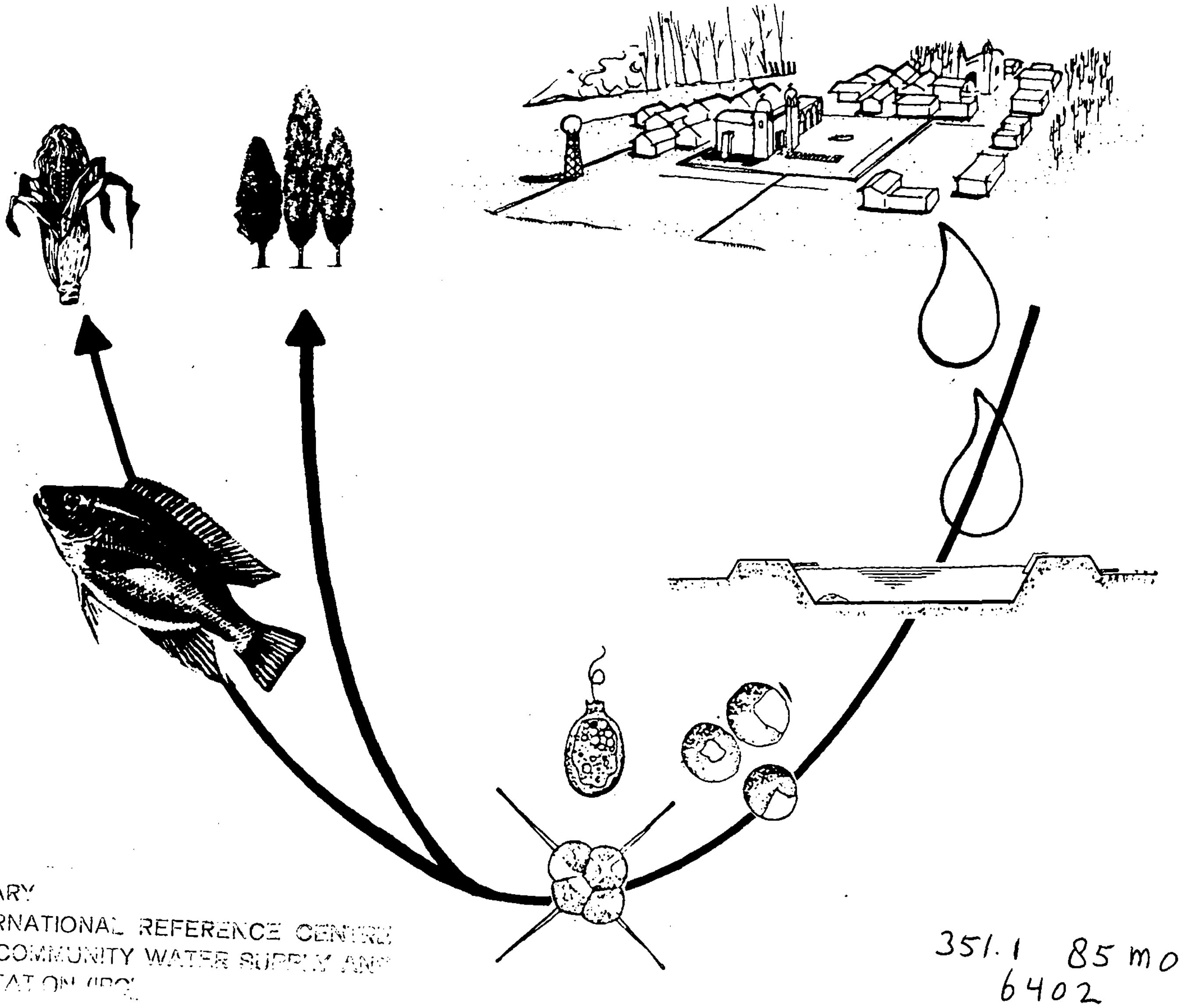
ITORING AND MAINTENANCE OF TREATED WATER QUALITY IN THE SAN JUAN LAGOONS SUPPORTING AQUACULTURE

(UNDP/WORLD BANK/GTZ INTEGRATED RESOURCE RECOVERY PROJECT GLO/80/004)

FINAL REPORT OF PHASES I-II



PAHO/WHO
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PAN AMERICAN CENTER FOR SANITARY
ENGINEERING AND ENVIRONMENTAL SCIENCES

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1. INTRODUCTION

1.1 General

The San Juan de Miraflores sewage reuse project is aimed at providing safe effluent for reutilization in agriculture and for other purposes, as shown in Table 1. The project began in 1961 with the construction of experimental waste stabilization ponds for the treatment of domestic sewage from nearby slum areas. These entered into operation in 1964, and the effluents have been reutilized for the irrigation of an area which today extends to over 500 ha with another 1300 ha planned for green belts with low irrigation requirements. Since 1977 CEPIS has undertaken extensive field and laboratory studies of the quality of the effluents, in cooperation with the Peruvian public health authorities, with the aim of evaluating the health risks of reuse and developing needed sanitary control measures for public health protection. Some of these studies are completed and have been reported (Yánez, *et al*, 1980; Yánez, 1983 and 1984), while additional investigations are underway or are being proposed in order to complete the analysis of effluent reuse. Figure 1 depicts the San Juan reuse eco-system, showing the components studied including those covered in the current research. The general site plan is shown in Figure 2.

Results of the previous investigations show that treatment in multicell ponds is efficient and that with adequate operation the ponds can produce effluents which meet common water quality guidelines for unrestricted irrigation with respect to parasites and indicator bacteria (WHO, 1973). Current studies are focused on the sanitary quality of fish and prawns produced in polishing ponds. Also, an evaluation is underway of potential groundwater pollution problems deriving from infiltration at the experimental site (Foster, 1982). Additional studies are proposed on the sanitary quality of agricultural products including bacteriological, virological and toxicological investigations of edible produce, together with an evaluation of the epidemiological and socioeconmic status of the user population (Bartone, *et al*, 1984).

The health, socioeconomic and environmental benefits resulting from safe, controlled reuse projects are many: the recovery of arid lands for agriculture and aquaculture; the creation of employment and settlement opportunities; increases in food production which can help resolve protein deficits and improve nutrition; the potential for recreational opportunities and amenities through the creation of parks and greenbelts; and the development of a viable alternative to other forms of sewage disposal and their corresponding pollution and public health problems. These benefits indicate that reuse may be one way of self-financing sewage disposal works.

TABLE 1

Reuse at San Juan Experimental Site

SILVICULTURE

- Creation of green belt
- Recreational parks
- Recovery of sanitary landfill site

AGRICULTURE

- Horticultural products (squatters)
  - vegetables, fruit trees
- Forage crops (squatters)
  - alfalfa
- Livestock watering (squatters)
- Animal feed for zoological park
  - papaya, banana, corn
- Commercial flower raising
  - roses

AQUACULTURE

- Fish culture
  - tilapia
  - giant Malaysian shrimp
- Aquatic plants for poultry feed
  - duckweed

In the case of Lima, which is situated in one of the world's driest coastal regions, reuse appears to be so promising that the Peruvian Government is considering a major development project to irrigate 5000 ha of desert land at the Pampas of San Bartolo using 5 m^3/s of urban sewage from the southern half of the city (SERPAR, 1981). The research results from the San Juan experimental site will serve to evaluate the sanitary implications of the larger San Bartolo project, and to formulate an appropriate sanitary code for such an extensive reuse scheme.

A preliminary socioeconomic survey was carried out on the population in direct contact with the San Juan experimental project (Matos Mar, 1984). Three different population groups have been identified. First there is the site worker population consisting of eight SERPAR workers responsible for the operation and maintenance of the site and of the ponds themselves, and for silviculture activities (principally eucalyptus forests and recreational parks) and experimental farming (animal food crops for the zoological park). Then there are sixteen farm families who have settled in the perimeters of the site itself, occupying 36.5 ha, and who irrigate with treated sewage effluents. These families, known locally as the precarios, are squatters who invaded the site during the 1960's and have managed to remain although under continued threat of eviction. The site authorities exercise little or no control over their use of the water nor over the cultivation or marketing of food or animal forage crops which they grow. Immediately adjacent to the experimental site two other groups have settled more recently comprising 51 families on 171 ha of desert land. Both these groups take water from raw sewage canals which are meant to irrigate a nearby greenbelt area, and use it for farmyard irrigation and livestock watering. In all 388 persons are estimated to be directly involved in sewage reuse activities.

These three local populations use the San Juan effluents, raw or treated, principally for agriculture. Most obtain water for drinking and domestic use from vendors who bring it in tanker trucks. Local water application rates for farm irrigation in the desert climate reach about 1 lps/ha, and water is applied by flood irrigation every 5-7 days. Crops are grown year-around. Typical productivity at San Juan is in excess of 500 tons/year and includes forage crops, fruit trees and horticultural produce (corn, potatoes, onions, tomatoes, squash, beans, celery and lettuce). All of the farm families without exception raise farm animals such as cows, pigs, goats, chickens and ducks.

Food crops are either consumed directly, sold to neighboring populations, or sold in truckload by the growers to intermediaries who in turn market them in nearby slum communities including the principal markets of San Juan de Miraflores and Villa María del Triunfo (Zapater, 1983; Matos Mar, 1984). Many small communities have grown up on the surrounding sand dune hillsides that completely encircle the San Juan site today.

Epidemiological data were analyzed by Campos (1984) for the populations in the districts producing the sewage and those consuming the produce from the reuse site. These data show that the most prevalent infectious diseases in these districts are: acute diarrheal diseases (principal agents are rotavirus, enterotoxigenic and enteropathogenic E. Coli, Campylobacter, Salmonella, Shigella); typhoid and paratyphoid fever; viral hepatitis; polio; and intestinal parasites such as Entamoeba histolytica and Giardia lamblia. The mortality and morbidity rates associated with these diseases in Lima are high. For example, deaths from acute diarrheal diseases in infants reach 12.8 per mil and represent the leading cause of child mortality in Peru (PAHO, 1982). The typhoid and paratyphoid fever rate in Peru is 1.79 per mil and is the highest in Latin America. Data for Lima for selected enteric diseases, often water-bourne, is shown in Table 2.

TABLE 2

Mortality and morbidity data for selected enteric diseases. Lima, 1980

(Total population = 4'542,437, under 5 years = 663,200)

CAUSE	DEATHS		REPORTED CASES	
	Total	5 yr	Total	5 yr
Acute diarrheal diseases	1,686	1,355	35,852	26,431
Typhoid fever	154	39	8,142	1,097
Hepatitus	50	9	2,574	1,156
Polio	15	12	121	117

Source: Campos (1984)

Data collected by Lucas (1980) on intestinal parasites near San Juan indicate that the rate of infection by Ascaris lumbricoides was 31.1% in a group of 61 subjects from families living and working at the San Juan pond site, while the rate for a group of 139 students from a local school 1 km from site was only 5.8%. Although the experimental design of the study does not permit hypothesis testing, these results indicate that a detailed study should be conducted of Ascaris lumbricoides transmission around the reuse site.

The above examples indicate a need to be especially cautious to avoid creating in the reuse process an additional link in the chain of transmission for enteric diseases. An epidemiological evaluation of the role of reuse in the transmission of the predominant pathogens, and the establishment of an adequately supervised sanitary code are essential conditions for continued and expanded reuse on the Peruvian desert coast.

1.2 Background

Human excreta and sewage can be used to promote the growth of aquatic plants and animals through the practice of aquaculture. Four main types of aquaculture are practiced: fishfarming, shellfish culturing, high-rate algae production, and aquatic macrophyte production (Mara, 1982). Freshwater fishfarming in ponds fertilized with human excreta and animal manure is widely practiced in many parts of the developing world, especially in Asia. Waste stabilization ponds receiving domestic sewage can be used advantageously for aquaculture systems to convert nutrients into plant, fish and shellfish biomass (Duffer, 1982). However, primary and secondary ponds for treatment seldom offer appropriate conditions for fish survival and growth, so that maturation or polishing ponds are required.

Aquaculture experiments have been incorporated into the San Juan research program. Fishculture experiences prior to 1977 have been summarized by Moscoso and Galecio (1978). During the initial phases of CEPIS' research program the National Agrarian University experimented with stocking Tilapia nilotica in secondary waste treatment ponds. Most of the prior work has sought to provide survival and growth data. A study has recently been initiated to evaluate the safety and efficacy of sewage grown duckweed (Lemnaceae) as a poultry feed (Paul Skillicorn and Robert Gillman, personal communication).

There are three areas of interest related to wastewater-aquaculture systems:

- the sanitary safety of aquaculture products;
- the viability of producing aquaculture products of economic value;
- the degree to which aquaculture affects effluent quality.

In the developing world aquaculture products are most highly valued for their contribution to increasing protein sources for improving the nutritional status of local populations. Economically viable reuse schemes for irrigation and for aquaculture can contribute indirectly to elevating the socioeconomic and health level of reuse worker families, and of local consumer groups. However, for such benefits to be realized food products must not contain unsafe levels of pathogenic organisms or toxic substances.

Risks of infections from eating foods grown with raw or treated sewage effluents depend upon the kind of crop and whether it is eaten raw; upon the handling of the food before and after cooking; and upon the cooking time-temperature relationship. Both food handling and cooking customs are cultural and educational characteristics whose influence on the design and operation of sewage reuse schemes must be determined on a case-by-case basis (Kalbermatten, et al, 1980). Chemical contaminants, such as pesticides and heavy metals, also present a potential health hazard because of their tendency to bioaccumulate in many edible plants and animals, particularly aquatic organisms (WHO, 1972).

The economic soundness of aquaculture will be determined by a number of factors, including the growth and production rates that can be achieved, production costs and the demand for the aquaculture products. In sewage aquaculture schemes fish may be stressed and growth or survival significantly reduced due to high concentrations of toxic un-ionized ammonia or detergents, or to frequent and prolonged low concentrations of dissolved oxygen. On the other hand production costs should be minimum as the incremental costs of modifying and operating waste stabilization ponds to support aquaculture should not be significant, and there should be little need for supplemental feed. Demand for the end products could be affected by local psychological attitudes toward growing fish in sewage even if it can be demonstrated safe from a public health view point.

Aquaculture systems can contribute to improved effluent quality. Floating macrophyte populations have been shown efficient in stripping nutrients and heavy metals from domestic sewage in ponds (Dinges, 1978; Wolverton and McDonald, 1979). It has also been demonstrated that a fishculture system was at least as efficient in treating sewage as a system of waste stabilization ponds without fish (Henderson, 1979).

Although aquaculture appears to be an appropriate technology for developing countries, the effectiveness of a combined sewage-aquaculture system for removing pathogenic organisms and toxic substances in tropical conditions has yet to be established. Results of research in temperate climates (Hejkal, et al, 1983; Reed and Bastion, 1980) indicate the feasibility of such schemes.

2. PURPOSE AND SCOPE OF RESEARCH

The present study was performed as part of a larger aquaculture research project to evaluate the production of fish (Tilapia nilotica) and prawns (Macrobrachium rosenbergii) in San Juan ponds receiving treated effluents. The overall project objectives were to determine the efficiency of the experimental wastewater-aquaculture system for controlling environmental conditions in the ponds in order to foster good fish growth, to demonstrate the economic viability of the treated sewage aquaculture system, and to evaluate any public health risks of human consumption of the fish and prawns.

The specific principal objectives of CEPIS participation in the project were:

- To conduct the necessary testing to evaluate the treated sewage quality in relation with established criteria for aquaculture;
- to determine the necessary waste stabilization pond pretreatment levels needed, in order to maintain required treated sewage quality in lagoons supporting aquaculture;
- to manage, monitor and maintain an adequate water quality in the pretreatment and aquaculture ponds.

Other secondary objectives were related to the working philosophy of CEPIS in all of its research projects and can be stated as follows:

- To assist the national authorities in training researchers, especially through in-service training;
- to disseminate information on aquaculture with undiluted treated sewage.

Other components of the project included fish growth and production studies by the National Agrarian University (UNA), and fish microbiology and parasitology by the Institute for Tropical and High Altitude Veterinary Research (IVITA). Site management was provided by the Park Service (SERPAR), formerly of the Ministry of Housing but now part of the Lima Water and Sewage Service (SEDAPAL).

The research program was developed in two phases: Phase I was carried out with the financial support of the UNDP/World Bank from April to December 1983; and Phase II was executed from March to August 1984 with funding assistance from the GTZ.

3. MATERIALS AND METHODS

3.1 Project site description

The San Juan waste stabilization ponds, which have operated continuously since 1964, consist of 21 lagoons occupying 20 ha. The ponds are divided into two batteries as shown in the site plan (Figure 2). The upper battery has six primary and four secondary ponds, and the secondary pond effluents go for agriculture, silviculture and park irrigation. The lower or experimental battery of eleven ponds was resequenced as shown in Figure 3 to create two series of ponds for conducting the aquaculture research (Series 1: P1-S1-T1-C1; and Series 2: P2-S2-T2-C2-Q2). A third series (P3-S3) produced irrigation water only and was not included in the experiments. The effluents of the two experimental series went to agriculture irrigation exclusively.

The San Juan complex receives sewage from three Lima slum areas - San Juan de Miraflores, Pamplona Baja and Ciudad de Dios. According to data supplied by SEDAPAL (Luis Salinas, personal communication) the population served is 108,500 out of a total of 193,000 inhabitants (59%). The average flow is 360 lps. Effluents are used to irrigate some 280 ha of forest and parks with another 1300 ha planned, and 220 ha of farmland. Most of the farmland within the perimeters of the experimental site itself has been occupied and operated since 1964 by squatter families.

For experimental purposes the two series of ponds were initially projected to receive average raw sewage loads of 250 to 350 Kg/ha-day of BOD_5. The primary, secondary and tertiary ponds were operated as continuous flow ponds with an average depth of 1.3 m while quaternary (C1, C2) and quintenary (Q2) ponds were operated as 1.0 m deep batchflow ponds periodically receiving only sufficient make-up water to compensate for evaporation and infiltration losses. Strictly speaking pond C2 was not batch flow since the make-up water for pond Q2 also was routed through it, but given the low additional flow involved C2 is considered as a batch operated pond for the purposes of this study. The primary and secondary ponds served as stabilization ponds for sewage treatment. At the average loading rates the primary ponds operated in the facultative mode (Yánez, 1982a), while all subsequent ponds were aerobic. The advanced maturation or polishing ponds were stocked at different intervals with fish and prawns as described by Moscoso and Nava (1984).

3.2 Field measurements

3.2.1 Flows

The total sewage flow to the experimental battery and the flow to each primary pond was measured continuously from May 1983 to April 1984 using Stevens type F water level recorders located over Parshall or Palmer-Bowlus flumes (Figure 3). The influents to ponds P1 and P2 were controlled by two dividing chambers calibrated to provide an estimated 350 Kg/ha-day average BOD_5 load to pond P1, and 250 Kg/ha-day to pond P2.

Water level measurements were made once daily in all the experimental ponds, and instantaneous flow measurements of effluents from ponds P1, S1, P2 and T2 were read using precalibrated triangular or rectangular weirs. To complete the information required to make flow balances in the two series of ponds, infiltration measurements were made in ponds S1, S2 and T2 by closing inlets and outlets and measuring water level changes over a period of 24 hours.

3.2.2 Daily observations

Routine daily field observations for all ponds were made in the mornings between 9 a.m. and 11 a.m. from May 1983 thru April 1984. Observations included meteorological conditions, pond appearance (color), odor, scum and floating matter, conditions of dikes, and presence of birds, insects and/or fish and prawns visible.

Also, daily data were obtained from a nearby meteorological station, including information on ambient air temperature, wind speed and direction, evaporation, solar radiation, hours of sunshine, precipitation and cloud cover.

3.2.3 Daily water quality measurements

Temperature and dissolved oxygen profiles in the fishponds were measured at approximately 10 a.m. and 2 p.m. each day during this period using polargraphic analysis (Yellow Spring Instruments, Model 57) with electrodes positioned at 20, 40 and 60 cm in the vicinity of the outlet structures. Surface pH measurements were taken at the same times using an Orion Research, Model 301 pH meter.

3.2.4 Diurnal water quality measurements

Temperature and dissolved oxygen profiles were recorded every two hours for periods of 3-5 days in fishponds on a rotational basis, by means of sets of temperature thermistors and DO electrodes positioned near the center of the ponds at 20, 40 and 60 cm, a multiple contact timer, a galvanic cell (Precision Scientific DO Analyzer) and a recorder (Esterline Angus, Model MS 401BB). At least two sets of diurnal measurements were made for each fishpond. Similarly, occasional diurnal cycles of pH were also recorded.

3.2.5 Primary productivity measurements

Primary productivity measurements were carried out four times in fishponds T1, C1, C2, and Q2 on a rotational basis during hours of greatest sunlight. Pairs of light and dark bottles filled with pond water at the center of the ponds from depths of 10, 20, 30 and 40 cm were suspended at those same depths for periods of one hour. Initial and final DO concentrations were measured using a specific electrode (Yellow Spring Instruments 5720A probe). In cases where DO values exceeded the upper range of the meter, samples were fixed and processed in the laboratory by the Winkler method. Net photosynthesis was estimated as mg/l of net oxygen production and converted to mg/m^2-hr of oxygen by integrating through the water column (Vollenweider, 1970; Hepher, 1962).

3.3 Laboratory water quality measurements

3.3.1 Weekly physical-chemical analyses

Daily water samples were collected near the effluent weirs from May 1983 to April 1984 in all fishponds at approximately 10 a.m. in plastic bottles of 1-l and acidified immediately with 1 ml of concentrated sulfuric acid. These samples were taken directly to the laboratory where they were analyzed for total ammonia nitrogen (NH_{3g} + NH_4^+) by distillation (Esparza, 1983). Dissolved un-ionized ammonia concentration was computed using the concurrent daily measurements of total ammonia, pH and temperature. The following formulas were applied for the calculations (Ferrara and Avci, 1982):

$$NH_3\text{-N total} = NH_4^+\text{-N} + NH_{3g}\text{-N}$$

$$NH_4^+ = NH_{3g} + H^+$$

$$K_a = \frac{[NH_{3g}]\,[H^+]}{[NH_4^+]}$$

$$NH_{3g}\text{-N} = \alpha\,(NH_3\text{-N total})$$

$$\alpha = \frac{1}{1 + 10^{(pKa-pH)}}$$

$$pKa = 10.5 - 0.032\ T$$

where T is the temperature in °C, and NH_{3g} refers to dissolved un-ionized ammonia gas.

A weekly sampling program was established during the same period for raw sewage and biweekly for all pond effluents. Composite samples were obtained using a 24-hour automatic sampler (Yánez, 1982b), in two bottles of 2-l each. One bottle contained 2 ml of concentrated sulfuric acid as a preservative, and the 24-hour composite sample was analyzed for total ammonia. During Phase I total organic nitrogen and soluble Chemical Oxygen Demand (COD) were also measured. The second bottle was packed in ice during

TABLE 3

Components of the Experimental Program and Analytical Techniques

PARAMETERS	UNITS	FREQUENCY	ANALYTHICAL METHOD
I. NON BIOLOGICAL			
A. Meteorological			
1. Wind velocity	Km/h	Daily	Data items 1-5 obtained from a nearby meteorological station
2. Wind direction	Degrees	Daily	
3. Air temperature	°C	Daily	
4. Evaporation	mm	Daily	
5. Solar radiation	cal/cm^2-day	Daily	
6. Infiltration	mm/day	Once	Change in level
B. Hydraulic			
1. Average flow	l/s	Daily	Flow recorder
2. Maximum hourly flow	l/s	Daily	Flow recorder
3. Maximum daily flow	l/s	Daily	Flow recorder
4. Water balance	mm	Monthly	By calculation
5. Level fluctuation	mm	Daily	Field measurement
6. Depth	m	Once	Field measurement
C. Physical factors			
1. Water temperature	°C		Electrometric (in situ)
a) Profile (20, 40, 60 cm)		Twice daily	
b) Diurnal		Hourly during 3-5 days	
2. Pond appearance	Qualitative	Daily	Field observation
3. Odor	Qualitative	Daily	Field observation
4. Scum and floating matter	Qualitative	Daily	Field observation
5. Vegetation on dike	Qualitative	Daily	Field observation
D. Physical factors			
1. pH	Units		
a) surface (20 cm)		Twice daily	pH meter
b) Diurnal		Hourly during 3-5 days	
E. Chemical factors			
1. Dissolved oxygen	mg/l		Galvanometric/Winkler
a) Profile (20, 40, 60 cm)		Twice daily	
b) Diurnal		Every hour during 3-5 days	
2. Chemical oxygen demand	mg/l	Bi-weekly in the first phase	Volumetric (Potasium dichromate)
3. Alkalinity	mg/l	Weekly	
4. Nutrients			
a) Organic nitrogen	mg/l	Weekly	Volumetric (Kjeldahl)
b) Ammonia nitrogen	mg/l	Weekly	Photometric (Nessler)
c) Nitrate nitrogen	mg/l	Occasionally	Cd reduction and diazotation
d) Orthophosphate phosphorus	mg/l	Weekly	Photometric (Ascorbic acid)
5. Detergents (MBAS)	mg/l	Weekly	Spectrophotometric methods
F. Biochemical factors			
1. BOD 20° C, 5 days	mg/l	Weekly	Electrometric method
2. BOD 20° C, 1,2,3,5,7 days	mg/l	Weekly in Phase I aquaculture ponds	Electrometric method
3. Primary productivity	mg-O_2/m^2-hr	Occasionally	Galvanometric cell
II. BIOLOGICAL			
A. Microbiological			
1. Total coliforms	MPN	Monthly	Multiple tubes (Lauryl triptose)
2. Fecal coliform	MPN	Monthly	Multiple tubes (E.C.)
3. Salmonella	MPN	Monthly	Multiple tubes (Selenite/Novobiocine)
4. Protozoa/helminths	Identification	Quarterly	Concentration; flotation with Zn sulfate and sedimentation with formalin-ether

TABLE 4

Preservation of samples and sample holding

Parameter	Volume required (ml)	Container	Preservative	Holding time
Alkalinity	100	P, G	Cool, 4° C	24 hours
BOD	1000	P, G	Cool, 4° C	24 hours
COD	50	P, G	H_2SO_4 to pH 2	7 days
Dissolved oxygen				
- Probe	300	G only	Det. on site	No holding
- Winkler	300	G only	Fix on site	4-8 hours
MBAS	250	P, G	Cool, 4° C	24 hours
Nitrogen				
- Ammonia	400	P, G	Cool, 4° C H_2SO_4 to pH 2	24 hours
- Organic	500	P, G	Cool, 4° C H_2SO_4 to pH 2	7 days
- Nitrate	100	P, G	Cool, 4° C H_2SO_4 to pH 2	24 hours
pH	25	P, G	Cool, 4° C Det. on site	6 hours No holding
Orthophosphate	50	P, G	Filter on site Cool, 4° C	24 hours

P = plastic
G = glass

the 24-hour sampling period and taken immediately to the laboratory for the analysis of soluble (filtered) BOD_5 for pond effluents and total BOD_5 for raw sewage. During Phase I measurements were also made of detergents (MBAS) and total orthophosphate phosphorus, as well as occasional raw sewage total nitrate nitrogen. In Phase II total and phenolphtalein alkalinity were added. All measurements were performed according to Standard Methods as summarized in Table 3 and described in the CEPIS laboratory manual (Esparza, 1983). Methods of preservation and holding times are given in Table 4.

