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DIURNAL UPTAKE OF AMINO ACIDS IN LAKE BALATON, KOROS BACKWATER RESERVOIR AND LIQUID MANURE FERTILIZED FISH POND (HUNGARY).

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ABSTRACT

The importance of organic matter utilization in natural waters was indicated by measuring heterotrophic activity. Amino acids are important as a source of organic nitrogen for heterotrophic bacteria. The diurnal uptake of amino acid was measured in situ, with single concentration, in three aquatic bodies with different degrees of eutrophication, Keszthely basin in Lake Balaton, Koros backwater reservoir, and liquid manure fertilized fish pond. Also physicochemical parameters and bacterial numbers were examined. Diurnal uptake of some amino acids was characterized by dramatic changes of velocity (increase or decrease) in the three ecosustems examined, associated with irregular fluctuations in bacterial numbers. The heterotrophic potential was affected by light and dark periods of the day. Results obtained in Lake Balaton were the lowest. Mean incorporation rate of amino acids was 2.072 µg dm^{-3} h^{-1}. Mean incorporation rates of the same amino acids in Koros backwater reservoir and liquid manure fertilized fish pond were 8.83 and 8.872 µg dm^{-3} h^{-1} , respectively. Incorporation rate, measured with short-term interval incubation, was greatly increased afternoon and early morning in Koros backwater reservoir, while one peak appeared during midnight in liquid manure fertilized fish pond. In Lake Balaton, peaks were recorded with different trends for amion acids.

INTRODUCTION

Natural waters contain many dissolved organic compounds such as monosaccharides, amino acids, vitamines, and fatty acids. In aquatic systems, these dissolved organic compounds are utilized by heterotrophic bacteria for obtaining energy and building new cells as well as for recycling inorganic nutrient (Wright and Burnison, 1979). In eutrophic or hypereutrophic aquatic ecosystems, this recycling has a very pronounced diurnal cycle.

The heterotrophic activity might be a good indicator for eutrophic condition (Albright and Wentworth, 1973; Burnison and Morita, 1974; and Gillespie, 1976). Albright (1983) reported heterotrophic bacterial biomasses, activities, and productivities within the Fraser River plume. Electron microscopy and biochemical measurements of heterotrophic activity were made on marine snow (Amy, 1987). Griffiths et al (1977) used two substrates that have the single-concentration method for determining relative microbial activity, and maximum potential rate of multi-concentration, he found that the measurement of substrate utilization at one concentration is roughly equivalent to using the heterotrophic activity method. The same results were obtained by Gocke (1977) who mentioned that in eutrophic or polluted regions, the values agree quite closely. Hence either one of methods can be applied. Hoppe (1978) published a review to summarize the results of most of tracer techniques and some problems concerning bacterial activity by autoradiography.

Investigating the role of amino acids incorporation in bacteria, it was necessary to know the behaviour and function of these bacteria during a lightdark period which produces different incorporation levels with a complete cycle of uptake during day time. The daily uptake is useful to determine the heterotrophic activities of aquatic systems especially in fish ponds.

The aim of this work is to measure the diurnal uptake of some amino acids in situ, as related to variations to time, and to obtain information as to the principle of heterotrophic activity during 24 hours.

MATERIAL AND METHODS

Description of the Areas

Lake Balaton is a shallow freshwater lake in central Europe, with a length of 77.9 km, a width of 7.7 km, average depth of 3.14 m and a surface permanently ruttled by wind. Sub-surface water samples (0.5 m) were collected at 2-hour intervals on 3rd and 4th of July, from about 3 Km off Keszthely Basin of Lake Balaton.

Koros backwater reservoir in Szarvas is situated in the southeastern region of Hungary. It is located west to the protected flood basin of the Harmas Koros River with 29.2 km length and 2.5 m. mean depth . The liquid manure fertilized fish pond has its water supply from the River Koros, with surface area of 1500 m^2 and average depth of 1 m. Water samples were collected from mid river and fish pond during 19th and 20th of May, and 10th to 11th of June, respectively.

