NETWORK OF AQUACULTURE CENTRES IN ASIA

NACA/WP/88/66 December 1988

PRELIMINARY STUDIES ON THE ANALYSIS OF BACTERIAL TYPES IN THE FISH PONDS APPLIED WITH FOUR KIND OF ANIMAL MANURE AND THE EFFECTS OF MANURING ON THE ECOSYSTEM & YIELD

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PRELIMINARY STUDIES ON THE ANALYSIS OF BACTERIAL TYPES IN THE FISH PONDS APPLIED WITH FOUR KIND OF ANIMAL MANURE AND THE EFFECTS OF MANURING ON THE ECOSYSTEM AND YIELD

ABSTRACT

The studies consisted of a continuous determination of the composition and change of aquatic bacteria and related ecological factors in the *fish* ponds applied with four kinds of manure of chicken, duck, pig and cattle, and the analysis of results using mathematical statistics and a minicomputer. The study shows that aquatic bacteria have very close relationship with many ecological factors in the fish pond such as dissolved oxygen, BOD, suspended substance, organic detritus, transparency, nutrient salts and plankton, which either shows direct or indirect ratio, and often play a decisive role in their existence and growth. It is thus obvious that the aquatic microorganisms have a very strong influence on the pond ecosystem. At the same time, aquatic bacteria, as the material decomposers and important natural feed for fishes, also directly or indirectly join the formation of autotrophic and heterotrophic food chains; it, in turn, affects the growth and yield of fish. Our studies also verify this through the analysis of the relationship between bacteria and fish yield and the determination of actual farming results of the fishes.

INTRODUCTION

The important role and significance of microorganisms in the recycling of the materials in the nature have been well known. Organic manure such as livestock manure is often applied in the integrated fish farming. The organic matter is mineralized into inorganic nutrient salts during the decomposition activity of aquatic microorganisms, especially aquatic bacteria, which provide material basis for further reproduction of feed organisms such as phytoplankton and zooplankton essential or the fishes. At the same time, a great quantity of bacteria is the \ill\ feed for the young fishes, and more important is that the bacteria s\ill\as feed for filter-feeding fish and omnivorous fish. According to the dies made in China and elsewhere, the fish yield gained from culture comes half to half nearly from autotrophic production with mainly algae and heterotrophic production with mainly bacteria. This indicates the important position occupied by the aquatic bacteria in the fish pond applied with manure.

The pond water body applied with organic manure such as manure of livestock is a semi-enclosed artificial ecosystem. From manure application to fish production, it goes through a complicated process of pond dynamics of growth and death of feed organisms, material recycling and energy flow, i.e., a process of continuous renewal of the artificial ecosystem to reach dynamic equilibrium. In order to understand this process for the dynamic increase of productivity in fish ponds, researchers have been carrying out studies during the past few years.

The aquatic bacteria not only serve in decomposing of material and energy transfer but also as a source of fish feed. Therefore, *the* rule and *position* of the aquatic microorganisms in the process of pond dynamics is of vital importance. It is therefore necessary to study the ecosystem of integrated fish farming, including ecology of aquatic bacteria.

The studies include the periodic determination (according to the manure application period) of the quantity, growth, death and species composition of aquatic bacteria and other major related ecological factors in the fish ponds applied with four kinds of manure of chicken, duck, pig and cattle and the preliminary analysis and studies on the effects of aquatic bacteria on ecological factors in the pond as well as the relationship with fish yield, by the utilization of statistics and minicomputer .

MATERIAL AND METHODS

a. Stocking species and ratio

Each of the ten 66.7 m^2 concrete ponds was stocked with 100 fingerlings at the rate of 45% silver carp Hypophthalmichthys molitrlx. 10% of bighead carp Aristichthys nobilis, 30% of crucian carp Carassius cuyieri, and 15% of common carp Cvprinus carpio. The culture period was 115 days.

The depth of pond .water was kept at about one metre. Water came from the Taihu Lake.

b. Manure application

The ten ponds were divided into five groups each consisting of two ponds. Four groups were applied with the manure of chicken, duck, pig and cattle respectively, and the fifth was used as the control group. One of the ponds in each group was chosen for water sample collection.

The manure was applied to the pond once a week at the rate of 21% of the fish weight (dry weight). It was mixed and stirred with the water evenly. The weight of fish in the pond was measured routinely in order to adjust the quantity of manure to be applied. During the period of the experiment, there was no application of grain, grass or other feeds.

