Bull. Inst. Oceanogr. & Fish. ARE, 14 (1) 1988: 31-42.

HETEROTROPHIC ACTIVITY OF DISSOLVED AMINO ACIDS IN WATERS ALONG THE EGYPTIAN MEDITERRANEAN COAST

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ABSTRACT

Inorganic nutrients fluctuate with the geographic location and the degree of pollution. Standing crops of phytoplankton range from 1500 cell dm^{-3} to 28050 cell dm^{-3} in sewage outfall of Anfoushy and Eastern Harbour, respectively. The incorporated uptake of five amino acids were studied at five locations. Activities varied and appeared to be affected by inflow of sewage and industrial waste. Concerning the incorporation of dissolved amino acids, the low incorporated V_{max} was recorded as 0.015 μg dm^{-3} h^{-1} for glutamic acid in El-Alamain. The highest incorporated V_{max} recorded was 1.394 μg dm^{-3} h^{-1} for glycine in Eastern Harbour. The turnover times of dissolved amino acids were used as reference to the eutrophication of sea water massrs. The slower rate is about 143 days indicating oligotrophic sea water in the western region. The mesotrophic and eutrophic characteristics were observed in Abu Qir Bay, El-Max and Eastern Harbour. Furthermore, the eutrophic sea water could be observed in semi-closed bay. The high heterotrophic potential was found in association with high number of phytoplankton.

INTRODUCTION

The Mediterranean sea is generally characterized by low tide, high evaporation and low biological production. There are few data available on the Mediterranean heterotrophic uptake. Conventionaly the eutrophication or pollution of the Mediterranean coastal waters are affected not only by organic materials inputs from sewage sources but also by inorganic waste products of industrial factories.

Analysis of heterotrophic potential has been applied on sea water by Parson and Stricland (1962); Vaccaro and Jannasch (1966); Banoub and Willams (1972); Dawson and Gocke (1978); Bolter and Dawson (1982); and Albright (1983).

The study of eutrophication in the Mediterranean is difficult, because of the rapid changes of physical, chemical, and biological factors. The heterotrophic microbial communities are considered as an important complementary process of water, and it has been suggested that the metabolic activity might be a valuable index to trophic conditions (Albright and Wentworth, 1973).

The present investigation is concerned with the study of heterotrophic activity of five amino acids at five different locations with different levels of pollution, located at about one kilometer offshore the Mediterranean coast, (Fig. 1). Only five amino acids were used in the present work and the respiration activity of microoranisms was ignored.

