

## HETEROTROPHIC ACTIVITY AND PHYSICO-CHEMICAL RELATIONSHIP IN EUTROPHIC SHALLOW WATERS, HUNGARY

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### ABSTRACT

The heterotrophic potential was estimated in four ecosystems with different degrees of eutrophication, namely; Lake Balaton, Koros backwater reservoir and two fish ponds, one fertilized by inorganic nutrients and the other by organic pig manures. The relationship between heterotrophic activity of amino acids and physico-chemical parameters, and also between bacterial numbers, chlorophyll-a and temperature were discussed in terms of correlation coefficients. Heterotrophic uptake was correlated with water temperature for methionine, lysine, tryptophan and  $V_{max}$  / bacterium. It was found that the activity per cell with chlorophyll-a was significant for glycine, glutamic acid, lysine and protein hydrolysate. Also, it was correlated with temperature for methionine, phenylalanine, lysine, tryptophan and protein hydrolysate. The activity per bacterium of different communities is attributed to the great substrate accessibility for bacteria. The fluctuation in the correlation coefficient indicates that the bacterial communities are not uniformly active and may differ in their physiological state.

### INTRODUCTION

The high level of biological activity and increase in nutrients loading to aquatic system, result in accelerating eutrophication. The function of bacteria in ecosystems is important for both the food chain and mineralization processes. Thus, it is essential to study their heterotrophic potential in situ.

The determination of uptake of  $^{14}\text{C}$ -labelled organic compound by natural heterotrophic microorganisms was initially used in studies of assimilation of organic matter in aquatic environments (Wright and Hobbie, 1966; Hobbie and Wright, 1968 and Allen, 1969). Lake Balaton is generally a shallow water lake, with an average depth 3.1 m, it is about 75 length and 8 Km width, its surface area is about 600 Km<sup>2</sup>. Koros backwater Reservoir is

29.9 Km length with mean depth 2.5 meters. The two fish ponds have a surface area of 1500 m<sup>2</sup> and an average depth of 1 meter. The fish pond fertilized with organic manure was supplied with 37.5 m<sup>3</sup> of liquid manure from pigsties during the growth season. Inorganic fertilized fish pond was treated with 150 Kg ammonium nitrate and 20 Kg super phosphate during the same season. The source of water supply for both ponds is Koros backwater Reservoir.

## MATERIAL AND METHODS

Water samples were collected from the four different aquatic systems with Ruttner sampler about 50 cm below the water surface. Samples were obtained from the middle of Koros backwater Reservoir, fish ponds and at about 5 Km offshore of the Keszthly Basin (Lake Balaton). The total numbers of bacterioplankton were determined by direct count using the method of Razumov (1932). The activity of bacterial population was measured by the modified method of heterotrophic potential (Wright and Hobbie, 1966). The method was introduced by Harrison *et al* (1971) for measurement of the rate of mineralization. Incubations were carried out *in situ* for 3 hours under sterile conditions. All amino acids were specifically labelled : glycine-1- <sup>14</sup>C, L-methionine (S-methyl- <sup>14</sup>C), DL-tryptophan-1 <sup>14</sup>C, with specific activities of 220.15 to 495.4 M Bq/mmole, protein hydrolysate with specific activity 1.48 G Bq/mg atom C, L-lysine 8.88 G Bq/mmole and L-valine 7.4 G Bq/mmole. All <sup>14</sup>C compounds were obtained from the Institute of isotopes of the Hungarian Academy of Sciences. The radioactivity was counted on a Beckman Liquid Scintillation Counter Model L S 100 C for 10 minutes for each channel. Chlorophyll-a was determined according to Strickland and Parsons (1968).

Correlation coefficient (r.) was calculated after testing of the 9 most common types for correlation and the best fitting was selected.

## RESULTS AND DISCUSSION

Heterotrophic activity of 4 shallow water bodies manifested a 19-fold of gross uptake glycine from 0.671 to 12.927 ug dm<sup>-3</sup> h<sup>-1</sup>, 12-fold range methionine from 1.213 to 15.119 ug dm<sup>-3</sup> h<sup>-1</sup>, 43-fold range of net uptake of tryptophan from 0.326 to 13.992 ug dm<sup>-3</sup> h<sup>-1</sup>, 30-fold range of lysine from 0.17 to 5.126 ug dm<sup>-3</sup> h<sup>-1</sup>, 8-fold range of valine from 0.289 to 2.42 ug dm<sup>-3</sup> h<sup>-1</sup>, 11-fold range of phenylalanine from 0.322 to 3.765 ug dm<sup>-3</sup> h<sup>-1</sup>, 38-fold of glutamic acid from 0.431 to 16.486 ug dm<sup>-3</sup> h<sup>-1</sup> and 40-fold range of protein hydrolysate from 0.403 to 19.628 ug dm<sup>-3</sup> h<sup>-1</sup>. Generally, 115-fold range of all amino acids examined fluctuated between 0.17 ug dm<sup>-3</sup> h<sup>-1</sup> for lysine and 19.628 ug dm<sup>-3</sup> h<sup>-1</sup> for protein hydrolysate.

