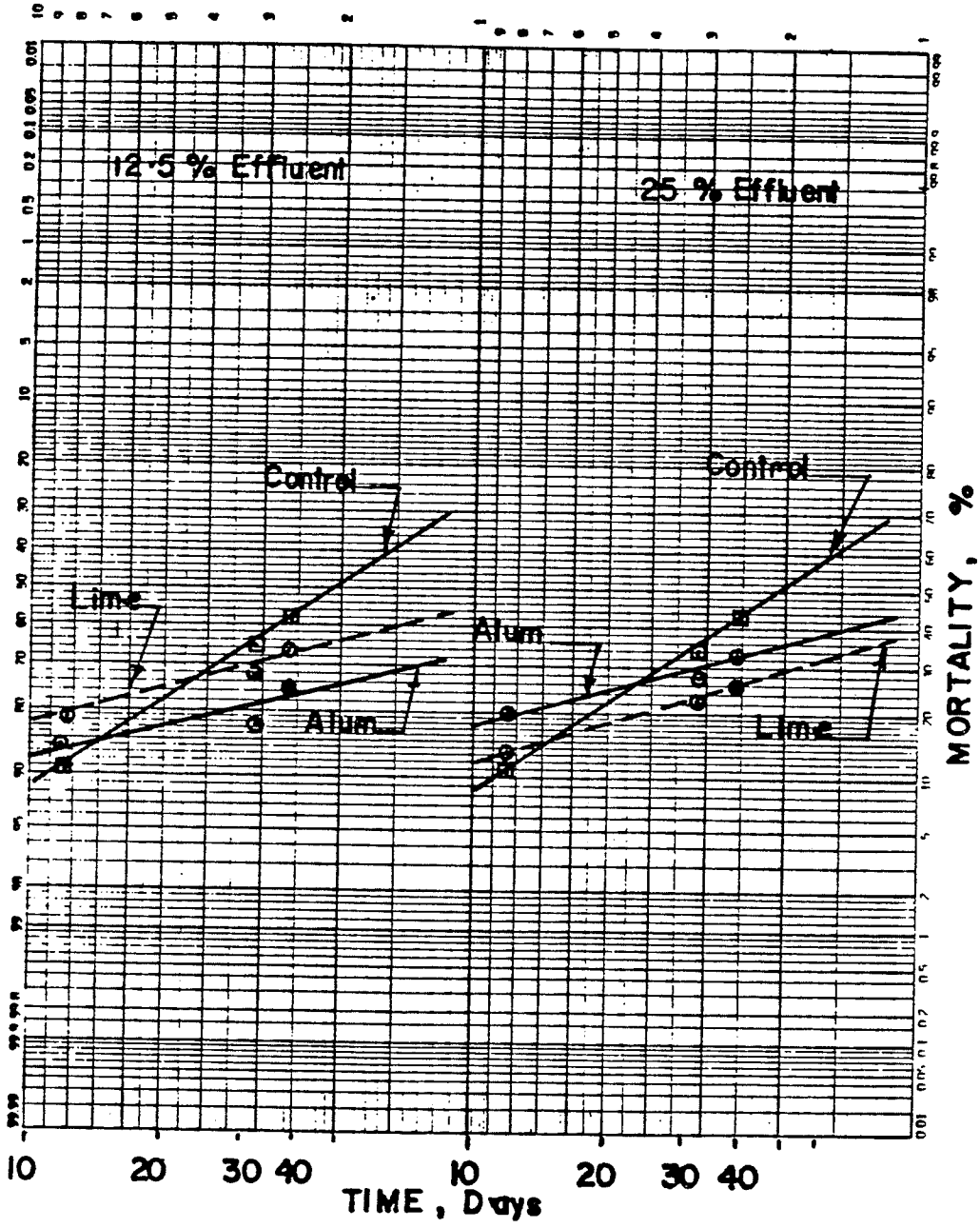

FIGURE 51. Log-probability record of mortality % versus time, days for 12.5% and 25% effluent respectively.

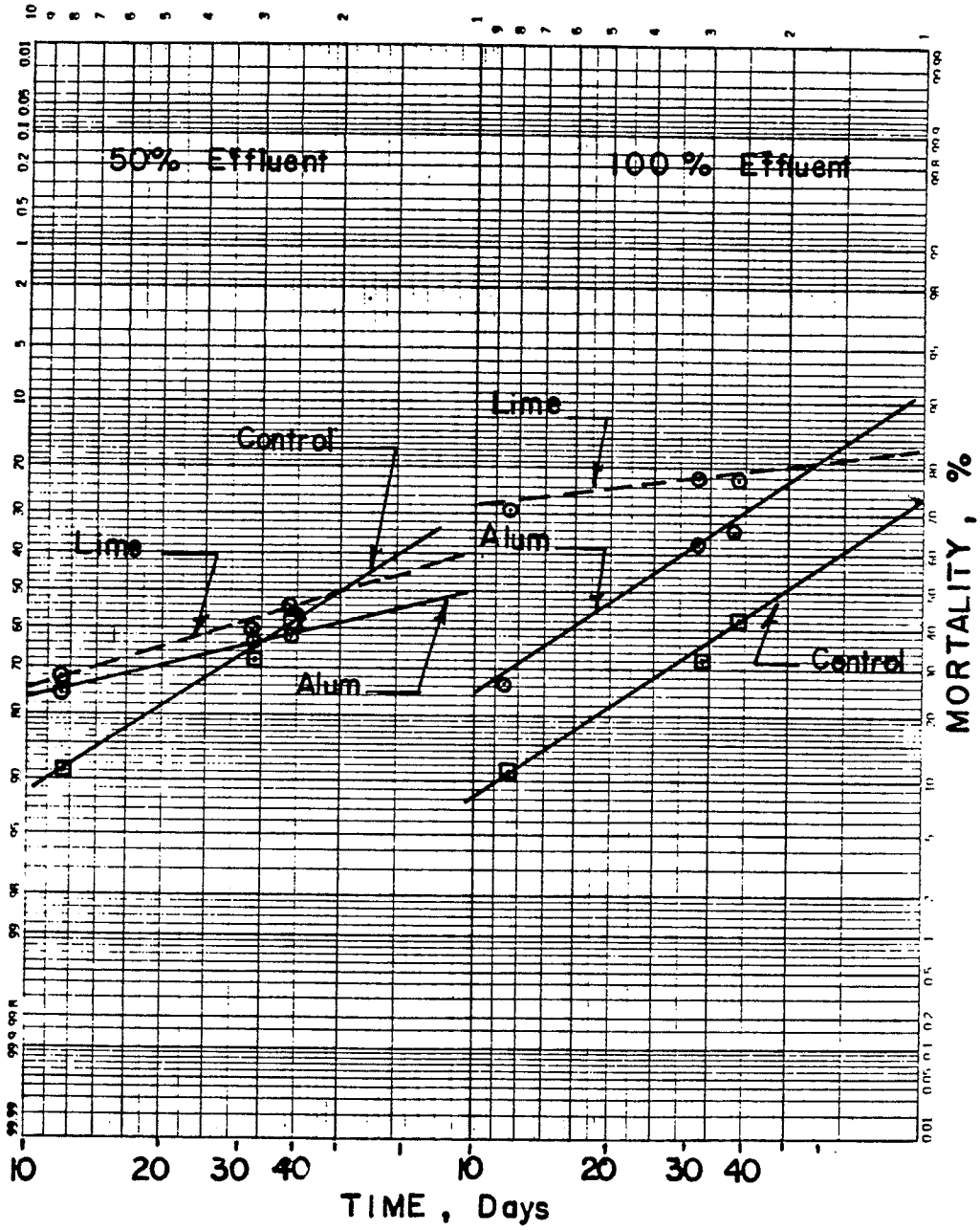

FIGURE 52. Log-probability record of mortality % versus time, days, for 50 and 100% effluent respectively.

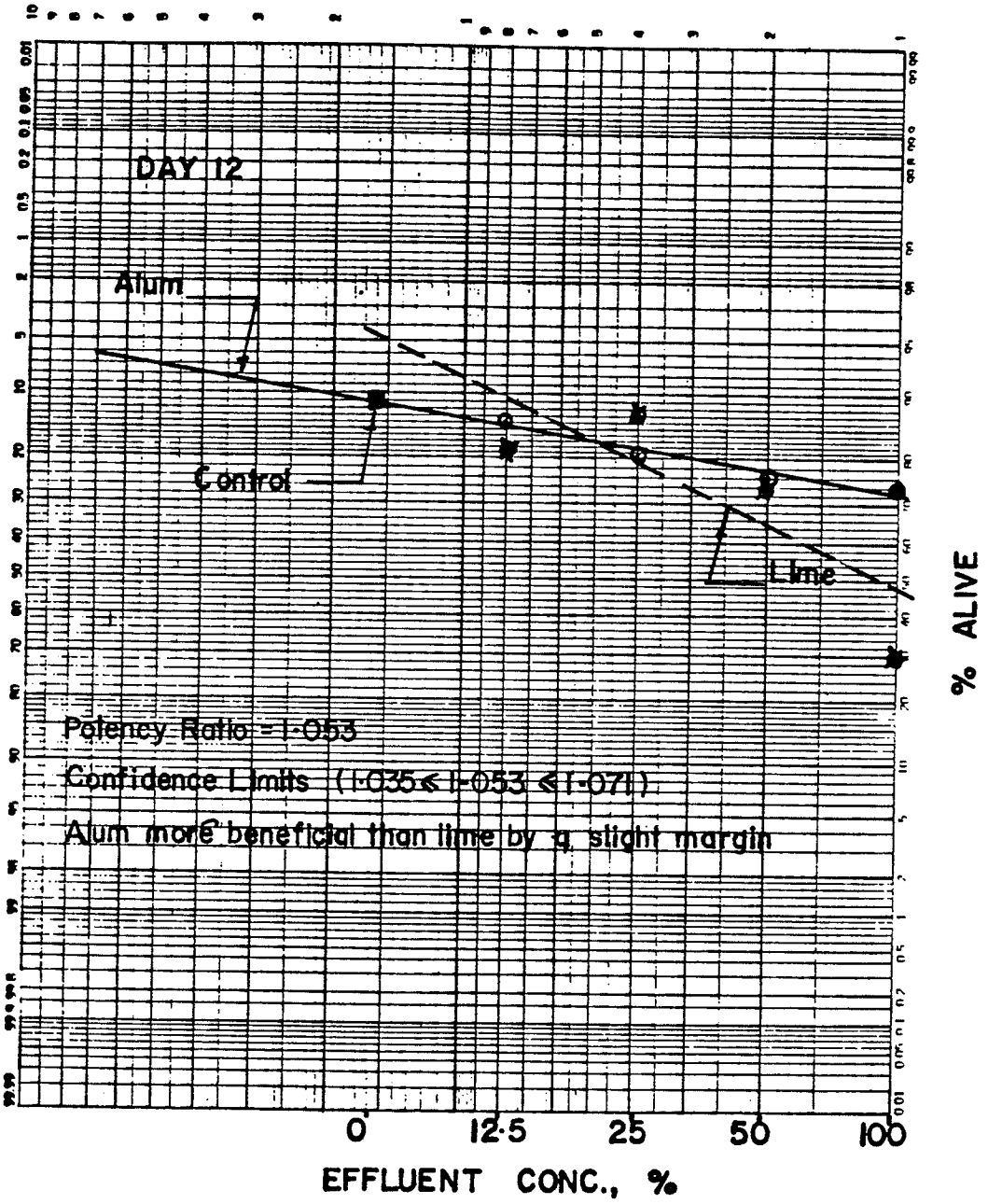

FIGURE 53. Log-probability curves plotting percentage of organisms alive on day 12 versus effluent concentration %. Curves used for statistical analysis by The Litchfield-Wilcoxon (45) Method.