Water temperature, and dissolved oxygen were measured by Dissolved Oxygen Meter Model 1510; pH was measured with pH meter (Type OP-107). Redox potential was measured by nA-mV meter (Type OH-801) and conductivity was determined with conductivity meter (Type OK-102/1).

Ammonia-nitrogen was determined by the method of Scheiner (1976). Nitrite and nitrate-nitrogen were determined by the method proposed by Strickland and Parsons (1968) after reduction of nitrate with cadmium. Dissolved phosphate was determined according to Robinson and Thompson (1948).

Dissolved free amino acid (DFAA) was detected by Beckman Amino Acid Analyzer, Modell 118, with "Single Column".

Total number of bacterioplankton was determined by direct membrane filter method of Razumov (1932).

Heterotrophic activity was measured using the method of Wright and Hobbie (1966), with single-concentration of labeled amino acid in situ. One concentration was added from U-C14 glycine with specific activity 431.79 M Bq/mmol (approximately 121.8 μg dm^{-3}); L-C14 methionine with specific activity 370.74 M Bq/mmol (approximately 121.4 μg dm^{-3}, and DL-C14 tryptophan with specific activity 220.52 M Bq/mmol (approximately 139.2 μg dm^{-3}) obtained from Isotopes Institute of the Hungarian Academy of Sciences.

For measuring uptake amino acid under sterile condition in situ, duplicate 100 ml aliquot of each sample was incubated in about 130 ml glass stoppered bottle for 2 hours in the dark. For each substrate one bottle fixed with 1 ml of Lugols was used as a blank. After incubation, the samples were filtered through 0.45 μm Sartorius membrane filter and washed three times with 10 ml sterile distilled water. Each filter was then placed in a scintillation vial contening 15 of Bray scintillation cocktail, and counted by Beckman L S 100 for 10 minutes for each channel. Quenching was Quenching was corrected by channel ratio method, to obtain disintegration per minute (dpm).

Correlation coefficient (r) was calculated after transformation of 9 different ways and from these the maximum value was taken into consideration.

RESULTS AND DISCUSSION

Diurnal Changes of Physicochemical Patterns

The physical and chemical parameters of the three aquatic locations examined are shown in Table 1. The temperature was higher, in liquid manure fertilized fish pond than in other aquatic locations. High dissolved oxygen concentrations was measured in Koros backwater reservoir with a mean value of 13.03 mg dm^{-3}, probably due to the high photosynthetic activity of plankton. The water was slightly alkaline in Lake Balaton and Koros backwater reservoir, pH fluctuated from 7.7 to 8.35. In liquid manure fertilized fish pond, the values of pH ranged between 7.4 and 7.6 with a mean value of 7.51, (Table 1).

Diurnal variation of pH were relatively low in the three aquatic locations, lower pH values were measured in liquid manure fertilized fish pond. This

is probably due to reduced photosynthetic activity of plankton. High redox potential (Eh) was recorded in Koros backwater reservoir with a mean value of + 496.5 mV. In liquid manure fertilized fish pond, the redox potential mean was + 381.9 mV. We may observe that recorded values of pH, dissolved oxygen and redox potential (Eh) are invariably lower in liquid manure fertilized fish pond probably due to bacterial degradation of dissolved and particulate organic matter load in water column.

Liquid manure fertilized fish pond was characterized by higher conductivity than Koros backwater reservoir which may be attributed to low water level, stagnation and evaporation.

Ammonia, nitrite, nitrate and phosphate were observed in noticeable concentrations in the three aquatic bodies, (Table 1 and Fig. 1). Higher levels of nutrient salts were recorded in liquid manure fertilized fish pond than in the other aquatic bodies due to the addition of pig manure.