The pronounced differences between Lake Balaton, Koros Backwater Reservoir and the two fish ponds represented a series with different degrees of eutrophication which was markedly higher in the fish ponds. This appears to be due to the heterotrophic potential ( $V_{max}$ ), rapid turnover rate and / or type of fertilizers applied.

The examination of ecological parameters was needed to assess the interpretation of our data on the heterotrophic activity. Correlations were also calculated for physio-chemical and biological parameters in the different water bodies examined. As shown in Figure (1), the correlations between the measured parameters ( $P < 0.05$ ) indicate that, the water temperature was negatively correlated with oxygen and positively correlated with total bacteria, seston and net uptake of glycine. It is known that, bacteria is positively related to seston content and that heterotrophic uptake is highly influenced by water temperature. The influence of phytoplankton primary productivity on the oxygen level is clear from the positive correlation between oxygen concentration and pH. Degradation of organic matter seems also to affect oxygen concentration. This is clear from the negative correlation between oxygen and ammonia. Phosphate which may be released through degradation of organic phosphorus, is also negatively correlated with oxygen. Chlorophyll-a is weakly correlated with net uptake of glycine which may support the assumption that the heterotrophic uptake of organic molecules is due to both bacteria and phytoplankton, but the uptake of phytoplankton is smaller than bacterial uptake (Wright and Hobbie, 1966).

Dissolved free amino acids (DFAA) are positively correlated with dissolved phosphate and negatively with nitrate, while they are positively correlated with net uptake of glycine and methionine which indicate bacterial utilization of dissolved free amino acids. The negative correlation between DFAA and nitrate may be due to the assimilation of the latter one by phytoplankton. When the organisms decompose as a result of oxidative bacterial action, they release nitrate and phosphate. The dissolved phosphate is correlated with ammonia, nitrite and uptake of both glycine and methionine, also it is correlated with  $V_{max}$ /bacterium. This correlation coincides with the phytoplankton blooms which consume big fractions of nitrate and phosphate. A weak correlation was also recorded between ammonia and nitrite. The correlation between nitrite and nitrate may be at least partially due to the effect of nitrification. It is not surprising that the dissolved organic nitrogen (DON) is not correlated with the other parameters due to the greater influence of allochthonous organic matter. and the different parameters.

For glycine the relationship between gross uptake and chlorophyll-a showed an intermediate correlation,  $P < 0.02$  ( $r = 0.5742$ ). While, the  $V_{max}$  with bacterial numbers was weak,  $P < 0.05$  ( $r = 0.4124$ ). Also,  $V_{max}$ /bacterium correlated with chlorophyll-a and percent of respiration with temperature

$P < 0.05$  ( $r = 0.4973$ ) respectively (figure 1). The most significant correlation was usually percentage respiration of glycine by bacterioplankton was usually

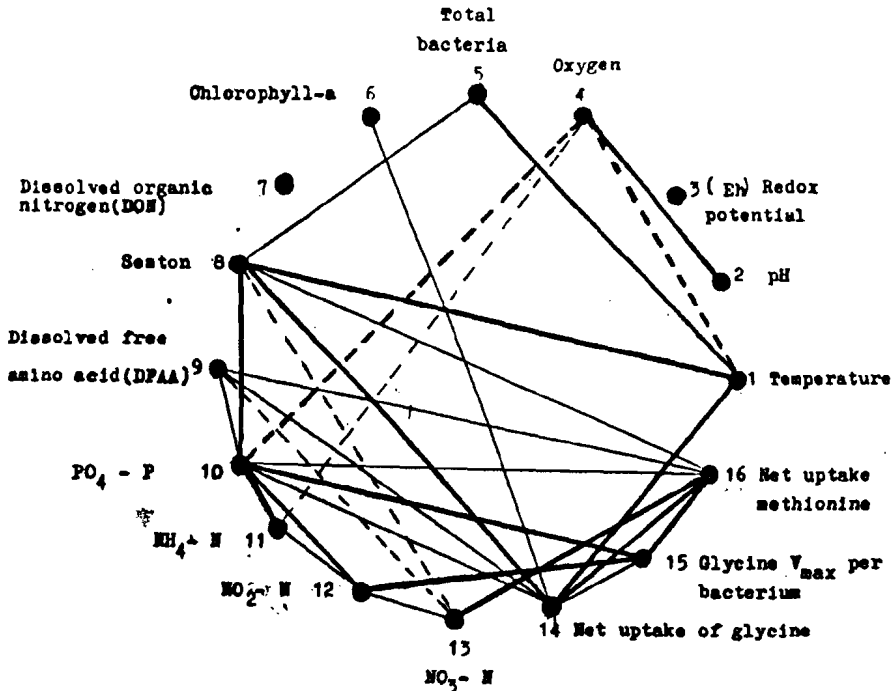


Fig. (1)  
Diagram demonstrating the relationship of the physico-chemical and biological parameters estimated for 29 samples.

- a- Strong correlation (r from 0.50 to 0.70).
- b- Weak correlation (r from 0.37 to 0.49).
- c- Negative correlation -----.

accompanied by increased amount of assimilation. Gouder (1980), mentioned that there was a significant correlation between the total number of bacteria and  $V_{max}$ .