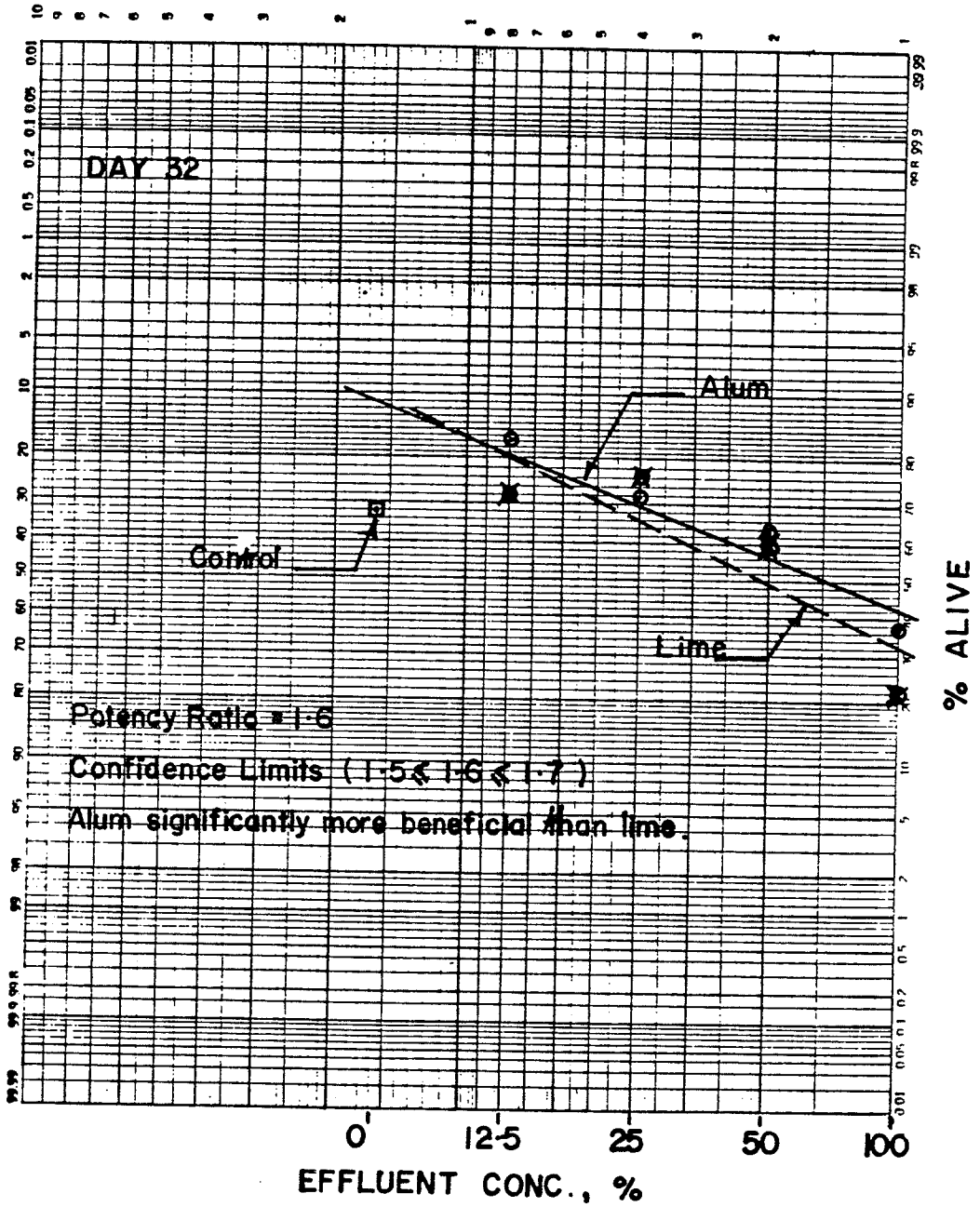

FIGURE 54. Log-probability curves plotting percentage of organisms alive on day 32 versus effluent concentration %. Curves used for statistical analysis by the Litchfield - Wilcoxon (45) Method.

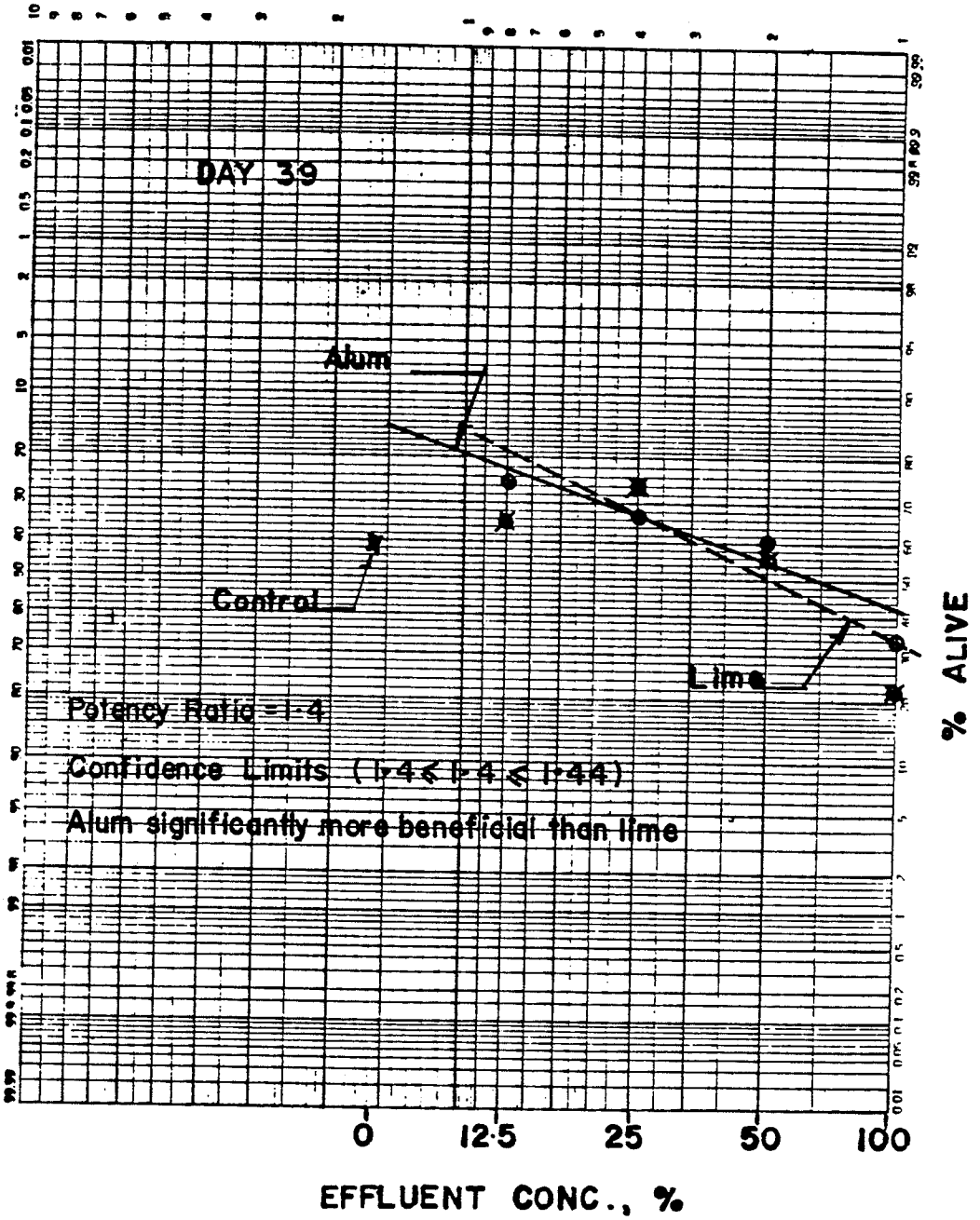

FIGURE 55. Log-probability curves plotting percentage of organisms alive on day 39 versus effluent concentration %. Curves used for statistical analysis by the Litchfield - Wilcoxon (45) Method.

	Effluent Concentration	Mean head to spine Lengths mm	Dunnett's (46) stat. analysis one-tailed test 95% confidence diminution, mm	Dunnett's stat. analysis two-tailed test 95% confidence diminution, mm	Statistical ranking based on a comparison of means (47) Group 5 most beneficial
Control	0%	1.96 2.14	Reference comparison zero mm	Reference comparison zero mm	Group 5
Lime Tertiary Effluent	12.5%	1.60 1.78	-.155 to -.565	-.126 to -.594	Group 4
	25%	1.30 1.27	-.555 to -.965	-.526 to -.994	Group 1
	50%	1.53 1.16	-.495 to -.905	-.466 to -.934	Group 2
	100%	1.32 1.06	-.655 to 1.065	-.626 to 1.094	Group 1
Alum Tertiary Effluent	12.5%	1.41 1.56	-.355 to -.765	-.326 to -.794	Group 3
	25%	1.38 1.28	-515 to -.925	-.486 to -.954	Group 2
	50%	1.37 1.46	-.425 to -.835	-.396 to -.864	Group 2
	100%	1.26 0*	-.585 to -.995	-.556 to -1.024	Group 1

TABLE 18. Statistical analysis and ranking of mean head to spine lengths of 25 organisms randomly selected from each replicate of all groups. Diminution ranges shown in mm for one-tailed and two-tailed tests at 95% confidence level.

* No life evident. Population eliminated by mysterious toxic circumstances.

	Effluent Concentration	Cumulative Progeny Production By Parent Group	Statistical Significance	Dunnett's (49) Confidence Interval at .05 Significance Level	Population Count on Day 39	Statistical Significance	Dunnett's Confidence Interval at .05 Significance Level
Control	0%	559 560	Comparison Standard	Comparison Standard	359 384	Comparison Standard	Comparison Standard
Lime Tertiary Effluent	12.5%	789 503	No Significant Difference	87 ± 2784	1024 977	No Significant Difference	629 ± 1993
	25%	2193 1137	No Significant Difference	1105 ± 2784	1251 859	No Significant Difference	684 ± 1993
	50%	900 2263	No Significant Difference	1022 ± 2784	1356 1831	No Significant Difference	1222 ± 1993
	100%	1556 2065	No Significant Difference	1251 ± 2784	2024 2757	Significant Difference	2019 + 1993
Alum Tertiary Effluent	12.5%	1141 1259	No Significant Difference	640 ± 2784	2097 1546	No Significant Difference	1450 ± 1993
	25%	1169 1448	No Significant Difference	748 ± 2784	1327 1602	No Significant Difference	1093 ± 1993
	50%	998 747	No Significant Difference	313 ± 2784	1222 1389	No Significant Difference	934 ± 1993
	100%	2275 766*	No Significant Difference	961 ± 2784	2140 0*	No Significant Difference	699 ± 1993

TABLE 19. Statistical analysis of cumulative progeny production by parent group and final population count using Dunnett's multiple comparison procedure for comparing several treatments with a control. Values shown for two-tailed tests with a .95 joint confidence coefficient.

* Final population eliminated by unidentified toxic event. Note a significant difference is found if first replicate is used as the average value. The Confidence interval is then 1769 ± 1933.

	Effluent Concentration	Birth rate =b for days 4 - 12	b days 12 - 32	b days 32 - 39	Average per diem birth rate $\bar{b}$	Statistical Significance	Dunnett's confidence interval at .05 Significance Level
Control	0%	.07 .05	.05 .05	.08 .06	.07 .05	Comparison Standard	Comparison Standard
Lime Tertiary Effluent	12.5%	.06 .05	.05 .04	.12 .10	.08 .06	No Significant Difference	-.081 to .101
	25%	.06 .04	.07 .05	.17 .15	.10 .08		-.061 to .121
	50%	.05 .06	.06 .08	.14 .18	.08 .11		-.056 to .126
	100%	.06 .05	.09 .08	.21 .24	.12 .12		-.031 to .151
Alum Tertiary Effluent	12.5%	.07 .07	.05 .06	.14 .11	.09 .08	No Significant Difference	-.066 to .116
	25%	.07 .07	.06 .06	.13 .14	.09 .09		-.061 to .121
	50%	.06 .07	.05 .06	.11 .15	.07 .09		-.066 to .116
	100%	.12 .09	.08 .07	.18 .06*	.13 .07*		-.051 to .131

TABLE 20. Interval birth rate analysis and statistical evaluation using Edmonson's (1968) Equation (41) and Dunnett's Statistical Evaluation (46).

* Day 32 to day 34. Population died because of unidentified toxic circumstances.

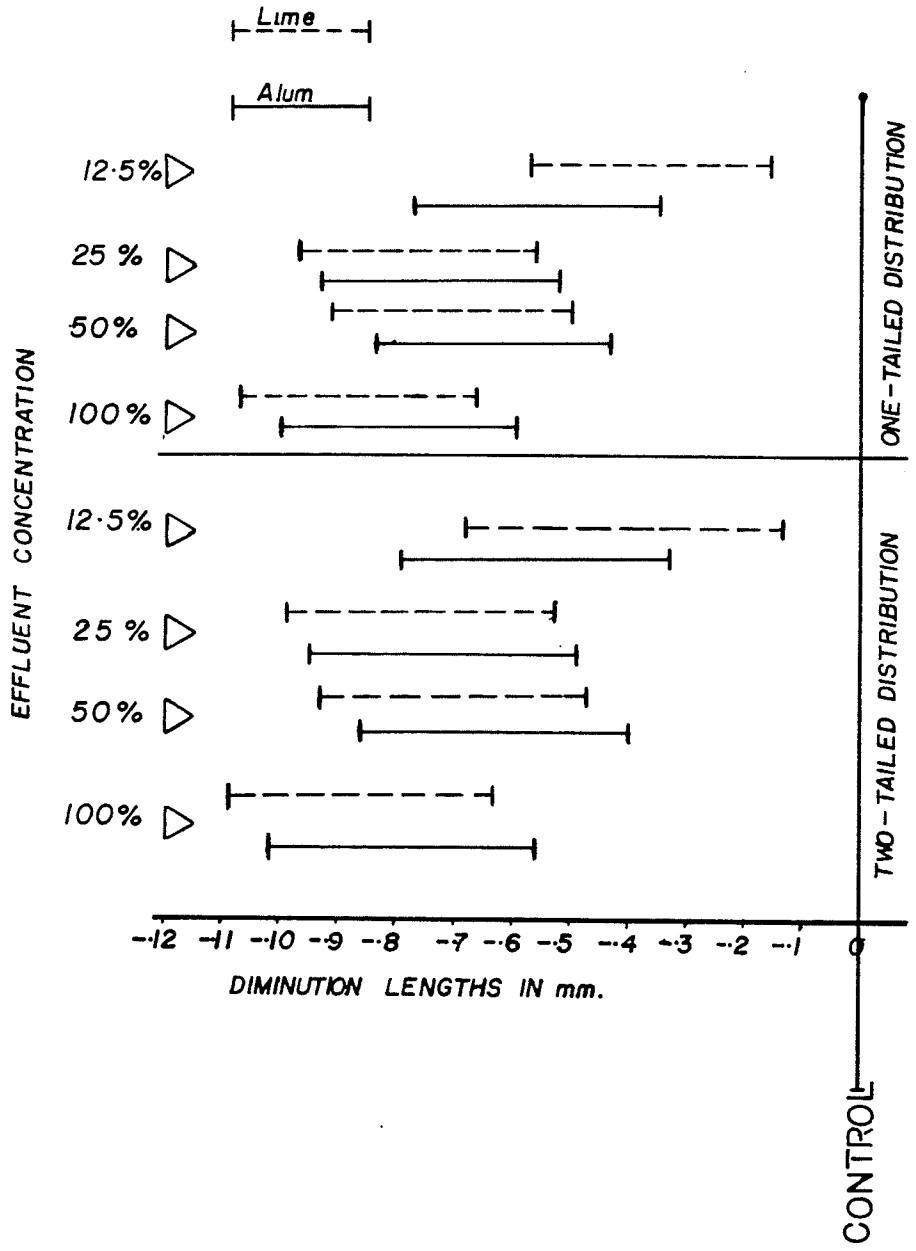

FIGURE 56. A comparison of the average head to spine lengths of 25 randomly selected organisms using the average head to spine length of the control group as reference point zero (0). Intervals shown for corresponding effluents for one and two-tailed tests shown in Table 18.