3.3.2 Monthly microbiological analyses

Water samples for bacteriologic analyses were taken monthly during the period June 1983 to June 1984 from all ponds and raw sewage in sterilized glass bottles of 125 ml. Water samples were assayed for total coliforms and fecal coliforms (E. Coli) using multiple tube methods as shown in Figure 4 (Mayo, 1983). From March to June 1984 Standard Plate Count and Salmonella MPN were also determined. Procedures for Salmonella enumeration included a multistep process as shown in Figure 5 (Mayo, 1983). Serotype identification and antibiotic sensitivity tests on all Salmonella isolates were made by the National Reference Laboratory for Enterobacteria.

Quarterly samples for parasitological analysis (protozoa and helminths) were taken in clean plastic bottles: 1-l for raw sewage, 2-l for primary and secondary ponds and 4-l for fishponds. The samples were processed in the laboratory as shown in Figure 6 (Mayo, 1983).

3.4 Sediments

Sediment sludge samples were obtained monthly from the first series of ponds during May to July 1984, using a 2" diameter PVC sharp-edge corer. Also, samples were taken from ponds T2, C2 and Q2 during March and April 1984. For bacterial analyses 50 g of sediment were mechanically mixed with 450 ml of buffer solution and immediately tested for Standard Plate Count, and for total and fecal coliforms MPN as described in Figure 4 above.

At first qualitative isolation of Salmonella was attempted, but the results were inconclusive so that the prepared sediment samples were also assayed for Salmonella spp. and enumerated by the multiple tube method as shown in Figure 5.

Diluted samples (200 g of sediment mixed with 1800 ml buffer solution) were likewise analyzed for parasites using the techniques already described in Figure 6.

3.5 Fish toxicology

A one-time sample of 6 fish (Tilapia nilotica) was obtained from pond C1 on 28 April 1984, and a homogenized sample of all muscle tissue prepared. From the total combined muscle tissue sample, approximately 100 g were used for analysis of water content, lipid content and fixed solids. For mercury measurement three 10 g samples were prepared by acid digestion and extraction, and analyzed by wet vapor atomic absorption spectophotometry. Lead, chromium and cadmium were determined using atomic absorption spectophotometry on three 5 g samples each which had previously been incinerated and diluted (50 ml, 10 ml and 5 ml).

Qualitative determinations of organochlorinated and organophosphorus pesticide were made using thin layer chromatography. From 500 g of homogenized muscle tissue, 2 g of lipids were extracted to provide subsamples for qualitative analysis using U.S. FDA methods (1977). Also, a sample was prepared and analyzed by gas chromatography for the identification and measurement of organochlorinated pesticides, using AOAC methods (Horwitz, 1980).

3.6 Data processing

A project data base was developed and implemented on CEPIS' microcomputer. Field and laboratory results were input monthly. Continuous flow recording data for raw sewage was digitized on 15-minute intervals and these results were used to compute average daily flows. Consistency tests and verification routines were applied to all data observations.

Statistical analyses were performed on the data to obtain means, standard deviations, and maximum and minimum values for the entire project period and on a monthly basis. Computer plots of daily and monthly means were generated for most parameters. Probability distribution curves were prepared for all weekly data.

The raw data, detailed statistical summaries and plots are included in Appendix I of this report.

Statistical summaries of data grouped by fish sampling and control periods were also provided to UNA at their request, for correlation with fish growth experimental results and are included in the UNA report (Moscoso and Nava, 1984).

4. RESULTS

4.1 Hydraulic and organic loadings

A daily water balance was obtained for each pond, and their hydraulic loadings computed. To carry out flow balances, infiltration rates were measured in ponds S1, S2 and T2. For the remaining ponds estimates of infiltration rates were taken from previous San Juan research projects (Yánez, et al, 1980). Table 5 summarizes the flows entering each pond; pond area, depth and volume; estimated infiltration rates; and nominal hydraulic detention time.

Raw sewage flow variability is shown in Figure 7 for ponds P1 and P2.

In order to calculate the average BOD_5 loadings on the ponds, the daily average flow for each pond was multiplied by the corresponding BOD_5 concentration interpolated from weekly sampling results. The average of these daily estimates is shown in Table 5 along with the resulting average surface loading rate for each pond.

Daily variability in the raw sewage BOD_5 concentrations and surface loads are also shown in Figure 7.

4.2 Physical-chemical parameters

4.2.1 Daily results

The averaged results, morning and afternoon, of the daily field measurements of DO, temperature, pH and total ammonia nitrogen are shown in Table 6 for all fishculture ponds, along with the computed average concentration of un-ionized nitrogen. The graphical presentation of the results for each fishpond, morning and afternoon, day-by-day for the entire study period, is given in Figures 9 through 18.

(In addition, the raw data for each pond is listed in Appendix A month-by-month, along with complete monthly statistical summaries and an overall detailed table of statistics for the entire study period).

4.2.2 Weekly results

Weekly data on BOD_5, total ammonia nitrogen, organic nitrogen, orthophosphate phosphorus, detergents (MBAS) and total alkalinity were averaged over the entire study period for all effluents. These results are summarized in Table 7.

(The detailed statistical report is included in Appendix B along with the raw data tables for each effluent.

4.2.3 Diurnal results

Typical diurnal patterns for DO and temperature are shown in Figures 19-22 for pond T1 for the day of 8 June 1983, along with complementary data on solar radiation, hours of sunshine and DO saturation levels.

4.3 Microbiological parameters

4.3.1 Parasites

Pond removal of enteric protozoa and helminths identified in raw sewage is reflected in Table 8 which gives the frequency of positive identifications in each pond effluent during the period of study.

TABLE 5

Hydraulic and organic loads on ponds. Average values May 1983 - April 1984

Pond	Inflow (lps)	Area (Ha)	Average Depth (m)	Volume (m^3)	Infiltration Rate (mm/day)	Hydraulic detention (days)	Influent BOD_5 (mg/l)	Surface BOD_5 Load [d] (Kg/Ha-day)
P1	29.7	1.20	1.3	15,600	7.6 [a]	6.1	155.7 [c]	333.2 [c]
S1	28.8	1.44	1.6	23,040	3.7	9.3	12.6	21.6
T1	28.1	1.49	1.3	19,370	6.1 [a]	8.0	11.7	18.0
C1	2.0	1.30	1.0	13,000	13.1 [a]	- [b]	20.9	2.5
P2	15.3	1.10	1.3	14,300	19.2 [a]	10.8	155.7 [c]	201.0 [c]
S2	12.8	0.88	1.3	11,440	14.1	10.3	10.6	15.5
T2	11.4	1.30	1.3	16,900	18.0	17.2	10.4	6.7
C2	1.2	0.49	1.3	6,370	10.0 [a]	- [b]	18.7	3.9
Q2	0.6	0.53	1.0	5,300	10.0 [a]	- [b]	7.8	0.9

a. Estimated (Yánez *et al*, 1980)

b. Operated as batch flow ponds

c. Total BOD_5, other values are soluble BOD_5

d. Average of computed daily loads.

TABLE 6

Environmental conditions in aquaculture ponds. Averages of daily samples May 1983 - April 1984

Pond	DO (mg/l)			Temperature (°C)			pH	Total Ammonia (mg-N/l)	Un-ionized Ammonia (mg-N/l)
	20 cm	40 cm	60 cm	20 cm	40 cm	60 cm			
				Morning					
T1	2.6	1.9	1.6	22.2	22.2	22.1	7.8	11.6	0.4
C1	4.6	3.6	3.1	23.5	23.5	23.5	8.3	2.1	0.2
T2	4.3	2.7	1.8	24.2	24.2	24.2	8.1	8.3	0.7
C2	5.3	4.3	3.7	23.4	23.4	23.4	8.6	1.8	0.3
Q2	4.2	3.0	2.3	23.8	23.8	23.7	8.5	1.5	0.2
				Afternoon					
T1	7.1	5.3	3.8	23.2	23.0	22.8	8.2	11.6	1.0
C1	11.4	9.3	7.1	25.4	25.2	24.9	8.7	2.1	0.5
T2	16.1	12.9	5.5	27.1	27.0	26.1	8.5	8.3	1.4
C2	13.4	11.1	7.4	25.2	24.9	24.5	9.1	1.8	0.6
Q2	12.8	9.6	5.8	26.0	25.7	25.0	9.0	1.5	0.5

TABLE 7

Environmental conditions in ponds. Average of weekly samples
May 1983 - April 1984

Pond	BOD$_5$	NH_3-N	Org-N	PO_4-P	MBAS	Alkal.
	(mg/l)					
Influent	155.7	31.4	18.1	3.10	1.38	270.5
P1	12.6	23.5	12.1	-	1.62	215.5
S1	11.7	16.6	12.0	-	1.46	196.9
T1	20.9	10.7	9.8	1.03	1.12	189.8
C1	16.5	2.6	10.7	0.55	0.94	141.0
P2	10.6	19.8	14.4	0.11	1.55	241.4
S2	10.4	16.8	9.8	0.16	1.78	185.6
T2	18.7	8.8	9.0	0.53	1.13	135.9
C2	7.8	2.5	10.5	0.45	0.85	116.6
Q2	6.8	1.6	10.8	0.78	0.98	121.2

TABLE 8

Frequency of positive results in enteric protozoa and helminths identification May 1983 - April 1984

Organism	Raw Sewage	Series 1 Effluents				Series 2 Effluents				
		P_1	S_1	T_1	C_1	P_2	S_2	T_2	C_2	Q_2
Protozoa										
Giardia lamblia	8/8	1/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Entamoeba coli	8/8	1/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Entamoeba histolytica	1/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Endolimax nana*	6/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Iodamoeba butchili*	8/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Chilomastix mesorili*	1/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Helminths										
Ascaris lumbricoides	5/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Trichiuris trichiura	3/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Hymenolepsis nana	7/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1

* Normally inhabiting the human intestine

TABLE 9

Frequency of positive Salmonella serotype identification*
March-June 1984

Serotype	Serogroup	Raw Sewage	Pond Effluents				Pond Sediments			
			P_1	S_1	T_1	C_1	P_1	S_1	T_1	C_1
S. paratyphi B	B	3/5	3/3	2/2	1/2	1/5	1/2	2/2	1/2	0/2
S. typhimurium	B	2/5	0/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. 4,5,12:-:-NM	B	0/5	1/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. montevideo	C_1	1/5	0/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. oranienburg	C_1	1/5	0/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. newport	C_2	1/5	2/3	0/2	1/2	0/5	1/2	1/2	0/2	0/2

*Preliminary results from the National Reference Laboratory for Enterobacteria.

4.3.2 Coliform bacteria

Average total and fecal coliform removal through the entire pond system are depicted in Figures 23 and 24 for Series 1 and Series 2 ponds respectively. Plots of the individual observations for the entire study period are shown in Figures 25-28.

(A table of the raw data and statistical summaries for coliform bacteria measurements is included in Appendix C).

In Figures 29-32 comparative values of total and fecal coliform concentrations in pond effluents and sediments are shown.

4.3.3 Salmonella

The Salmonella serotypes identified in the raw sewage and Series 1 pond effluents and sediments are shown in Table 9 together with their frequency of positive test results observed for the months March-June 1984. The enumeration of Salmonella spp. in effluents and sediments of the same ponds is shown in Figure 33 for the period May-June 1984.

4.3.4 Standard Plate Count

Standard Plate Count results are shown in Figure 34 for raw sewage and for pond effluents and sediments during the period March-April 1984.

4.4 Fish toxicology

The combined sample of 6 fish weighed 3669 g and yielded 1057 g of muscle tissue (28.8%). The results of the analysis of the homogenized sample of the muscle tissue are shown in Table 10.

Also, the results of the gas chromatographic analysis for organochlorinated pesticides showed only one minor peak of low concentration (0.001 ug/g) which was not identifiable but appeared similar to Aldrin, and could be an isomer. Figure 35 shows the resulting chromatograph for the fish sample, and the comparison chromatograph of pesticide standards.

4.5 Primary productivity

The results of primary productivity measurements in fishponds during the months June-October 1983 are shown in Table 11, together with the corresponding daily solar radiation. The overall average primary productivity for the four fishponds was 1,645 mg-O_2/m^2-hr with pond C2 manifesting considerably higher productivity than the remaining three ponds.

TABLE 10

Results of analyses on fish muscle sample (1057 g)
May 1984

Analysis	Result
Bromatological analysis	
Water content	80.3%
Lipid content	0.4%
Fixed solids	1.2%
Trace metal analysis	
Mercury	0.03 µg/g
Lead	0.26 µg/g
Cadmium	not detected (0.01 µg/g)
Chromium	not detected (0.1 µg/g)
Pesticide analysis (qualitative)	
Organophosphorus	negative (0.2 µg/g)
Organochlorinated	negative (0.2 µg/g)

TABLE 11

Primary productivity in fishponds
June-October 1984

Pond	Date	Water Temperature (° C)	Solar Radiation (cal/cm^2-day)	Primary Productivity (mg-O_2/m^2-hr
T1	29 June	22.0	198	1,025
	30 June	23.0	115	420
	24 August	22.2	225	1,940
	25 August	21.0	91	885
C1	29 June	22.0	198	1,225
	30 June	23.0	115	630
	23 August	21.0	87	1,650
	26 August	19.9	128	1,375
C2	4 October	21.6	215	4,840
	5 October	20.7	167	1,430
	24 October	21.5	174	1,655
	26 October	21.6	179	3,475
Q2	5 October	20.4	167	3,345
	7 October	-	172	220
	24 October	21.5	174	1,000
	26 October	21.6	179	1,195

5. DISCUSSION

5.1 Organic loads and removal

Although the initial plan called for surface loading rates of 350 and 250 Kg-DBO$_5$/ha-day on the primary ponds, P1 and P2 respectively, the actual applied surface loading averaged 333 Kg-BOD$_5$/ha-day on pond P1 and 201 Kg-BOD$_5$/ha-day on pond P2 (Table 5). In general, the San Juan wastewaters had an average concentration of 155.7 mg/l, which is considered low for domestic sewage in tropical zones.

In spite of these reduced average loads, transient raw sewage overloading in excess of 800 Kg-BOD$_5$/ha-day (see Figure 8) and ensuing water quality problems did arise during the month of February 1984 as a result of unauthorized tampering with the raw sewage inlet structures of both pond series by local farmers. This event, which is discussed further below, caused major perturbations in water quality and gave rise to fish kills in pond T2. An analysis of weekly data revealed that soluble BOD$_5$ levels in T2 shot up to 44.9 mg/l during this period, compared to an average concentration in pond T2 of 18.7 mg/l. The surface loading during this incident reached 20 Kg-BOD$_5$/Ha-day in T2 compared to an average load of 6.7 Kg-DBO$_5$/ha-day.

The average removed loads achieved in the primary facultative ponds were 307 Kg-BOD$_5$/ha-day in pond P1 (92.2%) and 188 Kg-BOD$_5$/ha-day in pond P2 (93.5%).

Although apparent BOD$_5$ reductions in subsequent ponds are low based upon inspection of Table 5, Yánez (1982, 1983) points out that the consistent load removal calculation for secondary and tertiary ponds should be based on the total applied BOD$_5$. Applying this criteria, and adopting a ratio of 3 between total to soluble BOD$_5$ for secondary and tertiary ponds at this low loading range based on data for San Juan presented by Rojas and Esparza (1983), the resulting load removals are as follows:

Pond	% Removed Load
S1	70.0
T1	45.8
S2	66.4
T2	47.5

Previously Yánez (1980) had found that the removal of BOD$_5$ loads in secondary ponds at San Juan averaged 76.5% for surface loading rates between 50 to 200 Kg-BOD$_5$/ha-day. At the consistently lower loadings used in this study secondary ponds appear not to do as well, and BOD$_5$ removal in tertiary ponds is greatly reduced. As an explanation, Reynolds et al (1977) suggest that the lower BOD removals of advanced polishing ponds are due to reduced bacterial biomass in those ponds. Since bacterial biomass is directly associated with the organic solids, which in turn settle out significantly in the primary ponds, the succeeding ponds do not always have an adequate bacterial biomass population to continue a high removal trend, and performance of the polishing ponds becomes erratic. In any case, multicell pond systems should be designed so that the primary ponds serve mainly for BOD removal, while polishing ponds are principally for pathogen control.

5.2 Ammonia

Total ammonia concentrations (NH_3g + NH_4^+) in the ponds decreased progressively through both series as shown in Tables 6 and 7. In batch-operated fishponds C1, C2 and Q2 average daily concentrations were 2.1, 1.8 and 1.5 mg-N/l respectively, while the corresponding computed un-ionized ammonia fractions during critical hours averaged 0.5, 0.6 and 0.5 mg-N/l. More importantly, of 734 daily measurements in these ponds (C1, C2 and Q2) only 7 observations (1%) of un-ionized ammonia exceeded 2.0 mg-N/l, with the overall maximum concentration being 4.22 mg-N/l.

In the continuous flow tertiary ponds, however, much higher values were observed. Daily total ammonia concentrations averaged 11.6 mg-N/l in pond T1, and 8.3 mg-N/l in pond T2. The corresponding un-ionized ammonia concentrations were 1.0 and 1.4 mg-N/l respectively, but approximately 14% of the daily values exceeded 2.0 mg-N/l. The majority of these high concentrations occurred in pond T2, many of them during the raw sewage overflow incident in February 1984 when fishkills were registered.

The fundamental site-specific mechanisms responsible for un-ionized ammonia toxicity to fish are pH, temperature, alkalinity and total ammonia concentration. Also, the sensitivity of individual species of fish is an important factor, as is the duration of the exposure period to high NH_3g levels. Szumski, et al (1982) have analyzed data presented by various researchers and conclude that a concentration of less than 0.08 mg-N/l of un-ionized ammonia at the gill surface of warm water fish is a conservative criteria. This translates to approximately 1 mg-N/l of total ammonia for San Juan conditions of pH, temperature and alkalinity. However, since tilapia are relatively tolerant to NH_3g toxicity (Redner and Stickney, 1979), a higher guideline value should probably be accepted. In the case of prawns the evidence is not so clear. Data reported by Armstrong, et al (1978) would indicate that larvae of Macrobrachium rosenbergii are not overly sensitive to un-ionized ammonia concentrations below 1 mg/l especially at pH above 8.0 units. However, both NH_4^+ and NH_3g are reported to be toxic to Macrobrachium so that the combined effect of total ammonia concentration should be taken into account (Redner and Stickney, 1979).

The findings of Moscoso and Nava (1984) tend to confirm that a higher guideline value may be warranted. The fish growth experiments at San Juan established that growth in the advanced, batch-operated ponds was very satisfactory, while in tertiary ponds it was only marginal. Fish showed indication of stress due to the high un-ionized ammonia levels in ponds T1 and T2. Also, during the February fishkill incident in pond T2 daily NH_3g

concentrations averaged 2 mg-N/l of un-ionized ammonia, while in ponds C2 and Q2 no fishkills were observed, even though the corresponding average daily NH_3g concentration rose to 1 mg-N/l during the same period. Moscoso and Nava hypothesize that at the higher NH_3g concentrations the fish in pond T2 were stressed and could not resist the diurnal dissolved oxygen depletion which occurs at night. In ponds C2 and Q2 with similar low dissolved oxygen levels in February, but lower NH_3g values, fishkills did not occur. Thus, based on the evidence at San Juan, satisfactory tilapia growth and survival is possible when average total ammonia is maintained at less than 2 mg-N/l, average un-ionized ammonia less than 0.5 mg-N/l, and short duration concentrations of un-ionized ammonia do not exceed 2 mg-N/l. Moscoso and Nava also reported good initial growth of prawn larvae in pond Q2 at the NH_3 concentrations occurring during Phase I of the study.

The data from this study show an overall systematic reduction of total ammonia concentration through the pond series, as is demonstrated in Table 7. The interpretation of this ammonia data is complicated by the many nitrogen transformations that can occur in ponds. Based on information on general nitrogen behavior the major mechanisms of ammonia addition or removal in the ponds may include:

- Ammonia loading rates,
- Biological uptake of ammonia,
- Sedimentation of organic nitrogen,
- Mineralization of organic nitrogen,
- Nitrification,
- Leaching of ammonia into groundwater, and
- Transfer of gaseous un-ionized ammonia to the atmosphere.

Many pond researchers consider that the main mechanism of ammonia removal may be assimilation into the algal cell, leading to the formation and precipitation of insoluble organic complexes (Meron, et al, 1972; Ferrara and Avci, 1982). As much as 20% of the cell biomass may be nonbiodegradable. Other researchers emphasize that volatilization of ammonia gas from the pond surface, especially at high pH and temperature and under well-mixed conditions, may provide for a significant amount of ammonia removal (Folkman and Wachs, 1972; Pano and Middlebrooks, 1982). Quantification of the relative importance of each mechanism has not been established, but both are generally accepted. On the other hand, most pond researchers agree that nitrification cannot be an important mechanism since pond conditions are not conducive to the development of the required nitrifying bacteria (Meron, et al, 1972).

In the case of San Juan insufficient data were collected to arrive at definitive conclusions regarding the volatization and release of ammonia gas. However, high pH and temperatures were maintained year around, and fairly steady wind action accross broad expanses of pond surface would be conducive to such releases. Also, several ponds have free falling effluent weirs and inlets where significant gas transfer could occur. On the other hand, stratified conditions in the ponds would serve to reduce the expected volatilization. With regard to biological uptake of ammonia and sedimentation of organic nitrogen, the following analysis of removal of Total Kjeldahl Nitrogen (TKN) is illustrative:

Pond	NH_3-N (mg/l)	Org-N (mg/l)	TKN (mg/l)	% Removal
Raw	31.38	18.07	49.45	-
P1	23.50	12.05	35.55	28.1
S1	16.57	11.95	28.52	19.8
T1	10.71	9.82	20.53	28.0
C1	2.46	10.69	13.15	35.9
P2	19.77	14.36	34.13	31.6
S2	16.75	9.81	26.56	22.7
T2	8.80	9.04	17.84	32.8
C2	2.52	10.48	13.01	27.1
Q2	1.63	10.84	12.47	4.

This table shows that the quaternary ponds C1 and C2 under more quiescent conditions play a role in the removal of TKN, the primary mechanism for this being through the sedimentation of organic nitrogen (Ferrara and Avci, 1982).