The gross rate of methionine showed a strong correlation with temperature and an intermediate one with bacterial number with  $P < 0.001$  ( $r = 0.6787$ ) and  $P < 0.02$  ( $r = 0.5034$ ) respectively (Figure 3). The high temperature led to high  $V_{max}$  values. Allen (1969), stated that  $V_{max}$  in an eutrophic lake was correlated with both total bacteria and temperature. The relationship between temperature and  $V_{max}$ /bacterium was weakly correlated,  $P < 0.1$  ( $r = 0.3783$ ), while, the higher degree of correlation was obtained between temperature and percent respiration,  $P < 0.001$  ( $r = 0.6788$ ). The relationship between temperature and microbial activity cannot be assumed to be causal but indirect (Bolter, 1982).

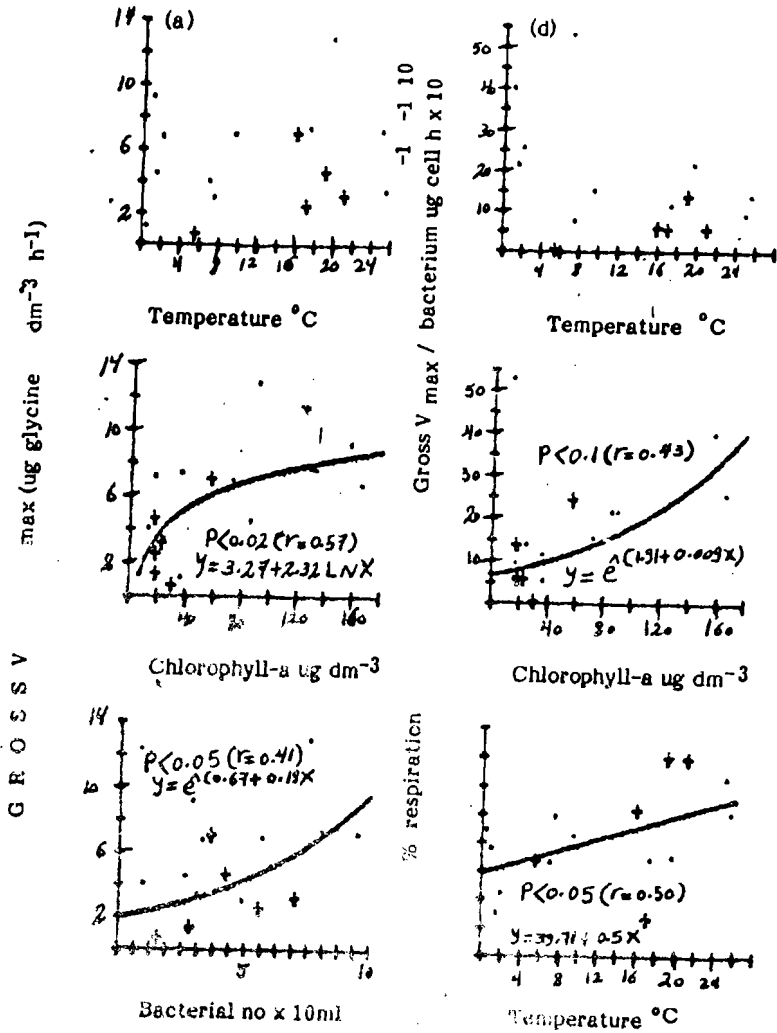


Fig. (2): Correlation of bacterotrophic uptake of glycine with the different parameters in the estimated areas (• = Karos backwater Reservoir; + = Lake Balaton; X = Fish ponds).