be conveniently referenced, should these actual numbers be of some usefulness. In some instances the graphical expressions of these tables have been presented in the main body of the Experimental Results. Tables 21 to 26 are contained in Appendix I.

8. DISCUSSION OF EXPERIMENTAL RESULTS

The order in which the Experimental Results are discussed is the same as the format used in Chapter 7; The Presentation of Experimental Results. Appropriate subsections will be used, where detailed discussion is required, to facilitate continued orientation. Although this study is only preliminary in scope, based on the data collected, some inferences will be attempted, within the text of this discussion, about the applicability of the findings to sanitary engineering practice.

8.1 The Exploratory Study

The results of the exploratory study, Table 12 and Figure 21 demonstrate that neither the lime nor the alum effluent was acutely toxic. In fact this study showed consistently higher mortalities, the more dilute the effluent. Curves for both effluents demonstrated parallelism, with the alum effluent performing better in terms of survival of Daphnia by fifteen (15) percentage points. Since the higher dosages illicited lower mortalities than the more dilute effluents, the application of the methods of Litchfield & Wilcoxon (45) was not essential for any predictive purposes. However, by application of the method one demonstrated that the lines through the points were of good fit and that the parallelism found was within the bounds of the experimental error. This application also showed that the two effluents differed significantly in potency,

with the lime effluent significantly more toxic than the Alum effluent and that, within the 19/20 confidence limits, the relative activity of the lime effluent was between 1.4 and 34.6 times that of the alum effluent. The results of the exploratory study suggested an investigation of chronic toxic effects. A medium to long range reproductive impairment study was selected.

The availability of food for survival was the factor causing higher deaths in the more dilute effluents. No food was given during the 96-hour bioassay study. The ten (10) organisms in each 600 ml aquarium were therefore dependent on the bacteria in their environment for survival. The tertiary treatment method, based on coagulation-precipitation, although effective in substantial bacterial reduction in the tertiary effluents (compared with the secondary effluent) would leave enough organisms to provide food over the four day period. Sufficient numbers of these bacteria could be assumed only for the higher effluent concentrations, the 10 and 100% effluents. Hence the best survival is shown for these effluents. The reconstituted water was sterilized before use. It therefore was of no significance in contributing any bacteria to the daphnids in the control group or in the diluted effluents. Since the higher dilutions contained more of this sterile effluent, organisms in the three lowest concentrations would have great difficulty in finding bacteria for nourishment. Whenever the energy expended by an organism in its quest for food, continues, over a sustained period, to exceed the energy intake derived from the food consumption, death results.

The higher toxicity of the lime effluent could be attributed to adverse pH effects, despite the three day equilibration period allowed

the effluents prior to the introduction of the organisms. These organisms were removed from the stock tank, where the pH was 6.5 and introduced into the various aquaria where the pH was in the 6.5 - 8.0 range in the case of the lime and 6.5 - 7.7 in the case of the alum effluent. These values are shown in Table 11. It has been reported that a pH change of two (2) units even over a period of 1 to 2 days may be detrimental to Daphnia (29). This abrupt change in the pH of the environment, most extreme in the case of the 10 and 100% lime effluents, could have been the factor responsible for the marginal increase in mortality noted for the lime effluents. The wide fluctuations in the pH during the lime tertiary treatment, could also have caused a reduction of the bacterial load.

8.2 Effluent Analysis Data

The effluent analysis data presented in Tables 13 through 16 is unremarkable except that the reconstituted water appears carbon deficient and the lime effluent low in magnesium. The total dissolved carbon in the reconstituted water was 4.2 mg/l, compared with 100.5 and 36.2 mg/l for the lime and alum effluents respectively. Metcalf and Eddy (12) report a minimum Carbon:Nitrogen: Phosphorus ratio, for well being of organisms in an algal community to be 100:5:1. This ratio is substantiated by Vallentyne (3) who gives the ratio 40C:7N:1P for every "100 units dry weight matter." Since the reconstituted dilution water appeared to be adequate in nitrogen and phosphorous content, the low carbon content would make carbon the limiting nutrient. The bioassay being static with periodic replacement, the lack of carbon in the reconstituted water,

which was used both as the control and for dilutions of the tertiary effluent series, would, as was demonstrated in the bioassay, severely limit the productivity of the control. This performance will be subsequently examined in more detail in section 8.4.

An examination of the post-experimental sewage analysis in Table 16 shows a 543% increase in the total dissolved carbon of the reconstituted effluent, and a 60 and 27% decrease for the lime and alum effluents respectively. These changes in carbon content are less dramatic for the dissolved organic carbon measure. For the reconstituted effluent an increase of 444% was noted. The lime and alum 100% effluents showed a decrease of 89 and 96% respectively.

Due to the limited availability of dissolved inorganic carbon the control sustained no algal bloom, as did the other effluent dilutions. The carbon increase noted was due to contributions added with the food preparation given the population every two days. In addition some contributions could have been incorporated from the atmosphere in the manner explained in the literature (3). The dramatic decrease in the inorganic carbon of the lime and alum effluents was due to the consumption by the algal blooms. In particular, the greater reduction in the alum samples reflect the more prolific algal activity noted for the alum effluents.

The alkalinity of the reconstituted water appeared to be marginal compared with the other effluents. In Table 13 the initial concentration of 6.0, as mg/l $CaCO_3$, for the control compared with 286 and 72 mg/l as $CaCO_3$ for the 100% lime and alum tertiary effluents respectively. This deficiency was eliminated by the addition of sodium bicarbonate in

an amount sufficient to boost the alkalinity of the reconstituted water to 12 mg/l as $CaCO_3$, recorded in Table 15. This preparation was then used to make up the dilutions of the lime and alum effluents.

The comparatively low sulphate content of the reconstituted water, 17.8 mg/l, was probably, in addition to its low dissolved inorganic carbon content and alkalinity, one of the factors contributing to the absence of a full scale algal bloom in the control aquaria. By contrast, the alum effluent, with a sulphate content of 258 mg/l, showed the first algal bloom. This bloom was sustained virtually throughout the experiment. As a result of this activity, the alkalinity in the alum effluent was virtually used up by the end of the bioassay study. Using the values in Tables 13 and 16 we have an average 94% reduction in total alkalinity for the 100% alum effluents. This more prolific algal activity is also seen in the very low dissolved inorganic carbon residues for the alum effluents, shown in an adjoining column of Table 16. The average residual of 1 mg/l represents a 96% reduction for the 100% alum effluents. The alum treatment reduced the total dissolved inorganic carbon from 68.6 to 25.9 mg/l. The algal bloom in the alum could have been sustained for a longer period if there was more dissolved inorganic carbon available.

By contrast, the 100% lime effluents with a sulphate content of 58 mg/l showed a belated algal bloom around day 22 of the bioassay. This bloom was sustained to the end of the schedule, predominantly in the 100% lime effluents. The residual alkalinities for the lime effluents in Table 16 show significantly higher concentrations than those for the alum effluents. For the 100% lime effluents a 77% reduction compared with a 94% reduction for the 100% alum effluents. The reduced algal activity in

the lime effluents is also demonstrated by the higher dissolved inorganic carbon residues present in the lime effluents; an average 89% reduction for the 100% lime effluents, versus a 90% reduction for the 100% alum.

The alkalinity of the control shows a four (4) fold increase mainly due to food additions and the absence of prolific algal activity due to the insufficiency of carbon and sulphate supply. The dissolved inorganic carbon residues show a 963% increase for these same reasons.

In further comparison of the effluents, the only other notable deficiency appears to be in the magnesium content of the 10% lime effluents; 0.8 mg/l compared with 15 and 4.2 mg/l for the alum and reconstituted dilution water respectively. Table 13 shows the magnesium content of the original secondary sewage sample to be 15.2 mg/l. The lime precipitation method was therefore effective in removing 95% of the magnesium versus 1% removal for the alum coagulation-precipitation method. The significance of this deficiency may be minimal since only trace quantities of magnesium are required for the sustenance of healthy algal cultures as is the claim of McKinney (51). However, in listing the macronutrients required for the support of healthy algal cultures, Standard Methods (23) includes the Mg^{++} ion, required at a concentration of 2.904 mg/l. It is therefore quite likely that the magnesium content in the lime effluents may have been insufficient to support a healthy algal/daphnid community.

8.3 Bioassay Effluent-Monitoring Data

8.3.1 Dissolved Oxygen

Figure 22 shows that all effluents, throughout the bioassay, were

kept in a high state of oxygenation. Up to day 18 most of the effluents, with the exception of the 100% alum were within the positive increase ranges for DO, shown in Table 6; 0.5 - 10 mg/l. From day 18 to day 39 the DO content was beyond this range for all of the effluents, the notable exception being the 100% alum effluent. The reason for this anomaly was the prolific algal growth in the 100% alum samples, beginning at day 9 and the corresponding high rate of progeny production as seen in figure 40. The high productivity in these aquaria depleted the algae (the preferred food of Daphnia) the inorganic carbon and available alkalinity. In the later stages of the bioassay with essential nutrients being rapidly depleted, with productivity being maintained at an average level of approximately 140 and with regular harvesting of organisms, the algae was probably in the senescent growth stage. From an examination of Figures 22 and 40 it can be seen that from day 32 the DO rises while there is a simultaneous decline in progeny generation. Due to a decline in the Daphnia population there are fewer grazers. The resulting denser growth produces the increase in oxygen noted. During the biossay none of the organisms appeared to suffer from flotation caused by the presence of oxygen bubbles under the carapace or in the gut.

The first appearance of the algae was linked to the incidental seeding of all aquaria during the introduction of neonates into the parent aquaria. The innoculum came from the natural algal growth in the stock tanks at the Freshwater Institute. Brown algae was first observed on day 22. Post experimental identification by the Botany Department (52) showed that the green sample was predominantly the blue green alga Oscillatoria with some brown alga, the diatom Navicula and the green alga

Chlorococcum interspersed. The brown sample was almost entirely the diatom Navicula.

Algae was proliferating as early as day 9 with the first observations made in the 12.5 and 25% alum replicates by day 2, closely followed by the 100% alum effluents which demonstrated the most prolific and sustained bloom. By day 22, most samples with the exception of the control aquaria demonstrated an increasing concentration of algae. The latest occurring blooms were observed in the 50 and 100% lime effluents.

8.3.2 Hydrogen Ion Concentration, pH

The pH, dissolved oxygen, ammonia and alkalinity states of a given aquatic body are maintained in a dynamic equilibrium that is temperature dependent. Temperature was constant at 20°C for this bioassay. The dynamism was affected by illumination and photosynthetic activity, the interrelationship with the animal population, and the combined effects of the flora and fauna on the nutrient pool. Figures 22 and 23 show a parallel trend in DO and pH. Beyond day 20 both measures show an increasing trend until the end of the study. The lime effluents in general show a consistently higher level of pH than any other effluent, with the exception of the control, which is marginally higher in pH and rises from 8.5 - 9 from day 32 to 39. All of the effluents; with the exception of the control, the 12.5, 25 and 50% lime effluents from day 32 to day 39, lie within the positive increase pH range. Between days 4 and 18 the control pH drops marginally below the lower limit of the 7 - 8.5 pH range given in Table 6. None of the effluents exceeded the extreme tolerance range of 6 to 9.4 shown in Table 6. This period of higher pH coincides

with increased productivity as can be seen in Figures 39 and 40; due to the parallel occurrence of log growth in the algae population. The generally poorer performance of the lime effluents in the earlier stages of the bioassay was probably due to the elevated pH to which the organisms were forced to acclimate. The 100% lime effluent proved the most difficult for the organisms. Eventually by day 23 acclimation appeared complete and the organism recovered in terms of productivity. The 50% lime effluent shows a similar productivity pattern, although the initial pH level was not as elevated as that for the 100% lime effluent. The pH under the conditions of this experiment could have been interfering with calcium assimilation or other vital biochemical processes. From day 23 a drastic decline in the pH of one of the 100% alum replicates was noted. This decline probably corresponded to loss of alkalinity in that replicate sample, disease, or other toxic event possibly toxins related to algal senescence. Peculiarly this event occurred simultaneously in the parent aquarium and its matching progeny aquarium. By day 34 only 4 organisms showed any signs of viability in the parent replicate. By day 39 all organisms in this set of replicates were dead.