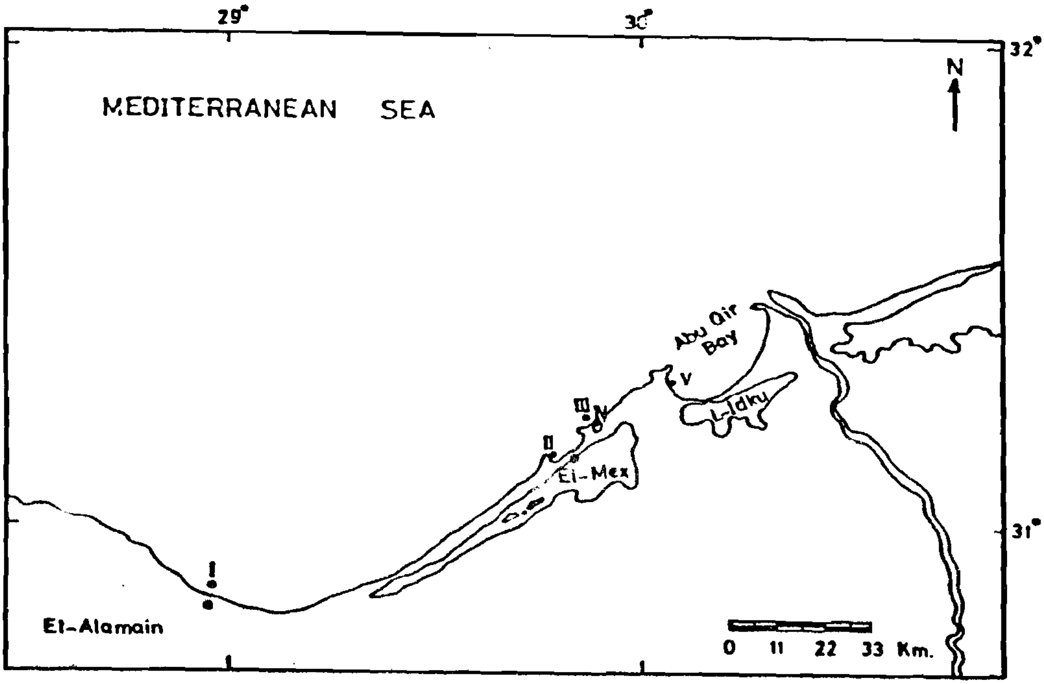

Fig. (1)
Area of investigation and location of sampling stations.

MATERIAL AND METHODS

1- Sampling

The water samples were collected from the different locations with Ruttner sampler about 50 cm below the seawater level. Samples were obtained from El-Alamain, El-Max, sewage outfalls at Anfoushy, Eastern Harbour and Abu Qir Bay during November and December, 1987. (Fig. 1).

2- Chemical analysis

Immediately after collection, the samples were filtered through Whatman GF/C glass fiber and the filtrates was analysed by standard methods.

3. Phytoplankton enumeration

The phytoplankton samples were enumerated per litre. All samples were preserved using few drops of Lugal's solution and allowed to sediment into 50 ml capacity. The quantitative enumeration of phytoplankton was made using sedimentation counting cells (capacity 2 ml).

Heterotrophic activity was to measure the enzyme kinetics at the actual temperature with short incubation time under sterile conditions (Wright and Hobbie, 1966). Increasing amounts of ^{14}C-labelled substrates were added to the original samples (Table 1). The uniformaly ^{14}C- labelled amino acids were obtained from the Institute of Isotopes of the Hungarian Academy of Science. For each substrate, ten 100 ml aliquots were incubated in galss-stoppered bottles of 130 ml capacity for 3 hours in dark in situ. Half ml of formalin was added to five of the bottles as a blank. After the incubation time, the samples were fixed with half ml formalin. 10 ml subsample from each bottle was filtered through 0.45 um Sartorius membrane filter. The filters were washed with an equal volume of filtered seawater. Each filter was then placed in a scintillation vial containing 10 ml of a standard fluor consisting of 0.01% POPOP and 0.4% PPO, and counted on L K B 120 Betaman Liquid Scintillation Counter.

Kinetic parameters were calculated by modified Lineweaver-Burk equation of Wright and Hobbie (1966):

$$t/f = (K_t + S_n)/ V_{max} + A/V_{max}$$

where,

t = incubation time in hours,

f = radioactivity assimilated of added ^{14}C- amino acids in disintegration per minute (dpm),

K_t = uptake constant equivalent to the Michaelis constant (ug dm^3),

S_n = the natural amino acid substrate in seawate (ug dm^{-3}),

V_{max} = the theoretical maximum velocity of uptake (ug $dm^{-3}h^{-1}$), and

A = concentration of added substrate (ug dm^{-3}).

Wright and Burnison (1979) mentioned that where A more **larger that** S_n, there is a basis for assuming that the added substrate will **not effect** the natural velocity.

TABLE 1
The substrate concentrations used for the uptake experiments.

Substrate	Substrate concentration ranges ($\mu g\ dm^{-3}$)	Specific activity in amino acids.
Glycine	2.793 - 18.96	220.15 to 495.
Glutamic acid	4.03 - 20.17	M Bq/ mmole
Methionine	4.13 - 20.63	
Phenylalanine	3.15 - 17.18	
Valine	4.5 - 22.48	7.4 G Bq/ mmole

Plot of t/f against A, the intercept on the negative abscissa is equal to (K_t + S_n) ug dm^{-3}, the reciprocal of the slope is $1/v_{max}$, while, the ordinate intercept is equivalent to the turnover time T_t hours.