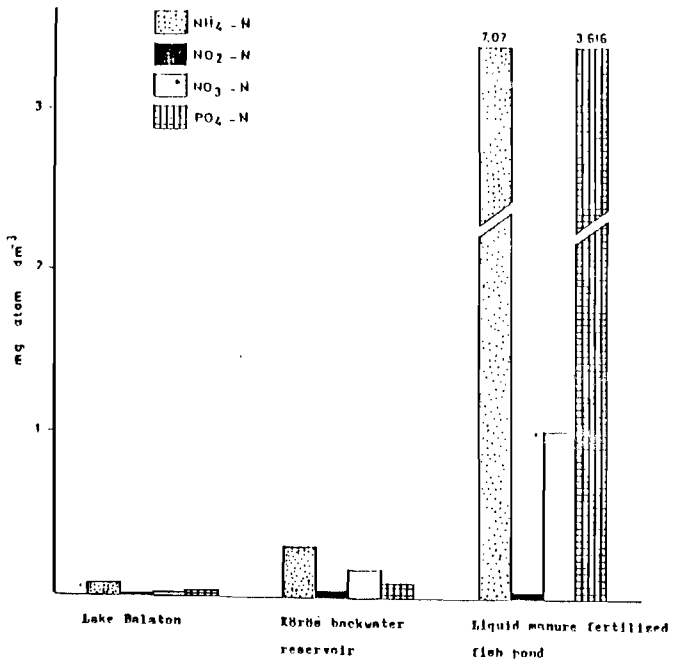

Fig. 1
Comparison between mean values of nutrient salts in the three aquatic bodies.

TABLE 1
Summary of physico-chemical parameters, bacterial number and incorporation rate of amino acids.

Ecosystem	Mean variables	Temp. °C	pH	Eh (mV)	Conductivity µS cm^{-1}	Oxygen mg O_2 dm^{-3}	Dissolved (mg atom dm^{-3})				Bacterial no.x10^6 cell ml^{-1}	Incorporation rate µg dm^{-3} h^{-1}		
							NH_4 -N	NO_2 -N	NO_3 -N	PO_4 -P		Glycine	Methionine	Tryptophan
Lake Balaton 3 and 4 July.	Mean	19	8.32	355.2	-	-	0.075	0.0017	0.020	0.029	3.67	0.133	0.744	1.195
	Minimum	-	8.12	202.0	-	-	0.040	0.0010	0.009	0.005	2.24	0.028	0.035	0.351
	Maximum	-	8.50	440.0	-	-	0.179	0.0060	0.056	0.090	4.74	0.266	1.818	2.524
Koros backwater reservoir,19 and 20 May	Mean	19.6	8.02	496.5	184.3	13.0	0.324	0.039	0.179	0.097	7.7	3.590	2.460	2.780
	Minimum	18.5	7.7	440.0	350.0	10.9	0.258	0.036	0.127	0.064	6.0	1.862	1.721	1.222
	Maximum	20.2	8.35	580.0	407.0	15.1	0.387	0.450	0.293	0.147	10.0	5.962	3.186	4.090
Liquid manure fertilized fish pond 10 lnd 11 June.	Mean	28.2	7.51	381.9	741.7	3.1	7.070	0.048	1.016	3.616	4.68	3.139	1.991	3.741
	Minimum	25.5	7.40	315.0	691.0	1.0	6.470	0.028	0.638	3.380	2.63	1.886	1.356	1.829
	Maximum	31.4	7.60	410.0	795.0	6.3	7.300	0.090	1.720	3.880	6.47	5.464	2.654	6.988

Diurnal Variation of Total Bacteria

Total bacteria was examined by direct counting. It ranged between 2.24 and 4.75 X 10^6 cell ml^{-1} in Lake Balaton, and between 6.0 to 10.0 X 10^6 cell ml^{-1} in Koros backwater reservoir. Total bacteria in liquid manure fertilized fish pond reached a maximum value of 6.47 X 10^6 cell ml^{-1}. Generally, the maximum first peak of total bacteria was recorded afternoon and second one during night, in Koros backwater reservoir. High number of bacteria was observed in Lake Balaton during afternoon and late morning, while in liquid manure fertilized fish pond maximum values were recorded during morning, (Table 1 and Figs. 2, 3 and 4).