The sudden demise of the Daphina in these matching 100% alum aquaria could possibly have been due to the effects of aluminum toxicity at low pH. Table 13 shows that on day 1, the aluminum concentration, in these aquaria was 0.236 mg/1. By day 28 the pH had dropped below 7.0 and was rapidly declining. Cronan (1979) notes that field and laboratory studies in the Adirondacs "have demonstrated that, in acidified waters, toxic conditions for fish may be produced by dissolved inorganic aluminum even at lake pH values that are not physiologically harmful" (53). In

particular brook trout showed a toxic response when exposed to synthetic acidic solutions and with aluminum concentrations in excess of 0.2 mg/l, in the 4.4-5.9 pH range. Significant sublethal effects were noted in the laboratory at "aluminum concentrations of 0.1-0.3 mg/l" (53). Biesinger and Christensen (1972) found that 0.32 mg/l aluminum in the pH range 6.5-7.5 produced 16% reproductive impairment in Daphina magna. A concentration of 0.68 mg/l produced 50% reproductive impairment in the same pH range. This was a 3-week chronic toxicity study (40). The diminution of the daphnids noted in the the higher alum concentrations may have been a sublethal effect attributable to aluminum toxicity at low pH. With most of the nutrients used up by day 28 of the bioassay, the full impact of this aluminum toxicity was probably borne by the organisms, since counter-ion effect would be significantly reduced at this stage. Normally counter-ion effect would lessen the impact of toxicity by sequestering some of the potentially toxic ions.

8.3.3 Conductivity

The conductivity monitoring generally reflects the total dissolved solids content of the effluents. As expected, the control by reason of its constitution, has the lowest conductivity. This, in contrast to the 50 and 100% effluents which have the highest level of conductivity. The trend of all curves shows no general increase in conductivity. There is no significant buildup of metabolites during the experiment. This implies that consumption matched food supply and product removal, in the form of the harvest of progeny. The general decline in the conductivity of the 100% lime effluent parallels a general decreasing pH trend from

day 22, as well as an increase in productivity, from the same date.

8.3.4 Hardness and Alkalinity

Figure 25 shows the generally higher hardness of the lime effluents despite the lowest free magnesium content. The large lime dose used in the tertiary treatment process created a dispersion of Ca^{++} ions which would have required special settling techniques because of colliodal behaviour. Despite the minimal free magnesium ion content, 0.8 mg/l, substantial quantities of magnesium contributed to the hardness spectrum in the form of magnesium hardness. The hardness of the effluents shows an increasing trend which reaches a relative maximum by day 28.

Figure 26 shows the overall higher alkalinity of the lime effluents and the trend in effluents, with the exception of the control, towards an alkalinity decrease as the study progressed. By day 42 the alkalinity of the alum effluents is virtually depleted. Productivity from these aquaria could no longer be sustained due to adverse pH changes caused by the loss of buffering capacity. By day 34, with the exception of the 12.5% alum effluents, all other alum effluents show a decline in productivity, whereas the lime effluents show an increasing productivity trend. This can be seen in figures 39 and 40. When the bioassay was terminated on day 39 the productivity in the lime aquaria was not yet declining. This could however be due to the initial lag in production shown by organisms in the lime effluents. Hence there was a difference in cyclic pattern that may have been dependent on the differences in effluent quality.

8.3.5 Total Dissolved Carbon

The carbon deficiency in the control has been previously discussed. The availability of inorganic carbon is more important than the organic carbon, since organic carbon in the form of CO_2 is generated as a by-product of bacterial metabolism and in limited transfer from the atmosphere. The initial and final sampling for dissolved carbon, figure 27, shows the trend towards depletion of the inorganic carbon in all but the control sample. The increasing concentrations of organic carbon in both parent and progeny aquaria on day 60 was due to the removal of all daphnids from the effluents. In the interval between this event and the effluent testing there was an increase in organic matter in solution.

8.3.6 Total Ammonia Nitrogen, as N

Figure 28 shows the general higher level of ammonia nitrogen in the alum effluents. This may be due to two events: 1) The higher pH of the lime effluents would favour the volatilization and evaporation of the ammonia, resulting in lower levels at the time of sampling; 2) The higher ammonia levels may also be a reflection of the increased productivity of the alum effluents up to day 24. The slope of the ammonia curve for the alum effluents, begins to decline by day 28. The higher levels of ammonia nitrogen can be an expression of increased putrescible organic matter. By similar argument, the Kjeldahl nitrogen would be higher for the alum effluents. The ammonia content of all samples, except the 100% alum on day one, is within the extreme tolerance range; 0 to 32 mg/l as NH_3, quoted in Table 6. The 100% alum effluents exceed the upper limit of the positive increase range, 0 - 17 mg/l in all instances.

8.3.7 Total Un-ionized Ammonia, as NH_3

The total un-ionized ammonia curves shown in figure 29 were based on the total ammonia values and the pH values at 20°C. Table 5 was then used to determine the actual values plotted. The values shown in Table 5 are based on the ideal case for distilled water. If the effluent impurities in the bioassay are taken into account, the values found for the un-ionized ammonia should be lower. The un-ionized ammonia content can be an index of crowding of organisms. Standard Methods (23) suggests that the upper limit, beyond which crowding may be inimical to the organisms, is 20 µg/l. However, this limit may pertain more so to fish populations and not organisms the size of microcrustaceans. Noteworthy is the fact that except on day 42, the control sample was below the suggested 20 µg/l limit when sampled. Samples for these tests were drawn immediately after removal and counting of progeny in the respective aquaria. The initial organism loading of all parent aquaria was 50 per 800 ml or a ratio 1:16. This is a higher loading than the 1:20 suggested optimum for laboratory conditions (29); however, with depletion due to mortality the effective loading was more in the order of 40 per aquarium or the optimum suggested ratio for laboratory cultures.

Noteworthy is the substantially higher un-ionized ammonia content of the alum effluents. Not only are the un-ionized ammonia concentrations an expression of the initially higher productivity of the daphnids, but they represent the increased productivity of the sulphate-fertilized algal blooms observed up to day 18 primarily in the alum effluents. The lime effluents show an increasing trend, commencing with day 15, towards increased algal activity and a corresponding increase in daphnid produc-

tivity.

8.4 Discussion of Presentations Generated from Bioassay Data

8.4.1 Toxicity

An examination of the toxicity curves for cumulative mortality, taken on days 12, 32 and 39, shows the alum effluents to be much less toxic than the lime effluents. A theoretical toxicity value can be found by extrapolation of the points beyond the 100% effluent concentration to meet the ordinate on right hand side. Accordingly Figure 30 can show that by day 12, the concentration of toxic matter in the lime effluent required to illicit 99.99% death would be 700% or seven (7) times the value producing the experimental level of death. By similar argument for the alum effluent a 1600% level of toxic matter, or 16 times the value producing the experimental level of death, would illicit 40% death. Table 27 summarizes these results for figures 30-32.

These results substantiate some of the findings of the exploratory study which did predict within the limits of 19/20 probability that "the lime effluents were significantly more active than the alum effluents, and that the relative activity was between 1.4 and 34.6 times that of the alum"(45). In addition, the progression of curves shown in figures 30 - 32 demonstrates pollution abatement and/or acclimation of organisms in the lime aquaria. The alum effluents demonstrate an increasing mortality for the same hypothetical toxic concentration, implying either a declining population due to some limitation in the effluents, or other environmental incident causing discomfort to the organisms and contributing to

Table 27 Estimated theoretical effluent toxicities producing level of death over interval shown.

Days	Lime		Alum	
	Potency level required	Mortality	Potency level required	Mortality
0-12	7 x (100%)	99.99%	16 x (100%)	40.0%
0-32	8 x (100%)	99.99%	16 x (100%)	99.2%
0-39	14 x (100%)	99.99%	16 x (100%)	99.5%

eventual death. Aluminum toxicity may be the cause, and may also account for the lack of abatement of the pollutant. The parent organisms sampled may also be at the end of their productivity cycle.

8.4.2 Performance of the Control

"Ten percent has been cited as an acceptable control mortality figure for invertebrates ... True assessment of effluent toxicity is difficult to determine when other factors appear responsible for mortality in controls" (54). By this criterion, the control used in the experiment performed poorly. By day 39 the average mortality in the control was 40%. The control was deficient in dissolved inorganic carbon and alkalinity both of which limited the growth potential of a healthy algal crop. The limited nutrients in the control effluents did produce healthy organisms but productivity was low. The control group population remained static at a healthy level, sustained on the limited available nutrients in the effluent and the Cerofood supplement. Excess death

could be attributed to an insufficiency of available nutrients. Figures 46 and 47 demonstrate this poor performance of the control. The final population characteristic shows an 85% adult population and only 15% young; by far the poorest performance of all effluents. In terms of the index described as the Viability differential statistic:

$$\text{Viability differential, \%} = 100 + \frac{\text{Final population count} - \text{Total progeny generated by parent group}}{\text{Total progeny generated by parent group}} \times 100$$

The poorest performers are the 25% lime effluent and the control group. The average progeny production, from figure 42, was 600. The average final population in each replicate control progeny aquarium, as seen in figure 43, was 400 neonates. The level of productivity for most aquaria was generally below par. This could be in part due to the influence of the dilutions made with the reconstituted dilution water used as the control.

A natural source water control, with the water screened by microfiltration and autoclaved to remove unwanted organisms, would have been a better alternative. This was not used primarily because of the difficulty inherent in locating such a water in January in Winnipeg. The nearest source, the Red River, was contaminated by secondary effluent discharge from the South End Sewage Treatment Plant. Access above the outfall was considered too hazardous, in addition to this water being already high in nutrients. The best control to be used in such studies would be of a sample taken from the target discharge area. This water

would be presumably well balanced with the basic macro and micro nutrients and would have natural buffering capacity. It would facilitate a truer prediction of ecological events.

Others have enquired about the lack of a parallel secondary effluent control. This was deliberately omitted, since it was well documented by McKinney (51) and other workers, that secondary sewage effluent has all the requirements to sustain a healthy bacterial and algal population. Indeed, oxidation ponds are known to sustain several healthy generations of Daphnia each summer. The only value in a secondary effluent control to this experiment would have been as a check for toxicity of the secondary effluent sample drawn from the South End Sewage Treatment Plant. If this were in question, easier verification could have been obtained via the BOD test.

8.4.3 Ephippial Production

If ephippial production is an indicator of stress or discomfort to Daphnia, as has been reported in the literature,(29), it would be apparent from Figure 36 that the maximum response to stress in this form was illicited by the more dilute effluents. This response to stress appears greater for the alum effluents. The statistical correlation data shown in Figure 36, is not significant for the alum effluents. The replicate correlation, -.32 is very poor, the beta regression correlation coefficient, .65, is not remarkable, and the regression significance, .51, is not strong. The statistics for the lime effluent curve are of greater significance. The correlation between replicates, .35, is weak, but both the beta regression correlation, .83, and the regression significance, .73, are fairly good statistics.

Conclusions based on this treatment of data should be at best tentative, since further testing is needed to determine the reproducibility of these results. One would have expected the higher concentrations to demonstrate the most stressful response. The converse is shown here. Indeed the 100% lime effluents generated the least ephippia. Examination of Figure 33 shows that the high strength effluents did produce high early stress by this criterion, but; they were able to acclimate relatively swiftly and by day 14, as seen in Figure 40, they were able to recover in terms of progeny generation. This mechanism of demonstrating stress response was not by continued ephippial production but by behaviour modification here expressed in stimulated productivity. This itself is a well documented response to stress (29).