Composition of the four kinds of animal manure was analysed and determined. (Table 1)

Table 1. Composition of the four animal manure

Unit : %

Item/manure of	Chicken	Duck	Pig	Cattle
Moisture content	27.76	74.45	75.05	80.30
Crude protein	25.00	19.87	18.13	13.40
Crude fat	8.52	9.19	8.90	7.90
Crude fibre	10.64	7.15	11.93	16.75
Total nitrogen	1.56	0.65	0.50	0.36
Total phosphorus	1.58	0.94	0.38	0.32
Total potassium	0.90	0.48	0.46	0.20

c. Water sample collection and determination

Water samples taken from the ponds from the day of manure application to the day before the next manure application in the first part of each month were examined. The details of water sample collection and determination are as follows:

1. Aquatic bacteria

The water sample was collected from a depth of 40-50 cm from the surface of several locations on the ponds using a 250 ml sterilized glass container as a collector.

a. The water sample was diluted with bacteria-free water and filtered with 0.3 urn filter membrane by vacuum drawing, before being treated with a solution of 50% carbolic acid and 5% erythrosin for the direct microscopoic counting to determine the number of total bacteria.

b. The water sample was then cultured for 48 hours at 25°C on beef extract-peptone -agar plate (pouring method) to determine the number of aerobic heterotrophic bacteria, using Gallenkamp-type colony counter.

c. Generic determination of the heterotrophic bacteria cultured on the plate mentioned above, after repeated separation and purification was carried out according to culture characteristics, form characteristics and some biochemical and physiogical characteristics using the normal bacterial classification methods followed at home and abroad. The statistics of the dominant colonies were also obtained.

2 Physico-chemical factors

a. The factors such as weather, air temperature, water temperature and pH value were recorded end determined.

b. A Secchi disc was used to determine the transparency of pond water, and YSI-57 Model Dissolved Oxygen Meter was used to determine the dissolved oxygen and BOD.

c. The normal methods were used to determine hydrochemical factors such as total nitrogen, total phosphorus, etc.

3 Biological factors

a. The normal methods were used in sampling and determining the biomass of phytoplankton and zooplankton.

b. The normal methods were used to determine the total amount of suspended matter in the water sample, which minus the biomass of phytoplankton and zooplankton was the weight of organic detritus.

RESULTS

a. The results of the weekly determination of aquatic bacteria from April to July (Figure 1) indicated that:

1 In the same period of each week the number of total bacteria in all the ponds varied. The number of total bacteria was the highest in the chicken manure applied pond, being 30.5 x 10^8/ml, and lowest in the control pond being only 0.01 x. 10^8/ml. The average value of total bacteria in the ponds was 11.05 x 10^8/ml in chicken manure applied ponds, 6.31 x 10^8/ml in duck manure applied ponds; 5.18 x 10^B/ml in cattle manure applied ponds.. 4.15 x 10^8/ml in pig manure applied ponds, and 1.7 xlO^s/ml in the control ponds.

In each period, the total number of bacteria in all the manure applied ponds showed a maximum peak. However, the composition of the total bacteria was very complicated, due to the influence of various kinds of manure applied. There were temporal differences in the occurences of the maximum peaks of the total bacterial number in different periods. In general, the maximum peaks appeared 3 to 5 days after the manure application; therefore, the growth and death of the total bacteria were not regular.

In the same period of each month and in each month the number of aerobic heterotrophic bacteria in all the ponds was also quite different from each other, bearing a sharp resemblance to the case of total bacteria (see Figure 2). The number of aerobic heterotrophic bacteria was highest in the chicken manure applied pond, being 27.8 x 10^3 /ml, and lowest in the control pond, being 0.03 x 10^3/ml. The average value of the aerobic heterotrophic bacteria was 7.93 x 10^3/ml in the cattle manure applied ponds, 3.9 x 10^3/ml in pig manure applied ponds and 0.93 x 10^3/ml in control ponds.

In each period, the number of aerobic heterotrophic bacteria in all the manure applied ponds showed two maximum peaks. The first maximum peak occurred on the first day after manure application. the second maximum peak occurred on the second and third days after manure application, and were usually higher than that of the first day. The first peak was due to the bacteria brought by the manure applied, and the second peak was due to further decomposition of the orgranic matter in *the manure* by heterotrophic bacteria in the water, which resulted in the mass