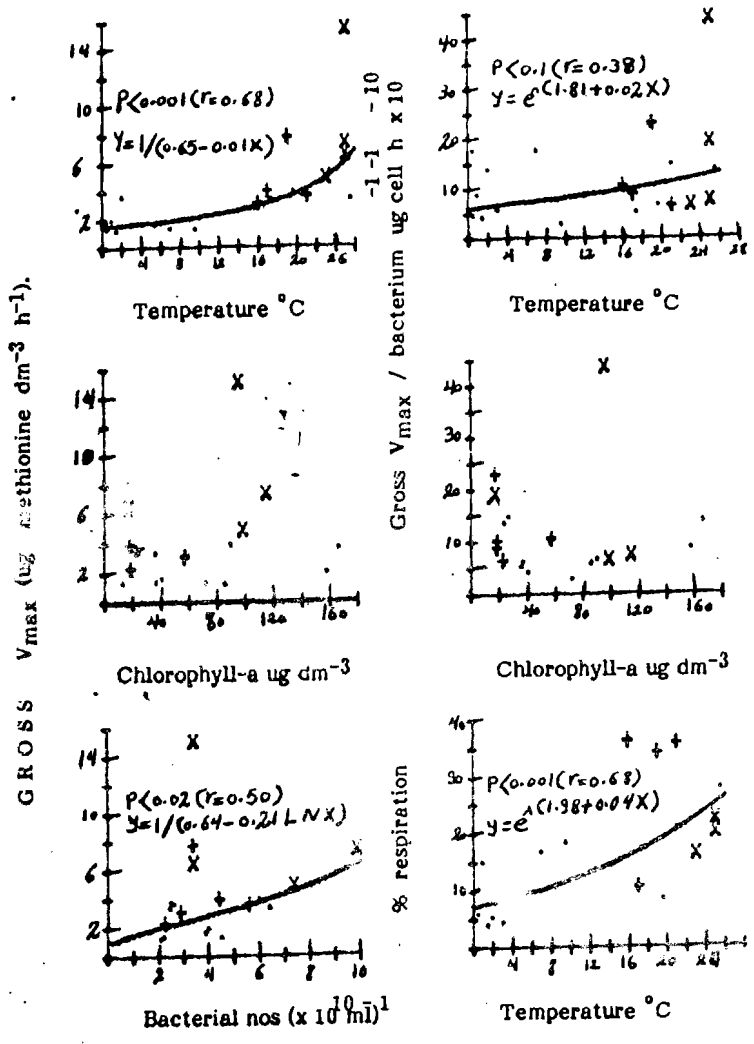


Fig. (3) : Correlation of beterotrophic uptake of methionine with the different parameters in the estimated areas ( . = Koros backwater Reservoir; + = Lake Balaton; x = fish ponds).

Lysine showed a strong correlation between gross  $V_{max}$  and temperature,  $P < 0.001$  ( $r = 0.80$ ), while temperature was weakly significant with  $V_{max}/bacterium$  and percent respiration,  $P < 0.05$  ( $r = 0.5529$ ) and  $P < 0.01$  ( $r = 0.4762$ ) respectively (Figure 4). Also, there was a relatively strong correlation between  $V_{max}/bacterium$  and chlorophyll-a,  $P < 0.05$  ( $r = 0.6664$ ). At the River Hull sites,  $V_{max}/bacterium$  was significantly correlated with water temperature (Goulder, 1979).

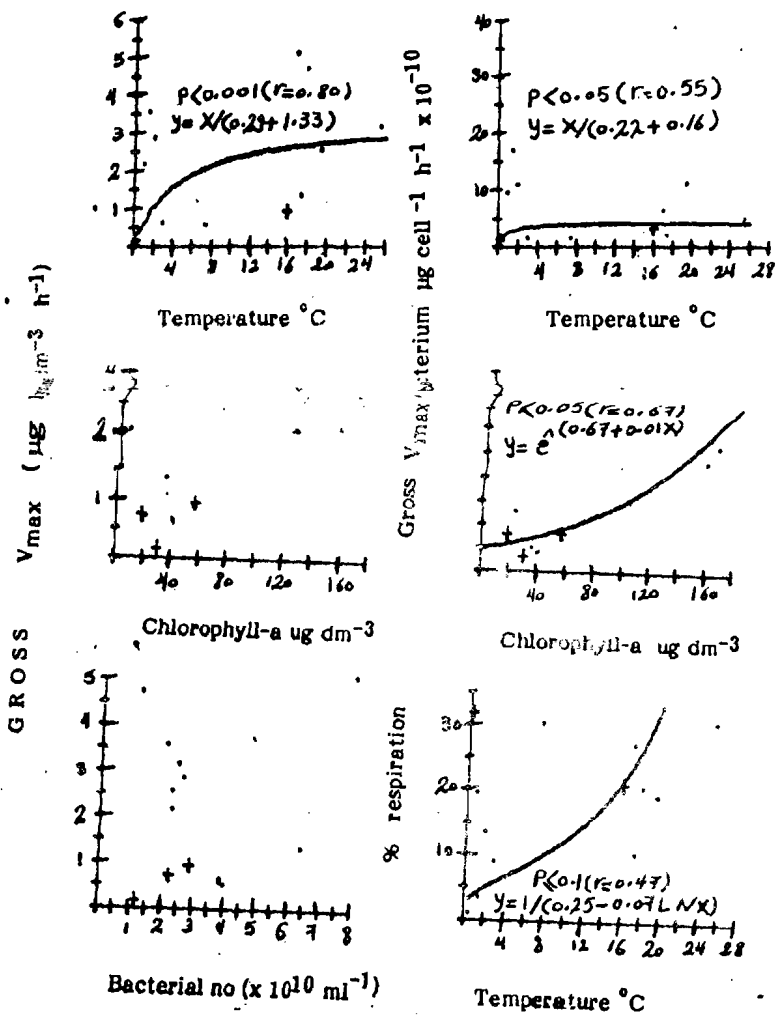


Fig. (4): Correlation of heterotrophic uptake of Lysine with the different parameters in the estimated areas (• = Koros backwater Reservoir; + = Lake Balaton; x = fish ponds).

Significant correlation of tryptophan was recorded for temperature and incorporated  $V_{\max}$ ,  $P < 0.02$  ( $r = 0.6339$ ) as shown in Figure (5). On the other hand the bacterial number with incorporated  $V_{\max}$  and the temperature with  $V_{\max}$ /bacterium were in weaker correlation,  $P < 0.1$  ( $r = 0.4973$ ).

There was significant correlations for phenylalanine between chlorophyll-a and gross  $V_{\max}$  and also between temperature and  $V_{\max}$ /bacterium,  $P < 0.05$  ( $r = 0.7076$ ) and  $P < 0.1$  ( $r = 0.5822$ ) respectively (Figure 6).

