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THE INVESTIGATION OF AST, ALT, GGT ENZYME ACTIVITIES IN FISH ORGANS AND PB, CD, AND AS LEVELS IN MUSSELS FROM BOSPHOROUS

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İstanbul Boğazındaki Balıkların Organlarında AST, ALT, GGT Enzim Aktiviteleri İle Midyelerde Pb, Cd Ve As Düzeylerinin Araştırılması

Özet: Son yıllarda Marmara denizindeki kirlenme büyük boyutlara ulaşmıştır. Bu durum sonucu pek çok balık türünde azalma veya ani kütlesel ölümler gerçekleşmektedir. Aynı zamanda kirleticilerin (ağır metaller, deterjanlar, pestisitler) besin yolu ile insanlara geçmesi insan sağlığı açısından önemli bir tehlike oluşturmaktadır.

Çalışmada halkımız tarafından en çok tüketilen balık olan istavrit materyal olarak kullanılmıştır. İstavritden alınan doku örneklerinde ALT, AST, GGT enzim aktiviteleri incelenmiştir. Örnekler spektrofotometrede analiz edilmiştir. Ayrıca çalışmada Boğaz kıyılarındaki kirlenmenin bir indikatörü olarak kullanılan midyelerde Pb, Cd, As düzeylerinin saptanması amaçlanmıştır. Örnek analizleri, X-Ray Spektrometri de (XRF) yapılmıştır.

Balık ve midye örnekleri İstanbul Boğazının belirli noktalarından 24 ay boyunca ayda bir alınmıştır. İstavrit karaciğer ve böbrek doku örneklerinde AST, ALT, GGT enzim aktiviteleri son 6 aylık dönemde istatistikii olarak anlamlı derecede artmıştır. ALT, AST, GGT enzim aktivitelerinde diğer dönemler arasında anlamlı farklılıklar saptanmamıştır. Midyelerde özellikle Pb konsantrasyonu dört dönem boyunca Cd ve As konsantrasyonlarına göre oldukça yüksek bulunmuştur

Summary: The pollution problems in Marmara Sea were increased gradually in recent years. As a result many kinds of fish were progressively decreased or sudden fish deathed in masses. At the same time the pollutants (heavy metals, detergents, pestisits e.g.) could be a potential hazard for human being according to degree of sea food consumption.

In this study, horse mackerel, one of the most popular kind of fish as food, were used as the main material. The enzymes activities of Aspartat transaminase (AST), Alanin transaminase (ALT) Gama-glutamily transpeptidase (GGT) determinations were done in tissues samples of horse mackerels. The samples were analysed by spectrophotometer.

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In addition this study aims at collecting information by using mussel as indicator about levels of Pb, Cd and As monitoring their changes during time at the coasts of Bosphorous. The samples were analysed by X-Ray Spectrometry (XRF).

Fish and mussel sample were collected from the selected point of the Bosphorous through out 24 months.

Liver and kidney of horse mackerels AST, ALT,GGT enzyme activities were increased in last six month periods. Nevertheless, there were no significant differences in AST, ALT, GGT activities, between all periods. Especially, Pb concentration was determinated higher according to Cd and As concentrations through out four periods.

Introduction

The Blast of population, high welfare rates, and especially industrial developments all underlie, as pronounced, the pollution in the sea-band around the Turkey main, as well as they do on global waters as a whole.

Studied from the 1950's on, in view of the pollution, witnessed in the Marmara sea and the connecting channels, Bhosphorus and Dardanel, evidently and ignominiously worsening by the time being, it has well and while long been recognised that the waste petrol materials deliberately or other wise dropped in to the sea waters and in particular some certain factors, though remote in origin, mostly caused the presentation (7,21,23,24).

In addition the organic chemicals, the most effective polutive agents which have so far caused to the present levels of pollution, enter in to the water in many ways. They are the products of domestics waste waters, liquid or solid industrial waste material, solid waste from the purification plantations, and of the decomposition of rubbish collected from working districts and/or inhabited areas as well as of the drafts from air born pollutes contaminated sea by means of natural ways (1,5).