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

RESULTS AND DISCUSSION

The results of physical and chemical aspects in this study have the common properties of low salinity in El-Max and sewage outfalls. These levels reflect the inland source inputs. The pH value and total alkalinity have the same trends except in, El-Alamain region. The noticeable nitrate, nitrite, ammonia, and phosphorus concentrations in seawater were obtained. The inorganic nutrients concentrations were low at El-Alamain. Remarkable high concentrations of inorganic nutrients were found in El-Max and sewage outfalls, (Table 2). This may be caused by the inflow of relatively fresh water and wastes of industrial product from the surrounding factories. The low concentrations of chemical parameters are more evident in oligotrophic seawater than in eutrophic seawater.

TABLE 2

Physical and chemical parameters of in the studied areas.

Environment factor	El-Alamain	El-Max	Sewage oufalls Anfoushy	Eastern Harbour	Abu Qir Bay
Temperature °C					
air	20	22	20	18.9	15.5
water	17	19	20	20.2	16.0
pH	8.0	7.68	7.73	7.84	7.84
Alkalinity (milli eq. dm^{-3})	2.78	4.73	4.59	4.49	3.48
Dissolved O_2ml dm^{-3}	5.03	3.7	6.21	6.28	4.92
Salinity ‰	38.9	35.37	34.04	38.8	38.31
NO_2 µg-at dm^{-3}	0.40	3.90	0.95	0.75	0.50
NO_3 µg-at dm^{-3}	1.46	14.62	5.43	4.88	3.40
NH_4 µg-at dm^{-3}	1.005	15.345	16.845	2.512	2.57
SiO_3 µg-at dm^{-3}	0.00	0.774	3.630	0.536	0.06
PO_4 µg-at dm^{-3}	0.02	2.647	2.172	1.143	0.19

In fact the phytoplankton population of the Egyptian Mediterranean waters has been investigated since 1956. All observations since 1965 have shown a drop in the magnitude of the diatom blooms, reflecting the drastic reduction in the Nile discharge (Halim, 1976). The distribution of phytoplankton is characterized by three large groups namely diatoms, chlorophyta, and cyanophyta, while dinoflagellates were recorded also as a rare form. Twenty three genera of diatoms were found. Dinoflagellates, chlorophyta, cyanophyta were represented by three or two genera only. Also, the members of chlorophyta and cyanophyta were rarely recorded.

El-Max and Eastern Harbour were the richest in total number of phytoplankton "19900 cell dm^{-3} "and " 28050 cell dm^{-3} ", respectively. The most dominant genera among all the diatoms recorded at the differnet regions in the western and eastern parts of the coastal Mediterranean water were **Melosira** Kutz; **Chaectoceros** Ehr, **Synedra** Ehr.; **Nitzchia** Hass.; **Rizosolenia** Ehr; **Thalassiosira** Grun and **Cyclotella** Kutz; also the green algae **Scenedesmus** Meyen were observed (Table 3).

The results of the measurements of amino acids uptake are given in Table 4.

The kinetic parameters of V_{max}, $K_t + S_n$ and T_t turnover time were estimated at each region. The heterotrophic potential (V_{max}) values fluctuated among water samples at the different stations (Table 4).

TABLE 3

Distribution of phytoplankton (cell dm^{-3}) of selected stations.

