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# Effects of Water Pollution in Lake Mariut on Gonadal Free Amino Acid Compositions in *Oreochromis niloticus* Fish

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**Abstract:** In the present report, we have determined the gonadal free amino acid compositions in *Oreochromis niloticus* collected from three sites in Lake Mariut: SE basin (less polluted), main basin (moderately polluted) and SW basin (highly polluted), in an attempt to develop sensitive biomarker to evaluate pollution effects from multiple sources. Variations of free amino acids (FAAs) were apparent in the polluted sites; total FAAs in testes and ovaries exhibited significant increases as compared to the less polluted site. This increase was attributed to increases in all individual amino acids including essential and non-essential ones. ANOVA indicated significant changes in all testicular FAAs, excluding phenylalanine. Levels of non-essential amino acids showed obvious alterations in ovaries. However, the increase in most essential amino acids, although insignificant, yet it was quite observable. In addition, the ratio of essential to non-essential amino acids was insignificantly decreased in gonads. This may be indicative of changes in protein metabolism.

**Key words:** Lake Mariut, water pollution, Oreochromis, gonads, free amino acids

## INTRODUCTION

In the last few decades, considerable works have been focused on the effects of pollution on different physiological parameters of fish (Larsson *et al*., 1985; Triebskorn *et al*., 2002; Carballo *et al*., 2005). In Egypt, most of the works have focused on treated fish with acute or sublethal doses of different pollutants (Hilmy *et al*., 1987; Ghazaly, 1991; El-Dib *et al*., 1996; El-Gendy *et al*., 1998). Few works, however, have been carried out on fish caught from disturbed natural systems. Adham *et al*. (1997 and 2001) discussed the physiological responses of *Oreochromis niloticus* fish caught from different sites of Lake Mariut with ranging degrees of physicochemical and organic criteria. Many parameters (e.g., cytochrome P450, stress proteins, biotransfomation enzymes, free amino acids, etc.) have been validated as beneficial biochemical markers.

Amino acids are considered one of the most reliable technique for the detection of changes in protein synthesis in cells and therefore, the protein pattern can be used as a criterion for the differentiation between several organs exposed to some pollutants. A considerable amount of information is available on the effects of toxicants and accessory factors on the FAAs of fish (Mikhopadhyay and Dehadrai, 1980; Sahana *et al*., 1986; Sreedevi *et al*., 1992; Reddy and Bashamohideen, 1995; Ahmed *et al*., 1995; Philip and Rajasree, 1996). In addition, there is also a strong evidence for toxicant induced alteration in FAA pool in fish from polluted habitats (Carr *et al*., 1991; Leatherland *et al*., 1998). The present study was designed to underline the usefulness of free amino acids in target organs (e.g., gonads) of fish as a sensitive indication of the metabolic changes associated with environmental perturbations in Lake Mariut.

## MATERIALS AND METHODS

**Study area:** Lake Mariut, located southwest of Alexandria, is the heavily polluted lake in Egypt and its water is pumped to the sea. It is now divided artificially into four basins, namely the lake proper, the fish farm, the southeast (SE) and the southwest (SW) basins.

**Sampling locations:** The locations of the sampling sites in Lake Mariut are shown in Fig. 1. Information about the health state of the lake is available (Massoud, 2003). The lake proper (sampling location II) represents the main basin of the lake. Sewage effluents (rate 42 500 $m^3$ $d^{-1}$) flush into the northern part of the main basin. Industrial wastes (rate 22 500 $m^3$ $d^{-1}$) are also discharged into the main basin at its northeastern corner. Another effluent source from El-Kalaa drain (rate 150 000 $m^3$ $d^{-1}$) is mainly loaded with agronomic wastes, which in turn consume considerable amounts of DO from ambient water during its degradation (El-Sharkawi, 1991). The surplus water from

