

Analytical, Nutritional and Clinical Methods

Determination of heavy metals (Cd, Pb) and trace elements (Cu, Zn) in sediments and fish of the Southeastern Aegean Sea (Turkey) by atomic absorption spectrometry

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Abstract

The Bay of Güllük in Southeastern Aegean Sea (Turkey) is very important by the potential of marine product in the Aegean Sea. There are various polluting elements in Güllük Bay. The current situation of the bay and impact of pollutants have become necessary. *Dicentrarchus labrax* are frequently used for human consumption. In this study, heavy metals (Pb, Cd) and trace elements (Cu, Zn) were analyzed in fish (*D. labrax*) and sediments in the Bay of Güllük by atomic absorption spectrometry. The average metal concentrations in the fish varied in the following ranges: Pb; <0.02–0.4, Cd; <0.01–0.04, Cu; <0.1, Zn; <0.5–7.2 mg kg⁻¹. In addition, seven sediment samples were analyzed and average concentrations of them were found as Zn; 80.8 ± 0.45, Cu; 25.2 ± 0.14, Pb; 20.0 ± 2, Cd; 0.560.08 mg kg⁻¹. The accuracy and precision of our results were checked by using International Certified Reference samples (fish: DORM-2, sediment: HISS-1).

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1. Introduction

It has been recognised for many years that the concentrations of metals found in coastal areas, whether they be in the dissolved or particulate phase, may be derived from a variety of anthropogenic and natural sources (Burrige et al., 1999). In most circumstances, the major part of the anthropogenic metal load in the sea and sea bed sediments and organisms has a terrestrial source from mining and intensive aquaculture and municipal wastewaters, untreated effluents, harbor activities, urban and agricultural runoff along major riv-

ers and estuaries and bays. On the other hand, the residual content of contaminants (metals and organics) in salmon diet constituents (fish), and of Cu, which is commonly used in antifouling chemicals for the treatment of net-pen cages, has drawn little attention regarding impacts to the marine environment (Burrige et al., 1999). The majority of feed/trace metal studies relate to salmon nutritional requirements (Berntssen, Kundebye, & Moage, 1999; Chou, Haya, Paon, Burrige, & Maffatt, 2002; Maage, 1994).

Much has been written on the sedimentology and geochemistry of surficial bottom deposits in the Aegean Sea (Balcı, Benli, & Kucuksezgin, 1990; Emelyanov, 1972; Ergin, Ediger, Bodur, & Okyar, 1990; Yemencioğlu, Yılmaz, Baştürk, Saydam, & Salihoğlu, 1998). However, no detailed information was available from the eastern Aegean margin, along the Turkish coasts. The objective

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of this study is to investigate the distribution and possible sources of metals in the surface sediments and fish of Güllük Bay in the Southeastern Aegean Sea (Fig. 1). Güllük Bay is potentially important area in terms of marine product within the Aegean Sea. Various materials in the Bay of Güllük are source of pollution. These can be summarized as; loads originating from domestic waste water, pollution caused by tourism activities, loads brought by the Sarıçay Stream, loads originating aquaculture activities, pollutant loads originating from Güllük Port activities and mining transferred from Güllük Port to the open sea. There is no industry in the area surrounding the Bay of Güllük and only a few small settlements such as Torba, Güvercinlik, Güllük. Their total population is less than 10,000 in winter. But there are intensive tourist activities in summer. Their total population is more than 100,000 in summer (Demirak, Balcı, Demirhan, & Tüfekçi, 2001).

The main aim of this work was to determine the total contents of Cd, Cu, Pb and Zn in fish and sediment collected from various sampling points of the Güllük Bay since this fish is an important component of the human diet in this zone. Also, concentrations values of some metals in sediment show pollution situation of area. So this information is very significant from the pollution of Güllük Bay. For this purpose the samples were dissolved using a microwave digestion method, proposed in this work, and the determination of metals was carried out by atomic absorption spectrometry (AAS) using flame (for Zn and Cu) and graphite furnace (for Cd and Pb) as atomization systems.