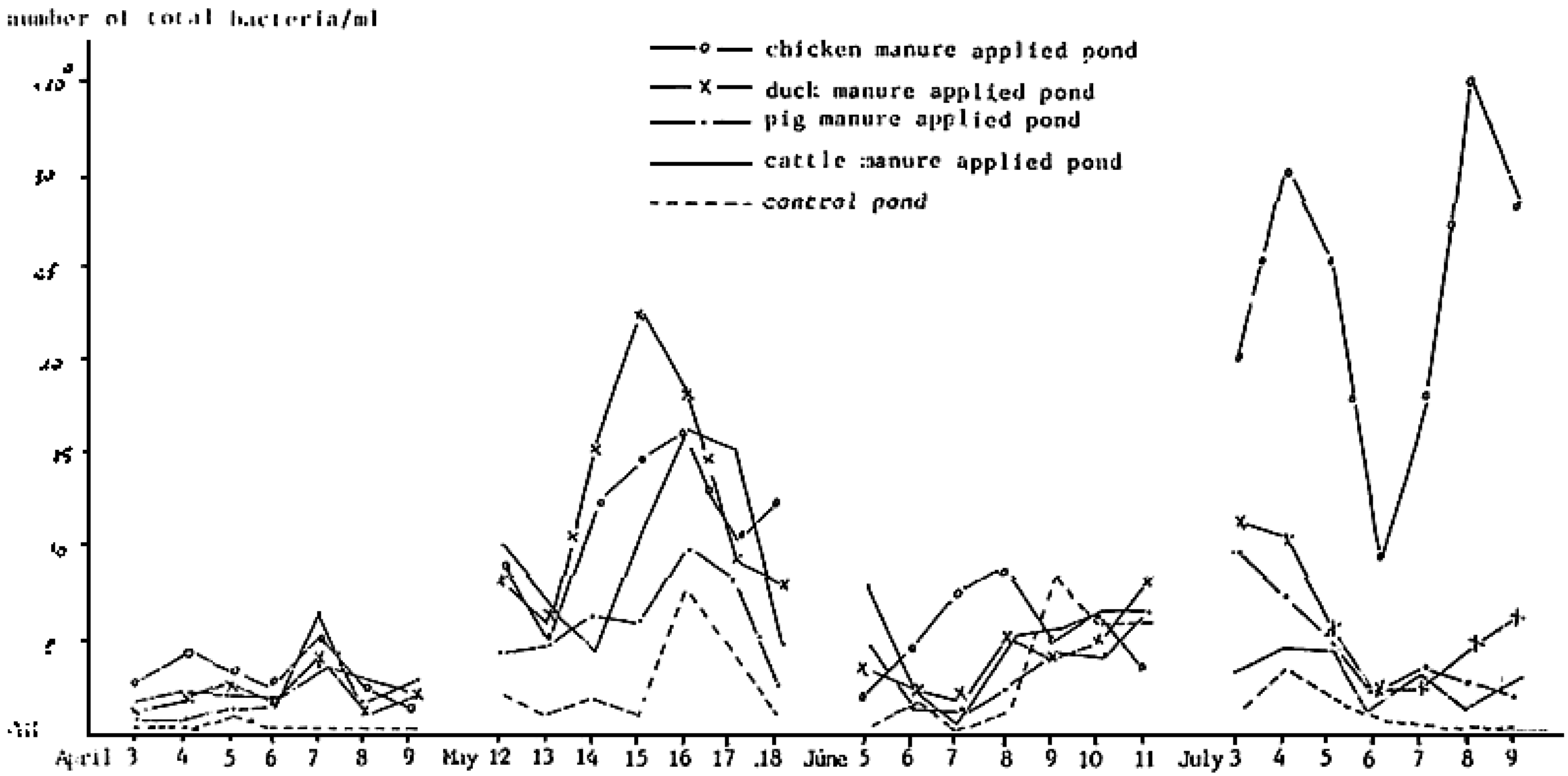


Figure I. The Change of Total Bacteria.