Thus, these organic chemicals, once arrived reactions limited by the physical and biological characteristics of the environment to have yielded in to new materials. Any water medium over imposed overcrowded with organic material, can be reproductive but only to a degree and can cause anaerobic biological medium which occurred due to the increase in oxygen consumptions (2,5). Not only the oxygen deficiency but also the evolution of new species tolerable to the altered medium occurs in marine life, especially being found mass production of green and red Algs. Studies on plantains shows ed. declines on numbers of fito and zooplakton species which provide the mains food especially for fish and other marine products(2) Which allows no life opportunity for the rest of living organisms, the removal of all new species from existence, and a slows down or a gall to any fish merriment's (2,5).

The Marmara, as is believed, composes of two layers of water, the upper thinner layer having much less salt concentration compared to the dancer lower one. Because of wave movement, streams and direct atmospheric contact; the upper layer can recover its

oxygen loss far a depth of 15 meters, while the waters 25-30 meter and more deeper take poor oxygen within the deficit limits of diffusion which happens between salty and unsalted surfaces of both layers(1).

The deep streams known to have existed in the system of Marmara and connecting water ways (Bosphorous and Dardanell) Also as the investigations indicated, the waste products deposited to the sea to diffuse into the water causes sea pollution (1).

Some researchers (7,10,13,20) point out that the present pollution leads to functional diversities in living organisms as well as to decreases in species and/or amount of fish, variations in the composition of fish populations and /or sudden fish deaths in masses.

Some reports (2,3) are, on heavy metal deposition in fish systems and taken by human as food, and on the effects of detergents on fish respiratory organs and the deformities in their gills cells. Pesticides, also cause as pointed out (3,4) nerve and liver diseases and decreases on reproduction and growth rates of fish.

Besides the organic pollution's, in sea, especially inorganic toxic elements (Pb, Cd, Mg, As) even in trace amounts, would take place into the structures of allges and other tiny living organisms. This inorganic toxic elements have been transferred to the fish that live on them then the human begins when they are later eaten as table food, with unhealthy life threading results. Recently the general interest to the trace elements and their unhealthy role in organisms, causing an increase in number of studies over the mussels in general (14,15,16,25). Since mussels is filtering water, they have long been considered as an indicator for the inorganic toxic elements, (heavy metals) in marine environment. Toxic elements Which trapped in mussels would show the level of pollution in the aqueous as an index of imperial data (14,16,17,25).

Although it has been known that some degrees of heavy metals concentrations are necessary, for enzyme activities, but when they exceed the their normal limits, they (Ag, Hg, Cu, Cd, Pb,) may lead, as pointed out (9) serious enzymatic inhibitions within the organisms. It is also reported that high concentrations of heavy metals whether needed for the organism are potential toxins and especially the elements Hg, Ag, Cu, Cd, Zn, Pb,Cr, Ni and Co are regarded as toxic elements (9). Although mass investigations on this matter have already been done for the Marmara sea and Bosphorus (14,15,16,25). Kut et all (16), points out that need for long termed, continuos studies on this line, Just to get certain knowledge about pollution.

Currently, what has been done at the developed countries by the scientist is to try to have evidence about pollution matter and water products as a main food resource, and try to developed some solution to subject under discussion (13).

In this study it was aimed to investigate periodic controls of water pollution in Bosphorous for two years and to determined levels of Aspartat transaminase (AST), Alanin transaminase (ALT) Gama-glutamily transpeptidase (GGT) in fish organs and of Pb,Cd, and As in mussels.

Material and Methods

The research was conducted on 360 horse mackerel and through 30 months. Within 24 months, each 15 horse mackerels and mussels, the samples were collected from the selected point (Rumeli Hisarı) of the Boshorous.

The collected fish sample was carried to the laboratory in the ice boxes. They were cut by the bistury from the median line to take the livers and kidneys. The sample of the tissues was weighted and 1g of it was homogenized with 2 ml , 0.25M serum physiologic and cut in to pieces in the ultratorax. The homogenate was santrifuge at 5000g. and it was diluted with the physiologic saline solution. Lastly, ALT, AST, GGT enzyme activities were determinated by spectrophotometrical method.