Two cusps are noted in this recovery pattern. The 50% effluents do acclimate as ephippial production decreases over the first 17 days, but in terms of progeny production, as seen in Figure 40, they begin their rebound much later than the 100% effluents, around day 24. The mortality curves, figure 35, for this bioassay show a gradual increase in mortality for the 12.5 and 25% effluents, an increasing rate for the 50% effluents and a very steep rate of increase for the 100% lime effluent over the first 12 days, followed by a decreasing rate of increase to day 32 and a levelling off to day 39. The 100% alum mortality rate is approximately intermediate between that observed for the 50% and 100% lime effluents, with a constant slope to day 32, followed by a levelling off to day 39. What is remarkable is that the organisms remaining in the aquaria suffering the highest mortality, in a relatively short interval, managed to acclimate and rebound with sufficient strength to maintain a high cumula-

tive level of productivity, as shown in Figure 41. The 100% lime effluent organisms in particular were able to outperform all other effluent organisms, in the testing period. Daphnia in alum effluents, with the exception of those in the 50% concentration, maintained competitive production levels. The anomalous behaviour of the 50% alum effluent in terms of progeny generation was matched only by the puzzling and poor performance of the 12.5% lime effluent. Figure 34 shows that, the 50% alum effluent in terms of ephippia release, maintained an increasing stress level from day 14 to day 39. The organisms in the 12.5% lime and 12.5% alum effluents showed an increasing stress from day 10 to day 39. The more highly stressed organisms in the alum effluent were more productive, as seen in Figure 41.

The higher overall stress experienced in the lime effluent was probably due to early caustic pH effects. With time this stress level attenuated to the point where the recorded ephippial releases were the lowest for the bioassay (from days 17 to 39) as seen in Figure 33. High sulphate concentrations and/or high aluminate concentrations; 258 and 256 mg/l respectively, were the probable cause of the discomfort to the organisms in the alum effluent. For both 100% effluents the following argument is being proposed:

1) The stress was so severe that only the organisms with the potential to adapt to the nuisance survived, and were forced into a response which would ensure species continuation by progeny generation at accelerated levels;

2) The virtual lack of ephippia produced by the 100% lime organisms during this period could possibly be attributed to the chemical charact-

eristics of the effluent, inhibiting the formation of ephippia. This characteristic was perhaps not present in the case of the 100% alum.

The rapid depletion of the limited carbon an alkalinity stores may have produced increasing stress on the organisms. The rapid increase in ephippial production from day 16 to 39 is shown in Figure 34. Progeny generation was, at this time, the lowest for most effluents.

8.4.4 Productivity

"The difference between life expectancy in nature and in captivity and the difference in fecundity per female as a function of the environment indicate that, under suitable conditions populations of organisms can increase at an exponential, logarithmic, or Malthusian rate."(55) The pattern of productivity plotted in figures 39 and 40 and the direct observations made during the bioassay substantiate this principle. The 12.5 and 25% effluents appear to demonstrate a logarithmic rate of productivity. The 50 and 100% lime effluents demonstrate an exponential rate of productivity from day 28. The control effluents because of the nutrient limitations maintatined a cyclic productivity pattern at a Malthusian rate.

The earliest indication of distress was observed in the low numbers of progeny produced in the lime effluents; more notably in the 50 and 100% concentrations. This low yield pattern was maintained over the first 17 days, and was in effect functional reproductive impairment. By day 6 approximately one sixth of the daphnids in the 100% line effluents were dead. Many of the surviving daphnids in these aquaria bore a peculiar black mottled pattern over their translucent bodies. No attempt was

made to examine them microscopically, since with the low initial sample number, this handling could have biased an eventual statistical evaluation of the bioassay. To avoid recording results which were in effect responses to the trauma of handling the organisms, the colonies were left alone except for monitoring, counting and feeding, to which each aquarium was exposed for a period of about five minutes every 48 hours, and in some cases every 72 hours. No time lapse was allowed before the introduction of the daphnids to the effluents. The parent organisms were initially transferred to the effluent as soon as temperature equilibration was achieved. Those in the more concentrated lime effluents were immediately exposed to the changes in pH which occurred over the first few days. Daphnia used in the four-day exploratory study were introduced into test concentrations which had been allowed to sit in the experimental chamber for three days.

The record of the adverse environment inhabited by the organisms in the 50 and 100% effluents, is demonstrated in Figure 35. By day 12, 25% of the organisms in the 50% lime and alum effluents were dead; while for the 100% effluents the alum organisms suffered approximately 25% mortality and the lime organisms about 70% mortality. The greater response required by the more adverse conditions, is indicated by the dramatic increase in the slope of the mortality curves for the 50 and 100% effluents over this 12 day induction period.

Figure 37 is a bar chart comparing the mean lengths of organism for all effluents. A comparison of this chart with the bar charts for progeny production and total population counts at the end of the bioassay; figures 42 and 43, shows that the larger mean sizes correspond to the

lower total numbers in each case. The inverse relationship between mean size and numbers is clear. Considering the biomass curves in Figure 50, this pattern may be explained on the basis of a combined response to stress and the fertilizer effect of the sewage effluents. The more dilute effluents were limited in essential nutrients. The more concentrated effluents were chemically unbalanced, but were eventually stabilized during the period of accelerated algal growth, directly stimulated by available fertilizing chemicals. In particular all the pictorial representations shown in the results demonstrate less variability for the alum effluents than for the lime effluents. The mean sizes, progeny production and total population counts are more uniform over all effluent concentrations, for the alum effluents. This may be the direct result of the more immediate vigorous growth of algae in the alum effluents triggered by sulphate fertilization. This held the advantage for the populations exposed to a more varied diet; a choice of natural forage, bacteria and Cerofood, the laboratory prepared nutrient. The biomass change over the effluent range is a gradual straight line expression ranging from .050 grams for the 10% effluent, to approximately .080 grams for the 100% effluent. The computation of the biomass is as per Marshall (41).

Biomass = Total Dry Weight of Organisms x 5

By contrast the lime biomass curve is described by a second order expression, with an upper critical inflection point about the 55% effluent concentration. This expression signifies a more dynamic response to environmental stress. Visually the adults in the lime effluents appeared to be generally undersized as is demonstrated by figures 37, 38 and 46. This effect was most pronounced in the case of the 100% repliactes, in

which organisms in addition to being small were of a peculiar opalescent hue. This could perhaps be due to the calcium content of the carapace. Was there a maturation delay or growth retardation caused by the precipitation of vital trace elements necessary for growth by the lime treatment of the secondary sewage effluent? The findings of Marshall (41), concerning the response of Daphnia galeata mendotae to chronic cadmium stress, may be here relevant: a reduction of average population numbers and biomass, and an increase in variability, survivability, turnover rate and adult fraction of the population.

The similarity in curves for the cumulative progeny production and the population biomass, figures 46 and 50 respectively are noted. This may imply that the stimulus effect which influenced progeny generation was maintained throughout the bioassay. The biomass expression, by computation, is indirectly a function of the progeny generation statistic.

In terms of total population on day 39, figure 45 shows the fertilizer influence on the effluents, with the alum showing a decided advantage in productivity over the lime effluents.

The bar graph, figure 46, presents the Final Population Characteristic. This expression has been devised to show the percentage yield in terms of a young to adult differential. Young defined as those organisms passing through the mesh size of side .75 mm, but retained on the smaller mesh size of side .113 mm. It is an index of maturation or growth retardation as influenced by environmental conditions. It gives some idea of the effect of varying effluent concentrations, on the organism. In figure 46 the control group shows the most bias in terms of adult population, because this population was being maintained at a basal level;

corresponding to the nutrient limitations, with a minimum of progeny generation and/or progeny survival. Alum effluents show the best balance. The lime effluents demonstrate the Type-4 Survivorship Curve situation, in which the toxicity affects mostly the young (55). Too high a proportion of young to adults implies either that adults are dying through stress, or more likely that progeny production is accelerated by a few acclimated adults to compensate for accelerated juvenile mortality. Examination of the progeny generation curves Figures 39 and 40, shows that with the exception of the 12.5% effluent concentration, which closely parallels the pattern of the alum effluents, the other lime effluents generate progeny consistently at higher levels than for all other effluents. The lime effluents are decidedly more stressful.

The bar graph shown in Figure 47, presents the Viability Differential, a parameter devised to demonstrate the percentage survival of organisms relative to the numbers of progeny generated. In this bioassay the size of the progeny aquaria was the limiting factor. Since all aquaria were of the same size, all populations were equally limited. The difference in population numbers would be therefore a direct result of the concentration of the tertiary effluents. The statistic is an index reflecting the ability to survive under the prevailing environmental conditions. An index of 100 indicates a survival rate equal to the progeny production rate. An index over or under 100 indicates a corresponding increase or decrease in survival rates.

Figure 47 shows a consistently good performance for all alum effluents and a poor performance for the control and 25% lime concentrations. The best performances are for the 12.5% alum and lime effluents and for the

50% lime effluent.

8.4.5 Numerical Turnover (41)

Table (17) and Figures 48-50 express the Numerical Turnover Rates. These functions are the birth rate, the rate of increase, the death rate, and the mean life expectancy. Their derivation is explained below:

The interval Birthrate, b, is based on Edmonson's (1968) Equation where: (41)

$b = \ln (1 + E)/D$ . . . for:

D, duration of development = 2.6 days
a value for Daphnia galeata mendotae at 20°C Hall (1964). This value is sometimes known as the Marshall Index and assumes that development is independent of stress, nutritional conditions and water chemistry and is a function only of temperature.

$E = \frac{\#\text{ of young produced}}{\#\text{ of females surviving}}$

The Rate of Increase, r, is based on a modified form of Edmonson's (1968) relationship where: (41)

$r = (\ln N_t - \ln N_{t-7}) / \Delta t$ . . . for t = the interval between censuses where N is the number counted at each census.

The Population Death Rate, d, is a combination of the birth rate and the rate of increase, where:

$d = b - r$

The Mean Life Expectancy is an expression of the death rate where:

Mean Life Expectancy = 1 / d

The birth rates plotted in Figure 48 show an increasing rate with

increasing effluent concentration. An increasing death rate is recorded in Table 17 and relates to an increase in effluent concentration. The mean life expectancy as a function of the effluent concentration ranges from 12.5-21 days for effluents below the 30% level of concentration. The organisms in the alum effluents attain a maximum of 15 days, whereas those in the lime effluents extended over the full range. These values show an increase over the range found for the control group of animals in the study on the "Response to Chronic Cadmimum Stress" done by Marshall (41), a range of 12-14 days at 20°C. By contrast the range for the control group in this experiment was, as shown in Figure 49, 15-22.5 days at 20°C. Figure 49 shows the organisms in the lime effluents below the 30% effluent concentration performing best in the mean life expectancy category. This may be related to the availability of vital trace elements and the ambient concentration of the Ca^{++}ion. The health of the organisms may be dependent on a critical biochemical synergistic relationship. A similar relationship may explain the marginally better performance of the alum effluents below the 30% concentration bench mark, compared with the steady decline from the 30 - 100% effluent range. Beyond the 30% level of concentration the ionic character of the effluents perhaps contributes to stress through adverse chelating effects. An examination of the birth rates in Table 17 shows that, compared to the rates found by Hall (1964) for Daphnia galeata mendotae at 20°C, .22-.34 day^{-1}(41), this bioassay performed badly for all categories of effluent.

8.4.6 Predictive Value of Bioassay

"An ecological law does not owe its validity to its indoor or outdoor origin but rather to its predictive value when confronted with actual problems" (55). The microcosm bioassay can be used for interpretation of the more dynamic "natural" environment. In particular an evaluation of the absence of predation may be relevant.

The populations adversely affected by the effluent did recover and appeared capable of re-establishing their vitality as the strength of the pollutant abated. However in an environment of continuous discharge or where predation was a factor, would this have been the case? Continuous discharges in the same zone could result in elimination of this species in a well-defined mixing zone, the niche to be perhaps filled by a species more tolerant of that particular pollutant. Predation would follow a different course. Seldom in nature is predation so severe as to completely eradicate the prey. In times of scarcity of prey if the range is not confined, the predator hunts where the prey is more numerous, and where the energy balance becomes more efficient. The dynamic aspect of the prey-predator relationship allows the prey adequate recovery time. Slobodkin(55) summarizes: "Typically, predators feed on the most abundant food species available at any particular time, and as soon as this shows any sign of scarcity they switch their feeding preferences." This may mean finding new forage grounds. By Lindeman's Rule, the base rate of chase energy expended in search must not exceed the energy intake through feeding.

The computerized curves used in this discussion, represent a probabilistic statement, based on the input information, that forecasts the likely path of the particular function. The more the data points

available to the computer, the more reliability can be assigned to the curve output by the computer. The computer consistently generates a curve or family of curves that will be a characteristic of both the effluent and the dilution water quality.

8.5 Log-Probability and Statistical Assessment Data

8.5.1 Mortality Versus Time

The mortality-time curves presented in Figures 51 and 52 were only based on three points. They may be relied upon only to give some useful indication since for every effluent the data points were in perfect alignment.

In Figure 51 it is evident that the control exceeds the 12.5 and 25% effluents in toxicity. The mortality-time function for the control group has the steepest gradient, implying the highest mortality; this by reason of its most severe nutrient deficiency, as previously noted. At the 12.5% effluent concentration level, the lime is the most toxic. At the 25% effluent level the alum is the most toxic. Figure 52 shows that at the 50% level the lime appears to be the marginally more toxic effluent with a gradient diverging to higher mortality. At the 100% effluent concentration level, the alum effluent has a slope parallel to and by far exceeding the control function in steepness. The bioassay was terminated on day 39. Within this time limit the lime was the most toxic effluent; but the alum effluents were becoming increasingly toxic because of too rapid a depletion of nutrients during the sustained period of

productivity. In the harvest of organisms from the parent aquaria nutrients were in effect being removed from the environmental pool; hence the depletion with time since apart from the Cerofood diet there was no other significant nutrient input. The bioassay was essentially a static bioassay. Replacement of fluids was limited to amounts lost during counting of organisms and the resupply of a total volume of 180 ml during the 39 day period; the volume drawn from each aquarium for analysis.