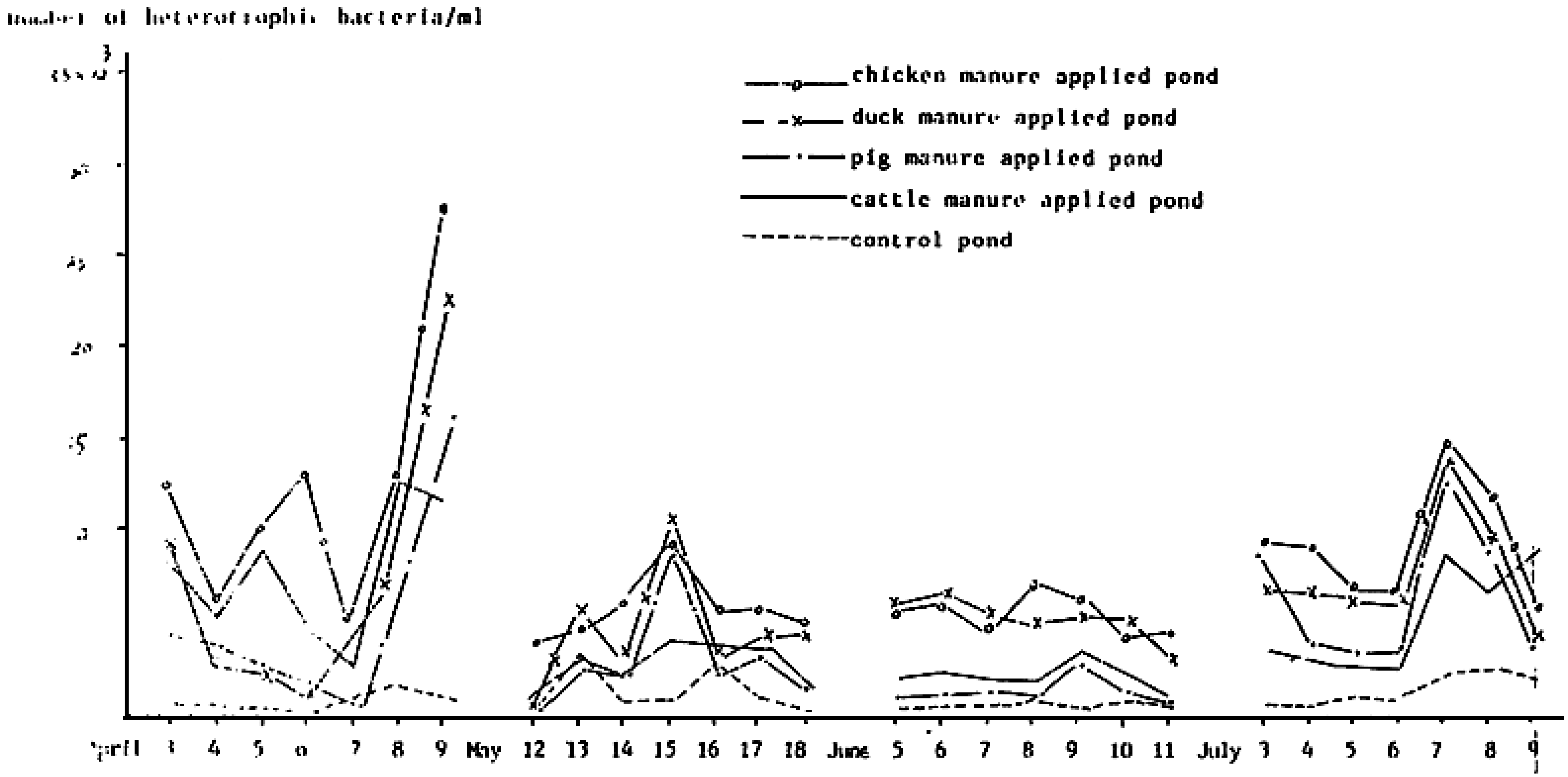


Figure II . The Change of Heterotrophic Bacteria

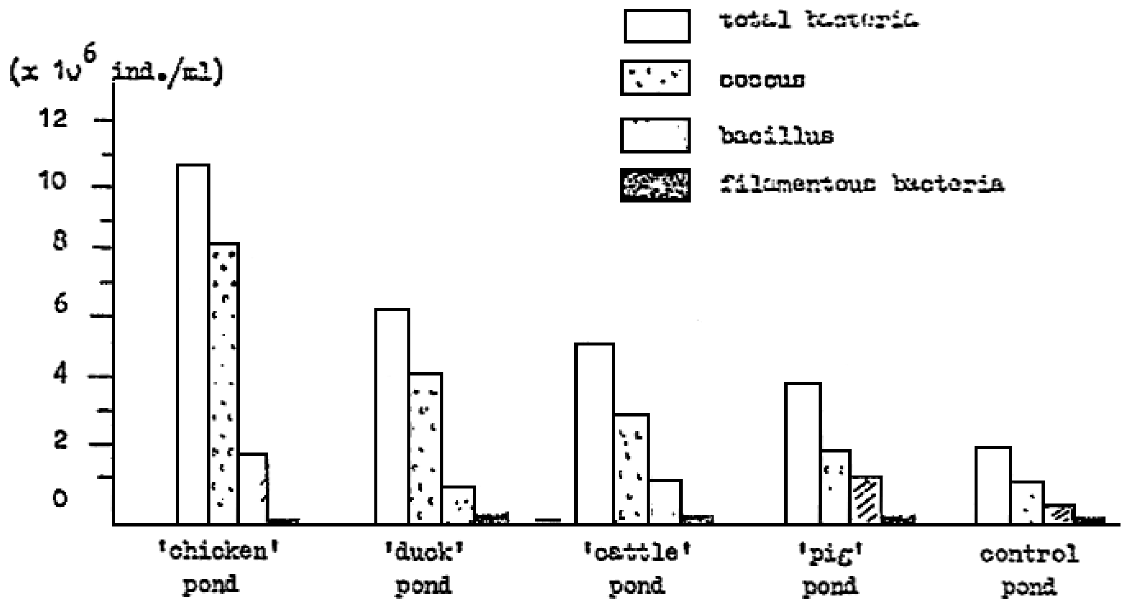


Figure 3. Composition and proportion of total bacteria.