2. Materials and methods

2.1. Apparatus

A GBC 9000 model flame atomic absorption spectrophotometer (FAAS) was used for the determination of copper and zinc. The maximum absorbance was obtained by adjusting the cathode lamps at the operation conditions shown in Table 1. A GF 3000 model graphite furnace atomic absorption spectrophotometer (GFAAS) was used for the determination of lead and cadmium. In GFAAS, all measurements were based on integrated absorbance whereas in AAS based on peak heights, both performed at 228.8 nm for Cd and 283.3 nm for Pb by using electrodeless discharge lamps system. The furnace program for determination of Cd and Pb by GFAAS are given in Table 2 with the instrumental setting. Argon 99.96% (v/v) was used as protective gas throughout in GFAAS.

2.2. Reagents

All reagents used were of analytical reagent grade (Merck, Germany). Acid washed glassware analytical grade reagents and double distilled deionized water was used throughout the experiments. Standard stock solutions of copper, cadmium, lead, and zinc were prepared from Titrasol (1000 mg l^{-1}). The working solution were freshly prepared by diluting an appropriate aliquot of the stock solutions. In order to check on the purity of the chemical used, a number of chemical blanks were

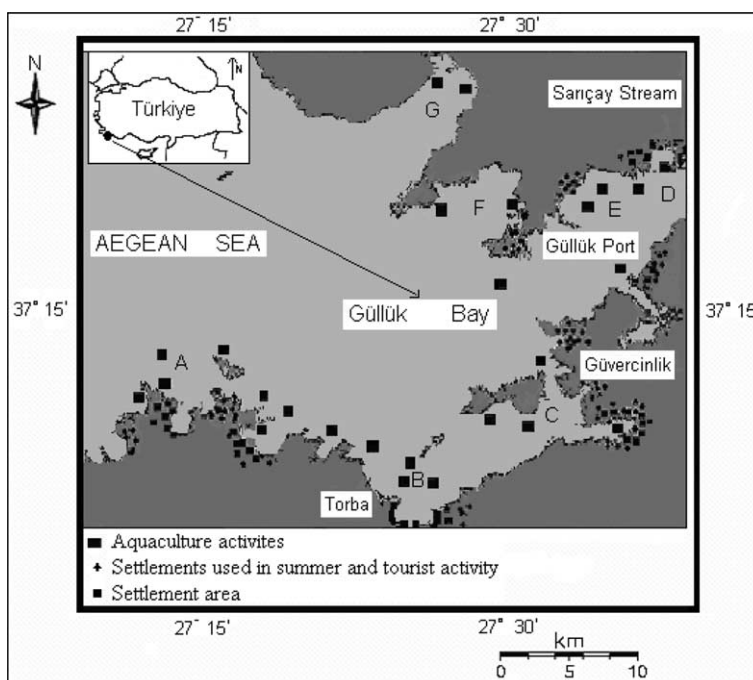


Fig. 1. Map of Güllük Bay and sampling sites.

Table 1
Flame atomic absorption spectrophotometer operating conditions

	Cu	Zn
Wavelength (nm)	324.7	213.9
Slit width (nm)	0.2	0.5
Sensitivity (µg/ml)	0.025	0.008
Flame type	Air–acetylene	Air–acetylene
Lamp current (mA)	3.0	5.0

Table 2
Heating programs for Cd and Pb in graphite furnace atomic absorption spectrophotometer

Step	Temperature (°C)	Ramp (s)	Hold (s)	Argon flow rate (ml min ⁻¹)
1	150	5	20	250
2	200	5	15	250
3	500 ^a , 800 ^b	10	20	250
4	1800 ^a , 2000 ^b	0	5	0
5	2200	1	3	250

^a Cadmium.

^b Lead.

run. There was no evidence of any contamination in these blanks.

The quality of data was checked by the analysis of standard reference material (fish: DORM-2, National Research Council, Canada; sediment: HISS-1, National Research Council, Canada).