The sample of the collected mussels shells was taken off and weighted wet and then dried by freezer drier. After that process, the dried samples separated from the sheaves and weighted again. Later, they were formed the pellet. The pellets were analyzed by X-Ray Fluoresans Spectrometry (XRF).

Results

This research that is studied to demonstrated how mussels and fishes has been affected by the pollution of Bosphorous. This was divided into four periods which was consisted six months.

I: Period: September 1995 - February 1996

II:Period: March 1996 - August 1996

III:Period: September 1996 - February 1997

IV:Period: March 1997 - August 1997

Standard deviations and averages of findings were shown at table I, II and III.

Table I: Pb, Cd and As levels of mussels (ppm).

	I: Period	II: Period	III: Period	IV: Period
Pb	5.23 0.9	9.31 ± 4.4	4.20 ± 1.3	4.35 ± 1.3
Cd	DLA	DLA	DLA	DLA
As	DLA	DLA	DLA	DLA

DLA: Under the limit of detection

Table II. AST, ALT, GGT enzymes activities of livers in horse mackerel (IU/g).

Parameters	AST (IU/g)	ALT (IU/g)	GGT (IU/g)
I. Periods	6.40 ± 0.36	3.70 ± 0.22	0.29 ± 0.26
II. Periods	6.11 ± 0.79	3.75 ± 0.41	0.31 ± 0.06
III. Periods	6.75 ± 0.45	3.80 ± 0.35	0.27 ± 0.09
IV. Periods	7.10 ± 0.52	4.15 ± 0.21	0.32 ± 0.08

Table III. AST, ALT, GGT enzymes activities of renal in horse mackerel (IU/g).

Parameters	AST (IU/g)	ALT (IU/g)	GGT (IU/g)
I. Periods	6.25 ± 0.53	3.49 ± 0.30	0.31 ± 0.02
II. Periods	6.08 ± 0.61	3.47 ± 0.32	0.34 ± 0.07
III. Periods	6.60 ± 0.56	3.85 ± 0.26	0.37 ± 0.05
IV. Periods	7.21 ± 0.45	4.15 ± 0.42	0.41 ± 0.02

Discussion

The purpose of the research was determined to show how mussels and fishes has been affected by the pollution in Bosphorous. In this research, mussels were selected because of being a good indicator of inorganic elements, horse mackerels were selected because of consume widespread in Turkey.

In this study it vas investigated the effects of the most toxic elements to the human health such as Pb, Cd and As. The main findings were as follows ; As, Cd were found under the limit of detection. The Pb concentration was found over the limits of the detection.

Results from this research are; in first 6 months, average of Pb concentration was 5,23 ppm, 12 months average was 7,27 ppm, 18 months average was 6,25 ppm, 24 months average was 5,77 ppm.

However, Kıratlı (14) reached different findings. he collected mussels from European and Anatolian lines of the Bosphorous and the Marmara Sea. He was suggested while the highest Pb concentration in mussels from European lines of Bosphorous is 20.74 ppm that the mussel's, which were collected from the Anatolia line, Pb concentra-

tion was 0.90-3.16 ppm. However, the Marmara sea's European line mussel's Pb concentration was 2.62 ppm.

In different countries of the World the acceptable findings have been from 0.5 to 6 ppm(19). The mussel's, which collected from the Bosphorous, Pb concentration was received to upper level of those findings. Kut et all(16), explained their findings determined for three years (1986-1989) as fallow : The averages of 1986 Pb concentration was, 8.11 ppm, year 1987 was 6.41 ppm year 1988 was 4.81 ppm.

In different countries of the World, the acceptable Cd concentration is between 0,1 to 5.5 ppm. As referred to this research average Cd concentration was found DLA, (under the limit of detection),in other words it was under 5 ppm. Thus, those findings were included the normal limits

Kıratlı (14) reported that, Cd values of mussels from the Bosphorous European line were changed between 1.03- 1.76 ppm, while the other line's values were 1.17-3.04 ppm. However European line of the Marmara Sea's values were changed from 1.84 to 4 ppm. Kut et al's (16) research which carried out at the Bosphorous reached these results : The average of 1986 Cd concentration was 4.21 ppm, the average of 1987 was 4.51 ppm and 1988 average was 3.41 ppm.