2. After culture, separation and purification, 203 pure bred strains of aerobic heterotropic bacteria were observed. The preliminary results of the bacterial classification indicate that,

a. Dyeing reaction: 99 strains 48.8%) of bacteria were Gram-positive, 92 strains (45.3%) were Gram-negative, and 12 strains (5.9%) were Gram-variable.

b. Classification: There were a total of 34 genera in various ponds, which included: Micrococcus Cohn, Achromobacter, Bergey et al, Lactobacillus Beijerinck, Cytophaga, Winogradsky, Streptococcus Rosenbach, Bacillus Cohn, Staphylococcus. Rosenbach, Corynebacterium. Lehmann et Neumann, and Arthrobacter. Connet dimmick.

3 After the culture and determination, the prominent colony for the water samples from various ponds was counted. (Table 2).

c The average values of the main physico-chemical factors, such as dissolved oxygen, BOD, transparency, total nitrogen, total phosphorus, etc., as well as the main biological factors such as phytoplankton and zooplankton, obtained by continuous determination in all the ponds during the four periods are shown in Table 3.

Table 2. Prominent colony of various ponds

Ponds applied with manure of	Genus of bacteria	Percentage
Chicken	Arthrobacter. Connet Dimmick	17.2
	Micrococcus, Cohn	14.3
	Aloaligenes. Castellani et Chalmers	9.0
Duck	Streptococcus, Rosenbach	15.8
	Micrococcus Cohn	12.3
	Lactobacillus, Beijerinck	10.6
Pig	Achromobacter, Bergey et, al	40.2
	Staphlococcus. Rosenbach	12.9
	Cytophaga. Winogradsky	12.9
Cat-tie.	Cytophaga,, Winogradsky	19.2
	Micrococcus. Cohn	12.8
Control	Micrococcus. Cohn	9.1
ponds	Aohromobacter. Bergey et. al	9.1

Table 3. Average value of physico-chemical and biological factors in various ponds

unit: mg/1

Items/ponds	Chicken	Duck	Pig	Cattle	Controlponds
Dissolved oxygen	8.60	9.60	8.80	11.30	12.40
BOD	5.80	4.80	4.10	4.20	1.70
Suspended matter	128.01	99.80	80.91	89.20	57.79
Organic matter	71.00	61.96	52.57	64.44	43.34
Transparency (cm)	44.67	36.52	47.31	41.72	79.42
Total nitrogen	6.79	4.17	4.16	3.90	3.41
Total phosphorus	0.434	0.094	0.067	0.024	0.019
Phytoplankton	38.70	22.55	20.61	19.15	12.84
Zooplankton	18.30	6.29	7.73	5.61	1.61

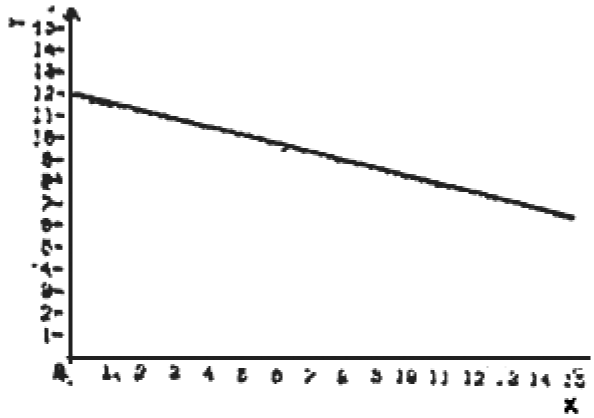
1. To find out the interactions between bacteria and pond ecological factors, we applied the biological mathematical statistics method in the analysis and check, and inputted the related data into a microcomputer for further calculation. The related regression equation and figures are as follows:

$$Y = a + bx$$

Where

a. interrelationship between bacteria and dissolved oxygen

Y = 12.061 + (-0.399)x
r = -0.7075 (showing notable inverse correlation)