An important additional control of ammonia in the fishponds was achieved by substantially lowering the ammonia loading rates through batch operation of C1, C2 and Q2, as is shown in the following table:

Pond	Applied load (Kg-N/ha-day)
P1	67.1
S1	40.6
T1	27.0
C1	1.4
P2	37.7
S2	24.9
T2	12.7
C2	1.9
Q2	0.2

It is difficult to assess the importance of other mechanisms. Leaching of soluble ammonia into pond subsoils could be significant since the average percolation losses in the pond systems was on the order of 10 mm/day. Leaching losses could range from as high as 3.8 Kg-N/ha-day in pond P2 to as low as .16 Kg-N/ha-day in Pond Q2. The mineralization of organic nitrogen to ammonia is also difficult to assess. This may occur through hydrolysis, bacterial action on particulate organics in the water column and sediments, or direct release of ammonia from cells due to zooplankton grazing. It is of interest to note that during the period of high ammonia concentrations in February 1984, which has been attributed above to organic overloading, massive blooms of crustacean zooplankton were also observed coincidentally in the second series of fishponds. This could also have helped contribute to the high NH_3 levels.

In summary, the most important mechanisms of ammonia removal in San Juan appear to be biological uptake followed by the sedimentation of organic nitrogen, and volatilization of un-ionized ammonia. The control of surface loading rates to the fishponds through batch operation was also an important factor for the success of the fishculture experiments. In future experiments provision should be made for fishpond aeration to break the thermal stratification and increase volatilization.

5.3 Dissolved oxygen, pH, alkalinity and temperature

The behaviour of dissolved oxygen, pH and temperature was essentially similar in all fishponds throughout the study period.

Diurnal dissolved oxygen displayed expected behavior for ponds. Phytoplankton photosynthesis activity often caused supersaturated DO conditions in the early afternoon extending into early evening. Nighttime biomass respiration lead to DO depletion, with the minimum concentration reached near dawn, and with rapid recovery once photosynthesis was resumed. The tertiary ponds had lower nighttime concentrations (near zero) and for more prolonged intervals. The batch operated fishponds showed average minimum nocturnal DO concentrations of about 2 mg/l, with an absolute minimum of about 1 mg/l at 60 cm depth. Thus fish in tertiary ponds were probably subject to oxygen stress for short periods each day which, in combination with high un-ionized ammonia concentrations could explain poor growth in those ponds, as reported by Moscoso and Nava (1984).

The diurnal behavior of pH was also controlled to a large extent by photosynthetic activity due to the removal of free and combined carbon dioxide from the water column by algae during daylight hours. The algae reduce the free CO_2 below its equilibrium concentration with air thus causing a rise in pH. As the pH increases the algae can extract CO_2 from bicarbonates and from carbonates releasing hydroxide ions until an inhibitory pH is reached, usually in the range of pH 10 to 11. During hours of darkness, algae are net producers of CO_2 because of their respiratory processes. This CO_2 production has the opposite effect and tends to reduce the pH. Diurnal variations in pH due to algal photosynthesis and respiration are common in ponds.

At San Juan on sunny summer days, afternoon pH values in fishponds often exceeded 10 units, while nighttime values dropped to 7 units. Average morning values ranged from 7.8 to 8.6. units, and average afternoon values ranged from 8.2 to 9.0 units during the study period. These diurnal pH variations, linked to algal photosynthesis, are important in determining the toxicity of ammonia to fish. As an example, at a pH of 8.5 only about 10% of total ammonia is in the toxic un-ionized form. However, at a pH of 10 over 80% of total ammonia is un-ionized NH_3g.

Alkalinity, because of its buffering effect on pH, has been shown to also affect the toxicity of NH_3g on fish (Szumski et al, 1982). For temperature and pH ranges similar to those at San Juan, un-ionized ammonia may be over twice as toxic at an alkalinity of 200 mg/l (as $CaCO_3$) as compared to an alkalinity of 100 mg/l. Because average alkalinity in the fishponds was higher in the tertiary ponds as opposed to batchflow ponds in each series (refer to Table 7), fish in the tertiary ponds would have been more affected by high NH_3g concentrations.

Temperatures in the fishponds are important for two main reasons. First of all, the toxicity of un-ionized ammonia to fish is directly proportional to temperature. Secondly, temperature effects greatly influence the efficiency of ponds for removing pathogenic bacteria. Each of these aspects will be discussed in turn.

Szumski et al (1982) have shown that temperature also is an important factor in evaluating the toxicity effects of NH_3g on warm water fish. At the average pH and alkalinity conditions of the San Juan ponds, fish are from 50% to 100% more sensitive to NH_3g concentrations at 30°C than at 20°C. Average seasonal water temperatures in the San Juan fishponds ranged from 20°C to 28°C so that a significant temperature effect on NH_3g toxicity is to be expected.

To summarize the discussion of environmental factors affecting warm water fish sensitivity to un-ionized ammonia in the case of San Juan, it is apparent that critical conditions can be expected during summer months (January to March) when photosynthetic activity and water temperature are at maximum levels. Also, these critical conditions can be expected to occur in the tertiary continuous-flow fishponds (T1 and T2) rather than the advanced batch-flow ponds (C1, C2 and Q2) because of significantly higher total ammonia and alkalinity levels. Given this combination of factors it is not unreasonable to expect that fish in ponds T1 and T2 would have been many times more affected by a summer shock ammonia load than they would have been in the other fishponds. Thus the conclusion of section 5.2 that the tertiary ponds are less adequate for fishculture activities is further reinforced.

Returning to the discussion of temperature effects on the ponds, Burgers (1982) and Yánez (1983) have presented in-depth analyses of temperature behavior in the San Juan ponds. The results of this study support their findings. Significant diurnal stratification was observed in all fishponds from October through May, with average temperature differences of 2°C between 20 cm and 60 cm. Burgers found that thermal stratification occured between 8:00 a.m. and 10:00 p.m., and that complete mixing of the ponds occurred during the nighttime hours. Yánez concluded that these diurnal thermal stratification events occurred on those days in which solar radiation exceeded 160 cal/cm^2-day with at least 6 hours of direct sunshine. Both authors also observed that pond water temperatures were consistently several degrees higher than ambient air temperatures, with water temperature seldom dropping below 19°C even when average daily winter ambient air temperatures were around 15°C. Thus the San Juan ponds offer a suitable temperature environment for warm water fish.

The effects of temperature on pathogen removal in the ponds occur in two opposed ways. Pathogen dieoff increases with increasing water temperature, but on the other hand the occurrence of thermal stratification events causes short circuiting in the ponds which can results in reduced detention times and pathogen breakthrough. Both of these phenomena are considered in greater detail in subsequent sections.

5.4 Detergents

The detergents used in Peru are principally of the nonbiodegradable type manufactured from dodecyl alkalbenzene sulfonates (ABS). The results of this study have shown that the average concentrations of detergents in the fishponds at San Juan ranged from 0.85 to 1.13 mg-MBAS/l. There were slight reductions in detergents through the pond series, most likely due to removal of ABS by foaming at pond outlets having energy drops. However, the difference in detergent concentrations between ponds C2 and Q2 (Table 7) was not statistically significant at the 95% confidence level. Chunga (1984) in a parallel study found that up to 60% of the detergents in the San Juan wastewaters could be removed by foaming, if necessary.

The concentration of detergents found do not appear to present any problem for fish survival. The median lethal dosis (LD_m) for ABS has been determined as 10 mg/l (Moscoso and Galecio, 1978). The U.S. Public Health Service (1968) recommends that the continuous exposure dosis should not exceed 1/7 of the 48-hour TL_m, and provides 96-hour TL_m data for tolerant fish species ranging from 7.4 to 22.0 mg/l. Thus an environmental concentration of 1.0 mg/l in fishponds for raising species such as tilapia and carp should be adequate. However, Moscoso and Galecio (1978) point out that sublethal concentrations of detergents may still reduce the growth of fish in ponds.

5.5 Detention times

The nominal or theoretical hydraulic detention times given in Table 5 have been shown to be overestimates of the real detention times since considerable short circuiting occurs in the ponds. Tracer studies were carried out at San Juan (Yánez, 1983) to estimate dispersion factors, and it was shown that real detention times were on the average considerably less than nominal detention times. Based on Yánez' results it is estimated that the true average overall detention period for the first three ponds is on the order of 13 days for Series 1 and 22 days for Series 2.

In some ponds tracer breakthrough was measured in the effluents within 2 hours. This suggests the importance of reliable pond design with respect to the location of inlets and outlets, and pond shape. Yánez (1984) recommends that maturation ponds for pathogen removal should be rectangular in shape with length-to-width ratios of from 2:1 to 4:1. Other solutions might include the use of baffled ponds or irregularly shaped elongated ponds.

5.6 Parasites

The data from this study show that complete parasite removal for both enteric protozoa and helminths was obtained in the primary treatment ponds (refer to Table 8). This is in agreement with previous results found at San Juan (Yánez, 1983; 1984), and with experiences on parasite removal in ponds in other countries (Gunnerson *et al*, 1984).

In order to insure maximum parasite removal, even in overloaded pond systems, Yánez (1983) recommends the use of baffled outlet weirs to prevent the solids breakthrough of floating from primary to secondary ponds. With this simple measure pond efficiency was found to increase significantly with respect to parasite removal. For assurance of complete protozoa and helminth removal from treated effluents Yánez (1984) also recommends the use of primary and secondary ponds with an overall nominal detention period of 20 days.

The principal mechanism of removal is thought to be by sedimentation of sewage solids. Typical survival time for protozoa in wastewaters and sludges is around 20 days, but some helminth ova such as *Ascaris* may survive for 1 year of more (Feachem *et al*, 1980). Thus the principal reservoir of parasites for the San Juan ponds should be the primary pond sediments and perhaps secondary pond sediments if solids breakthrough were to occur. Based on the evidence of effluent samples there is no reason to suspect the accumulation or storage of human enteric parasites in fishpond sediments. However, in the case of ponds C2 and Q2 which were formerly highly loaded secondary ponds prior to being converted to fishponds, the existing sediments might still contain viable helminth ova. IVITA (Noe *et al*, 1984) reported positive identification of protozoa cysts and helminths ova (possibly non-viable) in the sediments of pond Q2, which can be explained as above. In no case, however, were human enteric parasites found in fish, thus giving support to the conclusion that such parasites were effectively removed in the treatment ponds prior to discharging their effluents to the fishponds.

5.7 Bacteria

The use of fecal coliforms (*E. Coli*) as an indicator of fecal contamination is well established. Furthermore, in previous studies at San Juan it was shown that fecal coliforms is a reliable indicator of the removal of pathogenic bacteria such as *Salmonella* and fecal streptococci in ponds

(Yánez et al, 1980; Yánez, 1983). Fecal coliforms also serve as an indicator of parasite removal in ponds. Therefore, other indicator bacteria in wastewaters such as total coliforms or standard plate count are not emphasized in this discussion.

Enterobacteria removal in tropical conditions using waste stabilization ponds has been shown to be more effective than in any other type of treatment process except for disinfection. Several authors indicate that using ponds with hydraulic retention times on the order of 20-25 days should produce safe effluents for reuse (Feachem et al, 1980; Gunnerson et al, 1984). Yánez (1983) has found that the net fecal coliform dieoff rate for the San Juan ponds can be estimated by:

$$K_{bt} = 0.841 \times 1.07^{T-20}$$

where K_{bt} is the net removal rate (1/days) and T is the water temperature (°C). A similar rate was found for Salmonella. Waste stabilization ponds, which generally are a cost effective and robust solution, represent an appropriate technology for pathogen control in reuse schemes in hot climates.

In this study the removal of fecal coliforms was progressive throughout the pond series during the study period (refer to Figures 23 and 24), averaging 99.99% in series 1 and 99.999% in series 2. Geometric averages for the ponds and cumulative removals were as follows:

Pond	Fecal Coliforms (MPN/100 ml)	Cum. Removal (%)
Raw	4.66×10^7	-
P1	2.76×10^6	94.1
S1	2.69×10^5	99.4
T1	2.87×10^4	99.94
C1	2.30×10^3	99.995
P2	3.35×10^6	92.8
S2	2.27×10^5	99.5
T2	1.67×10^4	99.96
C2	5.10×10^2	99.999
Q2	1.58×10^2	99.9996

In pond C1 and C2, 99% of the measurements (42 of 46) were below 10^4 MPN/100 ml, with a maximum observed value of 2.4×10^4 MPN/100 ml. In pond Q2, 18 of 21 observed data (86%) were less than 10^3 MPN/100 ml, with the maximum observed value being 2.4×10^3 MPN/100 ml. Thus, the performance of the batch-flow fishponds was good with respect to fecal coliforms. Results in pond T1 were also consistent, with all observed data below 10^5 MPN/100 ml. In pond T2, however, during the period of excessive loading in February 1984 a peak value near 10^6 MPN/100 ml occurred.

It is important to note that during the latter period the fishpond C2 did not manifest a similar fecal coliform peak, again indicating that good water quality conditions were maintained in the batch flow ponds in spite of a temporary system overload.

A final consideration regarding fecal coliform removal is pertinent. This discussion is centered on the San Juan ponds as they have been operated with three continuous flow treatment ponds in series. Prior research by Yánez (1984) shows that it could be possible to obtain adequate bacterial quality (10^4MPN/100 ml) in secondary or tertiary ponds, after parasite removal, by management of those ponds in batch or unsteady-state mode. This is an area which merits further research because of the implications it has for reducing land areas required by maturation ponds.

The data available for the series 1 ponds in May and June 1984 confirmed Salmonella reductions on the order of 99.99%, the same as for fecal coliforms. This supports previous findings at San Juan that fecal coliforms are a good indicator of Salmonella removal (Lloyd, 1982; Yánez, 1984). Effluent concentrations of Salmonella in the fishponds were on the order of 0.1 MPN/100 ml. Preliminary serotyping results from the National Reference Laboratory for Enterobacteria (Table 9) identified the survival of S. paratyphi B in the fishponds. Although the results are not yet available for this phase of research, during previous studies antibiogram tests were carried out on Salmonella serotype isolates. The results established that all of the serotypes were resistant to a majority of the antibiotics tested (Yánez, 1983). This poses a potential public health problem since it implies that the Salmonella serotypes have acquired genetic resistance factors and may be more difficult to destroy in a waste stabilization pond environment (Kish and Lampky, 1983; Grabow et al, 1973).

The public health interpretation of these results in the fishculture project is difficult because little information is available to establish guidelines. Buras (1982) has suggested that a threshold concentration of about 10^4 bacteria per ml exists, above which detectable bacteria may penetrate into the muscle tissue of fish. Although inconclusive, the use of fecal coliforms as an indicator tend to confirm that at the lower concentration found in the San Juan fishponds no apparent invasion of fish muscle tissue occurred (Noe et al, 1984).

In fishpond sediments, indicator bacteria concentrations were found to be two or more orders of magnitude greater on the average than in effluents. This was generally true for total and fecal coliforms, standard plate count and Salmonella. It must be kept in mind that the comparison was made on the basis of 100 grams of sediments versus 100 ml of effluent, but this should not affect the relative orders of magnitude. Thus the sediments appear to act as a reservoir for bacteria in the ponds. It is important to observe, however, that only two sediment samples were analyzed for each indicator bacteria so that these results should be interpreted with care.

As in the case of pond effluents, no guideline values were found which could assist in the interpretation of these results. They seem to be acceptable for fishculture purposes based on the San Juan experience.

5.8 Trace metals and pesticides

The results of the trace metals and pesticide determinations do not indicate any accumulation of toxics in tilapia grown in the San Juan ponds. However, these results are limited and should be treated with reservation for three reasons. First of all only one composite sample was analyzed, thus it is only indicative and certainly not statistically significant. In the second place the sewage treated at San Juan is basically domestic in origin, therefore the results should not be extended indiscriminately to larger reuse projects such as San Bartolo, where industrial sewage is also to be reused. Finally, tilapia is probably not a good choice of organism to monitor for bioaccumulation of toxics in the food chain because of its extremely low lipid content (0.4%). Prawns or water hyacinths would be better screening organisms (Bartone *et al*, 1984).

5.9 Primary productivity

Average peak hour primary productivity in fishponds during winter months was 1,645 $mg\text{-}O_2/m^2\text{-}hr$ (see Table 11). Estimating daily productivity by using a half sine curve approximation with a 12-hour photoperiod yields an average daily value of:

$$P = \frac{24\ P_{max}}{\pi} = 12.6\ g\text{-}O_2/m^2\text{-}day$$

with the maximum daily value being 37.0 $g\text{-}O_2/m^2\text{-}day$. It is interesting to note that the average corresponding solar radiation was 161.5 $cal/cm^2\text{-}day$, and the average water temperature was 21.5°C, which can both be considered relatively low for a tropical zone. Data summarized by Yánez (1982b) for other tropical zones indicate a range of annual average productivity values of 12.8 to 43.2 $g\text{-}O_2/m^2\text{-}day$ for high-rate ponds.

Thus the results from San Juan maturation ponds compare favorably with average productivity for tropical high-rate ponds, even during the unfavorable period of winter insolation and temperature.

5.10 Site management

The experiences obtained in Phases I and II of this aquaculture project point to several problems with the research site. Some of these have already been discussed by Moscoso and Nava (1984) and concern pond design. All ponds used in the fishculture experiments were originally designed and used as waste treatment ponds. For fishculture purposes there was inadequate provision for rapid draining of ponds to permit efficient harvesting. Also, there was no opportunity to clean the ponds used for fishculture prior to beginning the experiments (draining, drying, cleaning, refilling and stabilization requires at least six months lead time), so that heavy benthal deposits were present in several ponds, notably C2 and Q2. This in turn constituted a significant oxygen sink which was particularly important during harvesting time as the water level was drawn down. It may have had an important impact on prawn survival.

For future experiments, the time and cost of cleaning the ponds and modifying the hydraulic structures to provide for rapid draining, would have to be compared to the cost of constructing proper fishponds. It is also likely that the San Juan ponds are simply too large for adequate control of fishculture experiments.

A management problem of a different nature arose between Phases I and II of the project when local farmers (precarios) tampered with the raw sewage inlet structures over a weekend, causing pond overloading and subsequent water quality problems leading to a fishkill in pond T2. The water quality problems are analyzed in the discussion above. The management problem was, unfortunately, one which occurs frequently at the site because of the open access which local farmers have to the hydraulic structures. There is no fence around the ponds to restrict access. When the farmers feel they are not receiving sufficient effluent for irrigation, they are prone to take matters into their own hands. To control this, every effort was made to insure farmers of an adequate supply of irrigation water and to maintain them informed of any modifications in effluent flows through a process of open communications. Also, a guard was normally employed to patrol the site at night, even though this was more for psychological effect rather than strict control because of the large open area involved and the difficulty of patrolling.

During the between-project period mentioned a number of overlapping problems occurred. Guard service was discontinued and CEPIS research staff was cut back to a minimum. Also, the SERPAR site supervisor was on annual leave. The result of this was a lapse in the normal communication and superveillance processes, and a tampering incident which was not detected for several days.

Several lessons can be derived from this experience. As a waste treatment plant the pond site should be fenced and access by unauthorized persons prevented. This responsibility corresponds to the ex-SERPAR (now SEDAPAL). The situation of the precarios also needs to be resolved. As a minimum, they should participate in deliberations on management decisions which will affect their water supply, through improved communications. Finally, when research projects are underway, the integrity of the experimental controls must be assured by tighter security measures.

5.11 Technology transfer

The project served as an important vehicle for technology transfer within Peru through the training of national staff working in SEDAPAL, SENAPA and the Ministry of Health, as well as in participating universities. The extent of training is shown in Annex 1 which includes a list of professionals, technicians and students trained at San Juan, as well as the titles of graduate theses produced as part of the San Juan research projects.

In addition to this report, a paper summarizing the aquaculture studies was presented in an international seminar (Bartone, 1984). Further publications and information dissemmination activities are planned for 1985 including a World Bank/GTZ seminar on aquaculture research in Peru, and two PAHO Regional seminars on wastewater reuse.

Finally, the transfer of the San Juan reuse experience to other coastal cities in Peru is underway with CEPIS collaboration, including Piura, Ica, Pisco and Trujillo.

6. CONCLUSIONS AND RECOMMENDATIONS

Three waste stabilization ponds in series operated in continuous flow mode with average loadings of 250-350 Kg-BOD$_5$/ha-day did not produce water of suitable quality for fishculture purposes in the tertiary continuous flow treatment ponds.

Advanced polishing ponds operated in batch mode (make-up water only) were suitable for fishculture purposes from the point of view of water quality. However, the San Juan pond design is inadequate for controlled fishculture experiments from an operational point of view. Specially designed fishponds are recommended for fishponds:

The key water quality parameter for fish growth and production appeared to be ammonia. For maximum loading of 350 Kg-BOD$_5$/ha-day it was possible to maintain low ammonia concentrations in batch operated polishing ponds given the pH, temperature and alkalinity conditions in the San Juan ponds. The following maximum ammonia concentrations are recommended:

Total ammonia (NH_3g + NH_4^+)	2.0 mg-N/l
Average un-ionized ammonia (NH_3g)	0.5 mg-N/l
Short duration NH_3g diurnal peaks	2.0 mg-N/l

Under the above conditions dissolved oxygen concentrations in the advanced fishponds did not present problems, even with normal diurnal variations and heavy benthal deposits.

Detergents do not appear to present problems for fish at San Juan. Fishpond values were generally maintained below 1 mg-MBAS/l of ABS detergents.

Complete protozoa and helminth removal was achieved in the primary and secondary treatment ponds, and human intestinal parasites presented no problem in fishponds.

Fecal coliform concentrations in the advanced fishponds were effectively maintained below 10^4 MPN/100 ml, at which level no problems were manifested in the fish. Fecal coliforms appear to be an appropriate indicator bacteria for pathogens in fishponds.

Good treatment pond design practice is vital for pathogen removal. Among factors to be considered are: the use of baffled outlet structures to prevent pathogen breakthrough with solids, and the proper positioning of inlet and outlet structures and pond shape to achieve adequate hydraulic retention times in tropical climates where ponds are subject to shortcircuiting due to thermal stratification.

While it is possible to manage water quality from a technical point of view, the human element cannot be forgotten. Experimental fishpond sites need adequate protection from external manipulation by neighboring populations and other effluent users.

Further research is recommended on. toxicological aspects of rearing fish in treated effluents, particularly if industrial sewage is being reused. Research on virological water quality of effluents should also be undertaken. Other investigations on the batch operation of maturation ponds for fecal coliform, removal, and aeration for ammonia control are suggested.

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Dr. Fabián Yánez collaborated in the review of the report manuscript and made many valuable contributions which are reflected in this final version. This research is, in fact, a continuation of a series of projects initiated and directed by Dr. Yánez when he was at CEPIS.