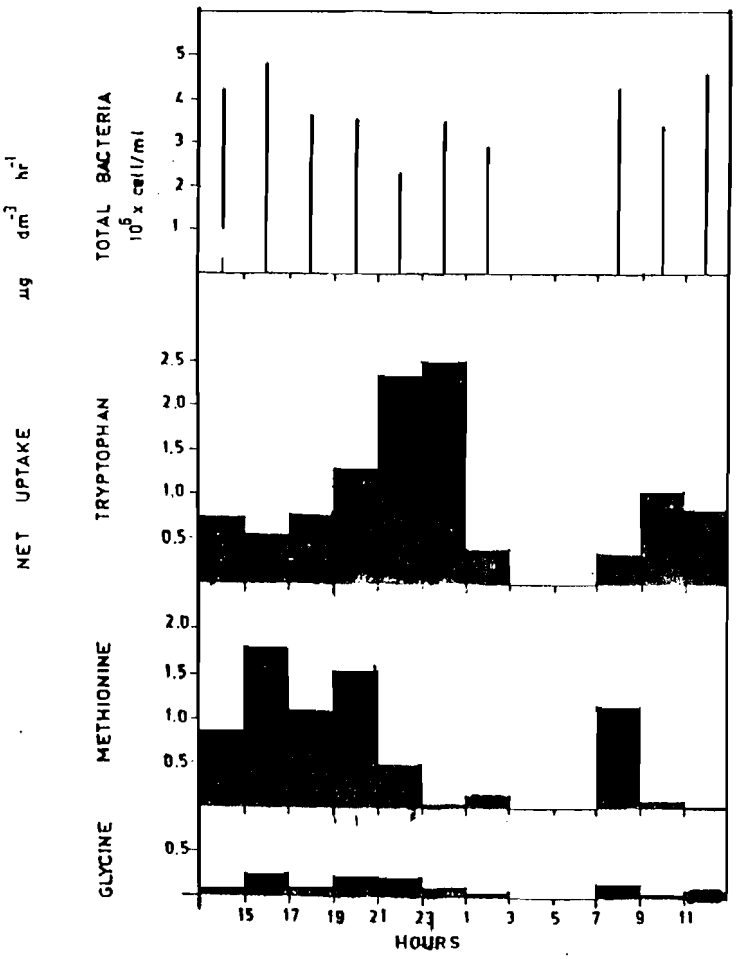

Fig. 2
Diurnal net uptake in Lake Balaton during 3 and 4 July

Total bacteria of Lake Balaton was significant with dissolved phosphate and pH while it was significant with dissolved oxygen concentration in Koros backwater reservoir. In liquid manure fertilized fish pond, no significance could be found with all factors, (Table 2).

TABLE 2

Relationship between bacterial and environmental variables, with significance test of correlation coefficients /r/: + $P_{10\%}$ = 0.4973, * $P_{5\%}$ = 0.5760, ** $P_{1\%}$ = 0.7079, *** $P_{0.1}$ = 0.8283, NS = no. significant.

Parameters	Temperature °C	PH	Redox Potential	Conductivity	DISSOLVED Oxygen	DISSOLVED Ammonia	DISSOLVED Nitrite	DISSOLVED Nitrate	DISSOLVED Phosphate
Lake Balaton									
Incorporated rate for glycine	-	NS	*	-	-	NS	NS	NS	***
Incorporated rate for methionine	-	NS	+	-	-	NS	NS	NS	NS
Incorporated rate for tryptophan	-	*	NS	-	-	**	NS	NS	NS
Bacterial numbers	-	+	NS	-	-	NS	NS	NS	**
Koros backwater reservoir									
Incorporated rate for glycine	NS	+	NS	+	NS	+	+	NS	*
Incorporated rate for methionine	NS	NS	NS	NS	NS	*	NS	NS	NS
Incorporated rate for tryptophan	NS	NS	NS	**	NS	**	NS	NS	NS
Bacterial numbers	NS	NS	NS	NS	*	NS	NS	NS	NS
Liquid manure fertilized fish pond									
Incorporated rate for glycine	+	NS	NS	NS	NS	NS	+	NS	**
Incorporated rate for methionine	NS	*	*	NS	NS	NS	*	NS	**
Incorporated rate for tryptophan	NS	+	+	NS	NS	NS	NS	NS	**
Bacterial numbers	NS	NS	NS	NS	NS	NS	NS	NS	NS

Diurnal Incorporation of Amino Acids in Lake Balaton

The diurnal uptake rate with fluctuation levels related to day time, the heterotrophic incorporation rate of glycine was recorded at low level ranging between 0.038 and 0.266 μg dm^{-3} h^{-1} with a mean value of 0.133 μg dm^{-3} h^{-1}. Two peaks were recorded due to changes of day time. The first one 0.266 μg dm^{-3} h^{-1} was recorded during 1500 - 1700 hs (afternoon), and the second 0.143 μg dm^{-3} h^{-1} in 700 - 900 hs (late morning), (Fig. 2).