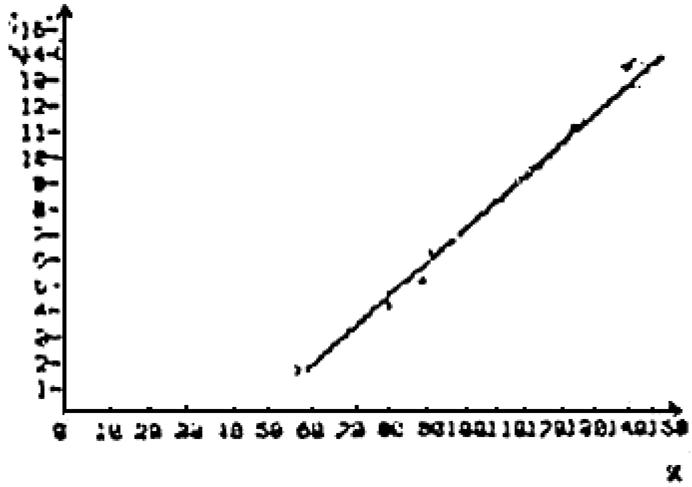


b interrelationship between bacteria and BOD

Y = 1.8480 + 0.483 x
r = 0.9133 (showing notable direct correlation)

c interrelationship between bacteria and suspended matter

Y = 47.9927 + 7.2823x
r = 0.9929 (showing notable direct correlation)



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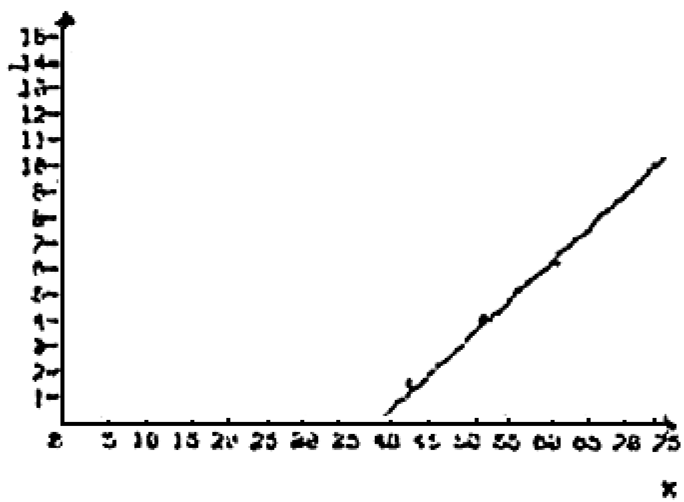
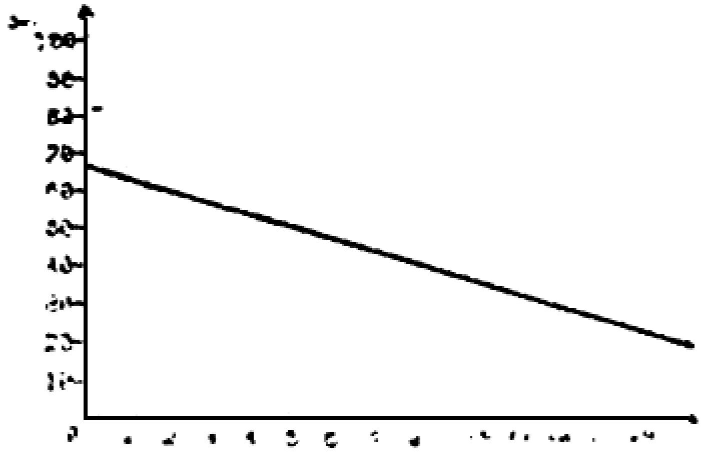
d interrelationship between bacteria and organic detritus

Y = 42.4915 + 2.8479X
r = 0.9085 (showing notable direct correlation)



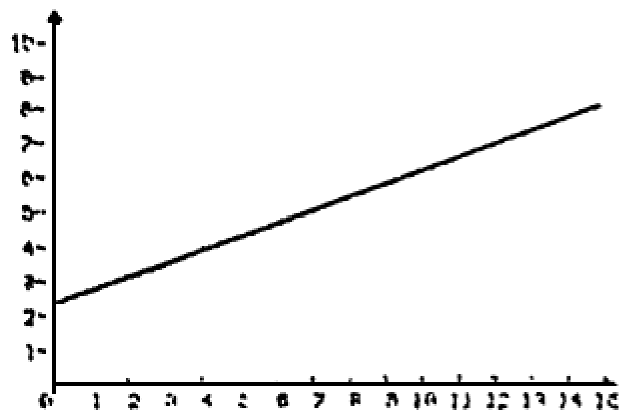
e interrelationship between bacteria and transparency

$$Y = 67.3140 + (-3.00300)x$$
$$r = -0.6229$$ (showing notable inverse correlation)