2.3. Sample collection preparation and digestion

In this study, seven sediment sampling sites for sediment samples were selected along the shore in districts receiving different sampling station (Fig. 1). Coordinates were determined by Global Positioning System (GPS). For each site samples, 3 surface sediments samples were collected and analyzed for their heavy metals (Pb, Cd) and trace elements (Cu, Zn) contents. Fish samples were collected from cages at 6 aquaculture farms and analyzed their heavy metals (Pb, Cd) and trace elements (Cu, Zn) contents.

The surface sediment samples were collected from the top 5 cm layer of bottom sediments using a Van Veen type grab sampler during 2001–2002. Four composite samples (3 grabs per sample) were collected from each site in the Bay of Güllük in August and September 2001, and March and June 2002. After collection, sediment samples were air dried for 4 days until to constant weight. Dried aliquots were ground using a mortar and pestle and sieved through 0.5 mm screen.

Immediately after collection, fish (*Dicentrarchus labrax*) samples were stored on ice in an insulated box and transferred to the laboratory and then were frozen at –21 °C until required for metal analysis. The samples

were pooled and freeze-dried for 10 days to a constant weigh for the determination of metal content, 0.5 g dry sediment samples and muscle samples of the fish.

The procedure used was that described for digestion of samples in the CEM Digestion Applications Manuel (Anon, 1994). A sample of 0.5 g was placed in a Teflon vessel (100 ml capacity) with water (10 ml), HNO₃ (5 ml), HF (4 ml) and HCl (1 ml). It was then subjected to treatment for 30 min, in a CEM Digestion System Model MDS 2000 (CEM Corporation Matthews, NC). Since power input, maximum 630 W, was interrupted when the pressure within the control vessel reached 0.828 Mpa, and since pressure generation within vessels was influenced largely by the organic matter content of samples, it was important on the grounds of safety to place the most labile sample in the control vessel. To facilitate this, samples were attended to in order of decreasing organic matter. Digests were treated with crystalline H₃BO₃ (2 g) to neutralize excess HF, transferred to polythene containers and made up to a weight of 53.95 g.

3. Results and discussion

3.1. Sediments

The heavy metal (Pb, Cd) and trace element (Cu, Zn) concentrations in sediments are shown in Table 3. The somewhat high Zn, Cd and Cu level in sediments from D and E sampling sites can be explained by the located Güllük Port and a lot of aquaculture farms. On the other hand, D and E sampling sites are close to mouth of Saray Stream. Most human-related metals (Cd, Cu, Zn) showed lowest in sediments from A sampling sites.

The Pb contents of the Bay of Güllük sediments are lower than the literature data (Table 4). It is the most likely that the concentration of Pb in Güllük Bay sediments related geochemical variables. The distribution of Zn and Cu are rather variable but comparable with that in adjacent marine regions. The results of Zn and Cu analyses done on the sediment at seven sampling sites on the Güllük Bay showed average Zn 80.8 ± 0.45 mg kg⁻¹ and Cu 25.2 ± 0.14 mg kg⁻¹. These levels are higher than those measured in sediment in the Gulf of Saros and the Northern Marmara shelf. But these levels are lower than in sediment from Erdek Bay (Northern Aegean Sea in Turkey) and Black Sea southern shelf.

3.2. Organisms

Table 5 shows the data for metal concentrations found in the fish (*D. labrax*) samples. Levels of Pb, Cd, Cu and Zn in the muscle of fish (*D. labrax*) were generally low in the Güllük Bay.