For glutamic acid the correlation between chlorophyll-a and gross  $V_{\max}$ /bacterium was relatively weak,  $P < 0.1$  ( $r = 0.6215$ ) as shown in Figure (7).

The correlation of protein hydrolysate was found to be stronger between temperature and gross  $V_{\max}$ ,  $P < 0.01$  ( $r = 0.9172$ ), while, the chlorophyll-a was in weaker correlation with  $V_{\max}$  and  $V_{\max}$ /bacterium,  $P < 0.05$  ( $r = 0.9500$ ). Also, the water temperature was weakly correlated with  $V_{\max}$ /bacterium,  $P < 0.1$  ( $r = 0.7293$ ) (Figure 8).

Results of the present investigation indicate that  $V_{\max}$  with water temperature showed no correlation for the uptake of glycine, phenylalanine and glutamic acid, but had a strong one for methionine, lysine and protein hydrolysate. Chlorophylla concentration was generally characterised by a high value of  $220.48 \mu\text{g dm}^{-3}$  in Koros backwater Reservoir as compared with the other aquatic bodies. The absence of correlation between  $V_{\max}$  and chlorophyll-a for the major amino acids may be related to the less competition of phytoplankton for these components. However, the correlation coefficient between  $V_{\max}$ /bacterium with chlorophyll-a was still significant for the major amino acids. Similarly, Ferguson and Palumbo (1979) found a correlation between chlorophyll-a and bacterial number. Spencer (1978) recorded also a significant correlation between chlorophyll-a and microbial adenosine triphosphate (ATP). The absence of correlation between bacterial number and the gross  $V_{\max}$  for some amino acids is related to the nature of bacterial communities which are not uniformly active and they may differ in their physiological state.

In the eutrophic system studied, the bacterioplankton was active in assimilation of dissolved free amino acids all the year round. However, such activities differed within the different seasons due to temperature variations (El-Sarraf, 1983). This is particularly true in the fish pond supplied with organic manure. Wide variations were also found in the correlations between  $V_{\max}$  and the other parameters. This conclusion agrees with the results of Morite *et al* (1977), Goulder (1980) and Bolter *et al* (1981). Morgan and Kalff (1972) found excellent positive correlation between  $V_{\max}$  and bacterial number, while Francisco (1970) found no clear correlation between  $V_{\max}$  and temperature in a stratified reservoir. Bolter and Dawson (1982), mentioned that no direct correlation could be established between the occurrence of dissolved organic matter and activity of heterotrophic organisms.



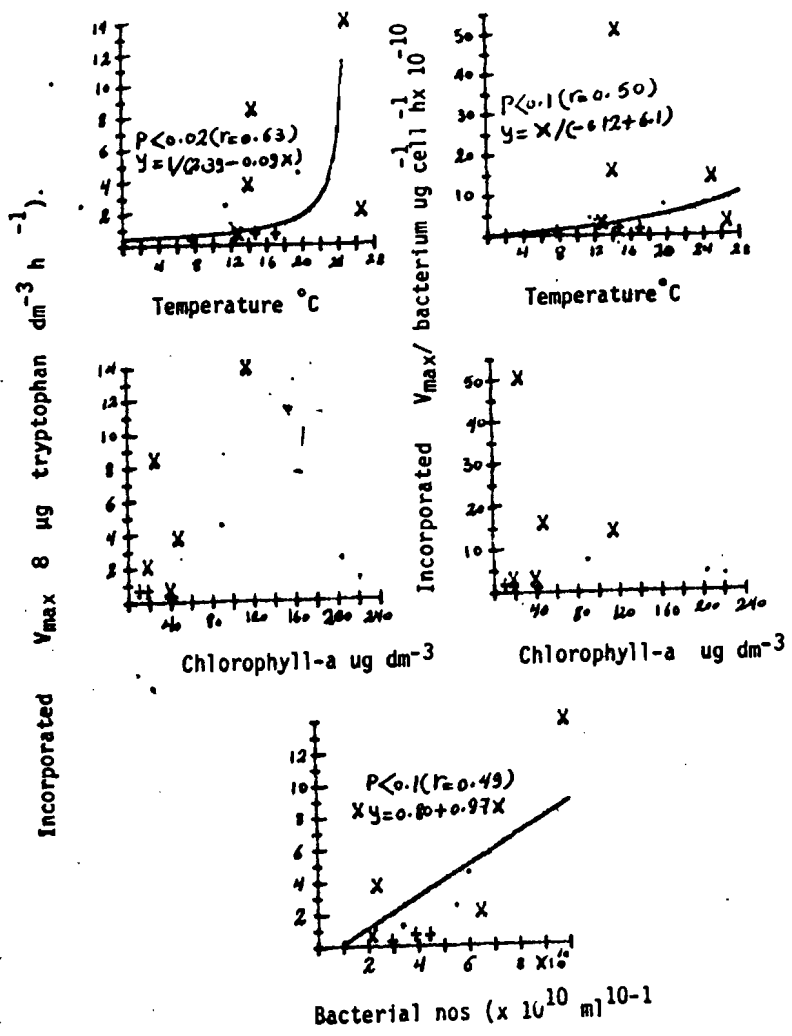


Fig. (5) : Correlation of heterotrophic uptake of tryptophan with the different parameters in the estimated areas ( . = Koros backwater Reservoir; + = Lake Balaton; x = Fish ponds).