The level of toxicity was not acute. The 12.5 and 25% effluent levels appear the most beneficial in the test range. The control group was the only function which when extrapolated produced an Effective Time - 84% (ET_{84}) statistic. This value projected that in 150 days at the rate expressed by the function, 84% mortality would have occurred in the control effluents. The low grade toxicity level, without the benefit of the ET84 statistic, implied that for all practical purposes there was no additional information to be gained by applying the method of Litchfield & Wilcoxon (45).

8.5.2 Percent Survival Versus Effluent Concentration

Application of the method of Litchfield & Wilcoxon (45) demonstrated that none of the effluents illicited the acute toxicity response, for which the method was devised. In all cases the ET16 statistic had to be estimated. For days 32 and 39 the dose ratio/slope function statistic was off the scale of the nomograph and could only be estimated.

On day 12, as per figure 53, the Litchfield-Wilcoxon (45) method gave the following information within the limits of 19/20 probability:

1) The curves deviated significantly from parallelism;

2) The lime and alum effluents differed significantly in potency;

3) The alum effluent was more beneficial by a slight margin;

4) The potency ratio, 1.053, was found within the following limits 1.035 to 1.071 implying that, at the level of test, the lime effluents appeared to be 1.053 times more toxic than the alum effluents.

On days 32 and 39 as per Figures 54 and 55 respectively the Litchfield-Wilcoxon method gave the following information within the 19/20 probability limits:

1) The curves deviated significantly from parallelism;

2) The lime and alum effluents differed significantly in potency;

3) The alum effluent was significantly more beneficial than the lime effluents;

4) The potency ratio on day 32 was 1.6, and lay within a 1.5 to 1.7 confidence interval. The lime effluents were, within the error margin, 1.6 times more toxic than the alum.

5) On day 39 the potency ratio was 1.4, within an error margin (1.4 to 1.44).

From this series of graphs, figure 53 - 55, if a common beneficial discharge limit were selected, based on the survival of organisms, the upper limit would be placed at the 25% concentration level. The functions for both types of effluent are approximately coincident at this point and produce survival percentages of 82, 68 and 68% for days 12, 32 and 39 respectively.

8.5.3 Tests of Significance

8.5.3.1 Analysis of Mean Head-Spine Lengths

Using the control group as reference and applying Dunnett's (46) Statistical Analysis (a multivariate analogue of the Student's-t distribution) for one- and two-tailed confidence intervals at the 95% level of test; the comparisons are presented in Table 18 and Figure 56. The control Daphnia were best in this category. The Daphnia in the 12.5 alum and lime effluents are the groups closest to the control.

Mean head to spine lengths were ranked by "A Comparison of Means" (45). The control was most beneficial, followed in order of benefit by the 12.5% lime, and next the 12.5% alum effluent. There were two groupings after these leaders. Next ranked was:

25% Alum, 50% Alum, 50% Lime.

Least beneficial was 25% Lime, 100% Lime and 100% Alum.

8.5.3.2 Significance of Progeny Production, Population and Birthrate

Cumulative progeny production by the parent group was tested for significance using the Dunnett's(46) statistic for a two-tailed confidence interval at a 95% level of significance. No significant difference was found between the control and the other effluents. The confidence margins are presented in Table 19.

The total population on hand on day 39 was analyzed using the same statistic and confidence levels. A significant positive difference in numbers was found in the case of the 100% lime effluents. Only one replicate was available for the 100% alum due to unexplained death of all the Daphnia in the other replicate. When the surviving replicate was used as an average of the two, a significant positive difference was found between this population and the control group. Superior numbers of

diminutive size found for the two worst performers in the head-spine length and mortality of parent Daphnia categories, was perhaps an adaptation to the adverse environment. The confidence margins are presented in Table 19.

Table 20, the Birth Rate Analysis by Dunnett's (46) statistic with a 95% confidence level for a two-tailed test, shows no significant difference between the birth rates of any groups when compared to the control.

9. CONCLUSIONS

The conclusions derived from the comparative bioassay study are:

1. This bioassay study has been preliminary in scope. There is need for further work in several areas for a closer scrutiny of these tentative results.
2. The lime and alum effluents were not acutely toxic. In terms of chronic toxicity, the lime effluents were significantly more potent than the alum effluents. In terms of statistically determined potency ratios the lime effluents were between 1.4 to 34.6 times more active than the alum effluents.
3. The lime and alum effluents caused changes in the response of the Daphnia to the environment. Both effluents produced a generalized fertilization effect on the community of flora and fauna. Whether the performance of these tertiary effluents would have surpassed the performance of a well- balanced control remains unclear.
4. With minor reservations, the alum effluents were more beneficial than the lime because they immediately promoted and sustained a standing crop of algae, that was food for Daphnia which graze and live in community with the algae.
5. Functional reproductive impairment was not clearly demonstrated. After a variable acclimation period, the organisms in the tertiary effluents demonstrated a population recovery in the form of

accelerated progeny production. This was most likely a response to the earlier and continuing stress. This bioassay could not differentiate between a population increase due to stress or to fertilizer effect.

6. The 100% lime effluents required the longest induction period and produced the highest early mortality; but the organisms recovered and sustained birth of progeny at a very high rate.
7. In terms of cumulative progeny production there was no statistically significant difference between the effluents.
8. The more concentrated effluents caused a pronounced diminution in the sizes of offspring, appeared to retard the growth and maturation of the progeny and sustained greater numbers of these organisms compared to the more dilute effluents.
9. The 100% lime effluents were the most toxic to the Daphnia. Early pH fluctuations may have been the cause of the high mortality in the 100% lime effluents.
10. The lime treatment of the secondary effluent reduced the magnesium from 15.2 mg/l to a residual of 0.08 mg/l in the tertiary effluent. Trace elements vital to the well-being of the microcrustacean may have also been similarly precipitated. To a lesser extent the alum precipitation may have had a similar effect on trace minerals.
11. The carbon and nutrient limited control exhibited a Malthusian rate of productivity.
12. The reconstituted water control provided the most beneficial environment in terms of mean life expectancy and mean head-spine length of organisms. This treatment was also the poorest in terms

of progeny production and total final population. The 12.5% lime and 12.5% alum tertiary effluents, in order, were the next best performers in terms of mean head-spine length of organisms.

13. The superior sizes of the organisms in the control and the known nutrient deficiency of the control may indicate that, were the control water adequate in terms of the chemical and nutrient requirements of a healthy algal culture, it could have sustained prolific production.

14. In conducting a bioassay study pertaining to a specific discharge environment, the safest policy would be to use as a control and dilution water, a screened and sterilized sample of the water taken from the intended discharge basin.

15. Before discharge into a given environment, the characteristics of each effluent should be carefully considered:

    (a) With 100% alum-treated effluents, the sulphate fertilization factor should be carefully scrutinized especially where the volume of effluent discharged would be significant, because the level of fertilization may be considerable.

    (b) The 100% lime effluents may cause early pH fluctuations, and require a long acclimation period before the microfauna become well adjusted. Also, there may be impairment of the normal biochemical functioning of microfauna, due to mineral deficiency, or other chemical interferences.

    (c) The alum treatment removes inorganic carbon in amounts that may be required for the continued sustenance of algae. An early decline in the vitality of the community of flora and fauna may

occur, due to the deficiency in inorganic carbon.

16. Holding ponds for tertiary effluent with a minimum retention time of 12 to 20 days are recommended to reduce the environmental hazard. Based on percentage survival, and life expectancy data, the upper limit for discharge, after adequate retention, would be a time-regulated volume, equivalent to a 30% effluent concentration.

10. SUGGESTIONS FOR FUTURE STUDY

The following suggestions for research have been made to bring into focus and/or clarify aspects of this bioassay study that are at the time of this writing in question.

1. The reasons for the mottling and high mortality among daphnids in the 100% lime effluents should be investigated.
2. A detailed study of ephippial production under the stressful conditions of this bioassay should be initiated to determine whether a cause-effect relationship exists that can be quantified.
3. A determination of the optimum alkalinity requirements of Daphnia magna should be conducted.
4. A study should be conducted on the effects of aluminum and turbidity on Daphnia magna to investigate whether optimum and minimum limits for well being exist.
5. The minimum magnesium requirement of Daphnia magna should be investigated.
6. Further comparative bioassay studies of chemicals commonly used in tertiary treatment, and their potential effects on the aquatic environment should be undertaken. Apart from lime, alum, ferrous sulphate and ferric chloride, such a study could include treatments using chemical combinations, for example, the lime plus sodium hydroxide system.

7. A study of the causes of growth retardation in organisms reared in the lime and 100% alum effluent should be launched and some attempt made to isolate the factors responsible. If an absent chemical is responsible, effluents may be fortified by the addition of required quantities so that immediate discharge could be attempted without environmental hazard.

8. A study should be launched to determine whether the bloom caused by the high sulphate content of alum effluents, exceeds the bloom to be expected from discharge of secondary effluents and which effluent is preferable in a given situation.

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12 APPENDICES

12.1 Appendix I

MISCELLANEOUS TABULATED EXPERIMENTAL DATA

	Effluent Concentration	Day 1 Parent Aquaria Dissolved Inorganic Carbon, mg/l	Day 1 Parent Aquaria Dissolved Inorganic Carbon, mg/l	Day 60 Parent Aquaria Dissolved Inorganic Carbon, mg/l	Day 60 Parent Aquaria Dissolved Inorganic Carbon, mg/l	Day 60 Progeny Aquaria Dissolved Inorganic Carbon, mg/l	Day 60 Progeny Aquaria Dissolved Inorganic Carbon, mg/l	Day 42 Turbidity J.T.U.
Control	0%	0.8	3.4	10	24	7	15	.24
		0.8	3.4	8	24	10	22	.40
Lime Tertiary Effluent	12.5%	11.8	4.4	13	25	7	14	.02
		11.8	4.4	16	21	9	14	.06
	25%	22.8	5.5	7	26	10	17	0
		22.8	5.5	13	33	13	17	.04
	50%	44.9	7.5	6	27	21	25	0
		44.9	7.5	12	49	20	24	0
	100%	88.9	11.6	7	33	12	36	0
		88.9	11.6	7	32	7	25	0
Alum Tertiary Effluent	12.5%	3.9	4.3	7	24	1	13	.22
		3.9	4.3	7	23	2	12	.21
	25%	7.1	5.1	1	14	1	10	.18
		7.1	5.1	1	14	1	13	.23
	50%	13.4	6.9	1	14	1	13	.22
		13.4	6.9	1	13	1	13	.12
	100%	25.9	10.3	1	22	1	22	.18
		25.9	10.3	1	54	1	29	.35

TABLE 21. Effluent monitoring for Dissolved Inorganic Carbon and Dissolved Organic Carbon in mg/l and Turbidity in Jackson Turbidity Units (J.T.U.) at times shown. Note, on Day 1 values for effluent dilutions were estimated from the 100% effluents and reconstituted dilution water by the weighted mean method.

	Effluent Concentration	Day 1 Total NH_3, mg/l	Day 1 Total Unionized NH_3, µg/l	Day 15 Total NH_3, mg/l	Day 15 Total Unionized NH_3, µg/l	Day 28 Total NH_3, mg/l	Day 28 Total Unionized NH_3, µg/l	Day 42 Total NH_3, mg/l	Day 42 Total Unionized NH_3, µg/l
Control	0%	4.94	18.3	1.24	4.6	1.24	9.9	0.41	115
		4.94	18.3	1.24	4.6	1.65	19.8	0.41	115
Lime Tertiary Effluent	12.5%	3.46	12.8	2.06	18.5	1.85	19.8	0.83	125
		3.38	12.5	1.98	19.8	1.40	42	0.58	162
	25%	1.65	6.1	0.41	5.33	1.24	46	0.41	53
		1.65	6.1	1.40	32.2	2.06	51.5	1.07	96
	50%	1.57	5.8	1.24	28.5	1.48	88.8	1.07	139
		2.06	7.6	0.58	17.4	1.48	133	1.65	248
	100%	2.47	9.1	1.40	84.0	1.40	70	1.24	136
		2.47	9.1	0.41	24.6	1.98	178	0.41	15.1
Alum Tertiary Effluent	12.5%	4.12	15.2	2.88	10.7	3.05	27	0.58	64
		5.36	19.8	2.88	14.4	3.3	16.5	0.58	45
	25%	6.18	22.9	5.77	28.9	5.77	104	1.65	99
		6.59	24.4	5.77	28.9	5.77	46	4.94	237
	50%	12.4	45.9	9.06	45.3	7.83	94	4.94	247
		12.8	47.4	9.89	49.5	7.83	391	4.94	183
	100%	25.6	94.7	16.9	152	16.2	194	13.2	488
		23.5	87.0	16.5	165	16.1	60	12.4	13

TABLE 22. Environmental monitoring for Total Ammonia as mg/l NH_3 and Total Unionized Ammonia as µg/l NH_3. Samples drawn from parent aquaria for interval testing as shown. Values for Unionized Ammonia found by use of Table 5 for ideal case using distilled water.