The lowest rate was 0.038 μg dm^{-3} h^{-1} during 900 - 1100 (late morning). These results may be attributed to the relatively high ruttling activity of wind and small amount and activity of bacteria.

Incorporation rate was significant with redox potential (Eh) and was in close correlation with dissolved phosphate, (Table 2).

The highest values of natural substrates (S_n) of glycine were 2.03 and 3.673 μg dm^{-3} during 1700 and 700 hs, respectively. This result was in agreement with Jorgensen (1982) who found that the assimilation rate of 15 μg C dm^{-3} h^{-1} was at midnight, when level of DFAA was highest.

The incoropration rate of methionine took the profile of that of glycine, with two peaks recorded at the same time as the glycine peaks. Maximum incorporation rates were 1.818 and 1.187 μg dm^{-3} h^{-1} during (1500-1700 hs) and (700-900 hs), respectively.

The results of DFAA of methionine at the same two peaks were 3.312 and 0.734 μg dm^{-3} during 1700 and 700 hs, respectively. The lowest level of incorporation rate was 0.035 μg dm^{-3} h^{-1} at noon (1100 - 1300). Incorporation rate was significant with redox potential.

Incorporation rate of tryptophan fluctuated between 0.351 and 2.524 μg dm^{-3} h^{-1}, with mean value of 1.195 μg $dm^{-3}h^{-1}$. It showed a small increase during 1300 - 2100 hs and reached a maximum peak during 2300 - 100 hs early morning. Incorporation rate then decreased rapidly during (100 300) and (700 - 900) hs, then increased again to a second relatively high peak at 900 - 1100 hs late morning, (Fig. 2). In Keszthely basin, situated in the great eutrophic part of the lake, the low uptake was recorded during strong windy days. This result agreed with Olah (1970) who found that the activity of population always increased on the day after as stromy one, and he concluded that, the bacteria were not carried up from the sediment to the water column, but in fact reproduced there. Padisak (1980), mentioned that, the productivity during the strom period was about half as much as after that time. Glycine and methionine were strongly correlated (r = 0.8399), while no apparant correlation was observed between glycine and tryptophan, and between methionine and tryptohan.

Diurnal Incorporation of Amino Acids in Koros Backwater Reservoir

The heterotrophic activity of glycine increased gradually from 900 to 1500 hs with value 3.650 μg dm^{-3} h^{-1}, then decreased again reaching a second peak during 300 to 500 hs early morning with maximum value of 5.962 μg dm^{-3} h^{-1}, (Fig. 3). Minimum incorporation rate has been observed during 900 to 1100 hs late morning with value of 1.862 μg dm^3 h^{-1}, (Fig. 3). This can be attributed to high total bacteria and activity, and the high degree of eutrophication than Lake Balaton, due to the higher uptake