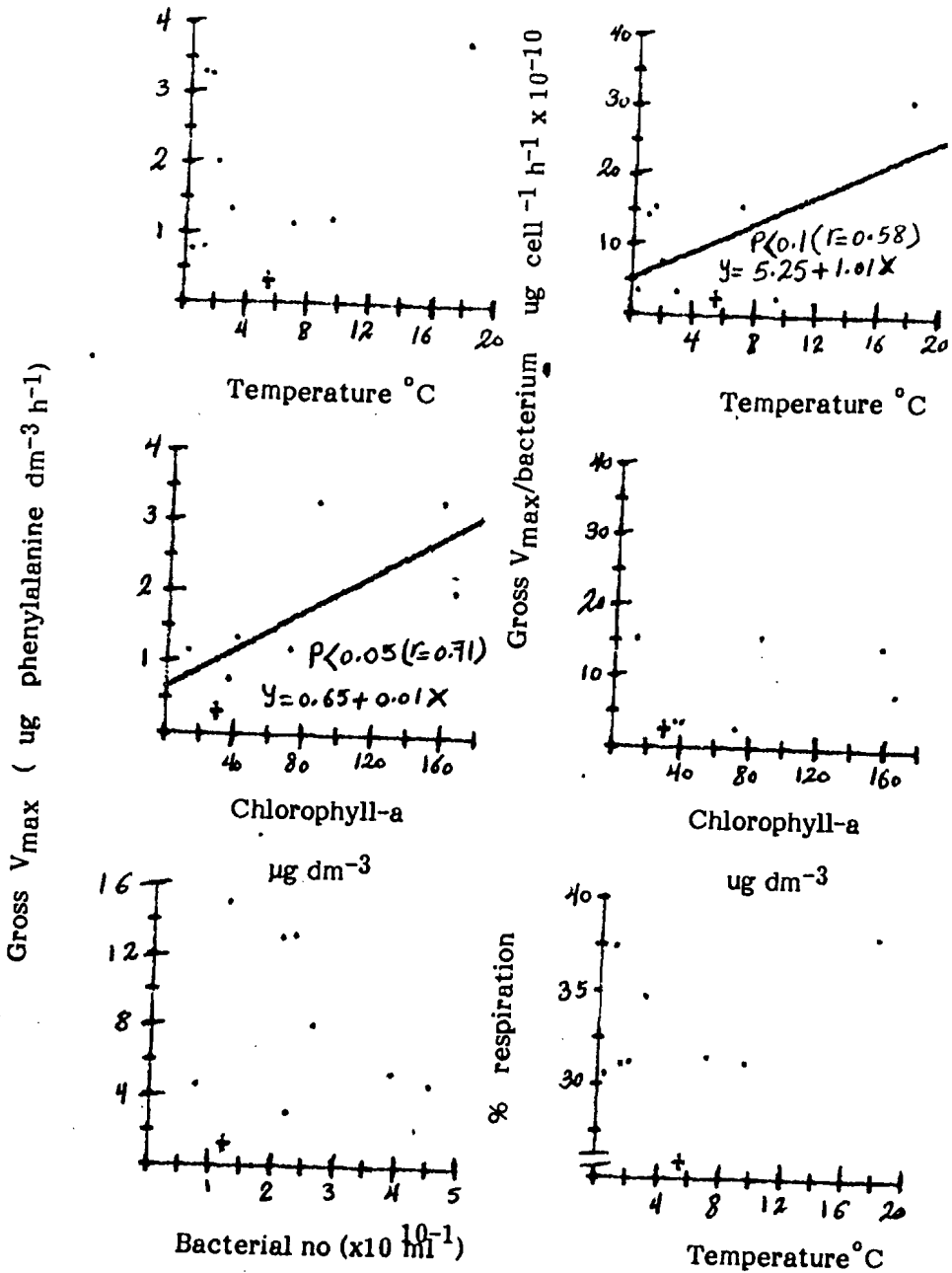


Fig. (6) : Correlation of beterotrophic uptake of phenylalan: with the different parameters in the estimated ar (. = Koros backwater Reservoir ; + = Lake Bals x = Fish ponds).