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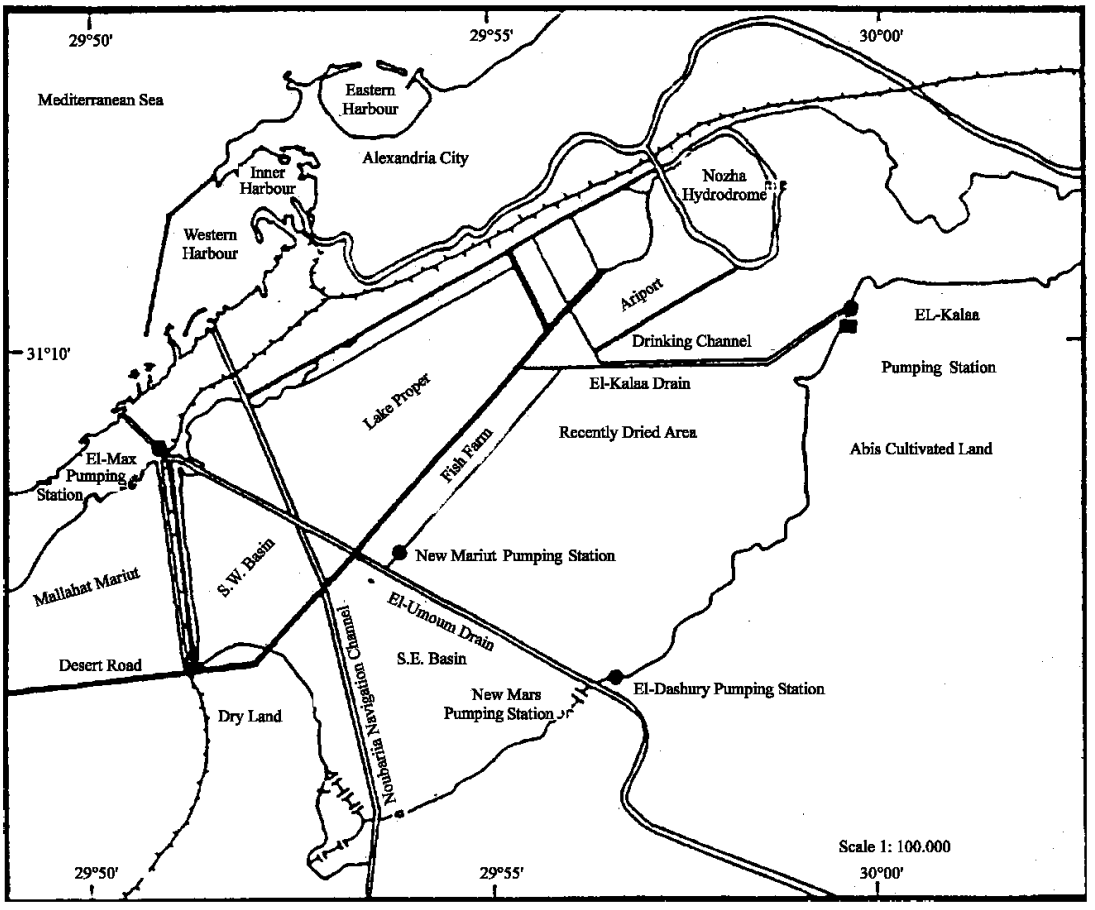


Fig. 1: Map of Lake Mariut showing sampling sites (I; SE basin, II; Lake Proper or main basin, III; SW or Merghim basin)

the lake proper is allowed to flow in to the lower reach of El-Umoum drain before pumping the mixed waters (6.8 $hm^3$ $d^{-1}$) to the Mediterranean Sea at El-Max (El-Rayis and El-Sabrouti, 1998). This pumping process maintains surface water level in the lake at about 2.5 m below sea level, to work properly as a recipient of different effluents. The untreated industrial and sewage wastes increased the heavy metals in the water and sediments of Lake Mariut (El-Rayis and Saad, 1990). According to Saleh *et al.* (1983), Adham *et al.* (1997) and Abdelmeguid *et al.* (2002), the SW basin (sampling location III) is contaminated with higher amounts of heavy metals in addition to mineral oils discharged by the cooling pipes of El-Nasr Petroleum Company. The reference location is the SE basin (sampling location I) receiving runoff water from the relatively unpolluted Noubariia canal and El-Umoum drain (Adham *et al.*, 1997, 2001).

**Sampling strategy:** Sexually maturing male and female *Oreochromis niloticus* were captured by close meshed nets from the selected locations. Twenty four fish (15-22 cm length and 73-180 g weight) from each location were randomly obtained between June and August 2003. The fish samples were transported alive to the laboratory in small tanks provided by oxygen pumps working with battery. Fish samples were then dissected; the gonads were excised quickly and stored at -20°C until analysis.