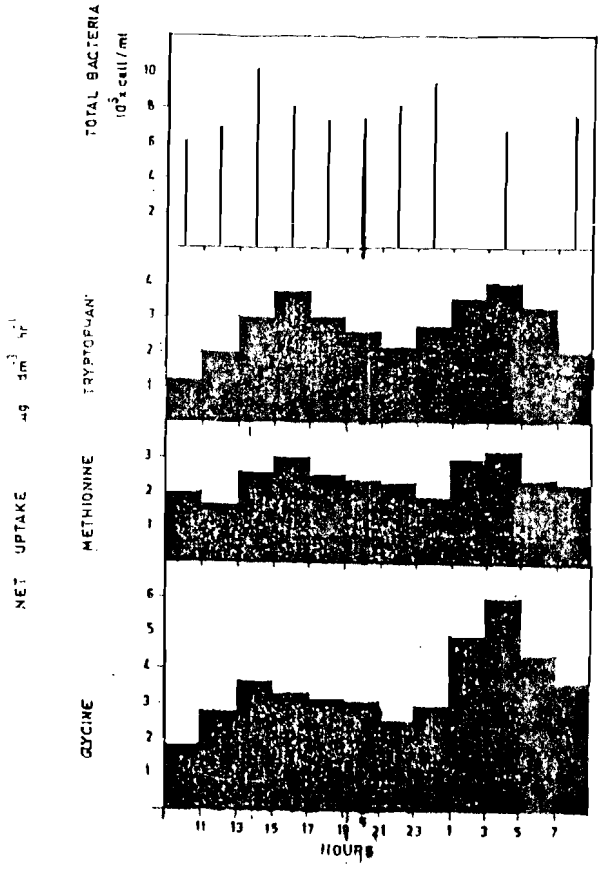

Fig. 3
Diurnal net uptake in Koros backwater reservoir during 19 and 20 May.

velocity. The second peak was characterized by a higher incorporation than the first one. We suggested that the bacterial activity was very high, while the number of bacteria was about 1.5 times larger than recorded in the second peak. Dawson and Gocke (1978) mentioned that the low bacterial biomass was capable of taking a large amount of substrate added. The large second peak was recorded in early morning with start sunrise. The heterotrophic activity was limited to day time, when significant uptake characterized the sunligh and declined with sunset and increased with sunrise aglan (Fig. 3). In general, the uptake rate prediced the actual heterotrophic activity of day time. Incorporation showed significant correlation between pII, conductivity, ammonia, nitrite and dissolved phosphate, (Table 2).

Diurnal incorporation rate of methionine increased from 1.721 to 3.186 $\mu g\ dm^{-3}\ h^{-1}$ with a mean value of 2.460 $\mu g\ dm^{-3}\ h^{-1}$. While two high peaks were frequently observed, during 1500 - 1700 hs afternoon and 300 - 500 hs early morning, the minimum level was recorded during 1100 - 1300 hs at noon. Generally, incorporation rates started from increasing toward the first peak and gently decline at 2300 - 100 hs early morning, and beginning sharply increased to reach the second peak. The second peak is slightly higher than the first. Incorporation rate of methionine was significant with ammonia.

Diurnal changes of incorporation rate of tryptophan showed the same profile as methionine. The rate of incorporation varied from 1.222 to 4.09 $\mu g\ dm^{-3}\ h^{-1}$ and was characterized by a second peak. Incorporation rate was strongly correlated with conductivity and ammonia. Highly significant regression relation ships were found between glycine and methionine (r = 0.7353), glycine and tryptophan (r = 0.8107) and methionine and tryptohan (r = 0.8273).

Diurnal Incorporation Rate of Amino Acids in Liquid Manure Fertilized Fish Pond

Incorporation profiles of glycine and methionine were presented in the same ways in day time, with one peak recorded during 1900-2100 hs. The daily cycle mean incorporation rates were 3.139 and 1.992 $\mu g\ dm^{-3}\ h^{-1}$ for glycine and methionine respectively. The lowest rates found were 1.889 and 1.356 $\mu g\ dm^{-3}\ h^{-1}$ during 300-500 hs. for glycine and methionine, respectively, (Figure 4). Incorporation rate of glycine was significant with temperature and nitrite, and showed a strong correlation with dissolved phosphate. Incorporation rate of methionine was strongly correlated with pII, redox potential and dissolved phosphate, while incorporation rate was also correlated with nitrite.

Incorporation rate of tryptophan ranged between 1.829 and 6.988 $\mu g\ dm^{-3}\ h^{-1}$. The daily rates of tryptophan characterized by two peaks, were recorded during 1100-1300 hs. and 700-900 hs. afternoon and late morning, respectively, while minimum rate level was observed during 2100-2300 hs in late night. The fertilized fish pond showed higher assimilation for amino acid. This resulted in a rapid growth phase following the stress caused

by fertilization. At the same time high quantity of algal excretion of dissolved organic matter has been produced (Fogg, 1971). The higher uptake velocity of amino acid recorded in fertilized fish pond is supposed to be due to higher activity of bacteria. Incorporation rate was weakly correlated with pH and redox potential, while it was strongly correlated with dissolved phosphate (Table 2 and Figure 4).