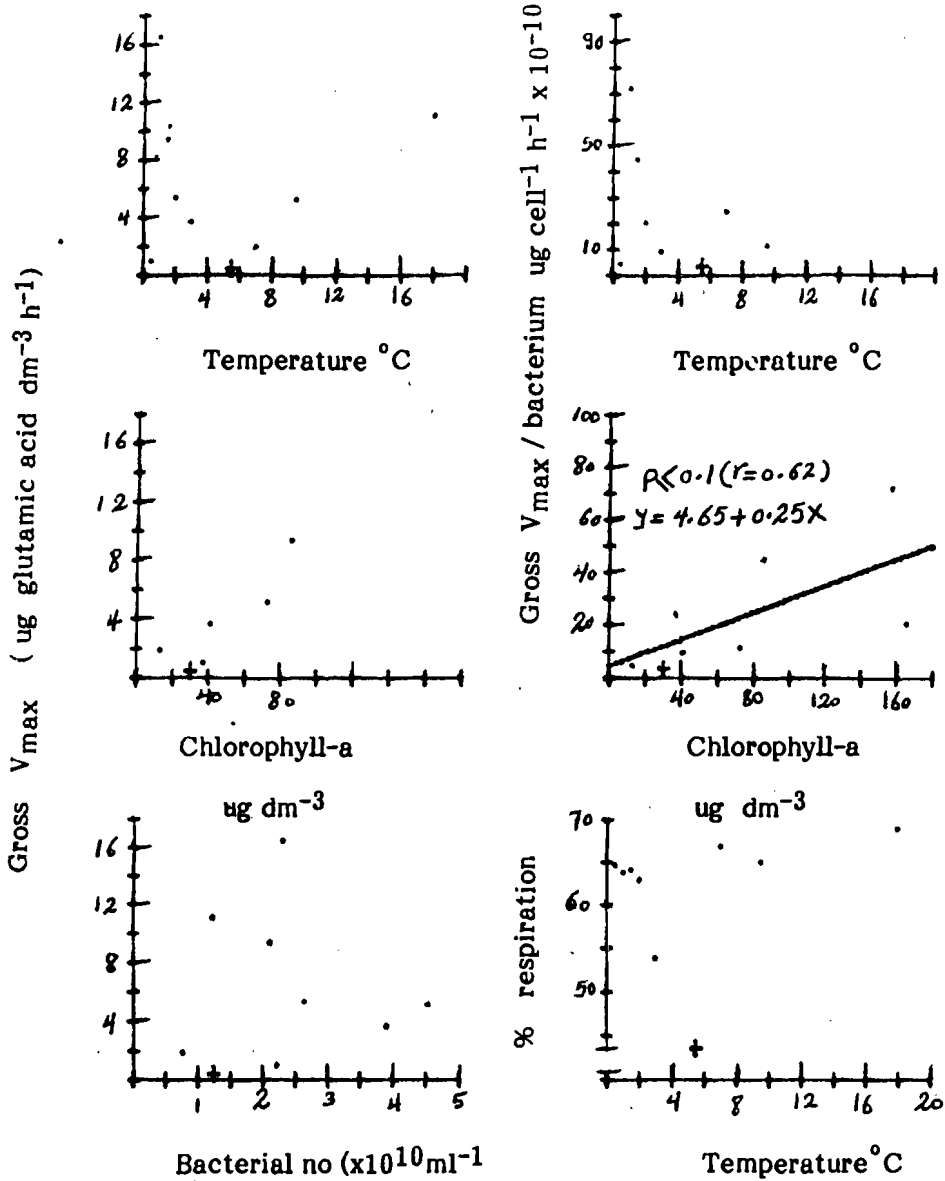


Fig. (7) : Correlation of beterotrophic uptake of glutamic acid with the different parameters in the estimated areas (. = Koros backwater Reservoir; + = Lake Balaton; x = Fish ponds).