In sum, it was found that the Cd concentration was under the 5 ppm and the value was closer to other researchers (14,15) results.

This research's findings of the As concentration are DLA. Kut et al. (16), explained average As concentration for 1986 was DLA , for 1987 was 21.6 ppm and for 1988 was 32.56 ppm . Thus, our findings showed normal limits.

During this research, in the sample of the mussel, which was taken from the same point, we have not been reached significant statistical differences in the average of 6 months, year and two years. Kut et al. (16) has found also that the concentrations of toxic elements in mussels between 1986-1988 have not significant change.

Chemicals, heavy metals, detegenates, pestisites e.g. get into natural water and may cause significant tissue damage in fish (4,10,113). The degree of increase in activity of cellular enzymes in sera depends primarily on the magniture and severity of cell damage (4,20,22). Furthermore pollutants may get into water combination with each other, causing additive harmful effects on the fish. The changes in the enzyme activities of LDH, GLDH, GOT have been used for demonstrating tissue damage in fish (3,4,20,22).

Nemcsok et.al. (20) pointed out that, adverse effects of environmental pollution have been demonstrated by the measurement plasma enzyme activities e.g. AST, ALT, AchE.

Determinations of the transaminas AST and ALT have proved useful in the diagnosis liver and kidney diseases in fish but as pointed out by researchers the activity of these enzymes in fish serum may very among species and normal levels must be evaluated for each species (22).

The tissue samples from horse mackerels one of the most popular kind of fish as food were used as the main material in the research. The average values, for AST, ALT, GGT enzyme activities to horse mackerel liver is show in table II, and to horse mackerel kidney in table III. No significant difference could be detected between early and late preparations of horse mackerel liver and kidney tissues under examination, any increase in enzyme activities having failed to give a sound idea for a duration of six months.

In spite of the ever increasing pollution found in the sea, the fact that the effects of the present pollution didn't reflect itself much in the parameters studied this might lead to an idea that while the amounts of fish have continuously decreases in number, living species, they might have adapted to the more polluted aqueous medium they have to present in.

References

1. Artüz,İ (1989): Marmara denizi ve boğazlar bölgesinde kirlenmenin tarihsel gelişimi ve bu günkü durumu. Marmara Denizi ve Boğazlarda Çevre Sorunları Sempozyumu say:49-52

2. Artüz,İ (1989): Marmara denizinde 1986-1988 Döneminde Yapılan Ekolojik Araştırma Sonuçları. Marmara Denizi ve Boğazlarda Çevre Sorunları Sempozyumu say:537

3. Astalos,B:, Nemcsok,J.(1985): Effects of Pesticides on the LDH Activity and Isoenzyme Pattern of Carp (Cyprinus carpio L.) Sera. Comp.Biochem.Physiol. Vol. 82 C-1, 217-219.

4. Astalos,B:,Nemcsok,J.Benedeczky,I., Gabriel, R.,Szabo,A.,Refaie,O.J.(1990): The effects of Pesticides on some Biochemical Parameters of Carp (Cyprinus carpio L) . Arc. Environ. Contam. Toxicol. 19,275-282.

5. Aydın,A. (1989): Organik Atıklar Nedeni İle Deniz ve Su Kirliliği. Marmara Denizi ve Boğazlarda Çevre Sorunları Sempozyumu say:37-42.

6. Başbakanlık Devlet İstatistik Enstitüsü Müdürlüğü (1987): Türkiye İstatistik Yıllığı. 208.

7. Baykurt,F., Yurdaer,G., Çalışkan,N.(1984): Marmara Denizinin Kirlenme Etüdü İstanbul Ünv. Çevre Sorunları Araştırma Merkezi - Ön Rapor.