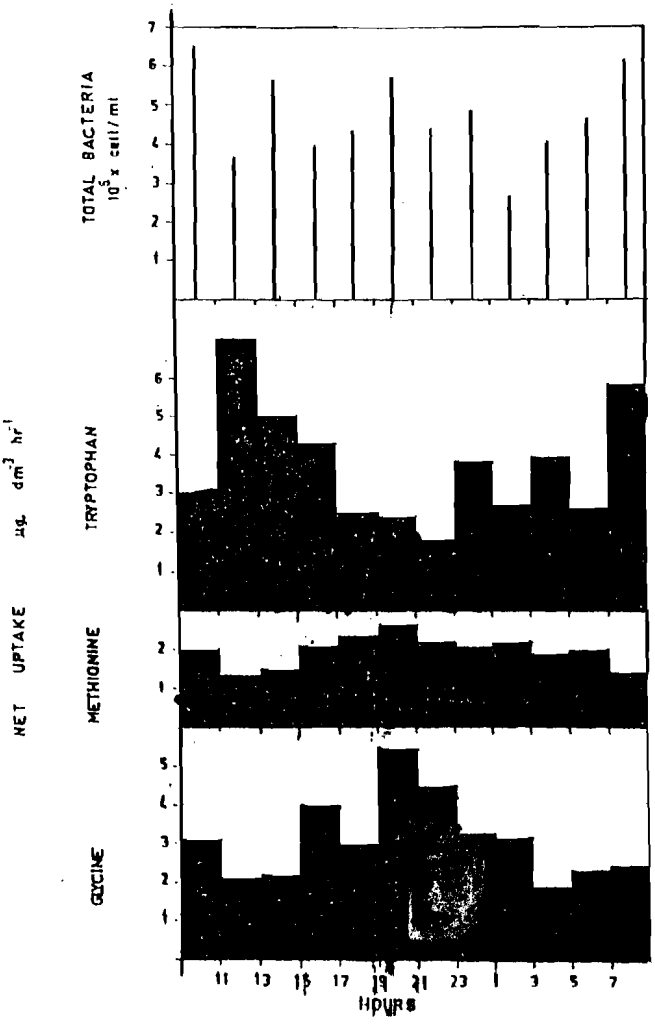

Fig. 4
Diurnal net uptake in liquid manure fertilized fish pond during 10 and 11 June.

Strong correlation coefficient was found between glycine and methionine (r=0.7827) while, intermediate negative correlation between glycine and tryptophan (r=-0.5666) was observed. Strong negative correlation was found between methionine and tryptophan (r=-0.8721). If we consider the mean incorporation rate values, we may observe that they are relatively comparable to the liquid manure fertilized fish pond and Koros backwater reservoir. This result could due to the fact that liquid manure fertilized fish pond supplied with water comming from Koros backwater reservoir. However, due to allochthonous bacteria, supplied with pig manure during fertilization of fish pond (pig manure, 37.5 m^3 during four months), bacterial activity in liquid manure fertilized fish pond is relatively higher.

Comparison of the physicochmical parameters and bacterial numbers showed great variations between the three aquatic bodies.

Similarly, incorporation rates for the three amino acids examined here were highly variable in the three aquatic bodies.

All these parameter may be useful for the regular observation of the changes connected to eutrophication. The diurnal uptake may add a clear picture to seasonal variations of heterotrophic activity in natural water.

The same basic pattern of decreased and increased heterotrophic activity was found in the three aquatic bodies. Due to the physiological conditions, these bacteria are capable to utilize amino acid substrate from water. Wright (1978) metioned that heterotrophic bacteria are not of similar activity, related to physiological differences that may well correspond with habitat changes. The diurnal variation of incorporation of glycine, methionine and tryptophan appeared to fluctuate from hour to other. These values were the same for the three sites examined, whereas the sites were widely different in environmental characteristics. Incorporation uptake was correlated to requirement of bacteria to energy from substrate. Morita et al.(1977) and Goulder (1980) concluded that there is no strong correlation between maximum uptake and ecological parameters.

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