During the course of the project the following persons, in alphabetical order, actively collaborated in the field and laboratory work:

- Maritza Aguirre
- Susana Chunga
- Antonio Evangelista
- Gonzalo Gallo
- Raymundo Lozano
- Enrique Quevedo
- César Reyes
- Richard Salazar
- Jorge Samamé
- Adolfo Torres
- Hernán Yupanqui

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FIGURE 1

San Juan Reuse Ecosystem

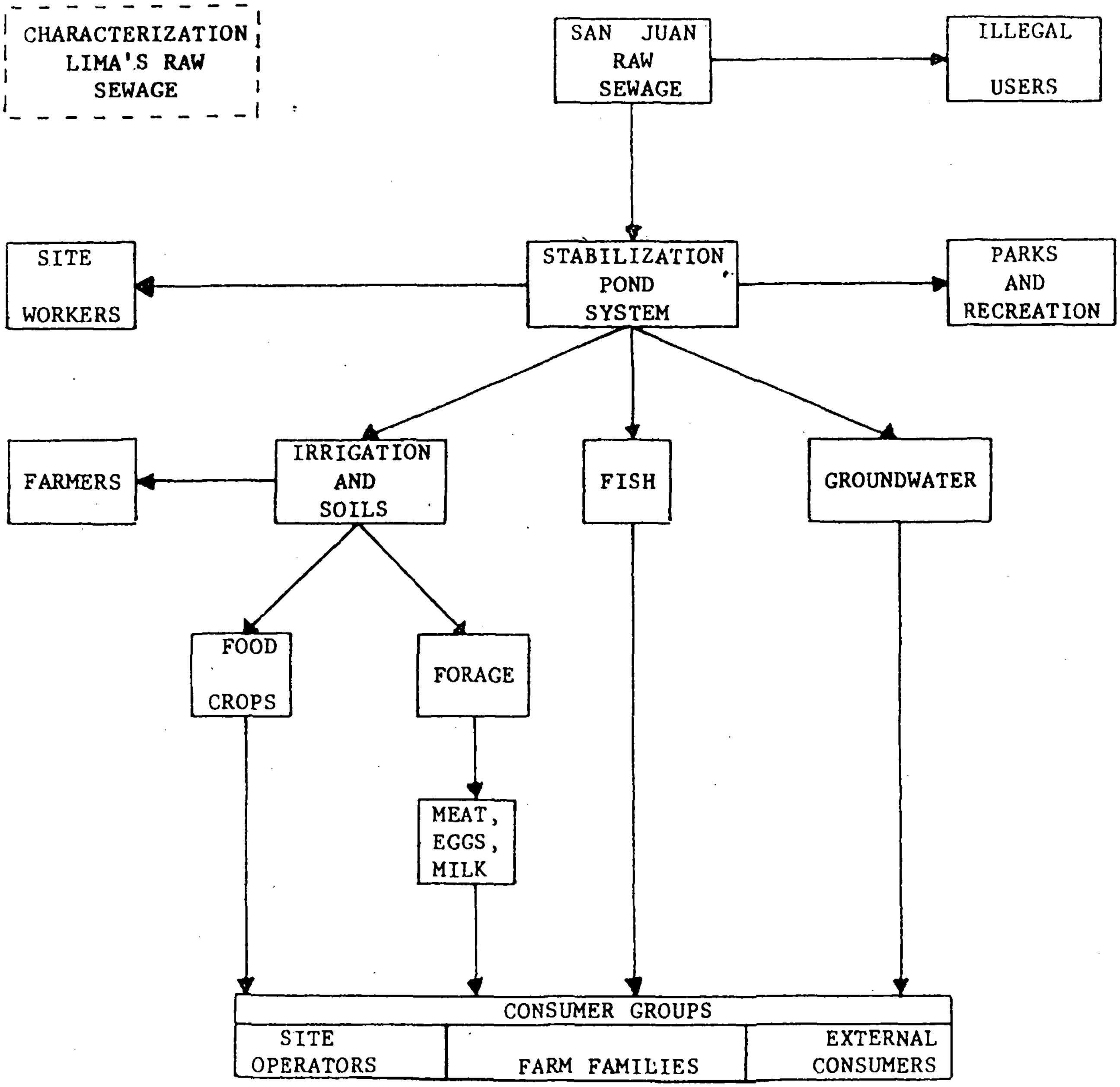


FIGURE 2

Map of the San Juan Effluent Reuse Project
Showing Cultivated Household Plots

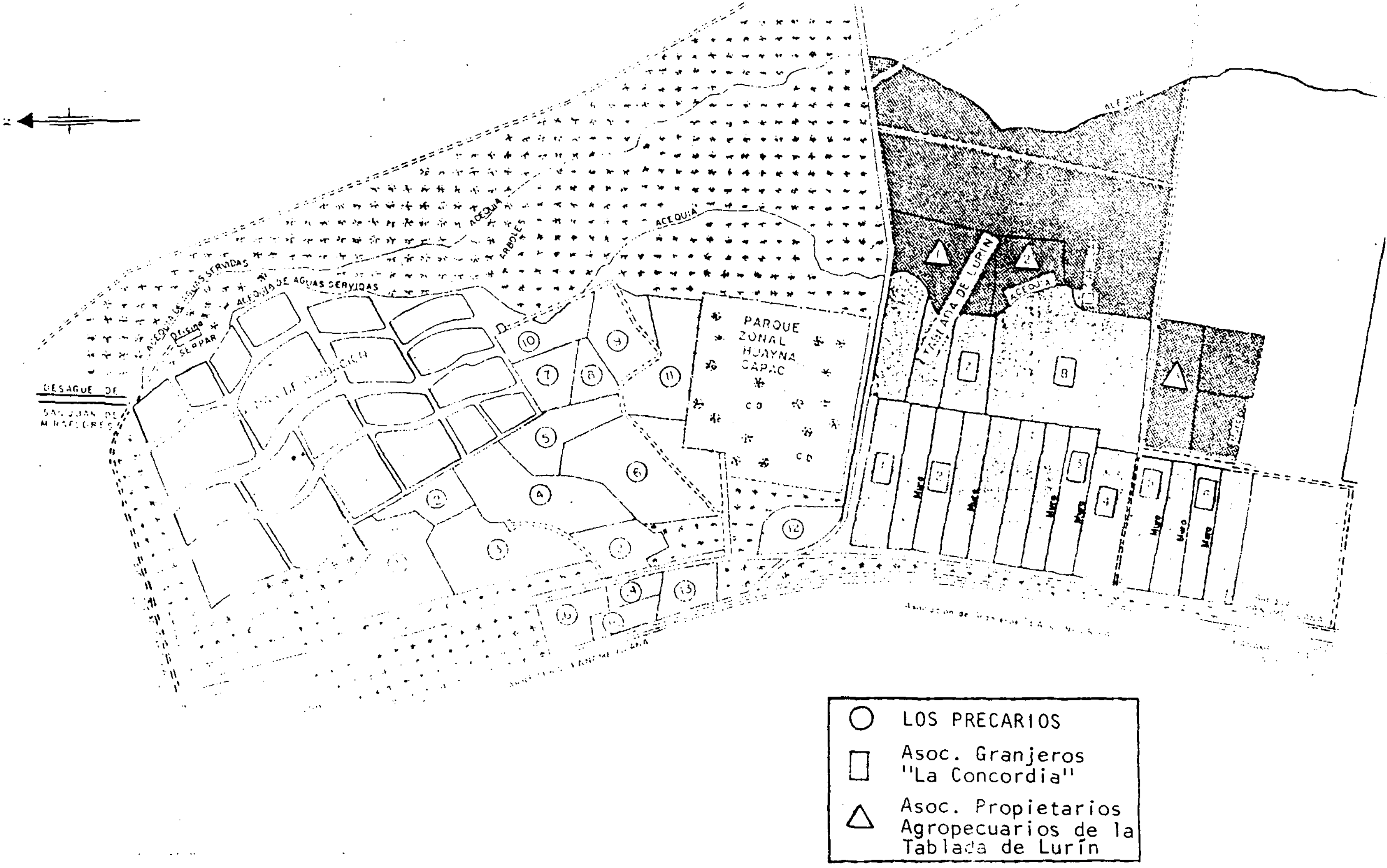


FIGURE 3

Pond Arrangement - Aquaculture Phase

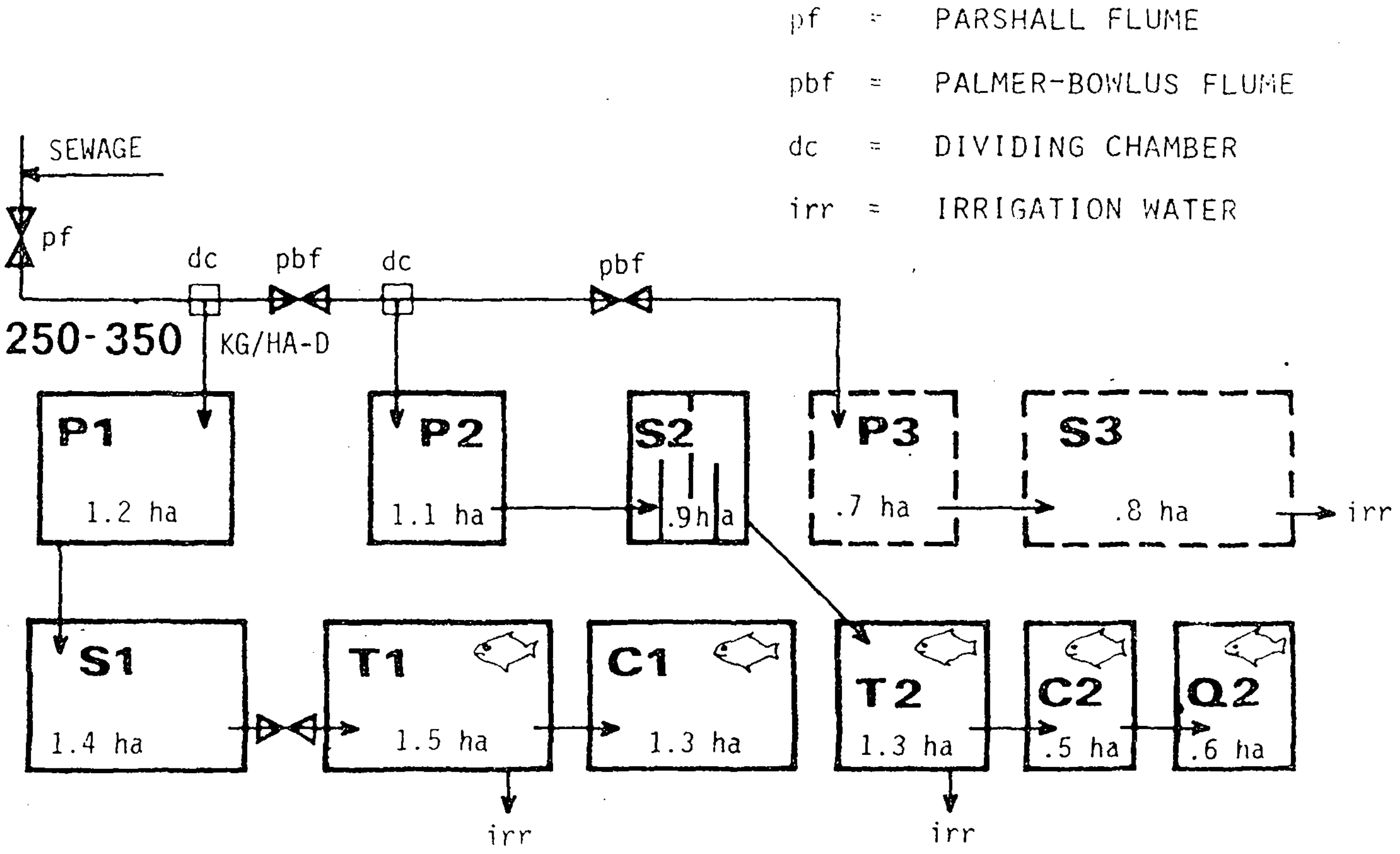


FIGURE 4

Total and Fecal Coliform Determination by Most Probable Number in Water and Sediments
(After May, 1983)

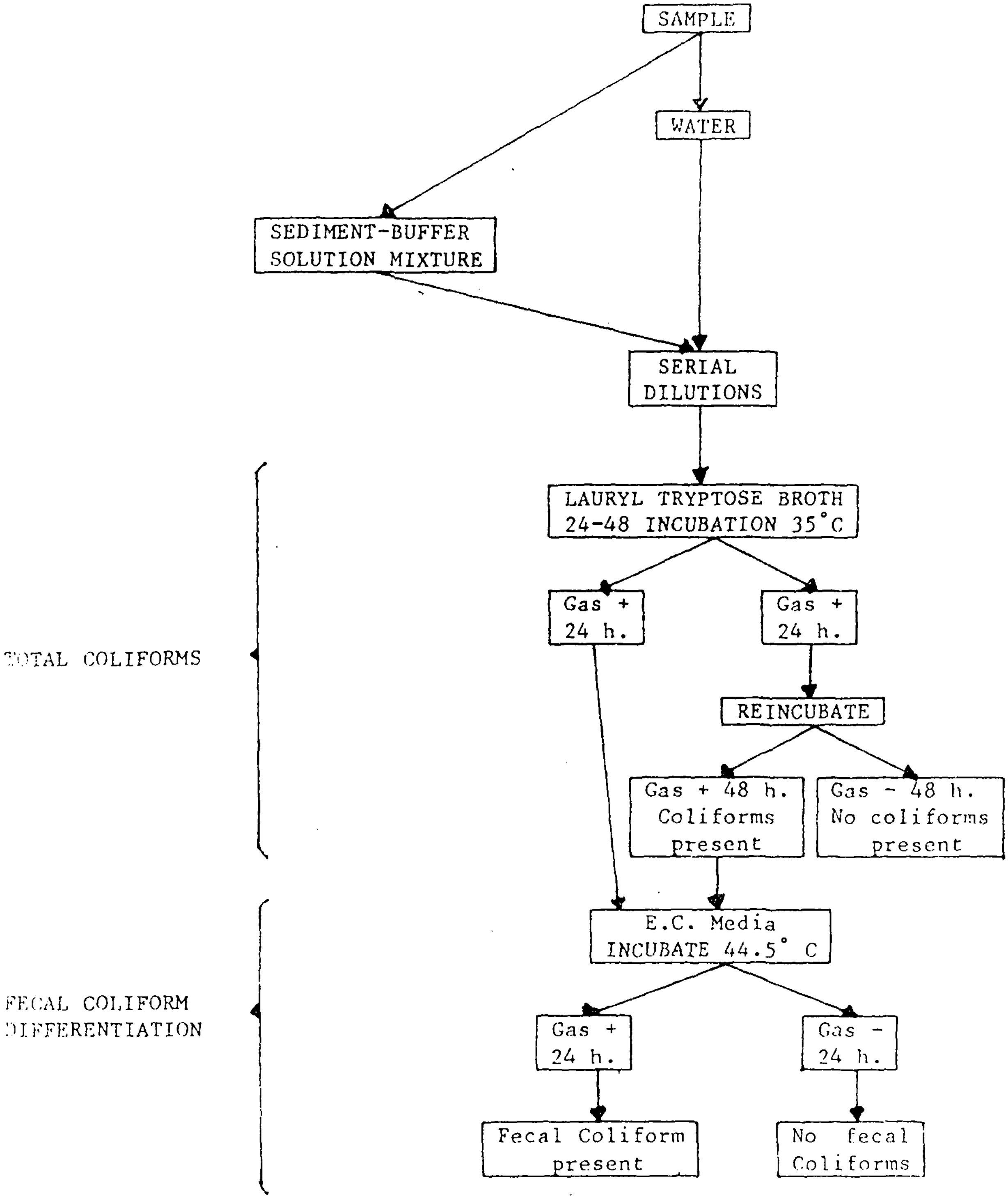


FIGURE 5

Salmonella Isolation and Quantification in Water and Sediments (After May, 1983)

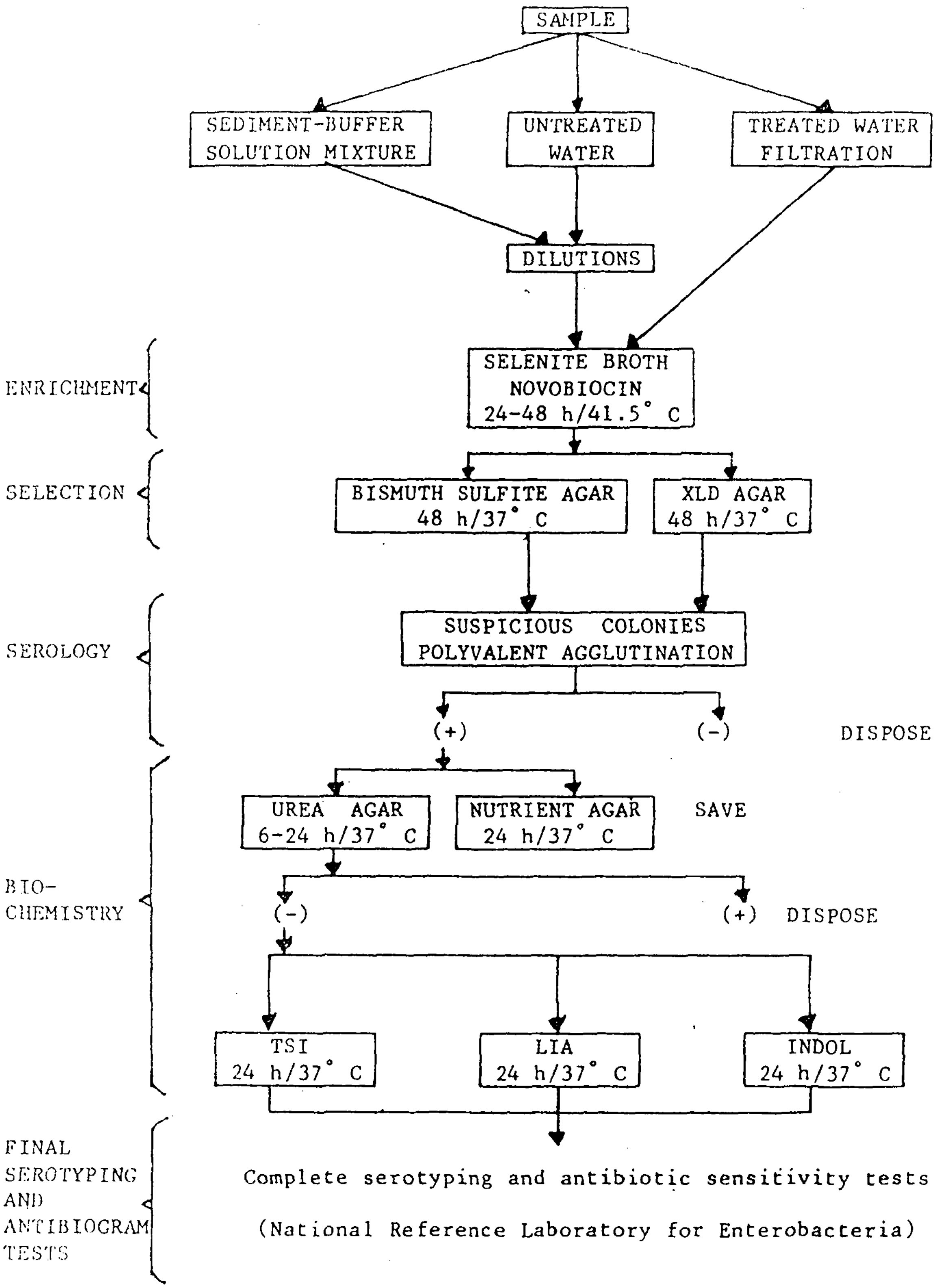


FIGURE 6

Quantitative Method for Protozoa and Helminths in Water and Sediments
(After May, 1983)

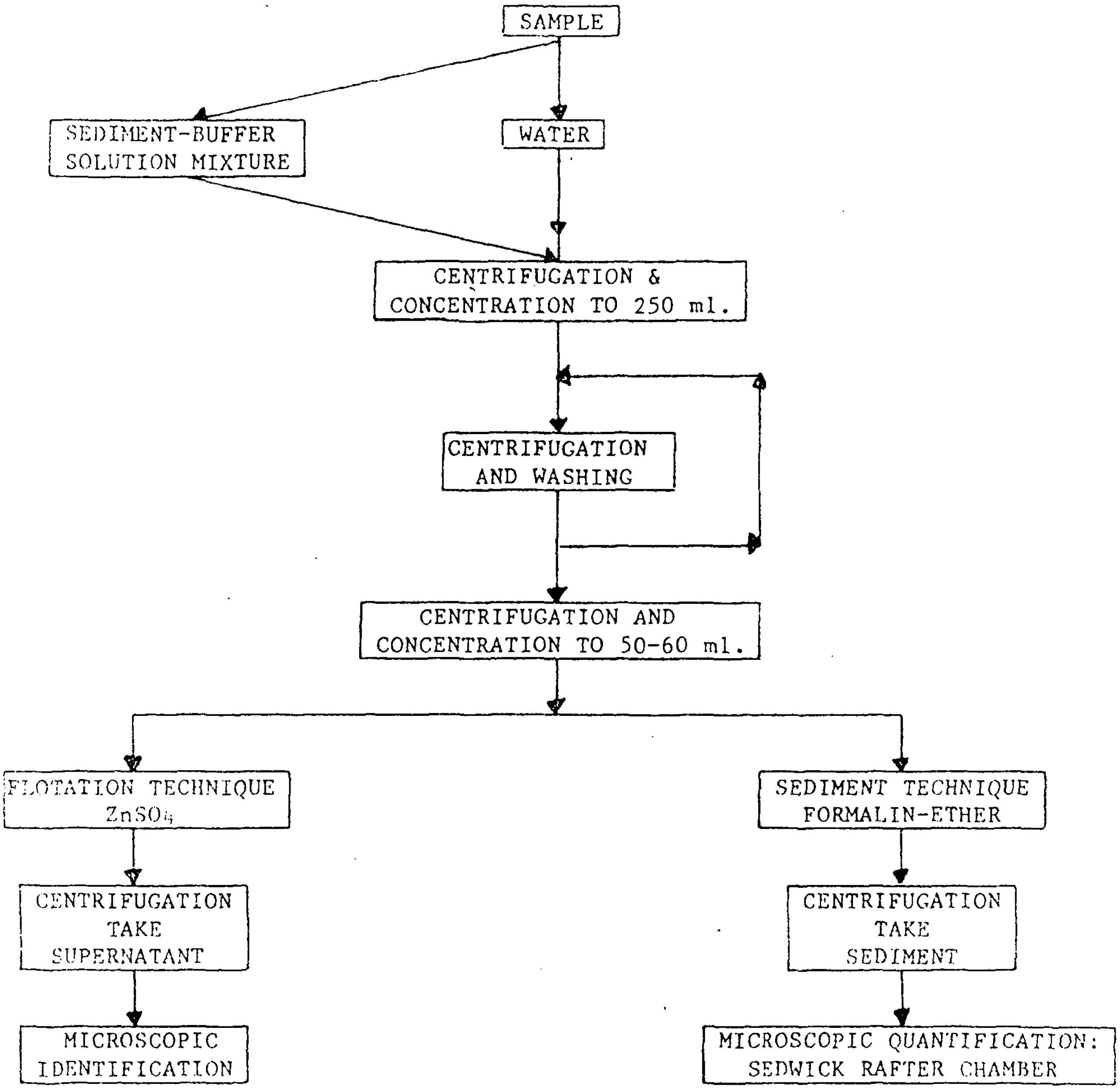


FIGURE 7. Daily Raw Sewage Flows to Ponds P1 and P2, May 1983-April 1984

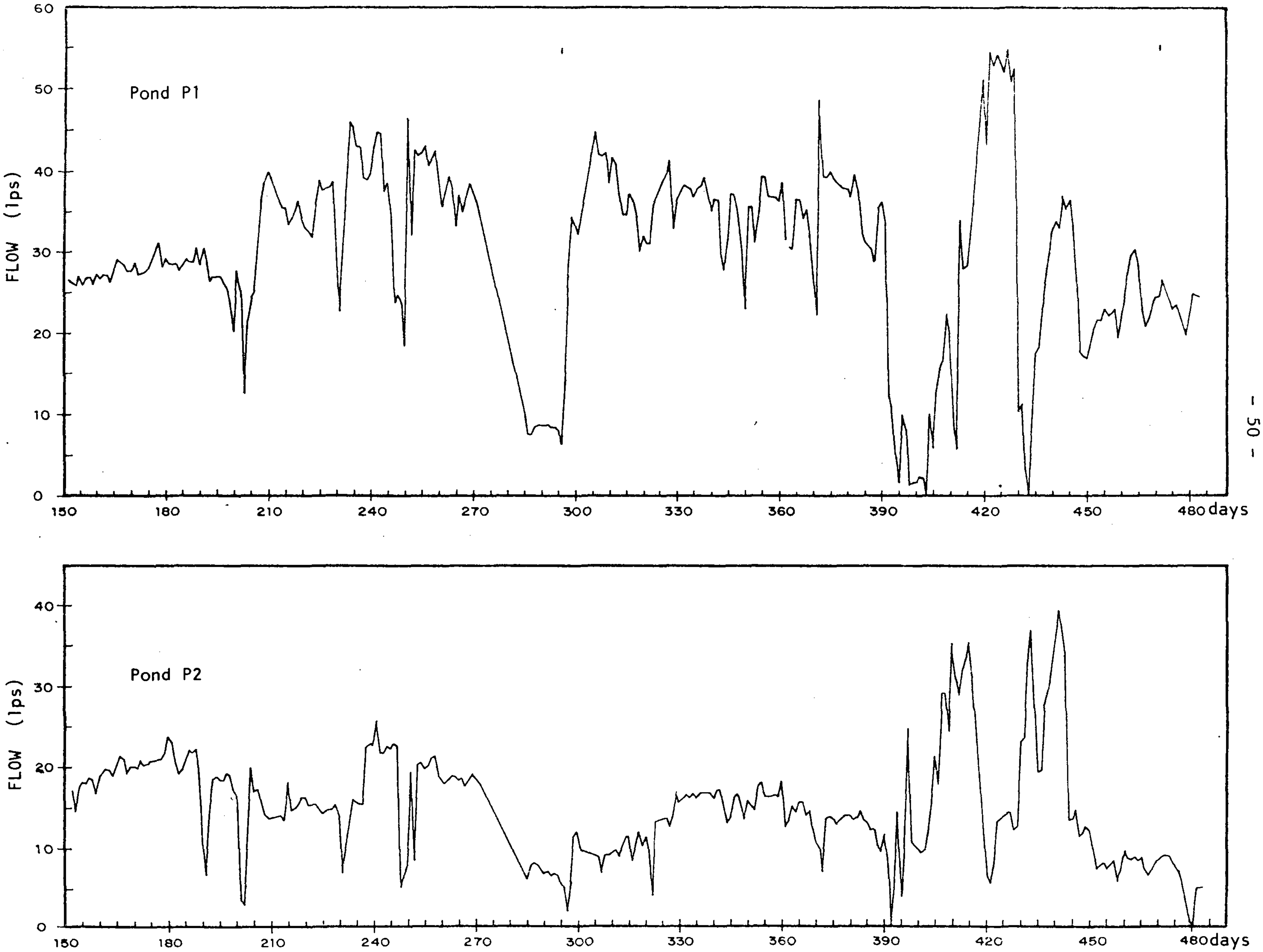
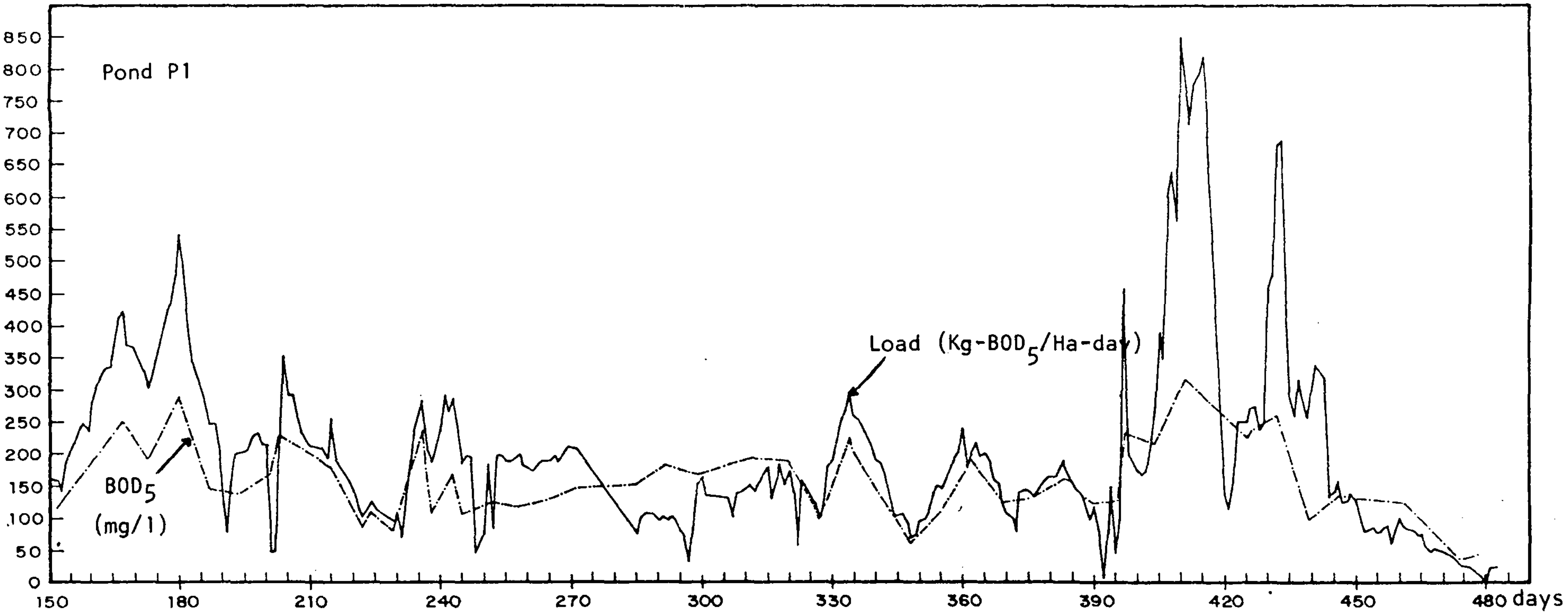