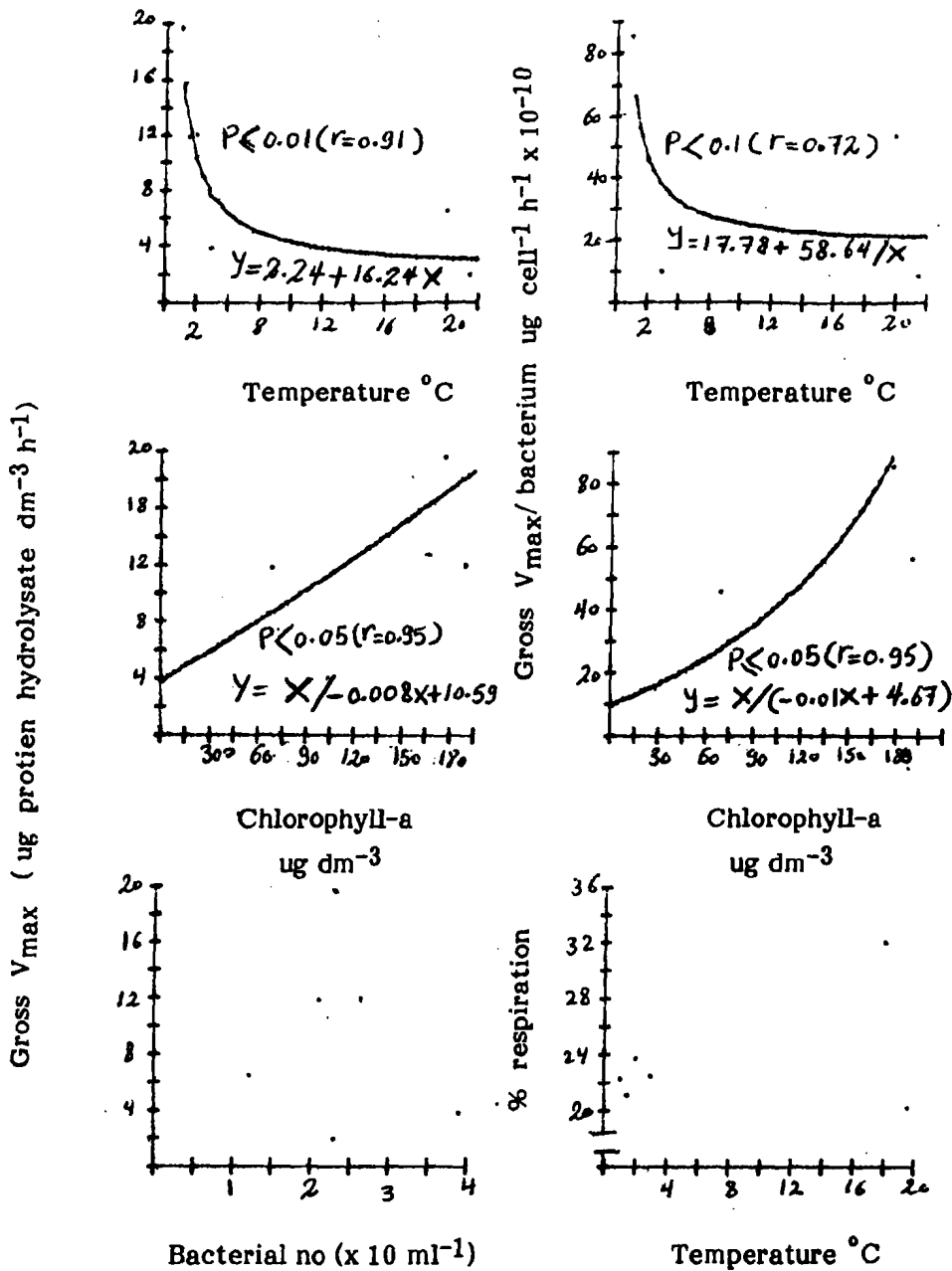


Fig. (8) : Correlation of beterotrophic uptake of protien hydrolysate with the different parameters in the estimated areas ( . = Koros backwater Reservoir; + = Lake Balaton ; x = Fish ponds).

## REFERENCES

- Allen, H.L. (1969). Chemo-organotrophic utilization of dissolved organic compound by planktic algae and bacteria in a pond. *Int Revue ges. Hydrobiol.*, 54 (1) 133.
- Bolter, M. and R. Dawson (1982): Heterotrophic utilization of biochemical compounds in Antarctic waters. *Netherlands J. of Sea Res.*, 16: 315-332.
- Bolter, M.; L. A. Meyer-Reil; R. Dawson; G. Liebezeit; K. Wolter and H. Szweringi (1981). Structure analysis of shallow water ecosystems: Interaction of microbiological, chemical, and physical characteristics measured in the overlying waters of sandy beach sediments. *Estuar., Coast. Shelf Sci.*, 13: 570-589.
- El-Sarraf, W.M. (1983). **Amino acids and microbial activity in Hungarian shallow waters**. Ph. D. Thesis, Hungarian Academy of Sciences, Budapest, Hungary, 219 pp.
- Ferguson, R.L. and A.V. Palumbo, (1979). Distribution of suspended bacteria in neritic waters south of Long Island during stratified conditions. *Limnol. Oceanogr.*, 24: 697-705.
- Francisco, D.A. (1970). **Glucose and acetate utilization by the natural microbial community in a stratified reservoir**. Ph.D. Thesis, Dept. Environ. Sci. and Engin. Univ. North Carolina, Chapel Hill., USA, 83 p.
- Goulder, R. (1979).  $V_{max}$  per bacterium and turnover time rate per bacterium for glucose mineralization in natural waters. *Current Microbiol.*, 2: 365-368.
- Goulder, R. (1980). Seasonal variation in heterotrophic activity and population density of planktonic bacteria in a Clear River. *J. Ecol.*, 68: 349-363.
- Harrison, M.J., R.T. Wright and R.Y. Morita (1971). Method for measuring mineralization in lake sediments. *Appl. Microbiol.*, 21: 698-702.
- Hobbie, J.E. and R.T. Wright (1968). A new method for the study of bacteria in lakes: description and results. *Mitt int. Verein. Limnol.*, 14: 64-71.
- Morgan, K.C. and J. Kalff, (1972). Bacterial dynamics in two high-arctic lakes. *Freshwat. Biol.*, 2: 217-228.
- Morita, R.Y., R.P. Griffith and S.S. Hayasaka (1977). Heterotrophic activity of microorganisms in Antarctic waters.-In: G.A. Liano. Adaptions within Antarctic ecosystems. *Proc. 3rd SCAR Symp. Ant. Biol.*, Smithsonian Inst., Washington D.C.: 99-113.
- Razumov, A.S. (1932). Direct method counting water bacteria. *Microbiologiya*, 1: 131-146.
- Spencer, M.J. (1978). Microbiol activity and biomass relationships in 26 oligotrophic to mesotrophic lakes in South Island, New Zealand. *Verh. Internat. Verein. Limnol.*, 20: 1175-1181.
- Strickland, J.D. and T.R. Parsons (1968). A practical handbook of Seawater analysis. *Bull. Fish. Res. Bd. Can.*, 167:311 p.
- Wright, R.T. and J.E. Hobbie (1966). Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology*, 47: 447-464.