	Effluent Concentration	Day 1 Parent Aquaria Total Hardness mg/l	Day 1 Parent Aquaria Ca^{++} Hardness mg/l	Day 15 Parent Aquaria Total Hardness mg/l	Day 15 Parent Aquaria Ca^{++} Hardness mg/l	Day 25 Parent Aquaria Total Hardness mg/l	Day 25 Parent Aquaria Ca^{++} Hardness mg/l	Day 42 Parent Aquaria Total Hardness mg/l	Day 42 Parent Aquaria Ca^{++} Hardness mg/l	Day 60 Progeny Aquaria Total Hardness mg/l	Day 60 Progeny Aquaria Ca^{++} Hardness mg/l
Control	0%	98	66	122	91	136	100	128	104	132	112
		98	66	122	91	136	105	112	92	144	116
Lime Tertiary Effluent	12.5%	108	80	138	127	158	145	148	116	144	120
		108	80	143	127	164	142	148	120	152	120
	25%	128	106	151	161	164	146	164	132	154	148
		128	106	163	161	184	152	172	144	168	144
	50%	172	138	202	209	220	200	204	172	216	192
		172	138	202	209	220	213	144	152	200	176
	100%	138	248	159	235	172	172	92	72	132	88
		138	248	129	235	124	132	132	120	92	88
Alum Tertiary Effluent	12.5%	96	72	124	89	140	113	144	104	152	76
		96	72	126	89	144	113	148	104	156	104
	25%	106	74	132	105	148	113	152	112	176	124
		106	74	140	105	160	128	156	116	160	116
	50%	126	82	155	118	172	127	172	116	192	140
		126	82	152	118	168	116	172	120	196	136
	100%	170	104	201	147	220	167	212	136	244	168
		170	104	196	147	212	142	208	144	236	160

TABLE 23. Effluent monitoring for Total Hardness and Calcium Hardness as mg/l $CaCO_3$. Magnesium hardness may be inferred by the difference. Initial values on day one for Total and Calcium Hardness were found by interpolation from 100% effluents and reconstituted water.

	Effluent Concentration	Day 1 Parent Aquaria T.Alk. as mg/l $CaCO_3$	Bic.Alk. as mg/l $CaCO_3$	$CO_3^=$Alk. as mg/l $CaCO_3$	Day 42 Parent Aquaria T.Alk. as mg/l $CaCO_3$	Bic.Alk. as mg/l $CaCO_3$	$CO_3^=$Alk. as mg/l $CaCO_3$	Day 42 Progeny Aquaria T.Alk. as mg/l $CaCO_3$	Bic.Alk. as mg/l $CaCO_3$	$CO_3^=$Alk. as mg/l $CaCO_3$	Day 60 Progeny Aquaria T.Alk. as mg/l $CaCO_3$	Bic.Alk. as mg/l $CaCO_3$	$CO_3^=$Alk. as mg/l $CaCO_3$
Control	0%	12	12	0	22	19.5	2.5	38	33	5	48	48	0
		10	10	0	21	16	5	32	24	8	58	58	0
Lime Tertiary Effluent	12.5%	46	46	0	36	30	6	46	39	7	52	50	2
		46	46	0	42	34	8	62	49	13	60	58.5	1.5
	25%	78	78	0	75	69.5	5.5	88	81	7	80	73	7
		80	80	0	71	66	5	78	72	6	86	83	3
	50%	152	152	0	128	122	6	104	98	6	132	130.8	1.2
		152	152	0	77	71	6	50	46	4	124	119.5	4.5
	100%	284	284	0	52	48	4	40	38	2	78	76	2
		284	284	0	94	91	3	76	74	2	56	53	3
Alum Tertiary Effluent	12.5%	16	16	0	13	13	0	10	10	0	14	14	0
		20	20	0	6	6	0	8	8	0	16	16	0
	25%	28	28	0	15	15	0	6	6	0	6	6	0
		28	28	0	19	19	0	10	10	0	10	10	0
	50%	42	42	0	8	8	0	8	8	0	12	12	0
		42	42	0	22	22	0	6	6	0	12	12	0
	100%	72	72	0	20	20	0	6	6	0	6	6	0
		72	72	0	2	2	0	0	0	0	2	2	0

TABLE 24. Environmental monitoring for Alkalinity as mg/l $CaCO_3$, in parent and progeny aquaria.

	Effluent Concentration	Time of Count in Minutes	Numbers of Young	Numbers of Adults	Total Numbers	% Young	% Adults	Viability Differential %
Control	0%	zero	60	299	359	17	83	64
		+615	57	327	384	15	85	69
Lime Tertiary Effluent Replicates Shown	12.5%	+420	558	466	1024	54	46	130
		+555	153	824	977	15	85	197
	25%	+645	1162	89	1251	93	7	57
		+1145	688	171	859	80	20	76
	50%	+800	547	809	1356	40	60	151
		+1060	1468	363	1831	80	20	81
	100%	+195	1845	179	2024	91	9	130
		+465	2645	112	2757	96	4	134
Alum Tertiary Effluent Replicates Shown	12.5%	+75	1364	733	2097	65	35	184
		+1105	449	1097	1546	29	71	123
	25%	+930	607	720	1327	46	54	114
		+1015	795	807	1602	50	50	111
	50%	+675	1100	122	1222	90	10	122
		+765	539	850	1389	39	61	186
	100%	+705	1281	859	2140	60	40	94
		*	*0	*0	*0			

TABLE 25 Final population count data. Duration 39 days. Final Population Characteristic shown as a percentage of young and adults in final population. Data for replicates.

$$\text{Viability Differential Index} = 100 + \frac{(\text{Final Population Count} - \Sigma \text{progeny generated by-parent group}) \times 100}{\Sigma \text{ progeny generated by parent group}}$$

* No life evident. Population eliminated by mysterious toxic circumstances. Noted after day 32.

	Effluent Concentration	Total population after 39 days	Population dry weight grams	Population ash weight grams	Population biomass = dry weight X5	Progeny produced in 39 days	Ephippia produced in 39 days
Control	0%	359	.0736	.0220	.368	559	72
		384	.1782	.0356	.891	560	35
Lime Tertiary Effluent	12.5%	1024	.0952	.0405	.476	789	30
		977	.1273	.0435	.637	503	54
	25%	1251	.1886	.0386	.943	2193	10
		859	.0706	.0256	.353	1137	39
	50%	1356	.1740	.0543	.870	900	18
		1831	.0964	.0317	.482	2263	18
	100%	2024	.1316	.0615	.658	1556	7
		2757	.1388	.0575	.694	2065	10
Alum Tertiary Effluent	12.5%	2097	.1383	.0508	.692	1141	22
		1546	.1740	.0570	.870	1259	88
	25%	1327	.1260	.0332	.630	1169	33
		1602	.1475	.0450	.738	1448	54
	50%	1222	.0872	.0426	.436	998	74
		1389	.1878	.0532	.938	747	14
	100%	2140	.1814	.0527	.907	2275	9
		*0				766*	13

TABLE 26. Summary of Total population on hand after 39 days, Dry weight, Ash weight, Population Biomass, Cumulative progeny produced by original parent group in 39 days, ephippia produced by parent group in 39 days.

* Note by day 39 the population in this replicate aquarium had disappeared. Simultaneous disappearance in parent aquarium unidentified toxic circumstances.

12.2 APPENDIX II

SAMPLE STATISTICAL ANALYSES

12.2.1. Test for Equality of Means

12.2.2. A Comparison of Means

12.2.3. Multivariate Analyses of Mean Head - Spine Lengths

12.2.4. Dunnett's - Statistic for Comparing Means With a Control

12.2. Sample Statistical Analyses

12.2.1. Test for Equality of Means (47)

MODEL

$$Y_{ij} = \mu_i + \varepsilon_{ij}$$

where Y_{ij} is the j-th observation of the i-th group;

μ_i is the mean of the i-th group; and,

ε_{ij} error is normally distributed with mean 0 and unknown variance σ^2.

To test H_o $(\mu_i = \mu_2 = ... = \mu_n)$ versus

H_a $(\mu_i \neq \mu_j$ for some ij)

$$\text{Correction factor} = \frac{(GT)^2}{N} = \frac{\chi^2}{N}$$

Raw data input: Head to spine measurements of daphnids randomly selected from all 18 progeny aquaria at the end of the experiment. Replicates combined as one sample ie. n = 50. For 100% alum where death occurred in one aquarium n = 25.

		Lime					Alum				
	Control	12.5%	25%	50%	100%	12.5%	25%	50%	100%	Row Manipulations
Σx_i =	102.6	86.2	64.3	67.1	59.5	74.6	66.4	69.8	31.4	Sum = χ = 621.9
$\bar{x}$ =	2.05	1.69	1.29	1.35	1.19	1.49	1.33	1.42	1.26	Average = $\bar{\chi}$ = 1.46
$(\Sigma x_i)^2$ =	10526.76	7430.44	4134.49	4502.41	3540.25	5565.16	4408.96	4872.04	985.96	
$n_i = \frac{\Sigma x_i}{\bar{x}}$	50.05	51.01	49.84	49.70	50.0	50.07	49.92	49.15	24.92	Sum = N = 424.66
$(\Sigma x_i)^2/n_i$ =	210.32	145.67	82.96	90.59	70.81	111.15	88.32	99.13	39.57	

Total Sum of Squares:

$$\sum_i \sum_j \chi_{ij}^2 - \frac{\chi^2}{N} = (97.92 + 116.71 + 65.03 + \ldots + 40.18) - \frac{(621.9)^2}{424.66}$$

$$= 47.21$$

Among-groups Sum of Squares:

$$\sum_i \frac{\chi_i^2}{\eta_i} - \frac{\chi^2}{N} = \frac{102.6^2}{50.05} + \frac{86.2^2}{51.01} + \ldots + \frac{31.4^2}{24.92} - \frac{(621.9)^2}{424.66}$$

$$= 27.77$$

Within-groups Sum of Squares = Total Sum of Squares - Among groups Sum of Squares:

$$= 47.21 - 27.77$$

$$= 19.44$$

Analysis of Variance Table:

Source	df	SS	MS	F
Treatment	8	27.77	3.47	74.26
Error	416	19.44	0.046731	
Totals	424	47.21		

To test H_o ($\mu_1 = \mu_2 = \mu_3 = \ldots = \mu_9$) versus

H_a ($\mu_i \neq \mu_j$ for some μ_{ij})

Sample F value :

$$F = \frac{3.47}{.046731} \equiv 74.3$$

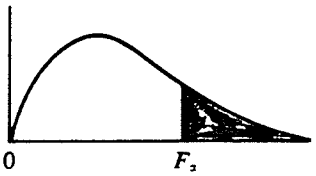
We reject null hypothesis if $F > F_\alpha$

We choose $\alpha = .05$

From Tables [28]

$$F_{.05,\ 8,\ 416} = F_{.05,\ 8,\ \alpha}$$

$$F = 1.94 < 74.3$$



Degrees of Freedom $\alpha = .05$

ν_1

ν_2	1	2	3	4	5	6	7	8	9
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04
120	3.92	3.07	2.68	2.45	2.29	2.17	2.09	2.02	1.96
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88

TABLE 28 Percentage points of the F distribution (50).

Decision: We reject the null hypothesis of equal means at the 5% level of test significance.

We accept H_a ie $(\mu_i \neq \mu_j)$

12.2.2. A Comparison of Means

1. Arrange means in ascending order of magnitude

   $\bar{x}_1, \bar{x}_2, \bar{x}_3, \bar{x}_4, \ldots, \bar{x}_n$

2. We should use the t - statistic but because of similarity we can use the normal distribution ie Z (48).

3. Finally group as to means difference.

$$Z = \frac{\bar{x}_2 - \bar{x}_1}{\sqrt{.046731}\ \sqrt{\frac{1}{\eta_1} + \frac{1}{\eta_2}}}$$

Means Test if:

$Z < 1.96$ implies no difference ie homogeneous

$Z > 1.96$ implies a difference. We start with $\bar{x}_2$.

We take $Z = \dfrac{\bar{x}_3 - \bar{x}_1}{\sqrt{.046731}\ \sqrt{\frac{1}{\eta_1} + \frac{1}{\eta_3}}}$

If $Z < 1.96$ continue for $\dfrac{\bar{x}_\eta - \bar{x}_1}{\sqrt{.046731}\ \sqrt{\frac{1}{\eta_1} + \frac{1}{\eta_n}}}$

If $Z = \dfrac{\bar{x}_4 - \bar{x}_1}{\sqrt{.046731}\ \sqrt{\frac{1}{n_1} + \frac{1}{n_4}}} > 1.96$

This implies a difference; we now use $\bar{x}_4$ as the new base.

$$Z = \frac{\bar{x}_5 - x_4}{\sqrt{.046731}\ \sqrt{\frac{1}{n_4} + \frac{1}{n_5}}}$$

Ranking:

Lime 100%	Alum 100%	Lime 25%	Alum 25%	Lime 50%	Alum 50%	Alum 12.5%	Lime 12.5%	Control
x_1=1.19	$\bar{x}_2$=1.26	x_3= 1.29	x_4= 1.33	x_5= 1.35	x_6= 1.42	x_7= 1.49	x_8= 1.69	x_9 = 2.05
n_1=50	n_2=25	n_3= 50	n_4= 50	n_5= 50	n_6= 50	n_7= 50	n_8= 50	n_9 = 50

$Z_1 = \dfrac{1.26 - 1.19}{\sqrt{.046731}\ \sqrt{\frac{1}{50} + \frac{1}{25}}} \equiv 1.32 < 1.96$ implies no difference.