**Amino acid compositions:** Samples of testes and ovaries were homogenized in exactly three times their weight in ice-cold distilled water. Crude homogenates (3 g) were deproteinized by the addition of 10 mL $g^{-1}$ sulfosalicylic acid (3.5%). The supernatant was collected after centrifugation at 3500 rpm for 15 min and stored at -16°C for amino acid analysis. Determinations of free amino acids in gonads were preformed using Beckman amino acid analyzer (model 119CL).

**Statistical analysis:** Analysis of variance (one way ANOVA) and least significant difference multiple-comparison test (LSD) were used to determine specific

differences in amino acid compositions between locations. Data were log-transformed as necessary prior to statistical analyses to normalize data and reduce heteroscedasticity. For all statistical tests, α was set at 0.05.

## RESULTS

**Testicular amino acid analysis:** Table 1 shows the concentrations of free amino acids (FAAs) and ammonia in gonads of male fish collected from the different sampling locations, along with a summary of statistical analyses.

In gonads of male tilapias from location I (the less polluted area), glutamic acid and alanine had the highest frequency proportions (16.5 and 12.9%, respectively), followed by aspartic acid, threonine, arginine, proline and serine comprising an average of 7.9, 7.1, 7.0, 6.5 and 5.1%, respectively. The relative contributions of the remaining amino acids varied considerably, ranging between 0.2-4.9%.

Multiple comparisons between means (LSD) revealed that the total FAA concentrations were significantly ($p<0.05$) higher in testes of fish collected from the polluted locations (II, III) than that from the reference area (location I), however, insignificant mean difference ($p>0.05$) was found between locations II and III. It was clearly evident that amino acid profile was altered by exposure to pollution. The increase in total FAAs detected in the polluted groups was attributed to increases in all individual amino acids, including essential and non-essential ones. One-way analysis of variance (ANOVA) indicated significant changes in all tested parameters among different sites, excluding phenylalanine. In addition, LSD showed that the contents of aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, valine, methionine, isoleucine and leucine were significantly changed between locations I, II and I, III. In the meantime, variations between the polluted groups were found to be insignificant. On the other hand, tyrosine showed one significant change between locations III and I while threonine was significant only between locations II and I. Among other quantified amino acids, lysine showed two significant variations between locations II, III and II, I. In addition, proline, histidine and arginine also displayed significant mean differences between the polluted locations as well as between locations III and I. Furthermore, the ratio of essential to non-essential amino acids (EAA/NEAA) showed insignificant variations. The highest value was found in location I (0.75), decreasing slightly in locations II and III (0.63 and 0.73, respectively).

Table 1: Concentrations of free amino acids (FAAs) in testes of the male Nile tilapia, *Oreochromis niloticus* caught from three locations in Lake Mariut during spawning period (Jun.-Aug.) ◙ and results of one-way analysis of variance (ANOVA) assessing the effects of site on these variables. Values with different superscripts are significantly different (LSD multiple range test, $p<0.05$). All data were normalized by log-transformation prior to statistical analysis