Genera	El-Alamain	El-Max	Sewage oufalls Anfoushy	Eastern Harbour	Abu Qir Bay
Diatoms:					
Amphora spp.	----	200	----	-----	-----
Asterionella sp.	----	-----	----	1000	1300
Bellarochia sp.	100	-----	----	500	-----
Gampylodiscus sp.	----	100	----	-----	-----
Chaetoceros sp.	----	3200	----	1000	2700
Cocconeis sp.	----	-----	----	100	-----
Cyclotella sp.	50	2000	----	500	100
Ditylium sp.	----	-----	100	300	-----
Gomphonema sp.	----	500	100	-----	-----
Grammatophra sp.	----	-----	----	-----	-----
Lithodesmium sp.	----	1400	----	700	-----
Lyomophora sp.	----	100	----	-----	-----
Mastogloia sp.	100	-----	100	-----	200
Melosira sp.	----	8100	----	18300	400
Navicula sp.	----	100	100	-----	-----
Nitzschia spp.	----	1500	----	800	900
Podosira sp.	----	100	----	-----	-----
Prorocentrum sp.	----	200	----	200	-----
Rhizosolenia spp.	2700	-----	----	-----	-----
Skeletonema sp.	----	-----	600	100	1400
Surirella sp.	----	-----	----	-----	100
Synedra sp.	----	200	----	-----	300
Thalassiosira sp.	----	400	----	4400	1000
Dinoflagellata					
Gymnodinium sp.	100	-----	----	500	-----
Peridinium sp.	----	-----	----	100	200
Chlorophytes					
Crucigena sp.	----	400	----	-----	-----
Scenedesmus spp.	----	1000	400	-----	-----
Euglena sp.	----	-----	----	-----	100
Gyanophytes					
Oscillatoria sp.	----	-----	100	-----	-----
Spirulina sp.	----	400	----	-----	-----
Total	3050	19900	1500	28050	9000

TABLE 4
The net uptake of amino acids by the heterotrophic bacteria along the Egyptian Mediterranean Coast.

Station	substrate	Net uptake V_{max} µg dm^{-3} h^{-1}	$K_t + S_n$ µg dm^{-3}	T_t hours	Correlation coefficient r.
El-Alamein	Glycine	0.017	18.392	1069.5	0.9892
	Valine	0.036	35.484	981.9	0.9834
4-12-1987	Glutamic acid	0.015	9.462	640.0	0.9977
	Methionine	0.051	19.172	375.0	0.9929
	Phenylalanine	0.038	13.697	363.0	0.9917
El-Max	Glycine	0.992	35.216	35.5	0.9856
	Valine	0.622	4.699	7.6	0.9937
	Glutamic acid	0.292	14.966	51.2	0.9977
6-11-1987	Methionine	0.791	45.976	58.1	0.9794
	Phenylalanine	0.646	21.216	32.5	0.9001
Sewage outfalls Anfoushy	Glycine	0.116	51.103	439.5	0.9583
	Valine	0.081	17.729	218.2	0.9976
	Glutamic acid	0.037	25.568	684.0	0.9807
24-11-1987	Methionine	0.057	20.050	352.9	0.9984
	Phenylalanine	0.063	14.378	229.5	0.9945
Eastern Harbour	Glycine	1.394	24.540	17.6	0.9892
	Valine	0.765	4.178	5.5	0.9903
	Glutamic acid	0.442	18.925	42.8	0.9919
16-11-1987	Methionine	0.986	49.492	50.2	0.9569
	Phenylalanine	0.629	8.202	13.0	0.9877
Abu Qir Bay	Glycine	0.395	50.259	127.2	0.9721
	Valine	0.225	7.324	32.6	0.9979
	Glutamic acid	0.087	21.537	246.2	0.9932
18-12-1987	Methionine	0.192	12.122	63.1	0.9945
	Phenylalanine	0.148	24.128	162.6	0.9905

The incorporated uptake of glycine varied from 0.017 µg dm^{-3} h^{-1} in Al-Alamain region to 1.394 µg dm^{-3} h^{-1} in the Eastern Harbour. The values of heterotrophic potential of glycine uptake varied 82-fold. The incorporated uptake of valine ranged from 0.036 to 0.765 µg $dm^{-3}h^{-1}$ in El-Alamain and Eastern Harbour, respectively, with 21 - fold. V_{max} of glutamic acid fluctuated from 0.015 µg dm^{-3} h^{-1} to 0.442 µg $dm^{-3}h^{-1}$, with varied V_{max} 29-fold at Al-Alamain and Eastern Harbour, respectively. Incorporated V_{max} of methionine ranged from 0.051 µg $dm^{-3}h^{-1}$ in AlAlamain region to 0.986 µg $dm^{-3}h^{-1}$ in Eastern Harbour with varied value 19-fold. The uptake of phenylalanine was 0.038 and 0.629 µg $dm^{3}h^{-1}$ in Al-Alamain and Eastern Harbour, respectively, with different value, 16-fold. This wide variation of the degree of different uptake rates is possible using this method. The explanation of the importance of the microbial utilization of dissolved amino acids and other organic material have been reported by Wright and Hobbie, (1966); Gocke, (1977); and ElSarraf, (1983) in natural waters. The heterotrophioc activity of amino acids in the present study can be classified into three categories according to the increasing microbial activity, (the Eastern Harbour>/ El-Max > Abu Qir Bay > Sewage outfalls El-Alamain.