FIGURE 8. Daily Organic Loading and Raw Sewage BOD_5 Concentrations, May 1983-April 1984



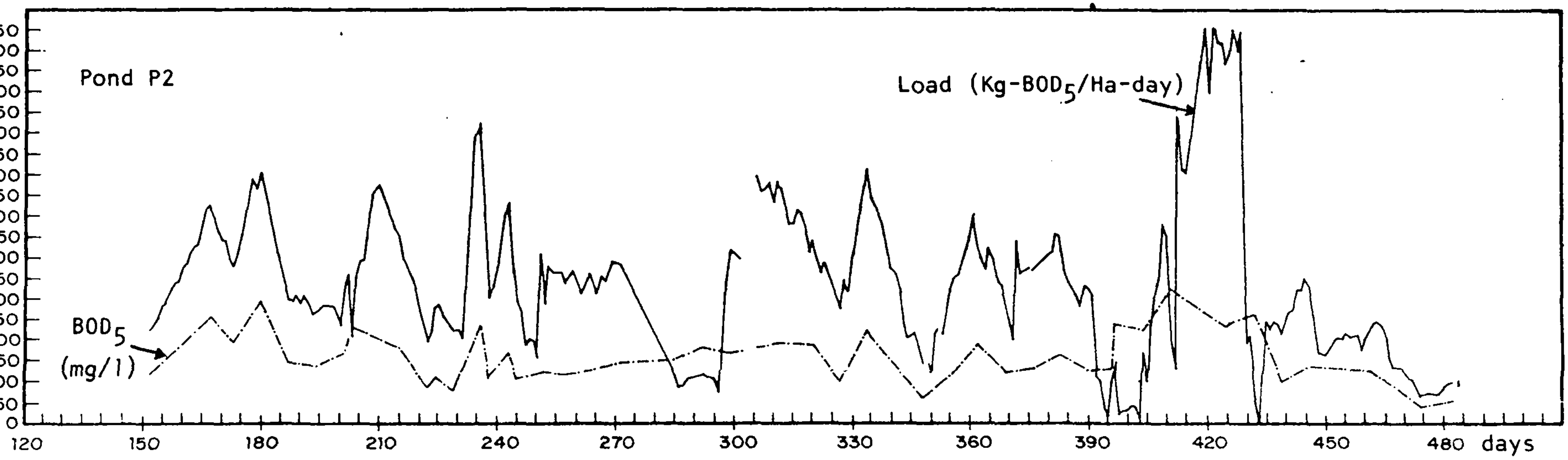
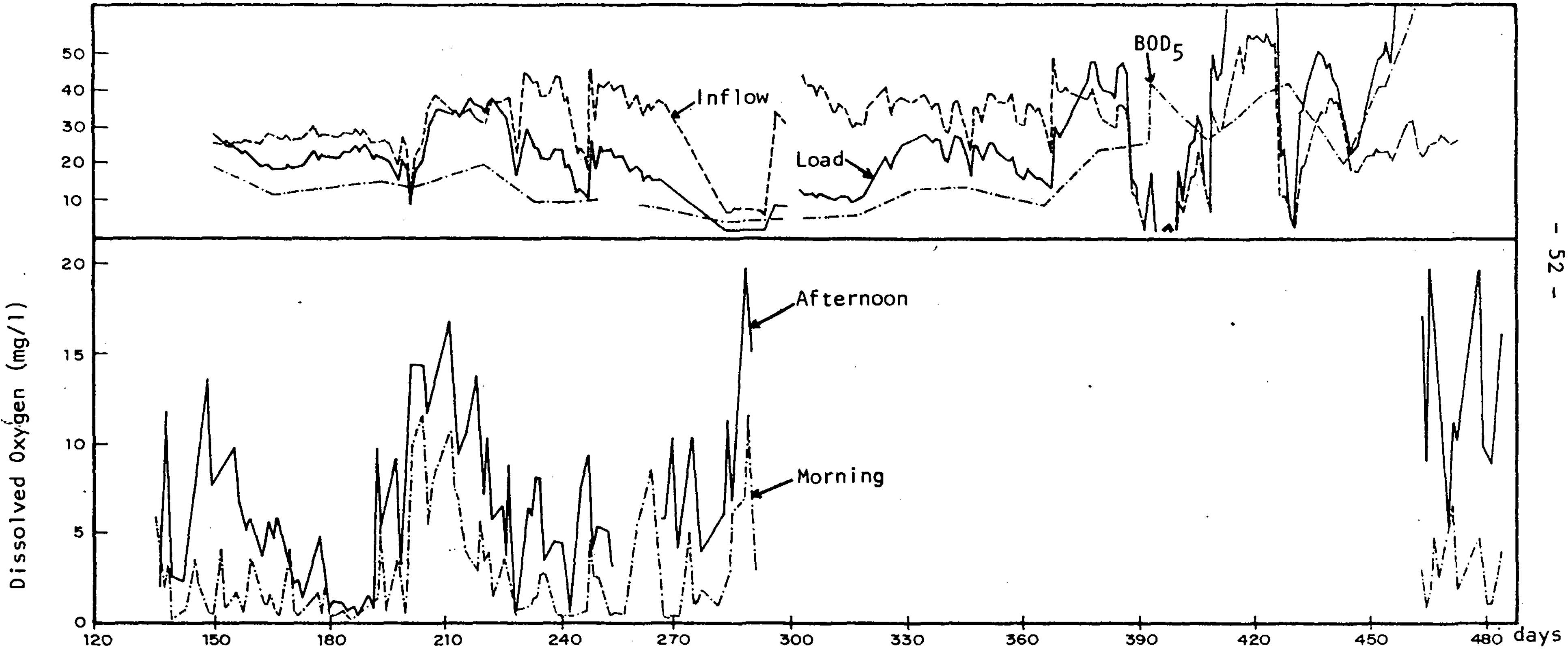


FIGURE 9. Daily Variations of Computed Inflow (lps), BOD_5 (mg/l), Load (Kg-BOD_5/Ha-day) and Dissolved Oxygen (mg/l), in Pond T1, May 1983-April 1984



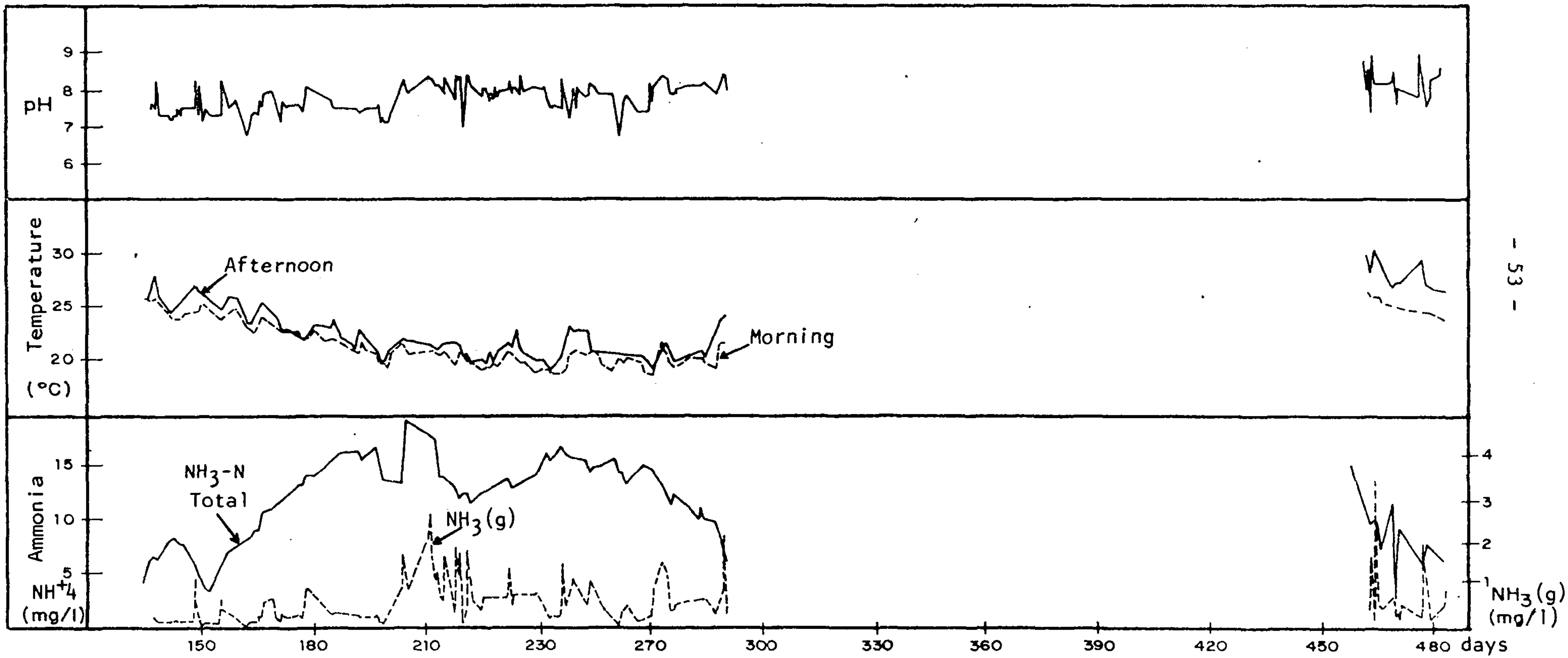


FIGURE 10. Daily Variations of pH, Temperature and Ammonia Nitrogen in Pond P1, May 1983-April 1984

FIGURE 11. Daily Variations of Computed Inflow (lps), Load (Kg-BOD$_5$/Ha-day) and Dissolved Oxygen (mg/l), in Pond C1, April 1983 - April 1984

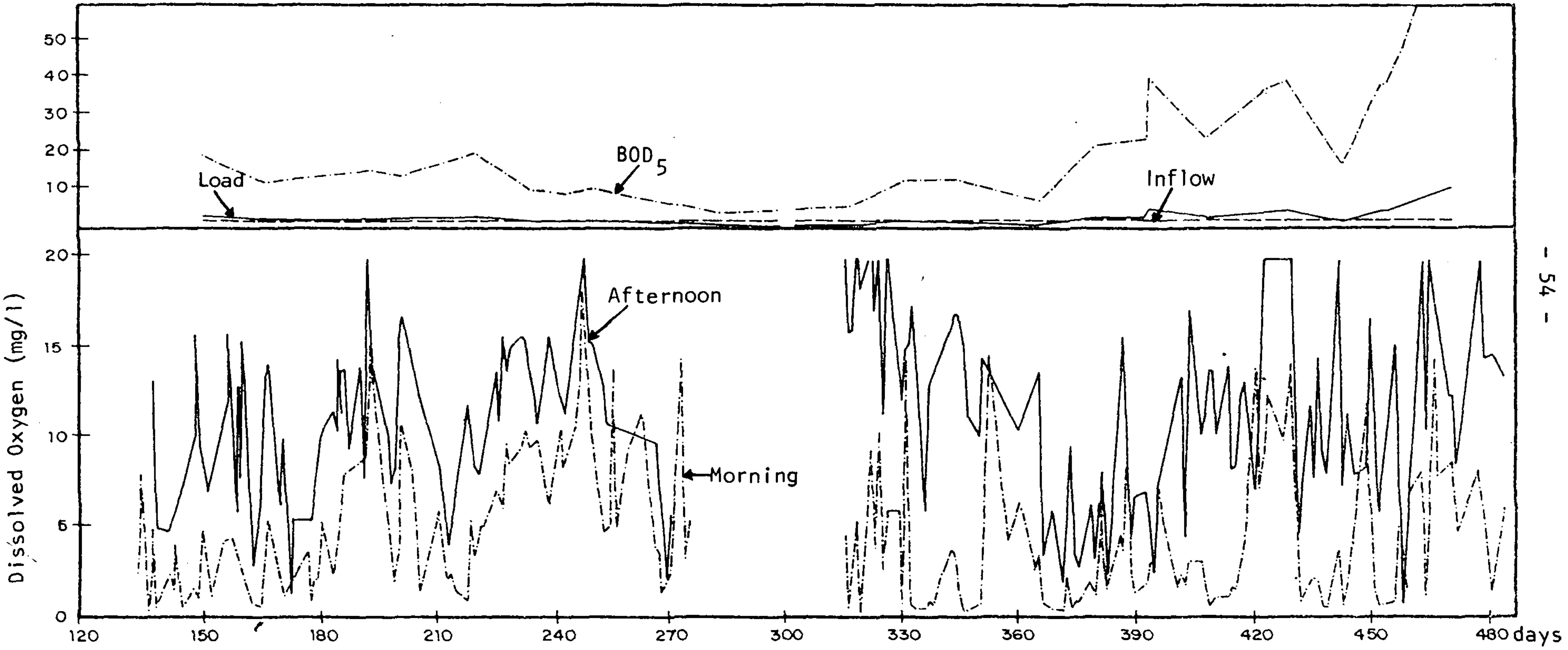


FIGURE 12. Daily Variations of pH, Temperature and Ammonia Nitrogen in Pond C1, April 1983-April 1984

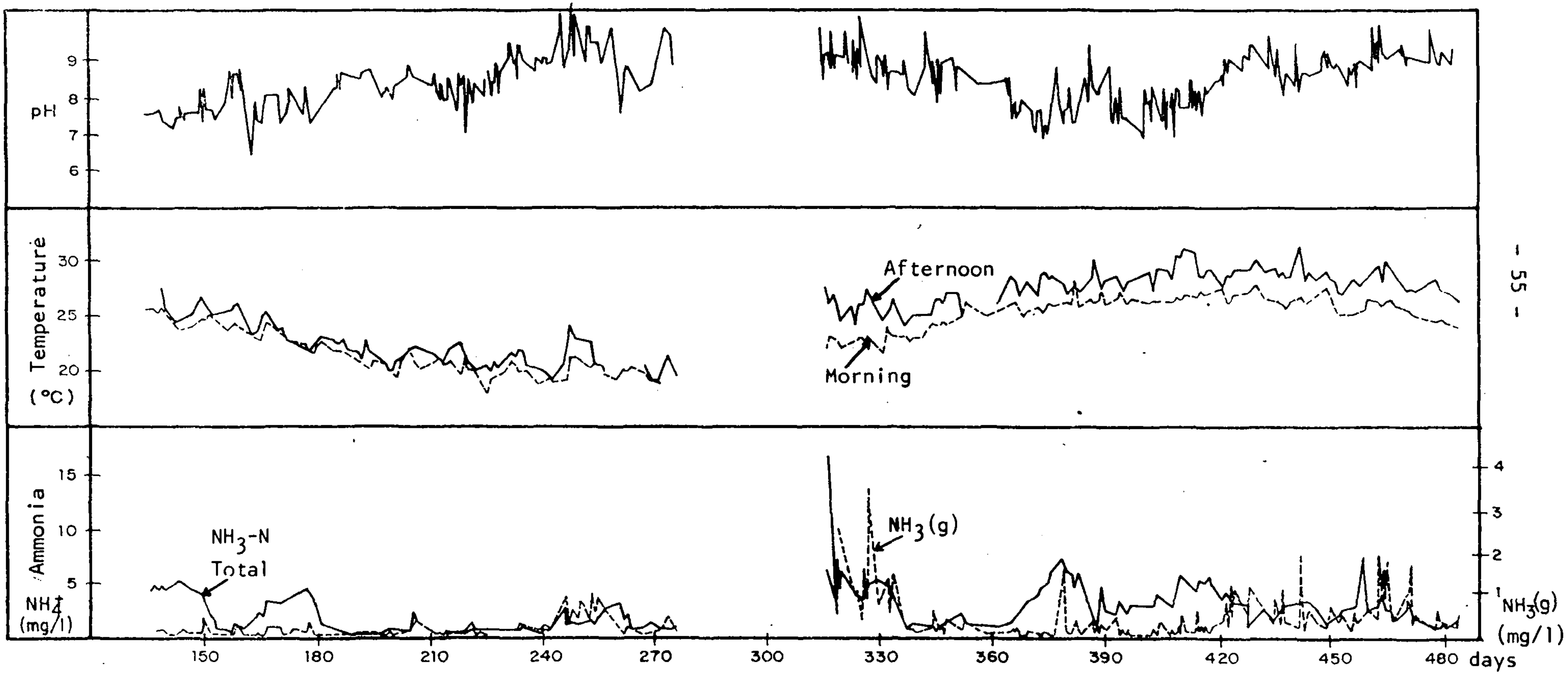


FIGURE 13. Daily Variations of Computed Inflow (lps), BOD_5 (mg/l), Load (Kg-BOD_5/Ha-day) and Dissolved Oxygen (mg/l) in Pond T2, April 1983 - April 1984

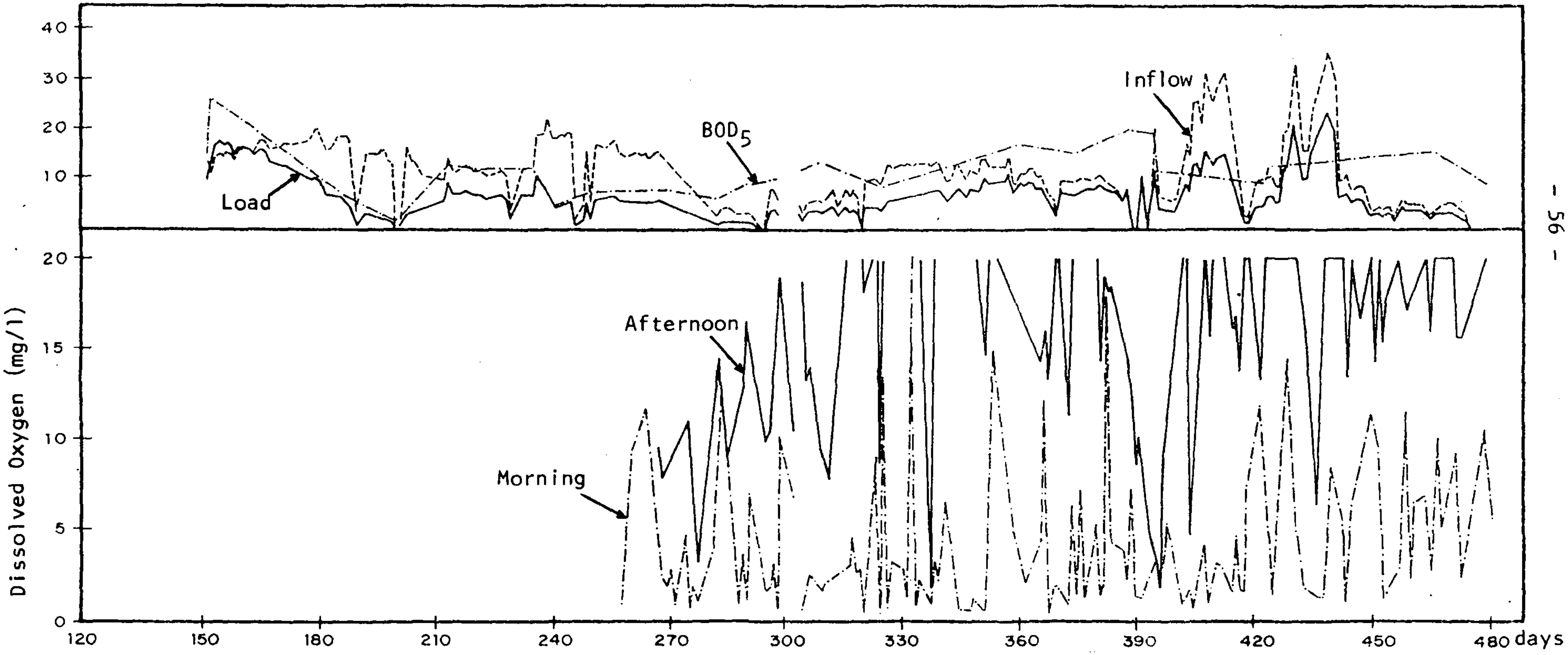


FIGURE 14. Daily Variations of pH, Temperature and Ammonia Nitrogen in Pond T2, April 1983-April 1984

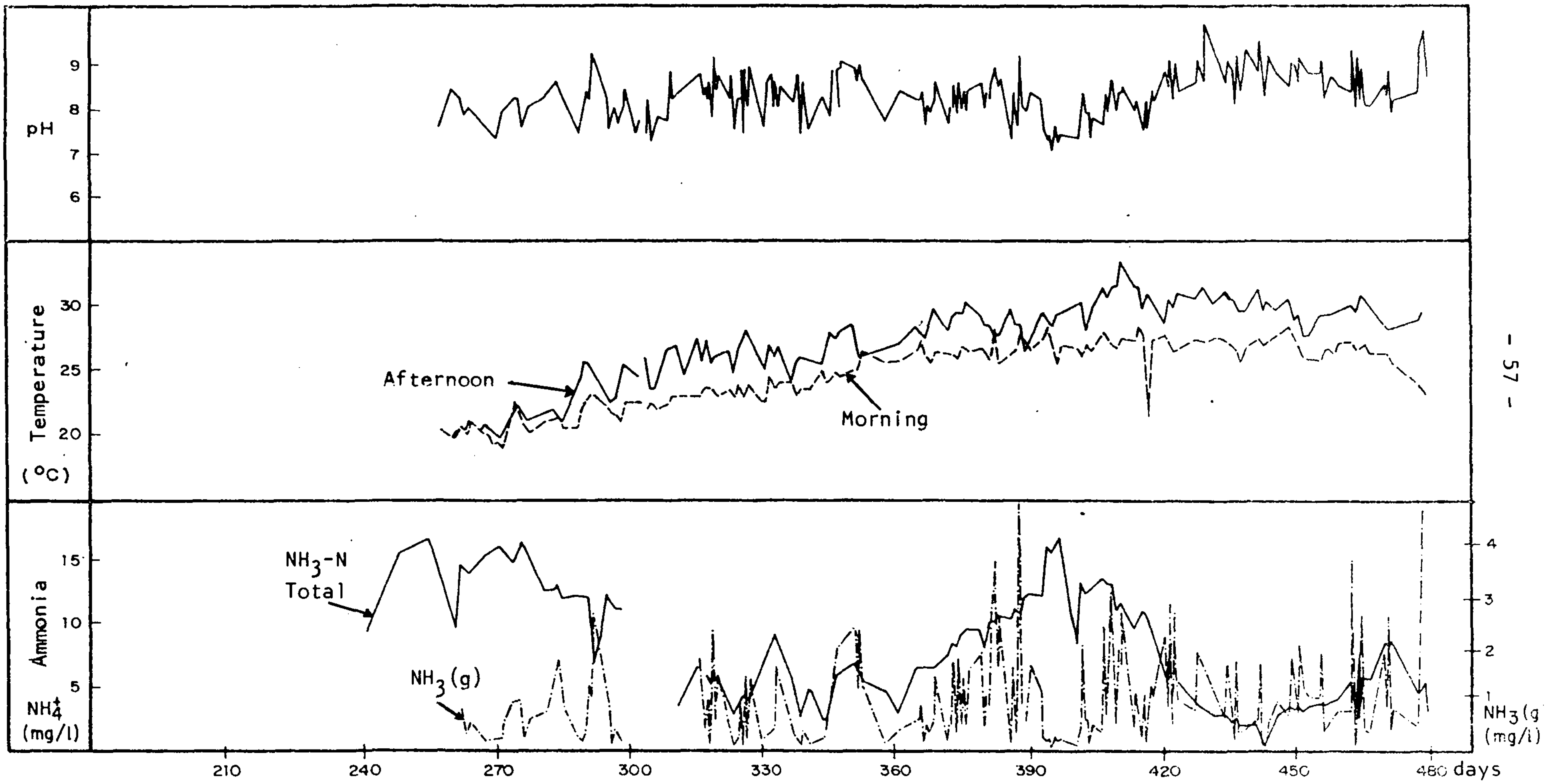


FIGURE 15. Daily Variations of Computed Inflow (lps), BOD_5 (mg/l), Load (Kg-BOD_5/Ha-day) and Dissolved Oxygen (mg/l) in Pond C2, April 1983 - April 1984