| | | | | One-way ANOVA | |
|---|---|---|---|---|---|
| Amino acids | Location I (Main basin) | Location II (Main basin) | Location III (SW basin) | F | p |
| Aspartic acid | 27.9±1.9$^{a}$ | 50.9±8.9$^{b}$ | 64.8±11.0$^{b}$ | 6.3 | 0.005* |
| Threonine† | 25.2±1.4$^{a}$ | 49.8±8.5$^{b}$ | 33.0±2.8$^{a,b}$ | 6.9 | 0.003* |
| Serine | 18.1±1.1$^{a}$ | 31.9±5.3$^{b}$ | 27.5±2.3$^{b}$ | 5.2 | 0.011* |
| Glutamic acid | 58.6±3.0$^{a}$ | 117.9±19.1$^{b}$ | 116.3±19.6$^{b}$ | 6.1 | 0.005* |
| Proline | 23.1±1.9$^{a}$ | 30.8±5.4$^{a}$ | 41.1±5.3$^{b}$ | 4.6 | 0.016* |
| Glycine | 15.1±0.8$^{a}$ | 26.0±4.2$^{b}$ | 21.3±1.6$^{b}$ | 4.1 | 0.025* |
| Alanine | 45.9±1.2$^{a}$ | 102.5±16.4$^{b}$ | 73.8±11.8$^{b}$ | 8.0 | 0.001* |
| Cysteine | 0.6±0.01$^{a}$ | 1.4±0.3$^{b}$ | 1.5±.0.5$^{b}$ | 4.4 | 0.020* |
| Valine† | 15.4±1.1$^{a}$ | 25.3±3.9$^{b}$ | 26.6±2.6$^{b}$ | 6.5 | 0.004* |
| Methionine† | 11.2±1.2$^{a}$ | 23.9±5.2$^{b}$ | 27.0±2.7$^{b}$ | 9.5 | 0.001* |
| Isoleucine† | 15.1±1.3$^{a}$ | 24.6±3.2$^{b}$ | 30.0±3.7$^{b}$ | 9.8 | 0.000* |
| Leucine† | 17.5±1.4$^{a}$ | 28.0±4.0$^{b}$ | 36.1±4.2$^{b}$ | 10.1 | 0.000* |
| Tyrosine | 12.6±0.7$^{a}$ | 18.5±3.2$^{a,b}$ | 22.1±2.3$^{b}$ | 5.8 | 0.007* |
| Phenylalanine† | 15.4±2.2$^{a}$ | 15.8±4.2$^{a}$ | 20.9±2.8$^{a}$ | 2.4 | 0.108 |
| Histidine† | 10.0±0.9$^{a}$ | 12.9±2.6$^{a}$ | 18.3±1.6$^{b}$ | 6.0 | 0.006* |
| Lysine† | 17.4±3.1$^{a}$ | 28.9±2.4$^{b}$ | 18.7±2.7$^{a}$ | 5.4 | 0.010* |
| Arginine† | 25.0±1.9$^{a}$ | 34.3±7.4$^{a}$ | 58.8±9.6$^{b}$ | 7.7 | 0.002* |
| Essential amino acids (EAA) | 152.2±19.9$^{a}$ | 240.2±32.3$^{b}$ | 269.4±28.5$^{b}$ | 5.6 | 0.008* |
| Non-essential amino acids (NEAA) | 201.9±14.0$^{a}$ | 383.2±66.7$^{b}$ | 368.4±36.3$^{b}$ | 8.6 | 0.001* |
| EAA/NEAA | 0.75±0.13$^{a}$ | 0.63±0.11$^{a}$ | 0.73±0.15$^{a}$ | 0.35 | 0.706 |
| Total FAAs | 354.1±23.0$^{a}$ | 623.4±98.7$^{b}$ | 637.8±81.1$^{b}$ | 6.4 | 0.004* |

◙ Data are presented as means±SE for 12 experiments and are expressed as mg/100 g tissue, *Statistically significant at 0.05 level of probability (p), †Essential amino acids

Table 2: Concentrations of free amino acids (FAAs) in ovaries of the female Nile tilapia, *Oreochromis niloticus* caught from three locations in Lake Mariut during spawning period (Jun-Aug.) ◙ and results of one-way analysis of variance (ANOVA) assessing the effects of site on these variables. Values with different superscripts are significantly different (LSD multiple range test, $p<0.05$). All data were normalized by log-transformation prior to statistical analysis