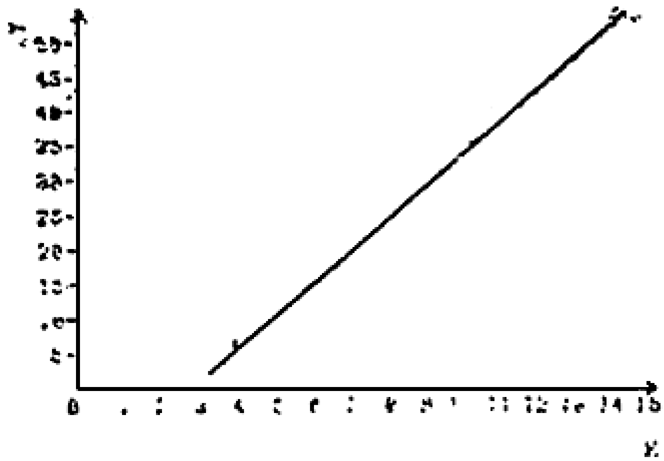
f interrelationship between bacteria. and *total* nitrogen

$$Y = 2.3520 + 0.3820X$$
$$r = 0.9404$$ (showing notable direct correlation)



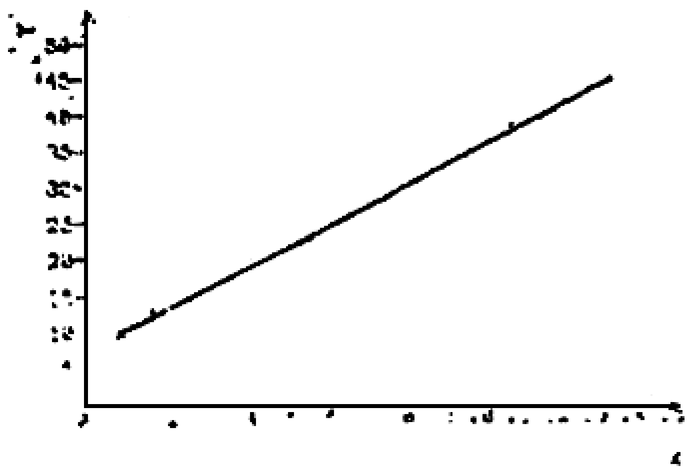
g. interrelationship between bacteria and total phosphorus

Y = 13.4750 + 4.6180x
r = 0.9156 (showing notable direct correlation)



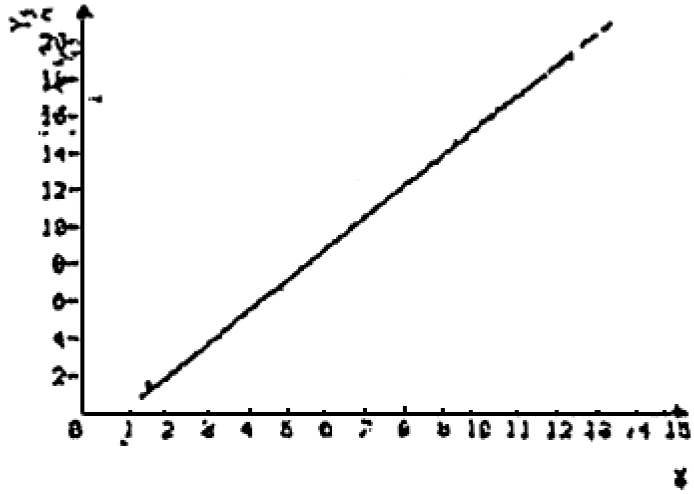
h. interrelationship between bacteria and phytoplankton

Y= 7.2780 + 2.7280x
r = 0.9789 (showing notable direct correlation)



i. interrelationship between bacteria and zooplankton

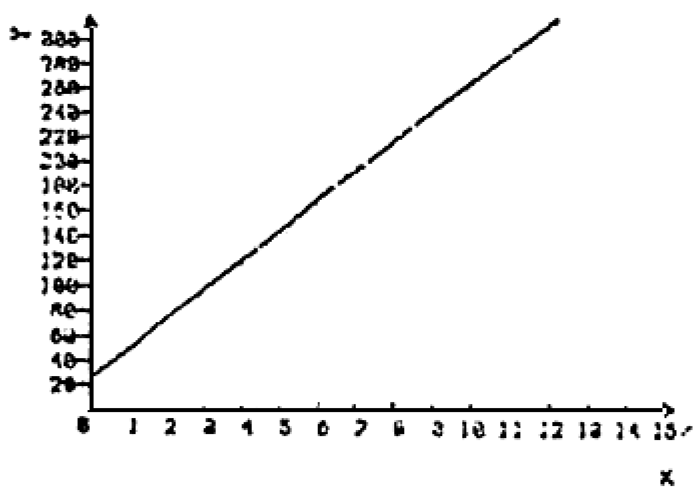
$Y = -1.7740 + 1.7500x$
$r = 0.9437$ (showing notable direct correlation)



2. To find out the interrelationship among aquatic bacteria which are also the decomposers of matters and transferers of energy and important as a natural feed for fish growth, we conducted the mathematical statistical analysis and used the microcomputer for determination of results. The regression equations and figures obtained were as follows:

interrelationship between bacteria and the fish yield:

$Y = 26.2500 + 24.1540x$
$R = 0.888294$ (showing notable direct correlation)



We also determined the interrelationship between bacteria and fish yield through the analysis and comparison of the actual results of the fish farming, (see Table 4).