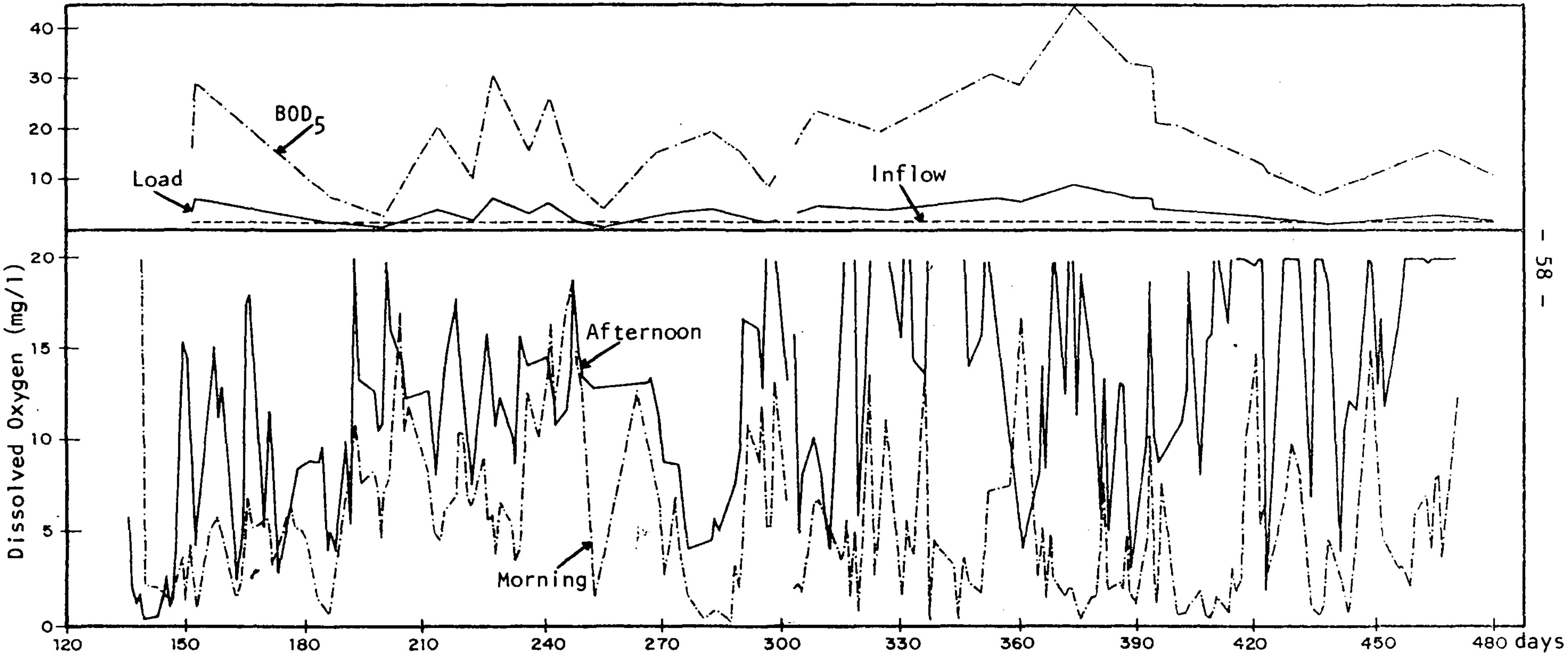


FIGURE 16. Daily Variations of pH, Temperature and Ammonia Nitrogen in Pond C2, April 1983-April 1984

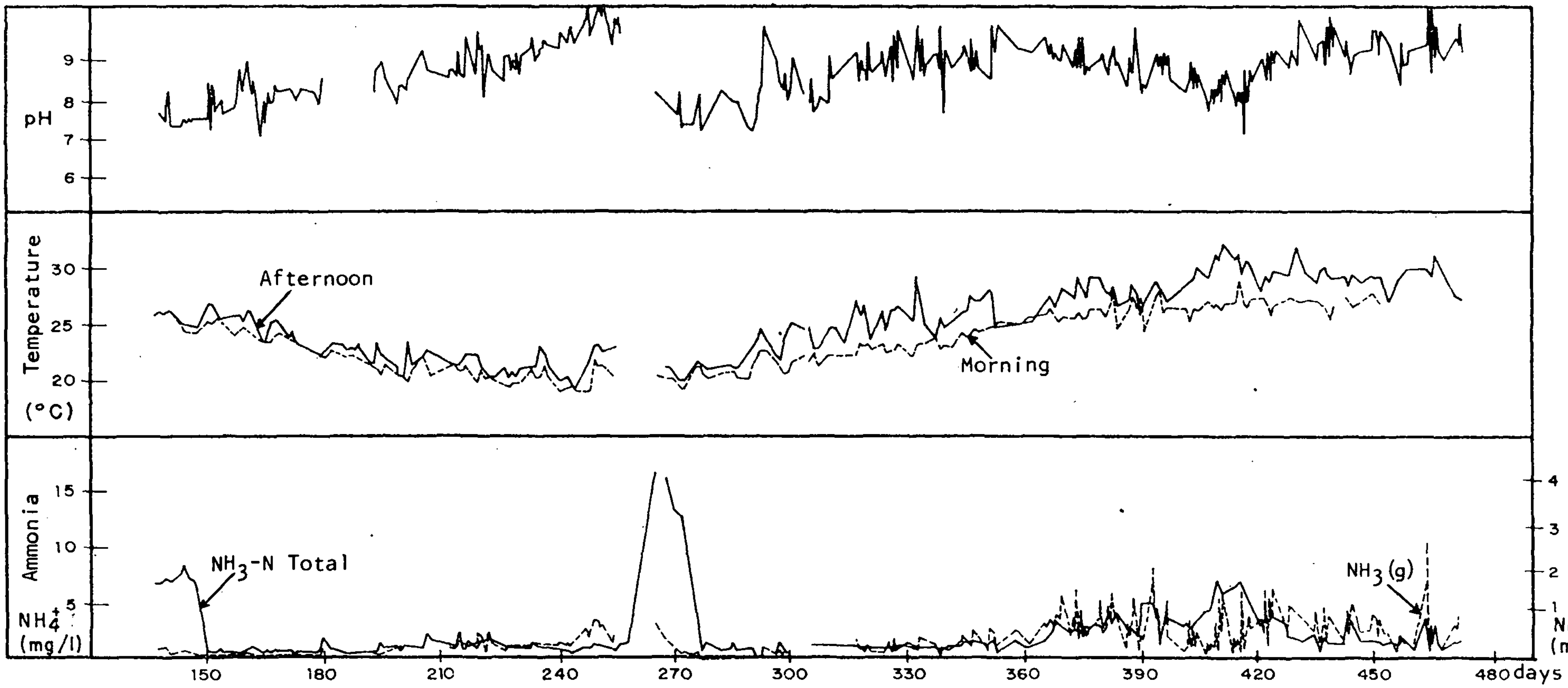


FIGURE 17. Daily Variations of Computed Inflow (lps), BOD_5 (mg/l), Load (Kg-BOD_5/Ha-day) and Dissolved Oxygen (mg/l) in Pond Q2, April 1983 - April 1984

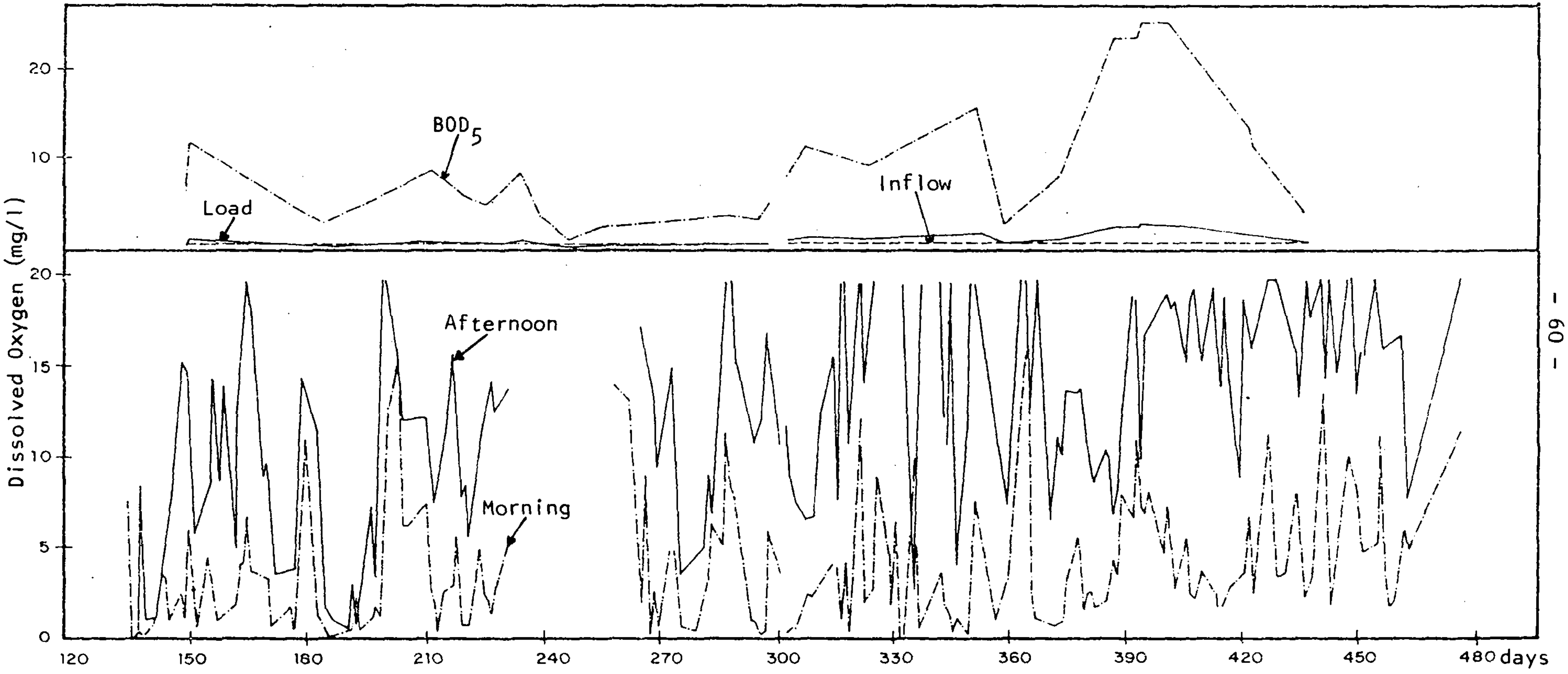
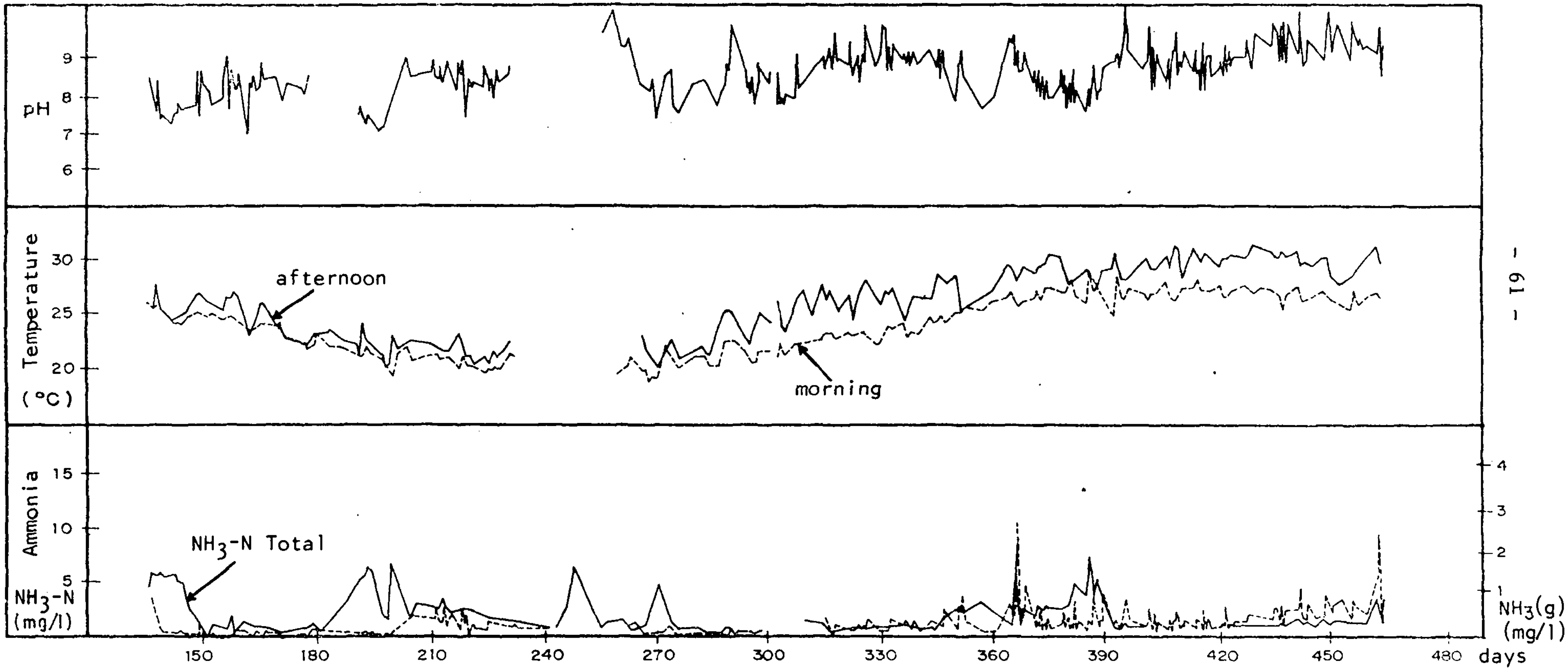


FIGURE 18. Daily Variations of pH, Temperature and Ammonia Nitrogen in Pond Q2, April 1983-April 1984



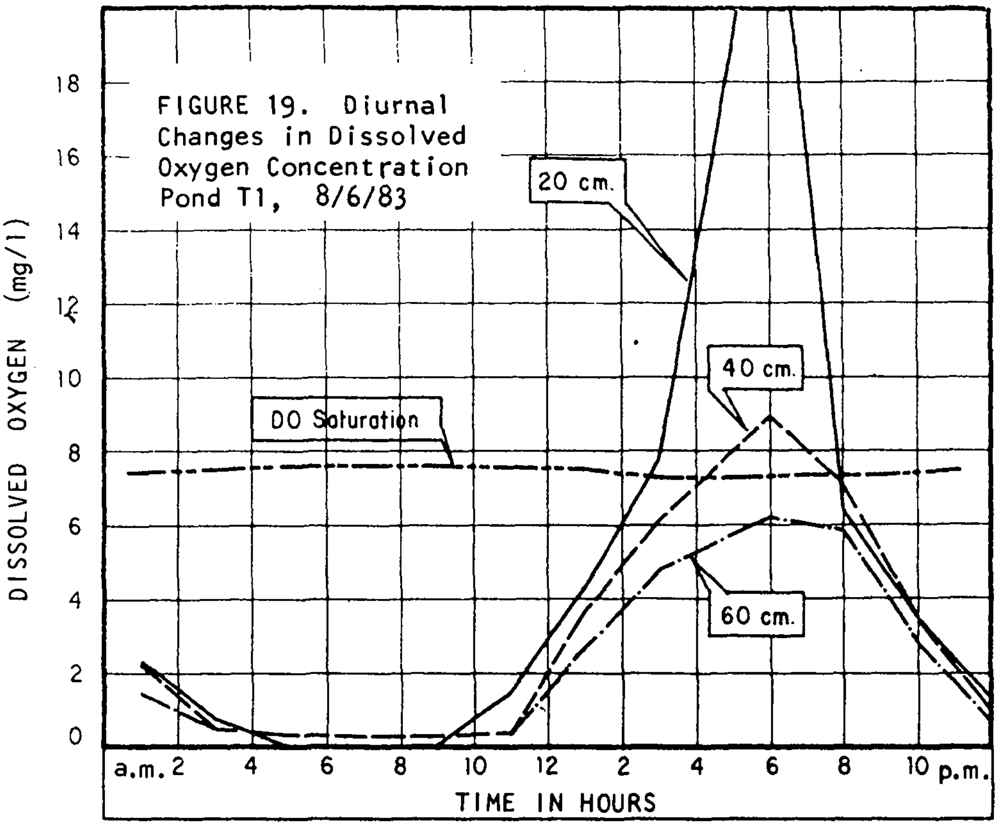


FIGURE 19. Diurnal Changes in Dissolved Oxygen Concentration Pond T1, 8/6/83

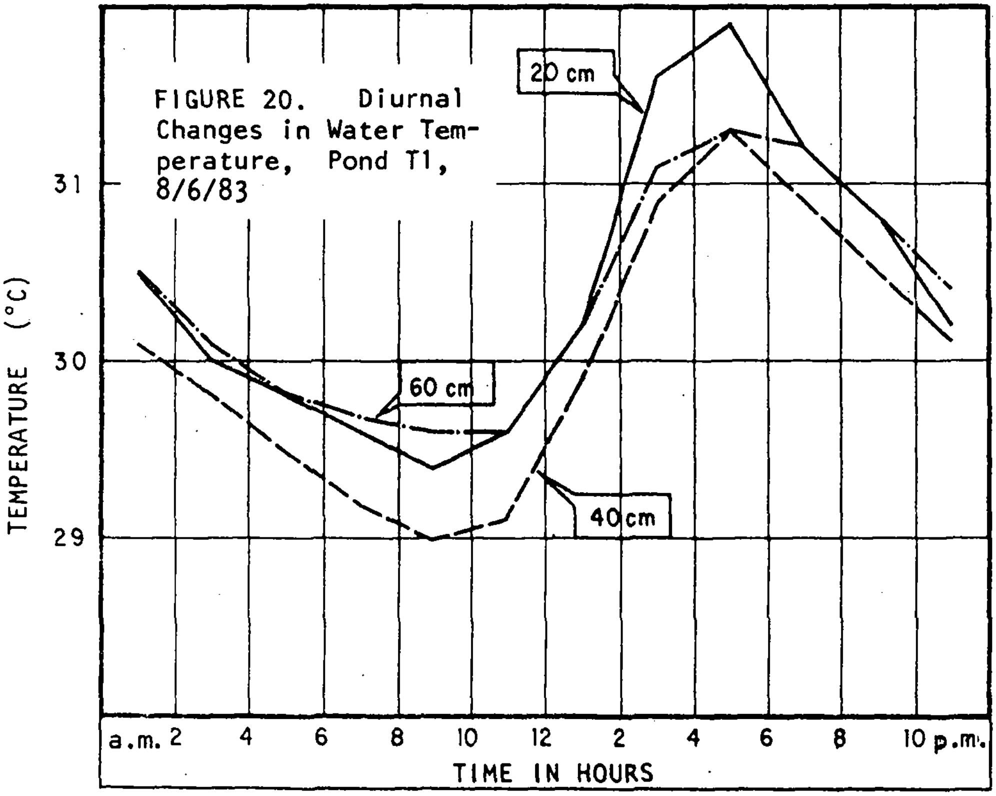


FIGURE 20. Diurnal Changes in Water Temperature, Pond T1, 8/6/83

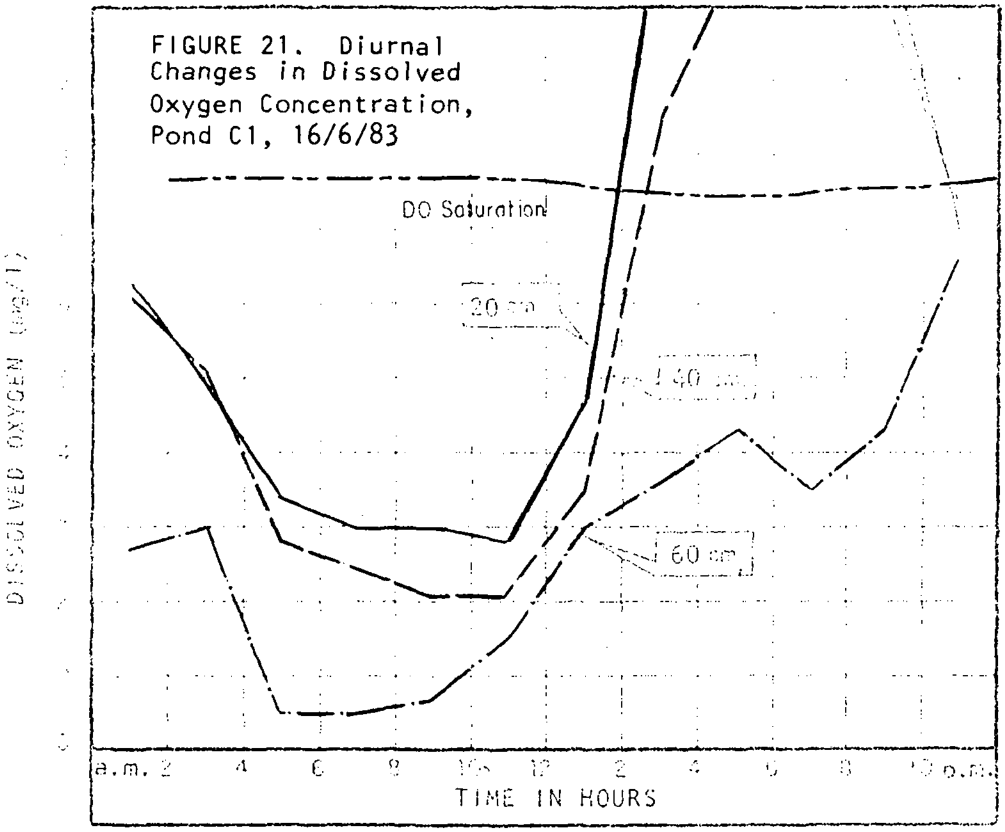


FIGURE 21. Diurnal Changes in Dissolved Oxygen Concentration, Pond C1, 16/6/83

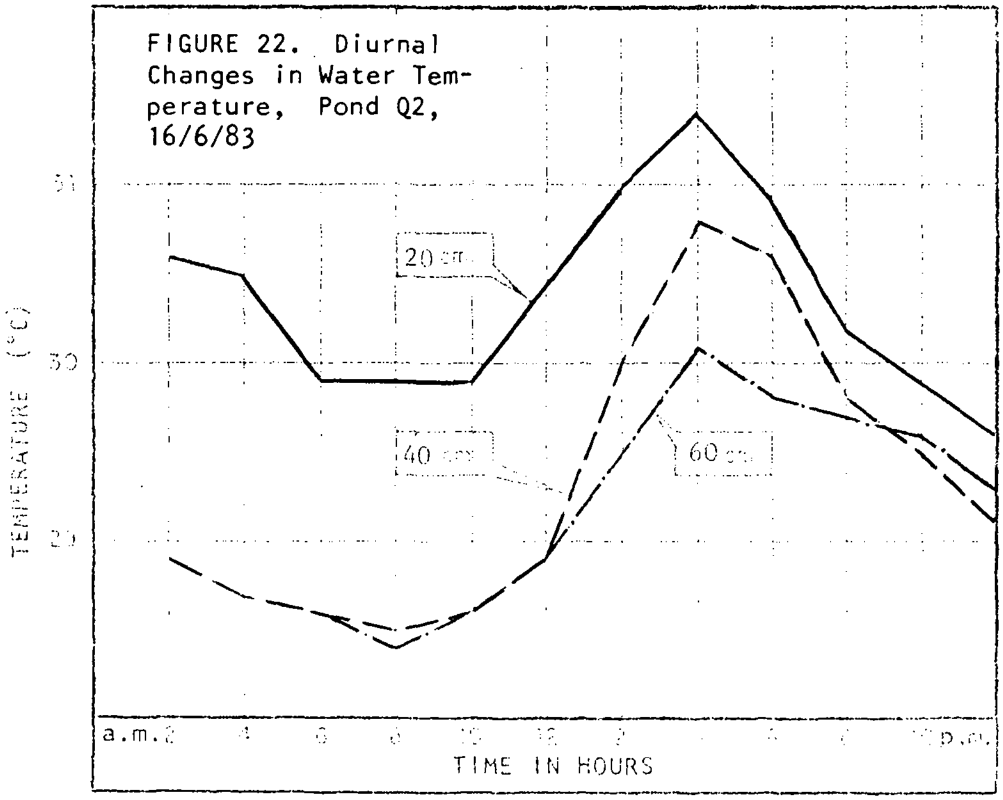


FIGURE 22. Diurnal Changes in Water Temperature, Pond Q2, 16/6/83

FIGURE 23. Concentration of Indicator Bacteria in Effluents, Series 1 (Geometric Average of Monthly Measurements, March 1983-June 1984)