| | | | | One-way ANOVA | |
|---|---|---|---|---|---|
| Amino acids | Location I (SE basin) | Location II (Main basin) | Location III (SW basin) | F | p |
| Aspartic acid | 27.1±3.0$^{a}$ | 34.5±1.8$^{a}$ | 61.4±7.8$^{b}$ | 15.5 | 0.000* |
| Threonine† | 38.6±2.6$^{a}$ | 51.3±4.1$^{b}$ | 60.9±9.5$^{b}$ | 4.4 | 0.021* |
| Serine | 23.3±1.9$^{a}$ | 34.3±2.9$^{b}$ | 38.3±6.1$^{b}$ | 5.2 | 0.011* |
| Glutamic acid | 57.2±5.9$^{a}$ | 86.0±17.4$^{a,b}$ | 135.0±29.6$^{b}$ | 5.7 | 0.008* |
| Proline | 17.2±2.1$^{a}$ | 29.9±3.1$^{b}$ | 35.3±7.0$^{b}$ | 5.4 | 0.009* |
| Glycine | 11.6±1.1$^{a}$ | 19.3±3.4$^{a,b}$ | 27.8±6.2$^{b}$ | 4.7 | 0.017* |
| Alanine | 33.6±3.0$^{a}$ | 56.5±9.2$^{b}$ | 69.6±7.4$^{b}$ | 10.7 | 0.000* |
| Cysteine | 2.3±0.3$^{a}$ | 3.2±0.4$^{a}$ | 3.1±0.5$^{a}$ | 1.3 | 0.298 |
| Valine† | 22.8±1.6$^{a}$ | 35.1±2.7$^{b}$ | 37.4±6.6$^{b}$ | 4.1 | 0.026* |
| Methionine† | 12.8±1.3$^{a}$ | 17.2±1.6$^{a}$ | 14.3±1.2$^{a}$ | 2.7 | 0.080 |
| Isoleucine† | 28.0±3.0$^{a}$ | 34.4±2.6$^{a}$ | 33.0±4.2$^{a}$ | 1.3 | 0.188 |
| Leucine† | 24.5±2.5$^{a}$ | 33.5±3.3$^{a}$ | 29.9±4.8$^{a}$ | 1.9 | 0.166 |
| Tyrosine | 15.2±1.3$^{a}$ | 17.8±0.8$^{a}$ | 19.0±2.0$^{a}$ | 1.7 | 0.193 |
| Phenylalanine† | 11.7±1.5$^{a}$ | 14.3±1.4$^{a}$ | 15.3±2.4$^{a}$ | 1.4 | 0.270 |
| Histidine† | 8.4±0.9$^{a}$ | 11.1±1.3$^{a}$ | 15.6±4.1$^{a}$ | 1.4 | 0.250 |
| Lysine† | 20.7±2.2$^{a}$ | 26.0±2.4$^{a}$ | 23.2±3.9$^{a}$ | 0.9 | 0.414 |
| Arginine† | 28.7±2.4$^{a}$ | 37.6±4.2$^{a}$ | 40.8±6.7$^{a}$ | 1.9 | 0.161 |
| Essential amino acids (EAA) | 196.2±18.4$^{a}$ | 260.5±25.7$^{a}$ | 269.4±35.2$^{a}$ | 2.1 | 0.140 |
| Non-essential amino acids (NEAA) | 187.5±18.3$^{a}$ | 281.5±34.8$^{b}$ | 389.5±56.2$^{b}$ | 8.0 | 0.001* |
| EAA/NEAA | 1.05±0.14$^{a}$ | 0.93±0.14$^{a}$ | 0.69±0.11$^{a}$ | 2.8 | 0.076 |
| Total FAAs | 383.7±32.9$^{a}$ | 542.0±54.5$^{b}$ | 658.9±48.9$^{b}$ | 12.4 | 0.000* |

◙ Data are presented as means±SE for 12 experiments and are expressed as mg/100 g tissue, *Statistically significant at 0.05 level of probability (p), †Essential amino acids

**Ovarian amino acid analysis:** Table 2 illustrates data for the concentrations of FAAs and ammonia in gonads of female fish caught from three sites in Lake Mariut, in addition to the results of one-way ANOVA and multiple range tests of mean differences (LSD).

In the reference specimens (location I), glutamic acid and threonine represented 14.9 and 10.1% of the total FAA and were the most abundant amino acids. Alanine, arginine, isoleucine, aspartic acid, leucine, serine, valine and lysine were the next most abundantand had relative proportions of about 8.8, 7.5, 7.3, 7.1, 6.4, 6.1, 5.9 and 5.4%, respectively. Each of the remaining amino acid represented between 0.6%-4.5% of the total FAA pool.

There were remarkable changes in gonadal amino acids of female fish collected from the three sampling locations, being elevated in the polluted groups. In turn, total FAAs were significantly increased in locations II and III when compared to location I. The variation in total FAAs between locations II and III was, however, insignificant. On the other hand, the levels of non-essential amino acids showed obvious alterations. Aspartic acid showed two significant variations between locations III, II and III, I. Moreover, serine, proline and alanine showed significant mean differences between locations I, II and I, III. In addition, glutamic acid and glycine exhibited one significant change between locations I, III. Other non-essential amino acids including, cystine and tyrosine revealed insignificant increases in location II and III as compared to the reference. In fish caught from the polluted areas, the increase in essential amino acids, although insignificant, yet it was quite observable. Threonine and valine were, however, exceptional. They showed two significant variations between locations I, II and I, III.

There were insignificant changes in the ratio EAA/NEAA among the different sites. The ratio decreased markedly in location III (0.69) as compared to locations II and I (0.93 and 1.05, respectively).