The total values of incorporated V_{max} of amino acids are 4.216; 3.343; 1.047; 0.354 and 0.157 µg $dm^{-3}h^{-1}$, respectively. Albright and Wentworth (1973) as well asa Carney and Colwell (1973) and El-Sarraf (1983) have noticed that, highly polluted areas show increased V_{max} values, whereas increased incorporated V_{max} were related to different levels of trophic status of water. High heterotrophic activity was found in association with high numbers of phytoplankton in the water. Rai and Hill (1981), found that highest values of V_{max} were accompanied with highest Chlorophyll-a values. Hobbie (1969), found that values above 10^{-3}mg were consistently found in polluted lakes. It appears that the heterotrophic activity is one of the highest sensitive characteristics of the relative amount of pollution in natural waters. The lowest incorporated V_{max} was recorded at Al-Alamain region far and free of domestic waste and industrial pollution. Therefore, it appears that Al-Alamain can be considered as a nonpolluted area. Higher incorporated V_{max} or microbial metabolism in Eastern Harbour probably allow allochthonous bacteria to tolerate the effect pollutant contamination more effectively.

The present study suggests that, it is possible to calculate the daily values of V_{max} as a function of substrates growth. Total incorporated uptake of glycine in all stations examined was 2.914 µg $dm^{-3}h^{-1}$. The daily assimilation was 69.936 mg glycine per cubic meter per day. The daily heterotrophic communities would be theortically constant, 41.496 mg valine; 20.952 mg glutamic acid; 49.848 mg methionine and 36.576 mg phenylalanine per cubic meter per day, respectively. Williams et al., (1976) found that total uptake of individual amino acids varied from "undetectable" to 1210 mg amino acid per cubic meter per day. Williams (1970), mentioned that the abundance of free amino acids like serine and glycine in seawater may cause low uptake. On the other hand, Gillespie (1976) and Iturriaga and

Zsolnay (1981) found that uptake of glycine has higher incorporation. A comparison of uptake rate of these amino acids found in seawater is shown in Table 5.

The incorporated V_{max} of El-Max and Eastern Harbour is highly significant, P> 0.02 (r = 0.9456), also it is significantly higher P> 0.01 (r = 0.9717) for seawage outfalls and Abu Qir Bay. The correlation coefficient between the Eastern Harbour and El-Max is P> 0.01 (r = 0.9467). Comparison of uptake potentials (V_{max}) of amino acid substrates at other parts of the world are listed in Table 5.

TABLE 5
Uptake of amino acids in the seawater of different ecosystems (μg dm^{-3} h^{-1}).

Ecosystem	Substrate	V_{max}	References
Western Mediterranean	Amino acid	0.002	Banoub and Williams (1972)
Baltic Seawater	Amino acid	0.071 - 3.19	Dawson and Gocke (1978)
Antartic Ocean	Glutamic acid	0.011	Gillespie et.al (1976)
	Phenylalanine	0.0054	
	Valine	0.011	
Prudhoe Sea	Glutamic acid	0.037 - 0.044	Griffiths et.al (1978).
Eastern Tropical Pacific Ocea	Glutamic acid	0.015	Hamilton and Preslan (1970).
Antartic Ocean	Glutamic acid	0.011	Morita et.al (1977)
Philippine Sea	Glycine	0.004	Seki et.al (1974)
	Glutamic acid	0.008	
Tokyo Bay	Glycine	9.8	Seki et.al (1975)
	Glutamic acid	8.1	
Shimoda Bay	Glycine	0.095 - 1.7	Seki et.al (1980)
	Glutamic acid	0.74 - 4.4	
Booth Bay	Glycine	0.11	Wright and Shah (1975)
South Eastern	Glycine	0.017 - 1.394	This study
Mediterranean	Valine	0.036 - 0.765	This study
	Glutamic acid	0.015 - 0.442	This study
	Methionine	0.051 - 0.986	This study
	Phenylalanine	0.038 - 0.646	This study