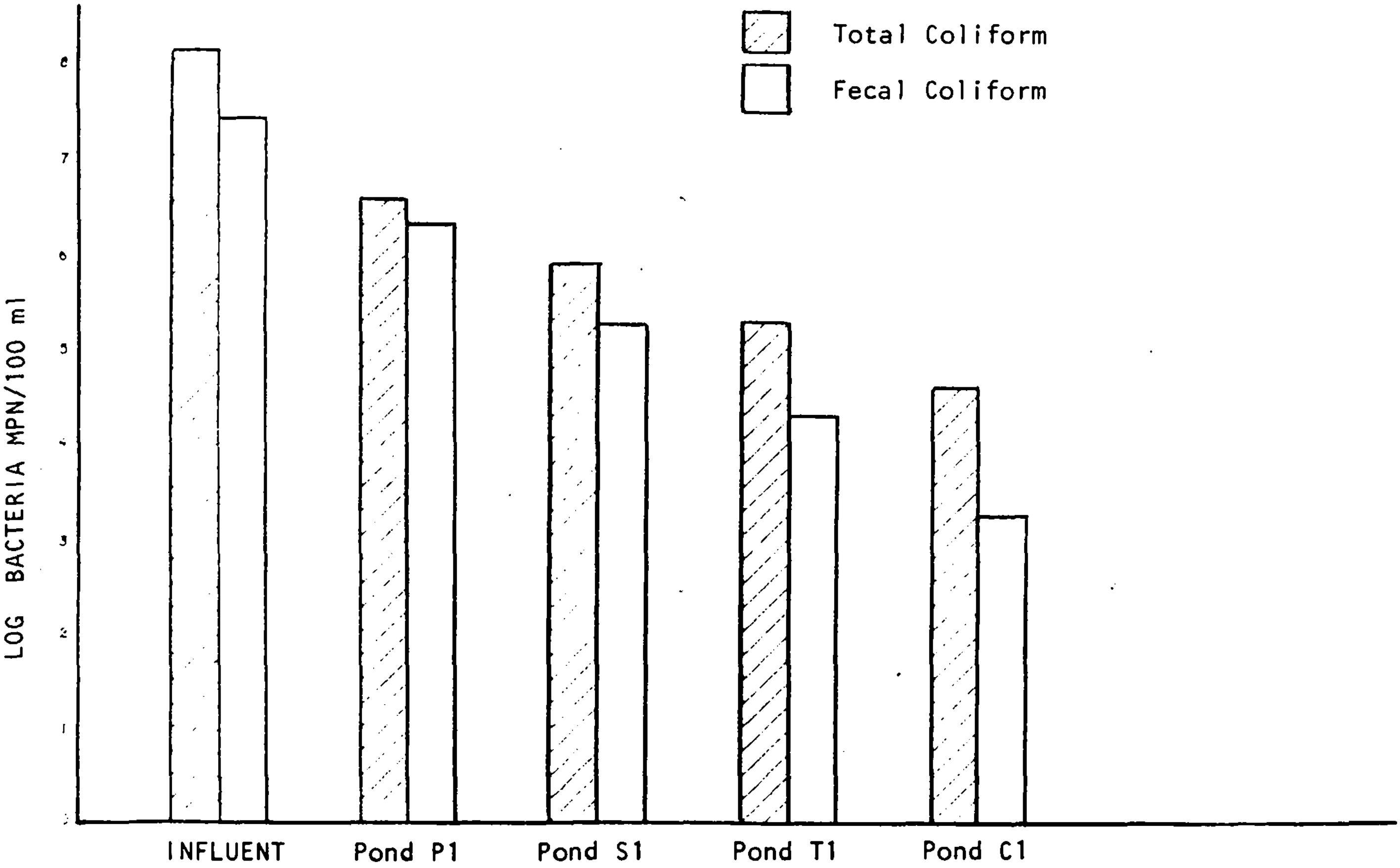


FIGURE 24. Concentration of Indicator Bacteria in Pond Effluents, Series 2. (Geometric Average of Monthly Measurements, March 1983-April 1984)

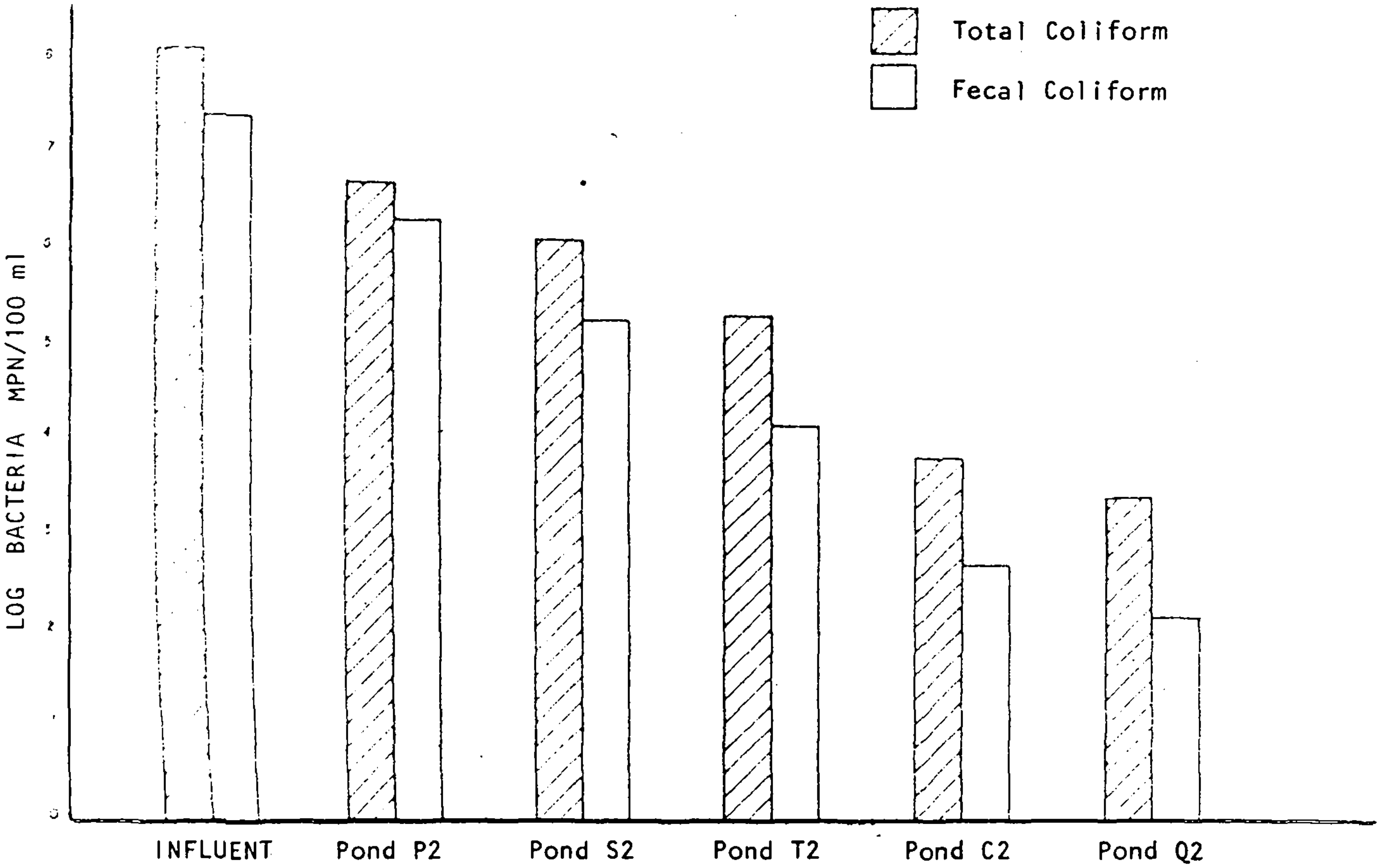


FIGURE 25. Total Coliforms in Pond Effluents, Series 1

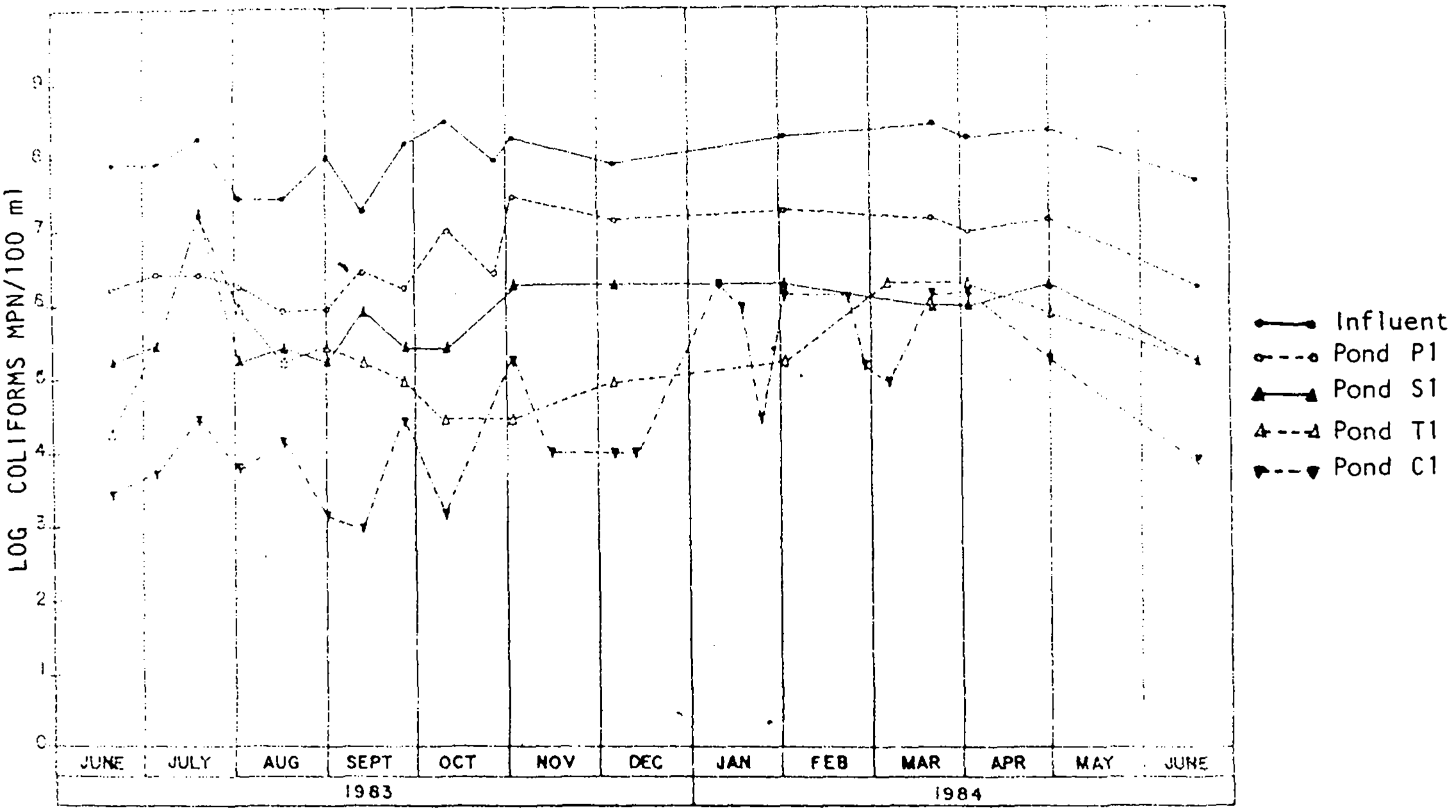


FIGURE 26. Fecal Coliforms in Pond Effluents, Series 1

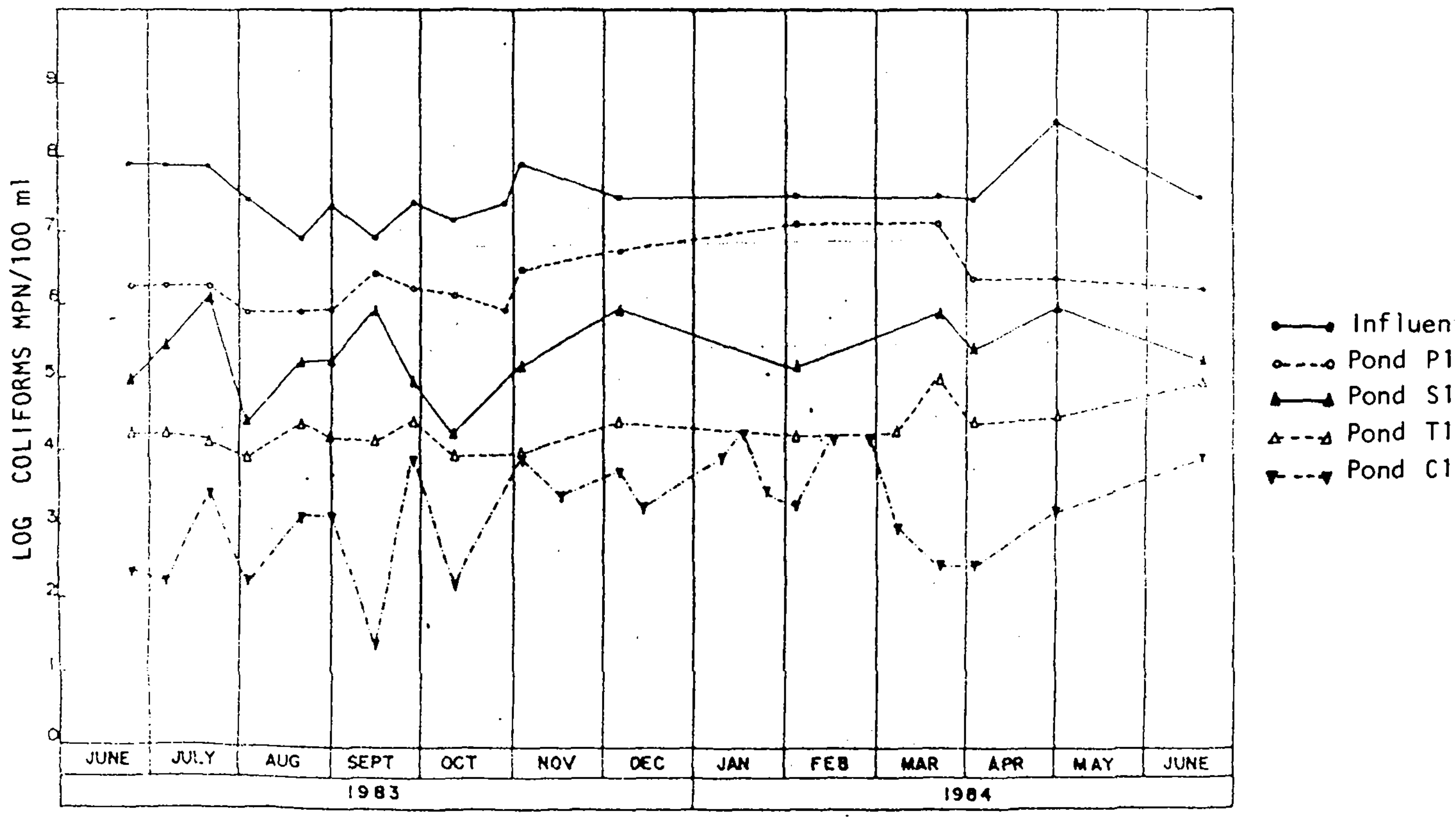


FIGURE 27. Total Coliforms in Pond Effluents, Series 2

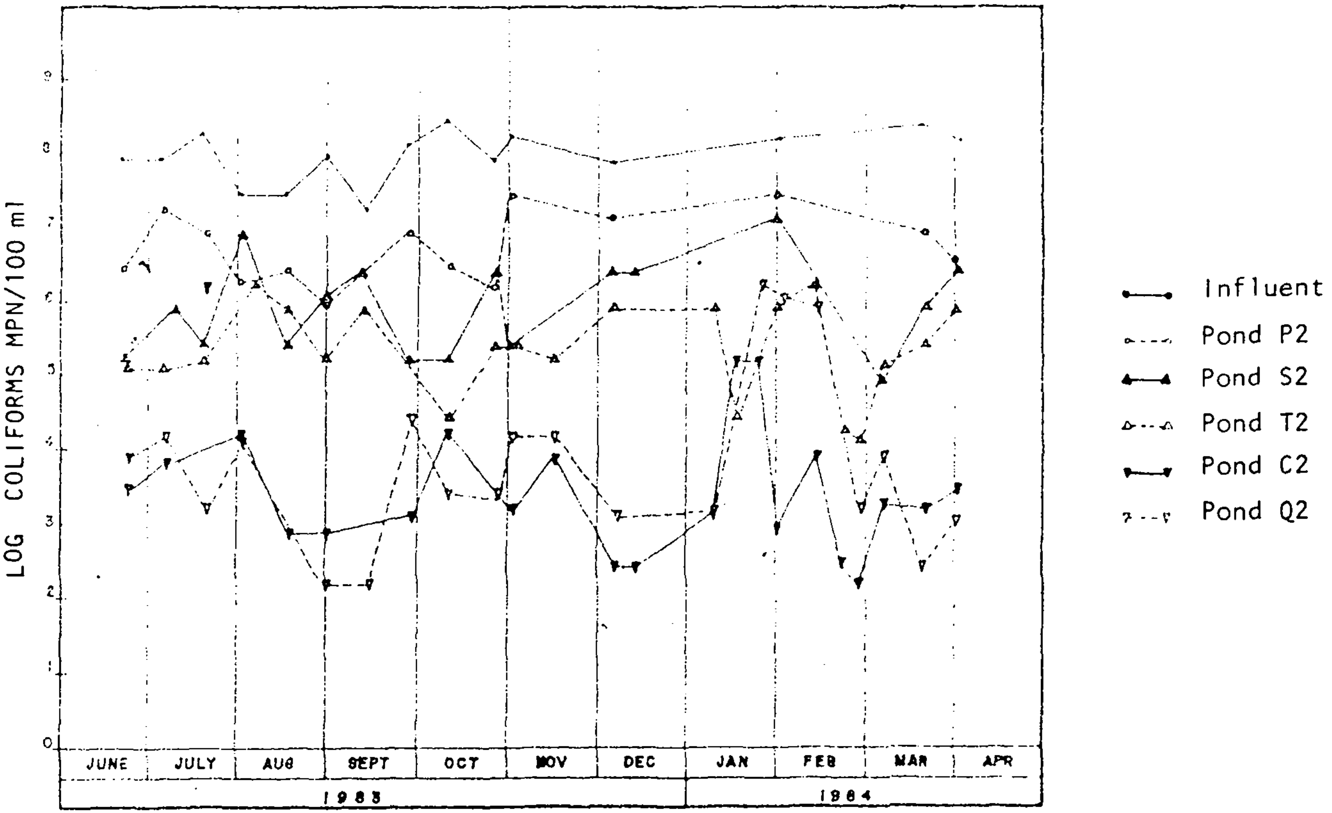


FIGURE 28. Fecal Coliforms in Pond Effluents, Series 2

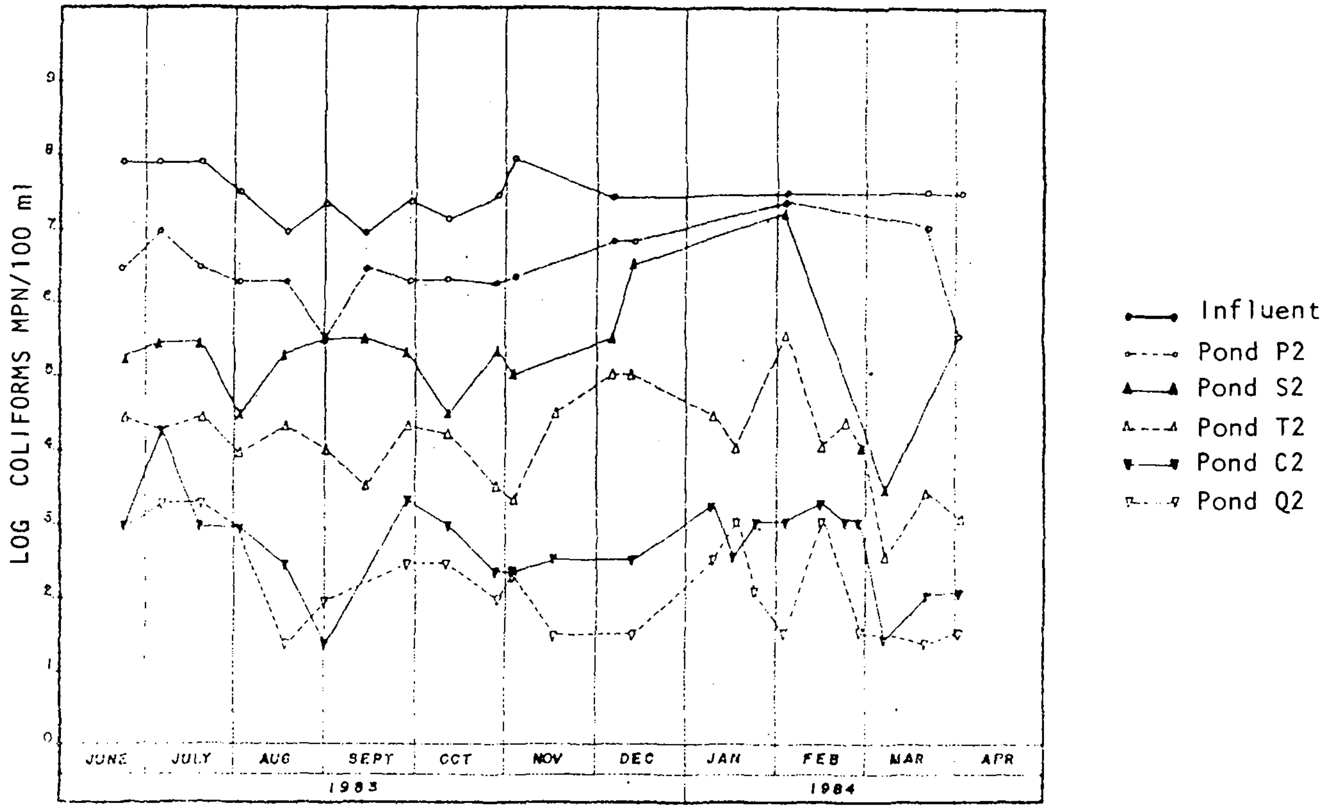


FIGURE 29. Total Coliforms in Pond Effluents and Sediments, Series 1, May-June 1984