## DISCUSSION

Two types of changes in the FAA pool of organisms normally occur under toxicant-induced stress. The first involves either an increase or decrease in the total FAA concentration. The second type of response results in alterations in specific amino acids (either increases or decreases), which may or may not cause changes in the total amino acid concentration. The present study indicated an increase in concentration of total FAAs in gonads of mature fish inhabiting areas with high rates of hazardous contamination (heavy metals at location II, heavy metals and petroleum oil at location III) as compared to location I. However, insignificant changes in total FAA concentrations were recorded between the

polluted groups. The increase in total FAAs recorded in the polluted areas was attributed to increases in the recorded individual amino acids. Results reported in the present study indicated that most of the individual FAAs in male gonads of fish from the polluted areas were sensitive to pollution and most of the recorded differences between these sites and the reference site were highly significant. Aspartic acid, isoleucine, leucine and arginine (in location III), threonine and alanine (in location II) and glutamic acid, cysteine and methionine (in both of the polluted groups) showed the highest increase, approximately one-fold. On the other hand, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine and valine were the most affected FAAs in female gonads, particularly those from location III, with the remaining amino acids showed insignificant increases. The decrease in the ratio of essential/non-essential amino acids detected in the polluted groups, although insignificant, may be an indicative of changes in protein metabolism. Protein degradation and subsequent utilization of the released amino acids for anaplerotic reactions and/or energy production represent an important mechanism for changes in the total FAA concentration as well as the pattern of individual FAA. For many invertebrates, amino acids may contribute significantly to the total energy budget of the organism (Gilles, 1970). Adenosine 5-monophosphate (AMP) and/or ADP are covalent modifiers of many of the enzymes involved in nitrogen metabolism, such as glutamate dehydrogenase (Stryer, 1981), so that changes in the energy status of the organism can modulate the oxidation of amino acids, thus altering the relative proportions or profile of the FAA pool. Therefore, under the increased energy demand associated with toxicant-induced stress, invertebrates may degrade proteins to augment the available energy supply, thus altering the FAA pool (Powell *et al*., 1982; Bhagyalakshmi *et al*., 1983).

Similarly, fish preferentially generate aerobic energy from protein catabolism. Sivarama-krishna and Radhakrishnaiah (1998) revealed an initial high proteolysis in the tissues of common carp, *Cyprinus carpio* on sublethal mercury intoxication with a recovery on long-term exposure. This was characterized by a progressive elevation in the levels of FAAs and the activities of protease and aminotransferases (AAT, AlAT) in the liver, brain and muscles. In addition, De Smet and Blust (2001) found that the concentrations of FAAs and the activities of proteases, AAT and AlAT were increased at day 4 in gills, liver and kidney of carp exposed to 4 and 20 μM Cd and in gills and kidney at day 29 in carp exposed to 4 μM Cd. They suggested that the observed proteolysis was intended to increase the role of proteins in energy production due to Cd stress. However, they also found that the increased activity of both aminotransferases was not found in gills during the lethal Cd exposure. Furthermore, Wood and Van Der Kraak (2003) provided the first evidence for a novel mechanism of follicular atresia in teleosts involving cathepsin-mediated yolk proteolysis confirmed by a significant increase in oocyte free amino acid content after 72 h culture in serum-free medium.

On the other hand, the environmental factors may generate a common signal, which in turn changes the gene expression of protein synthesis. One of the possible alterations could be the induction of heat shock proteins (hsps). Theodorakis and Morimoto (1987) found that, under stressful conditions, hsps are strongly induced and the synthesis of other proteins is inhibited. They also showed that hsps make up less than 1% of all proteins. Accordingly, Tang-Chun *et al*. (1998) in their work on the levels FAAs in the plasma of workers exposed to heat, CO and heat+CO, suggested that the inhibition of the synthesis of other cellular proteins might thus lead to a relative surplus of free amino acidsand a subsequent increase in their free plasma levels. Increased levels of various hsps have been measured in tissues of fish exposed to environmental contaminants, such as heavy metals (Heikkila *et al*., 1982; Misra *et al*., 1989; Williams *et al*., 1996; Duffy *et al*., 1999), polycyclic aromatic hydrocarbons (Vijayan *et al*., 1998) and pesticides (Hassanein *et al*., 1999).

In conclusion, the influence of pollution in Lake Mariut could be highly negative to the aquatic biota and affect the biotic integrity of the lake and thus needs to be urgently addressed. Pollution of the lake should be monitored regularly and by means of an integrated approach, including investigation of fish and macroinvertebrates. This level of monitoring could produce sufficient information to implement better management strategies, which would reduce the present negative impacts.

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