Table 4. Comparison of the effects of four animal manure on fish farming

Items/pond	Chicken	Duck	Pig	Cattle	Control
Total bacteria (average daily amount) individual/ml	11.05xl0^{8}	6.31xl0^{s}	4.15xl0^{B}	5.18xl0^{s}	1.7xl0^{s}
Heterotrophic bacteria (average daily amount) individual/ml	7.93xlO^{3}	5.8xl0^{3}	3.9xl0^{3}	4.24xl0^{3}	0.93xl0^{3}
Conversion rate of manure dry weight	2.28	2.32	2.17	3.15	0
Body weight increment compared with that when stocked	3.6	3.2	2.9	2.5	0.86
compared with that in control pond	4.4	3.8	3.6	2.9	1.0
Net yield of fish kg/mm	129.8	106.8	100.9	70.9	-6.3

Note: a.the culture period is 115 days
b.15 mu is equal to 1 ha

3. We conducted the analysis of some closely-related biological factors using the polybasic linear regression equation, in order to understand further their interrelationship with the bacteria.

$$Y= a_0 + b_1 X_1 + b_2 X_2$$

a. interrelationship between total bacteria, total nitrogen and total phosphorus

$Y = 5.6632 - 0.6339 X_1$ (total N) $+ 24.3523 X_2$ (total P)

$r = 0.9890$ (showing notable direct correlation)

b. interrelationship between total bacteria, phytoplankton and zooplankton

$Y = -6.0131 + 0.7055 X_1$ (phytoplankton - $0.5530 X_2$ (zooplankton)

CONCLUSION

1. In order to further the studies on fluctuation performance and the interrelationship of many ecological factors in the ponds applied with four kinds of animal manure, we changed the usual method of periodic sampling and determination for every 10 days, 15 days or even one month and adopted the new method of simultaneous and continuous sampling and determination of the aquatic bacteria and related ecological factors according to the manure application period. It is beneficial for further understanding of the characteristics of the types of aquatic bacteria and their regulation of growth and death as well as the effects on the pond ecosystem and fish yield.

2. The results of the continuous observation and determination of bacteria in the fish ponds applied with the manure of chicken, duck, pig and cattle for the four periods shows that under the condition of fixed amount of manure application, the number of total bacteria and heterotrophic bacteria as well as their composition, prominence and the proportion of types are different due to the differences in the kinds of manure. The important reason for the differences in the biomass and type composition may be closely related to the nutritional source of the aquatic bacteria i.e. livestock manure, besides the mutual restriction and effects of the related ecological factors in the pond water. The determination shows notable differences in quality of the four kinds of animal manure which directly caused the ecological difference for the aquatic bacteria.

3. The manured-pond water body is a semi-enclosed artificial ecosystem. The aquatic bacteria are not only the decomposers of materials but also the transferrers of energy in this ecosystem. The recycling of elements such as carbon, nitrogen, phosphorus, iron can only be conducted through decomposition and transfer. It continuously promotes the process of pond dynamics. Therefore, it has a direct or indirect influence on the existence and reproduction and ecological factors in the ponds. The experiment consisted of four periods of continuous determination; analysis of the determination results showed that the aquatic bacteria had notable direct or indirect correlation with many ecological factors and that there existed very close interrelationship between them. Therefore it is evident from these results that the aquatic microorganisms have very deep influence on the pond ecosystem, and their function and position are obviously very important.

4. After the organic manure is applied to the fish pond, a series of decomposition activities will take place by the action of aquatic bacteria autotrophic and heterotrophic food chains will be formed to the converted into fish body weight at the end. The aquatic bacteria themselves are the important natural feeds for many kinds of fishes, as well as the starting point and major member of the heterotrophic food chain; therefore, they effect the growth and yield of fish decomposition and food chain as well.Our determination and analysis on the relationship between aquactic bacteria and fish yield showed that there is notable correlation between them The pond applied with chicken measure showed best results giving a higher

body weight increment and yield of fish and a lower conversion rate of manure than any of the other ponds. The results from the ponas applied with duck manure, pig manure and cattle manure ranked second,, third and fourth, respectively. The control pond showed minus value because the fish grew very slowly and suffered higher mortality than those in the manured ponds.

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