Table 3

Heavy metal and trace element concentrations (mg kg⁻¹, dry wt) in sediment samples (n = 3)

Stations	Pb ^a	Zn ^a	Cu ^a	Cd ^a
A	19.2 ± 2	30.5 ± 0.45	27.6 ± 0.14	ND ^b
B	19.2 ± 2	34.1 ± 0.45	26.6 ± 0.14	ND ^b
C	20.2 ± 2	34.1 ± 0.45	20.0 ± 0.14	0.53 ± 0.08
D	21.1 ± 2	158.9 ± 0.45	28.0 ± 0.14	0.69 ± 0.08
E	21.1 ± 2	110.1 ± 0.45	30.0 ± 0.14	0.65 ± 0.08
F	19.2 ± 2	96.4 ± 0.45	20.0 ± 0.14	0.47 ± 0.08
G	20.1 ± 2	101.8 ± 0.45	24.3 ± 0.14	0.45 ± 0.08
Average	20.0 ± 2	80.8 ± 0.45	25.2 ± 0.14	0.56 ± 0.08

^a All results are given as mean value standard deviation of three determinations.^b Below detection limit.

Table 4

Comparison of metal levels (mg kg⁻¹) in this study with the literature data for surface sediments

Regions	Ref.	Pb	Zn	Cu	Cd
Bay of Güllük	a	20	81	25	0.56
Gulf of Saros	b	22	73	19	–
Northern Marmara	c	24	71	21	–
Erdek Bay	d	40	125	28	–
Black Sea	e	34	87	49	–
İzmir Bay	f	23–52	45–114	13–49	–
Eastern Aegean Sea	g	28–55	25–72	8–28	0.14–0.35

^a (This study).^b Sarı and Çağatay (2002).^c Çağatay et al. (1996).^d Balkis (1998).^e Yücesoy (1991).^f Balci and Türkoğlu (1993).^g Balci and Küçük (1994).

According to the UK Food Standards Committee Report and Institute of Turkish Standards (ITS) tolerance level in fish (muscle) in Turkey, Zn levels in food and fish (muscle) should not exceed 50 mg kg⁻¹ (Cronin et al., 1998; ITS, 2000). These limits for Zn were not exceeded in the muscle of any of the fish analysed in this study. The highest concentrations found for these elements in muscle were approximately seven times lower than the legislated level for Zn. Zinc ranged between undetectable to 7.2 mg kg⁻¹ in the study area. We can therefore conclude that this metal present no problem for the consumption of muscle of this fish.

The correlation coefficient between the concentrations of Zn in sediments and the concentrations of Zn in the muscle of fish (*D. labrax*) is very low ($R^2 = 0.097$) at the Güllük Bay. These levels can be showed that concentrations of Zn in sediments are slightly related fish farming.

It should be emphasized that Pb and Cd are accumulated in human tissues and hence they are harmful to human health (Van Oostdam et al., 1999). It is known that most human exposure to Pb is from food. The maximum levels of muscle Pb and Cd in this study are signif-

icantly lower than the PTWIs (permissible tolerable weekly intakes) for these elements (Table 5) (FAO/WHO, 1989; WHO, 1993) and do not constitute any threat for fish consumers. Turkey legislation establishes maximum levels for Pb and Cd of the metals studied, above which human consumption is not permitted: 0.1 mg kg⁻¹ for Cd, 5 mg kg⁻¹ for Cu and 0.5 mg kg⁻¹ for Pb (all expressed in wet mass) (ITS, 2000). These limits for Pb, Cd and Cu were not exceeded in the muscle of any of the fish analyzed in this study.

The concentrations of these metals in muscle studied were lower than the maximum levels set by law (Table 5) and, therefore, the muscle of all the fish analyzed was fit for human consumption in Turkey. There was no correlation between the lead and cadmium and Copper content in the sediments and in the muscle of the fish (*D. labrax*). We can conclude that metal concentrations in the muscle of some fish (*D. labrax*) cannot be useful as bioindicators of the degree of pollution in marine ecosystems.