$Z_2 = \dfrac{1.29 - 1.19}{\sqrt{.04673}\ \sqrt{.04 + .04}} = 1.64 < 1.96$ implies no difference

$Z_3 = \dfrac{1.33 - 1.19}{.0611} = 2.29 > 1.96$ implies a difference.

$Z_4 = \dfrac{1.35 - 1.33}{.0611} = .3271 < 1.96$ implies no difference.

$Z_5 = \dfrac{1.42 - 1.33}{.0611} = 1.47 < 1.96$ implies a difference.

$Z_6 = \frac{1.49 - 1.33}{.0611} = 2.61 > 1.96$ implies a difference.

$Z_7 = \frac{1.69 - 1.49}{.0611} = 3.2 > 1.96$ implies a difference.

$Z_8 = \frac{2.05 - 1.69}{.0611} = 5.88 > 1.96$ implies a difference.

Grouping; from a standpoint of size the highest group is most beneficial.:

Group 1	Group 2	Group 3	Group 4	Group 5
100% Lime	25% Alum	12.5% Alum	12.5% Lime	Control
100% Alum	50% Lime			Group
25% Lime	50% Alum			

12.2.3. Multivariate Analysis of Mean Head - Spine Lengths (49).

		Lime				Alum			
	Control	12.5%	25%	50%	100%	12.5%	25%	50%	100%
Sum of lengths, mm	102.6	86.2	64.3	67.1	59.5	74.6	66.4	69.8	31.4
Observation = η	50	50	50	50	50	50	50	50	25
Sample Mean = $\bar{x}$	2.05	1.69	1.29	1.35	1.19	1.49	1.33	1.42	1.26
Variances = σ^2	.0854	.0398	.0232	.0420	.0396	.0339	.0510	.0786	.0309

for p, number of treatments excluding control = 8

for N, number of observation per sample = 25.

$df = (\Sigma N_i) - (p + 1) = 425 - 9$

$= 416$

TABLE 29 Table of t for two-sided comparisons between p treatment means and a control for a joint confidence coefficient of P = 95%. (49).

	p, Number Of Treatment Means (Excluding The Control)								
d.f.	1	2	3	4	5	6	7	8	9
5	2.57	3.03	3.39	3.66	3.88	4.06	4.22	4.36	4.49
6	2.45	2.86	3.18	3.41	3.60	3.75	3.88	4.00	4.11
7	2.36	2.75	3.04	3.24	3.41	3.54	3.66	3.76	3.86
8	2.31	2.67	2.94	3.13	3.28	3.40	3.51	3.60	3.68
9	2.26	2.61	2.86	3.04	3.18	3.29	3.39	3.48	3.55
10	2.23	2.57	2.81	2.97	3.11	3.21	3.31	3.39	3.46
11	2.20	2.53	2.76	2.92	3.05	3.15	3.24	3.31	3.38
12	2.18	2.50	2.72	2.88	3.00	3.10	3.18	3.25	3.32
13	2.16	2.48	2.69	2.84	2.96	3.06	3.14	3.21	3.27
14	2.14	2.46	2.67	2.81	2.93	3.02	3.10	3.17	3.23
15	2.13	2.44	2.64	2.79	2.90	2.99	3.07	3.13	3.19
16	2.12	2.42	2.63	2.77	2.88	2.96	3.04	3.10	3.16
17	2.11	2.41	2.61	2.75	2.85	2.94	3.01	3.08	3.13
18	2.10	2.40	2.59	2.73	2.84	2.92	2.99	3.05	3.11
19	2.09	2.39	2.58	2.72	2.82	2.90	2.97	3.04	3.09
20	2.09	2.38	2.57	2.70	2.81	2.89	2.96	3.02	3.07
24	2.06	2.35	2.53	2.66	2.76	2.84	2.91	2.96	3.01
30	2.04	2.32	2.50	2.62	2.72	2.79	2.86	2.91	2.96
40	2.02	2.29	2.47	2.58	2.67	2.75	2.81	2.86	2.90
60	2.00	2.27	2.43	2.55	2.63	2.70	2.76	2.81	2.85
120	1.98	2.24	2.40	2.51	2.59	2.66	2.71	2.76	2.80
inf.	1.96	2.21	2.37	2.47	2.55	2.62	2.67	2.71	2.75

Calculation of Average Variance = S_2

$$S_2 = \frac{\sum_{i=1}^{25}\sum_{i=1}^{25} x_i^2 - \left(\frac{102.6^2}{50} + \frac{86.2^2}{50} + \frac{64.3^2}{50} + \ldots + \frac{31.4^2}{25}\right)}{(\Sigma N_i) - (p+1)}$$

$$= \frac{957.95 - (899.61 + 19.72)}{416}$$

$$S^2 = .0928$$

$$S = .3047$$

Estimated standard error of a difference between two means

$$= S\sqrt{2/N}$$

$$= .3047\ \sqrt{2/25}$$

$$= .0862$$

Allowance, $A = ts\sqrt{2/N}$

Using Table 29 for p = 8 and df = 416 find t = 2.71 for 95% confidence.

$$A = 2.71 \times .3047\ \sqrt{2/25}$$

$$= .2336$$

Therefore for two-sided limits the experimenter can conclude with 95% confidence that the mean head to spine lengths of the organisms for the two processes are depressed, compared with those of the control, by the following amounts in mm.

Treatment mean - Control mean ± A

Lime				Alum			
12.5%	25%	50%	100%	12.5%	25%	50%	100%
-.1264	-.5264	-.4664	-.6264	-.3264	-.4864	-.3964	-.5564
-.5936	-.9936	-.9336	-1.0936	-.7936	-.9536	-.8635	-1.0236

Calculating A for one sided limits.

Using Table 30, p = 8, df = ∞, t = 2.38 for 95% confidence.

$$A = 2.38 \times .3047 \sqrt{2/25}$$

$$= .2051$$

For one-sided limit we can conclude with 95% confidence that the mean head to spine lengths of the organisms for these processes are depressed compared with those of the control of the following amounts in mm. Treatment mean - Control mean ±A

Lime				Alum			
12.5%	25%	50%	100%	12.5%	25%	50%	100%
-.155	-.555	-.495	-.655	-.3549	-.515	-.425	-.585
-.565	-.965	0.905	-1.065	-.765	-.925	-.835	-.995

12.2.4. Dunnett's t-Statistic for Comparing Means with a Control (46)

Analysis of cumulative progeny production:

η = number of trials per batch = 2

k = number of treatments (including the control) = 9

df = degrees of freedoms for MS_{error} = (ΣN_i) - (p + 1)

p = number of treatments (excluding the control)

$df_{(methods)}$ = k - 1 = 8

H_o = progeny production in all groups (equal)

H_a ≠ progeny production

TABLE 30 Table of t for one-sided comparisons between p treatment means and a control for a joint confidence coefficient of P = 95%. (49).

	p, Number Of Treatment Means (Excluding The Control)								
d.f.	1	2	3	4	5	6	7	8	9
5	2.02	2.44	2.68	2.85	2.98	3.08	3.16	3.24	3.30
6	1.94	2.34	2.56	2.71	2.83	2.92	3.00	3.07	3.12
7	1.89	2.27	2.48	2.62	2.73	2.82	2.89	2.95	3.01
8	1.86	2.22	2.42	2.55	2.66	2.74	2.81	2.87	2.92
9	1.83	2.18	2.37	2.50	2.60	2.68	2.75	2.81	2.86
10	1.81	2.15	2.34	2.47	2.56	2.64	2.70	2.76	2.81
11	1.80	2.13	2.31	2.44	2.53	2.60	2.67	2.72	2.77
12	1.78	2.11	2.29	2.41	2.50	2.58	2.64	2.69	2.74
13	1.77	2.09	2.27	2.39	2.48	2.55	2.61	2.66	2.71
14	1.76	2.08	2.25	2.37	2.46	2.53	2.59	2.64	2.69
15	1.75	2.07	2.24	2.36	2.44	2.51	2.57	2.62	2.67
16	1.75	2.06	2.23	2.34	2.43	2.50	2.56	2.61	2.65
17	1.74	2.05	2.22	2.33	2.42	2.49	2.54	2.59	2.64
18	1.73	2.04	2.21	2.32	2.41	2.48	2.53	2.58	2.62
19	1.73	2.03	2.20	2.31	2.40	2.47	2.52	2.57	2.61
20	1.72	2.03	2.19	2.30	2.39	2.46	2.51	2.56	2.60
24	1.71	2.01	2.17	2.28	2.36	2.43	2.48	2.53	2.57
30	1.70	1.99	2.15	2.25	2.33	2.40	2.45	2.50	2.54
40	1.68	1.97	2.13	2.23	2.31	2.37	2.42	2.47	2.51
60	1.67	1.95	2.10	2.21	2.28	2.35	2.39	2.44	2.48
120	1.66	1.93	2.08	2.18	2.26	2.32	2.37	2.41	2.45
inf.	1.64	1.92	2.06	2.16	2.23	2.29	2.34	2.38	2.42

$$t = \frac{\bar{T}_j - \bar{T}_o}{\sqrt{2MS_{error}}/17}$$

$$= \frac{\bar{T}_j - \bar{T}_o}{\sqrt{MS_{error}}} \quad \text{for } \eta = 2$$

		Lime				Alum			
	Control	12.5%	25%	50%	100%	12.5%	25%	50%	100%
	559	789	2193	900	1536	1141	1169	998	2275
	560	503	1137	2263	2065	1259	1448	747	766
T_j	1119	1292	3330	3163	3621	2400	2617	1745	3041
$\Sigma(\chi_j^2)$	626081	875530	6102018	5931169	6685361	2886962	6350227	1554013	5762381
$\bar{T}_j$	559.5	646	1665	1581.5	1810.5	1200	1308.5	872.5	1520.5

$G = \Sigma(T_j) = 22328$; $\Sigma(\Sigma\chi^2) = 36773742$

(1) $= G^2/k\eta \equiv (22328)^2/18 = 27696644$

(2) $= \Sigma(\Sigma\chi^2) \equiv 36773742$

(3) $= (\Sigma T_j^2)/\eta \equiv (1119^2 + 1292^2 + \ldots + 3041^2)/2$

$= 31013965$

$SS_{methods} = (3) - (1) = 3317321$

$SS_{error} = (2) - (3) = 5759777$

$SS_{total} = (2) - (1) = 9077098$

Source of Variation	SS	df	MS	F
Methods	3317321	8	414665.13	.64793
Experimental Error	5759777	9	639975.22	
Total	9077098	17		

From Table 29, $T_{.95\ (F,\ 9)} = 3.48$

$$t = \frac{646 - 559.5}{\sqrt{639975.22}} = .108$$

Since $.108 < t_{critical} = 3.48$ we accept H_o

$$\text{Allowance, } A = t_{.95}\ \sqrt{2MS_{error}/n}$$

$$= 3.48\ \sqrt{639975.22}$$

$$= 2784$$

$$\text{Confidence Interval} = \bar{T}_j - \bar{T}_o \pm$$

$$\text{For 12.5\% Lime CI} = (646 - 559.5) \pm 2784$$

$$= 84 \pm 2784$$

Hence at the 95% level of test we can state that there is no significant difference between the progeny production in the control group and the 12.5% lime effluent.

This iteration is continued for all groups.

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14. VITA

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1. Born April 25, 1944, at Cocoyea Village (San Fernando) Trinidad, W.I.
2. Attended Naparima Boy's High School, Paradise Hill, San Fernando, from January 1957 to December 1963.
3. Worked as a High School Teacher at A.S.J.A. College, and San Fernando Secondary School, until September 1966.
4. Attended the University of Manitoba from September 1966 to September 1969. Graduated with Bachelor of Science degree in Chemistry and Zoology, October, 1969.
5. Worked as the Pathologist's Assistant at the Victoria General Hospital, Winnipeg, from December 1970 to September 1973.
6. Enrolled in Civil Engineering at the University of Manitoba, from September 1973. Graduated with B.Sc. Civil Engineering, April 1977. Held summer positions as surveying Instrument man, Site Inspector and Engineering Aid with Underwood McLellan and Associates and the City of Winnipeg. Completed course work and thesis research in M.Sc. programme (Sanitary Engineering) by June 1978. Employed by the City of Winnipeg Parks and Recreation Department as The Contracted Projects Supervisor, from May 1978 to December 1980.
7. Married, one child.