The sum of the constant K_t and the natural substarte concentration S_n exhibit no clear direction. Instead $K_t + S_n$ values fluctuate from one region to another. Burnison and Morita (1974), found that the viaues of $K_t + S_n$ for amino acids generally, did not change over the year, with exception of glycine, serine, alanine, and asparagine which widely changed. The lowest values were recorded in El-Alamain region, while the highest values were observed in both sewage outfalls and El-Max region. Krambeck (1979) stated that the overall K_t is a function of all V_{max} values of intermediate enzymes in the reaction chain, while V_{max} is directly proportional to the extent of enzyme persuade. The lowest values were recorded in El-Alamain, whereas the highest values were found in both El-Max and sewage outfalls. It can be concluded that the values of $K_t + S_n$ increase with the degree of eutrophication and did not reliable trends, (El-Sarraf, 1983).

Turnover time "T_t" is the time required for a complete removal of natural substrate present in the water. The longest turnover time was observed at El-Alamain, while the shortest one was recorded in Eastern Harbour. Jorgenson (1982) found that, amino acid turnover time is imporatnt in the carbon flux of the estuary. Hobbie and Wright (1965) mentioned that, T_t varied from 0.5 h in a polluted pond to over 5000 h in cold oligotrophic lakes. Furthermore, Wright and Hobbie (1966) recorded that, the turnover time is a reliable parameter for metabolic constituents. On the other hand Rai and Hill (1981) found that T_t fluctuated from 120 h to 36000 h, with an average annual T_t of about 6600 h. The turnover times of amino acids would reflect the microbial activity related to the trophic status of seawater.

The correlation coefficient of T_t between El-Max and Eastern Harbour is highly significant, $P > 0.02$ (r. = 0.9297).

According to Seki and Nakano (1981), the organic compounds are divided into three catagories relative to their abilities of heterotrophic utilization. According to the classification mentioned by them, El-Alamain and sewage outfalls can be considered as oligotrophic regions (143 days and 80 days, respectively). The oligotrophic status could be attributed to the dispersive currents and open seawater. It is worthy to mention that Johannes (1967) calculated a T_t of one month for surface dissolved free amino acids in the open ocean. This was confirmed experimentally by Gocke (1977), who calculated the largest differences in estimated T_t values in oligotrophic waters. Abu Qir Bay is considered as a mesotrophic water (26 days) which may be explained by the semistagnant water situation. El-Max and Eastern Harbour are classified as eutrophic water masses (8 and 5.4 days, respectively) which might be due to the enrichment by organic matter produced by discharge of sewage and industrial wastes.

The interpretation of chemical, phytoplankton, and heterotrophic activities imply to the following conclusion:

1- The Egyptian coastal Mediterranean water at Alexandria is affect by many factors, e.g. converging and/ or diverging currents, inorganic nutrients, industrial wastes as well as flow.

2- The freshwater discharged from Rosetta mouth is probably dispersed westward by the surface current circulating Abu Qir Bay in winter (Technical report no 2/1, 1979).

3- El-Max region is greatly affected by water discharged from the pumping station. This discharged water is characterized by varying rates and turblances, as well as autochthonous heterotrophic bacteria.

4- It seems that physical and chemical oceanographic parameters highly affect the lateral distribution of the excretion of the phytoplankton used for heterotrophic bacterial utilization in cyclic formation.

5- It is important to say that heterotrophic activity is reliable in the classification of trophic aquatic systems in addition to the role of the marine microorganisms.

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