The heavy metal concentrations in several fish species from Black Sea and Aegean Sea were determined (Güner, Dinçer, Alemdag, Çolak, & Tüfekçi, 1998;

Table 5
Heavy metal and trace element concentrations (mg kg⁻¹, dry wt) in the muscle of fish samples (*Dicentrarchus labrax*) (n = 3)

Regions	Ref.	Samples	No. of fish	Pb	Cd	Cu	Zn
ITS tolerance level in fish in Turkey							
Güllük Bay	1	All fish	24	0.5	0.1	5	50
Black Sea	2	<i>D. labrax</i>		<0.02–0.4 ^a	<0.01–0.04 ^a	<0.1 ^a	<0.5–7.2 ^a
Black Sea	3	<i>Whiting</i>		<0.05	<0.02	4.54	30.2
Black Sea	4	<i>Anchovy</i>		<0.05	0.1	2.21	35.7
Black Sea	4	<i>Whiting</i>		0.088 ± 0.009	0.0131 ± 0.001	1.3 ± 0.1	3.3 ± 0.2
Aegean Sea	5	<i>M barbatus</i>	6	0.008 wet wt	0.00044 wet wt	–	–
Aegean Sea	6	<i>M barbatus</i>	10	0.012 wet wt	0.00093 wet wt	–	–
Aegean Sea	7	<i>M barbatus</i>	10	0.150 wet wt	0.00091 wet wt	–	–

¹ This study.

^{2,3} Topcuoglu et al. (2002).

⁴ Güner et al. (1998).

^{5–7} Küçüksezgin et al. (2002).

^a All results are given as mean value three determinations.

Table 6
Concentrations of metals found in Standard Reference Material DORM-2 and HISS-1 from the National Research Council, Canada (all data as means ± standard errors, in mg kg⁻¹ dry wt) by FAAS and GFAAS (n = 3)

	Certified values (mg kg ⁻¹)	Measured values ^a	Recovery (%)
<i>DORM-2 (fish)</i>			
Cd	0.043 ± 0.008	0.044 ± 0.009	102
Pb	0.065 ± 0.007	0.068 ± 0.008	105
Cu	2.34 ± 0.16	2.27 ± 0.17	97
Zn	25.6 ± 2.3	24.7 ± 2.4	96
<i>HISS-1 (Sediment)</i>			
Cd	0.024 ± 0.009	0.022 ± 0.010	92
Pb	3.13 ± 0.40	3.13 ± 0.42	100
Cu	2.29 ± 0.37	2.27 ± 0.39	99
Zn	4.94 ± 0.79	4.99 ± 0.75	101

^a Mean of three parallel determinations ± standard deviation of measurements.

Küçüksezgin, Uluturhan, Kontas, & Altay, 2002; Topcuoglu, Kurbaşoglu, & Güngör, 2002). No similar published data in scientific literature. For this reason, it is impossible to write any conclusion about metal levels in the marine fishes. At the same time, the comparison of heavy metal and trace element concentrations in fish with other studies carried out in different marine environments are also difficult because of differences in fish species, types of tissue analyzed, methodologies and other factors.

3.3. Quality assurance

The accuracy and precision of our results were checked by analyzing standard reference material (fish: DORM-2, National Research Council, Canada; sediment: HISS-1, National Research Council, Canada). Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 96% and 105% for fish and 92% and 101% for sediment (Table 6). All metal concentrations were quoted as mg kg⁻¹ dry weight.

4. Conclusion

The somewhat high concentrations of Cu, Cd and Zn in sediments at the D and E sampling sites can be thought to have resulted from anthropogenic influence. But Aquaculture operating sites are not valid indicators for sediment metal components in Güllük Bay.

The results of this study supply valuable information about the metal contents in fish from different sampling stations of the Gulluk Bay. This can be considered as a bioindicator of the environmental contamination in this zone by estimating the bioavailability of metals to the marine biota. Moreover, these results can also be used to test the chemical quality of the marine food, in order to evaluate the possible risk associated with their consumption by humans. In relation to this, the low metal contents found in all the studied fish samples are insufficient to cause toxicological effects on human health when these fish are included in the diet.

On the other hand, Güllük Bay is hardly polluted but requires regular monitoring of fishes, particularly near the new fish farm settlement. The metal concentrations

found in this study were lower than those found in polluted areas of Aegean and Black sea.

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