8. Bulum, L., Mengi, A.(1993):Alabalıklarda (Salmo gairdneri) Serum Glutamik Oksalasetik Transaminaz (GOT) ve Serum Glutamik Piruvik Transaminaz (GPT) Enzim Düzeyleri Üzerine Çalışmalar.İ.Ü.Vet.Fak.Dergisi. Cilt 19. Sayı 1. Istanbul

9. Bryan,G.W. (1976): Heavy metal contaminationin the sea. In: Marıne Pollution. Academic Press İnc. London. 185-302.

10. Casillas,E., Ames,W. (1986): Hepatotoxic Effects of CCL4 on English sole (Parophyrs vetulus): Possible Indicatör of Liver Dyfunction. Comp.Biochem. Physiol. Vol.84 C.2 397-400.

11. Devlet İstatistik Enstitüsü (1991). Istanbul

12. Erentürk,N., Saygı,N., Esen,N., Başsarı,A., Seddigh,E., Topçuoğlu,S. (1989):Halkımızın En Çok Tükettiği Balıklardan Olan Horse mackerel, Kolyoz ve Lüferde Toksik Element Miktarları. III. Ulusal Nükleer Bilimler Kongresi, Bildiri Kitabı.

13. Folmar,L.C. (1993): Effects of Chemical Contaminants on Blood Chemistry of Teleost Fish: A Bibliography and Synopsis of Selected Effects. Envir. Toxicol. Chem. Vol. 12.337-375.

14. Kıratlı,N. (1997): Marmara Denizi Kıyılarınındaki Midyelerde Ağır Metal Tayini (Doktora Tezi).

15. Kıratlı,N., Yıldızdağ,H.G.(1992): Marmara Denizinde Ekonomik Su Ürünlerinde Ağır Metal (Hg,Zn) Miktarı. TÜBİTAK DEBAG-2 say:88.

16. Kut,D., Esen, N., Erentürk, N., Saygı, N., Başsarı, A., Seddigh, E., Topçuoğlu,S. (1989):İstanbul Boğazı'nda Tutulan Karides ve Midyelerde Toksik Element Seviyeleri. III. Ulusal Nükleer Bilimler Kongresi, Bildiri Kitabı.

17. Kut,D., Esen,N., Topçuoğlu,S.(1992): Boğaziçi, Marmara ve Karadeniz' de Yakalanan Deniz Organizmalarında Se (Selenyum) Seviyeleri. Istanbul

18. Morgan,R.P., Fleming,R.F., Rasing,V.J., Heinle,D.R.(1973): Sublethal Effects of Baltimor of Harbor Water on the White Perch.Chesapeake Sci. Vol.14, 1, 17-27.

19. Nauen, E.,(1983): Compılation of Legal Limits for Hazardous Substances. FAO Fisheries Circular. No: 764 Roma. 1-100.

20. Nemcsok,J.G., Hughes,G.M. (1988): The effects of Copper Sulphate on Some Biochemical Parameters of Rainbow Trout. Environ . Pollut. 49, 77-85.

21. Öztürk,S.(1990): Tarım İlaçları. Hasad Yayıncılık ve Reklamcılık. Istanbul. 174-175

22. Saandnes, K., Lie, Q., Waaagbo,R.(1988): Normal Ranges of Some Blood Chemistry Parameters in adult farmed Atlanyic Salmon, Salmo Salar. J. Fish.Biol. 32, 129-136.

23. Topçuoğlu, S., Ünlü, Y., Bulut, M., Sadıkoğlu, N.(1992): Küçükçekmece Gölünde Deterjan Kirliliği ve Toksititesi. Kimya ve Kimya Mühendisliği Sempozyumu. Istanbul.

24. Topçuoğlu, S., Kut, D., Erentürk, N., Saygi, N.(1990): Hamsi, Lüfer, Atlantik Uskumrusu ve Yunus Balıklarında Toksik Element Seviyeleri. II. Ulusal Halk Sağlığı Kongresi. 22-25 mayıs 1990. Istanbul.

25- Uzunören, N.(1987): Ağır Metallerle Kirlenmiş Sulardan Toplanan Midyeler ve Benzeri Deniz Ürünlerinde Ağır Metallerden Arsenik ve Türevleri ile Kirlenme Düzeylerinin Saptanması. (Doktora Tezi). İst.Üniv.Vet.Fak.