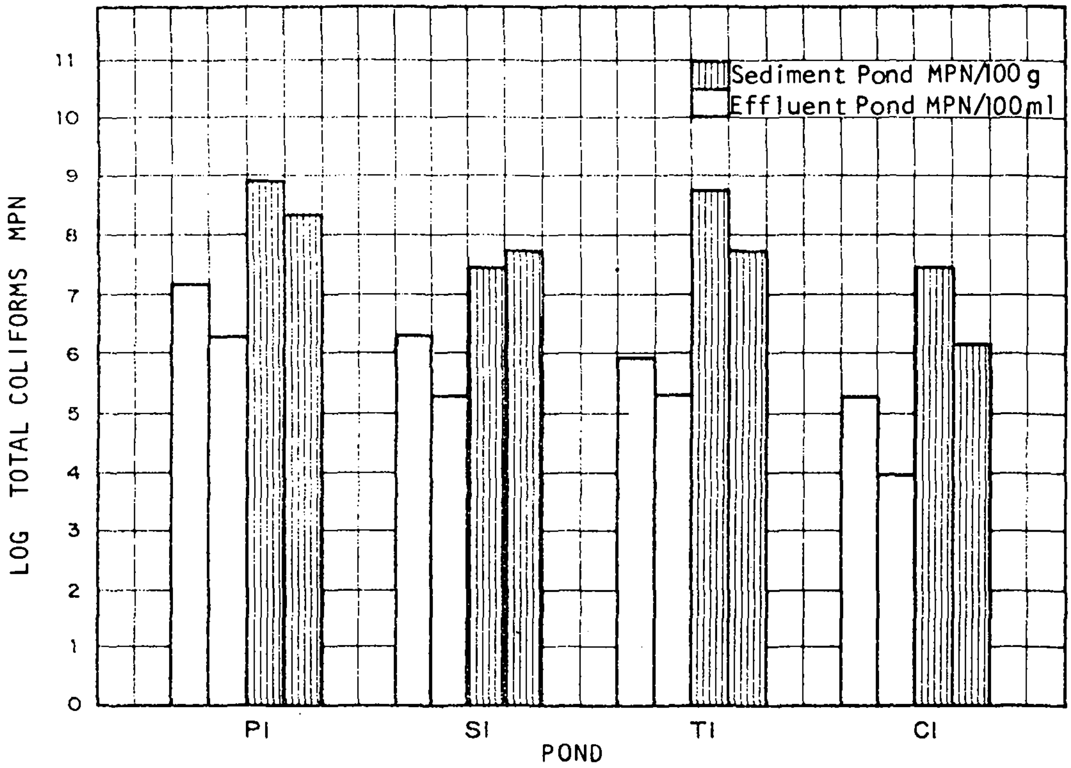


FIGURE 30. Fecal Coliforms in Pond Effluents and Sediments, Series 1, May-June 1984

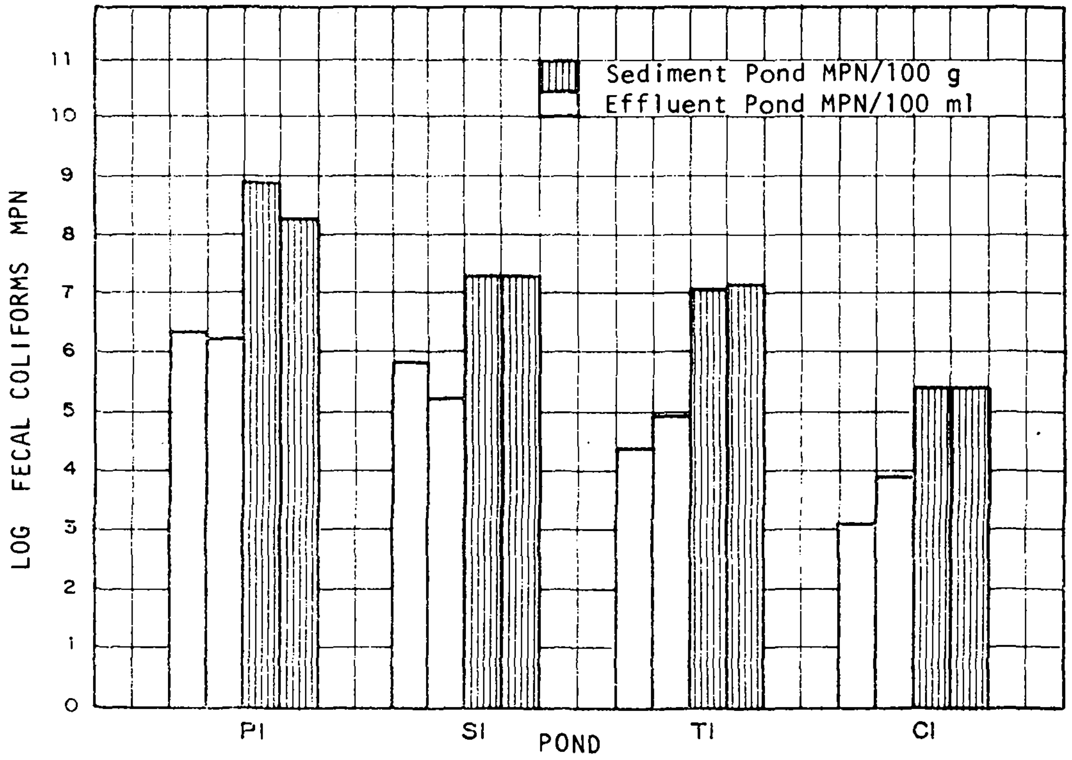


FIGURE 31. Total Coliforms in Pond Effluents and Sediments, Series 2, May-June 1984

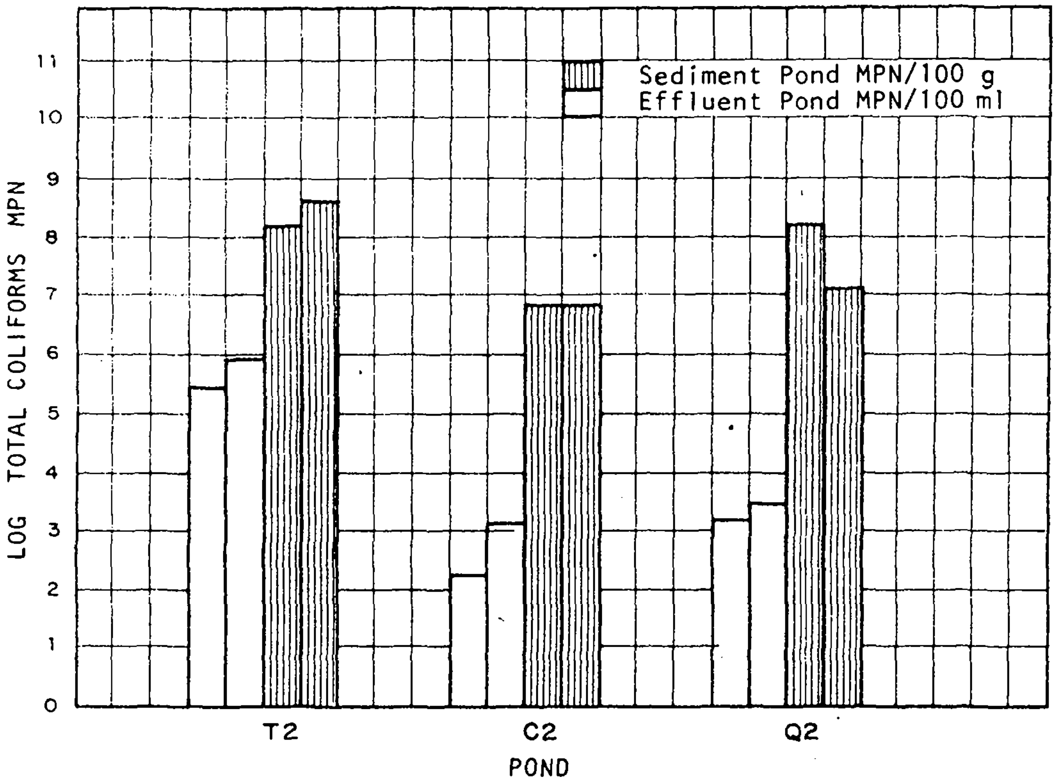


FIGURE 32. Fecal Coliforms in Pond Effluents and Sediments, Series 2, May-June 1984

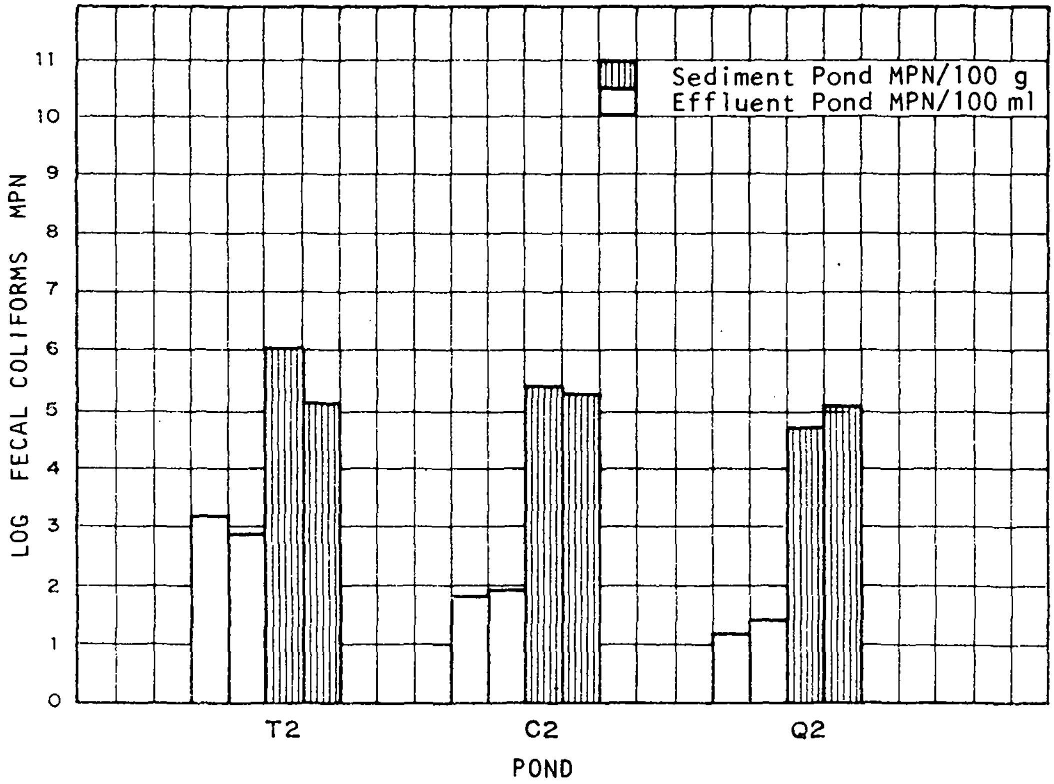


FIGURE 33. *Salmonella sp* Concentration in Pond Effluents and Sediments (May-June 1984)

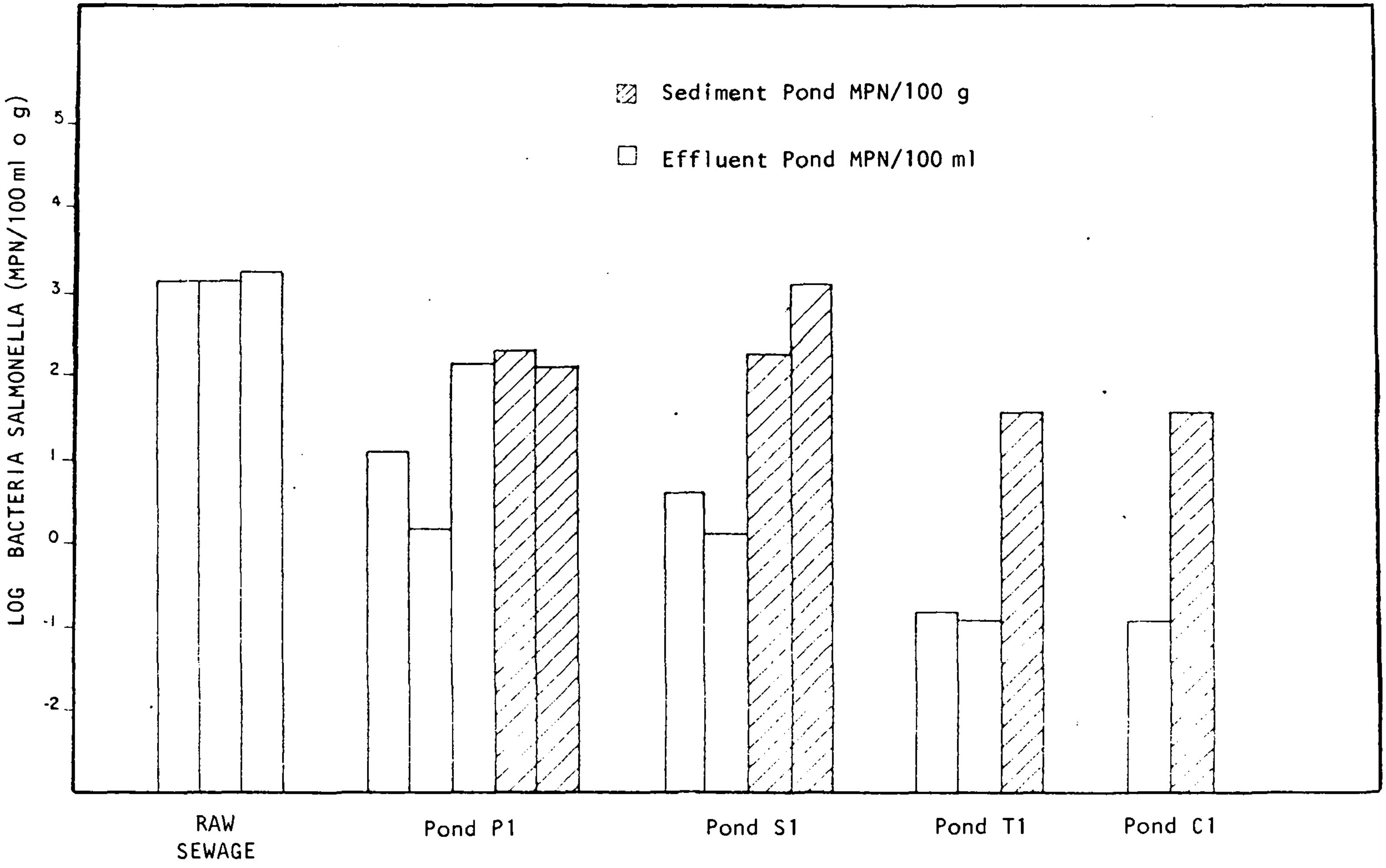


FIGURE 34. Bacteria Standard Plate Count (SPS) in Pond Effluent, Pond and Sediments (May-June 198[illegible])

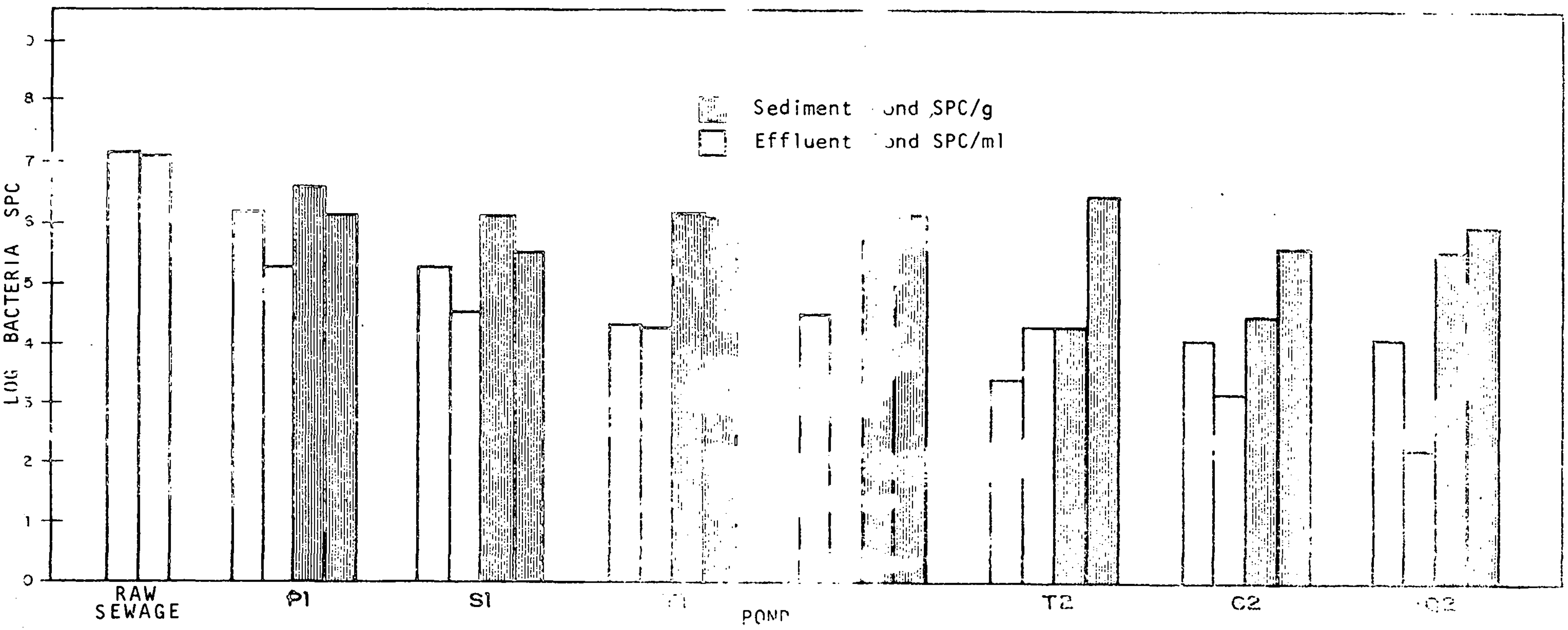
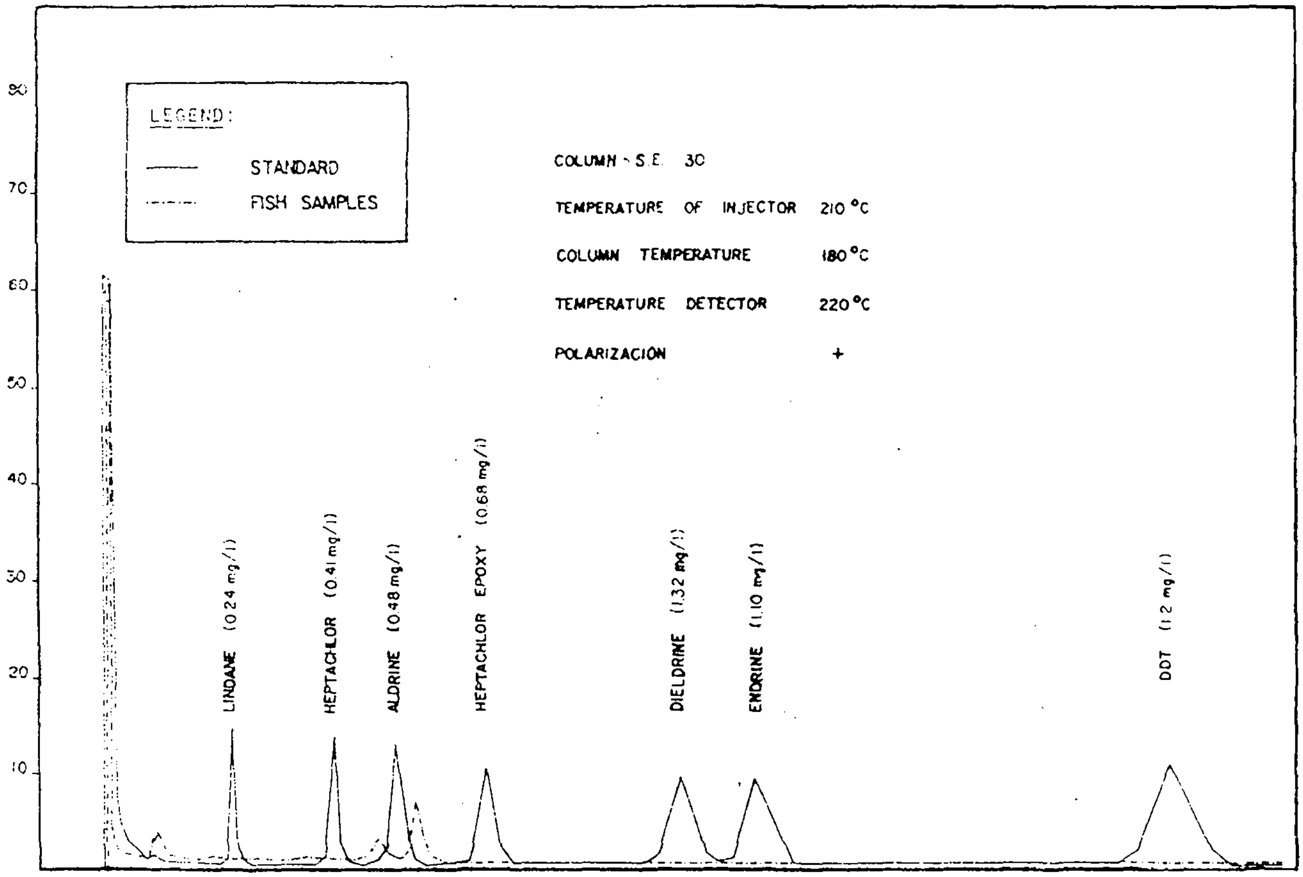


FIGURE 35. Sample and Standard Chromatographs for Organochlorinated Pesticides



ANNEX 1

LIST OF PROFESSIONALS, TECHNICIANS AND STUDENTS WHO PARTICIPATED IN CEPIS TRAINING PROGRAM WITHIN THE SAN JUAN RESEARCH PROGRAM

Professionals:	Country:	Period:
- Blg. María Lupe Galeano	Colombia	1 month
- Mr. Jorge Halife Scarza	Mexico	1/2 month
- Blg. César Lazcano	Peru	1 month
- Blg. Carmen Lucas*	Peru	6 months
- Eng. Magda Mateo	Peru	10 months
- Chem. Heriberto Meza	Paraguay	1 month
- Blg. Gabriela Moeller	Mexico	1 month
- Eng. J.C. Moscoso	Peru	2 months
- Eng. Mauricio Pardón*	Peru	4 months
- Eng. Danivel A. Pavón	Argentina	1 month
- Eng. Javier Pino	Bolivia	1 1/2 months
- Blg. Esther Robles	Peru	1/2 month
- Eng. Otto Rosasco	Peru	1 month
- Blg. Carmen Juárez	Peru	1 month
- Blg. César Jorge Taboada	Peru	1 month
- Blg. Haydeé Valenzuela**	Peru	18 months
- Blg. Mary Valverde**	Peru	2 + 3 months
- Mr. Tadeo Vitko* **	Peru	4 months
- Blg. Antonio Evangelista**	Peru	5 months
- Blg. Maritza Aguirre**	Peru	4 months
- Eng. Viviana Liberal**	Argentina	1 week
- Eng. Oscar Adolfo Torres**	Argentina	1 week
- Chem. Luz Castro* **	Peru	4 months
- Eng. César Barrientos	Guatemala	1 month
- Chem. Richard Salazar**	Peru	3 weeks

Technicians:		
- Mr. Luis Paz	Peru	3 months
- Mr. Enrique Quevedo**	Peru	10 + 6 months
- Mr. Hernán Yupanqui**	Peru	3 + 6 months
- Mr. Jorge Samamé**	Peru	6 months
- Mr. J. Martínez**	Peru	6 months
- Mr. Raymundo Lozano**	Peru	5 months

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* Started their training as students but were later recruited as associate researchers

** Participated in the San Juan Aquaculture study (World Bank/GTZ/CEPIS)

Students:	Country:	Period:
- Mr. Hermenegildo Ascencio	Peru	4 months
- Mr. José Ascue	Peru	8 months
- Ms. Livia Benavides	Peru	3 months
- Ms. Lizette Burgers	Holland	6 months
- Ms. Ariana Carughi	Peru	2 months
- Ms. María del Pilar Castillo	Peru	8 months
- Mr. Susana Chunga**	Peru	3 + 12 months
- Ms. Helen Espinoza	Peru	4 months
- Mr. Felipe Livia	Peru	6 months
- Mr. Luis Malarín	Peru	4 months
- Mr. Leonardo Merino	Peru	8 months
- Mr. César Reyes* **	Peru	4 months
- Mr. Gonzalo Gallo* **	Peru	4 months

Theses with CEPIS support:

- Mr. Felipe Livia. Thesis for a Sanitary Engineering degree. "Estructuras Hidráulicas en Sistema de Lagunas de Estabilización". National University of Engineering. Sanitary Engineering Program. 1982

- Ms. Carmen Lucas. Thesis for a Microbiology degree. "Estudio de Tres Técnicas Parasitológicas y su Evaluación en el Distrito de San Juan de Miraflores". Universidad Particular Ricardo Palma. Biology program. 1980

- Ms. Lizette Burgers. Masters thesis. "Temperature Behaviour in Stabilization Ponds Under Tropical Conditions". University of Wageningen, Holland. 1982

- Ms. Susana Chunga. Thesis for a Chemical Engineering degree. "Remoción de Detergentes en Aguas Residuales Domésticas Tratadas en Lagunas de Estabilización para su Empleo en Piscicultura". Universidad Nacional Mayor de San Marcos. Chemical Engineering Program. 1984

- Mr. Gonzalo Gallo. Thesis for a Biology degree. "Aislamiento e Identificación de Salmonella en los Terrenos Aledaños a las Lagunas de San Juan". Universidad Ricardo Palma. Biology Program. 1985

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* Started their training as students but were later recruited as associate researchers

** Participated in the San Juan Aquaculture study (World Bank/GTZ/CEPIS)