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Cyanide determination in the Black Sea and Istanbul Strait seawater, fish and mussel

Karadeniz ve Istanbul Boğazı denizsuyu, balık ve midyelerinde siyanür tayini

Kasım Cemal Güven, Tuncay Gezgin, Selma Ünlü, Erdoğan Okuş, Ayhan Uysal and Ertuğrul Doğan

Institute of Marine Sciences and Management, University of Istanbul
34470, Vefa, Istanbul, Turkey

Abstract

In this work the cyanide contamination was investigated in seawater, fish and mussel in Turkish coasts following the cyanide accident in Baia gold mines in Feb. 2000. The determination of cyanide was conducted according to Csikai and Barnard (1983) method slightly modified by us. Seawater sample was analysed after lyophilization or after addition of NaOH directly. Fish and mussel samples were extracted with water then analysed. The cyanide amount varied 0.13-3.01 µg/L in the Black Sea (K1,K2,K3), 0.11-0.34-µg/L in İğneada, 0.25-1.56µg/L in Kilyos and Rumeli Feneri and 0.09-1.74 µg/L in Istanbul Strait. The highest levels of cyanide found in seawater of Turkish coasts is lower than the specified regulation limit of 5 µg/L.
The cyanide was found in talbot 0.0052µg/g collected from Rumanian and 0.032µg/g from İğneada coasts, 0.0093µg/g in gray mullet, 0.017µg/g in surmullet, 0.0072 µg/g in small blue fish , 0.00µg/g in silversid and 0.013 µg/g in flesh and 0.054µg/g in gut in common braem (Aegean Sea), in mussel. 0.028 µg/g collected from Garipçe, 0.056µg/g in Rumeli Feneri.
The amounts of cyanide in fish and mussel are considered negligeable to be toxic for human consumption.
This is first record on cyanide analysis in Turkish coasts.

Keywords: Cyanide,the Black Sea,Istanbul Strait, seawater, fish, mussel

Introduction

An accident occured in Feb. 2000 at the gold mines of Baia, Rumania, when cyanide solution of approx. 22 mil. gals ran off to Tissa river, thereby to Danube. Large quantities of dead fish were observed in the river. This accident caused great concern in the Black Sea countries. After the accident, a report by the 'Coordination of the Regional Environment and Water Inspectorate, Ministry of Environment and Water of Bulgaria, gave the results for cyanide content on the Black Sea coasts of Bulgaria in 28 and 29 Feb. 2000 and 1–5 March 2000 (Anonymous a). Cyanide is used in metallurgical extraction of gold and in various electroplating operations. The industries use cyanides and they are a source of cyanide contamination in aquatic systems. Municipal seawage systems discharge generally with or without treatment to river or seawater. For elimination of cyanide the proposed methods for pre-treatment are alkaline chlorination, electrolytic oxidation, ion-exchange, ozonation and radiation. A potential source of pollution of the seawater is the dumping of cyanide content by waste tankers (Duursma and Marchand, 1974).

Sodium cyanide is being used on reefs in the Asia–Pasific region to capture live fish for the aquarium industry. Considerable progress has been made in combating cyanide fishing in the Philippines by training fishers in alternative harvesting methods (Jones *et.al.,* 1999). Toxicity of cyanides to Australian prawn, Penaeus monodon, which is widely distributed in Australian waters was studied. NaCN and cyanide complex $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ found in wastes of blast–furnace operations and their toxicity were compared, the NaCN alone showed greater mortalities of P. monodon. The toxicity of the solution of iron–cyanide complexes, toxicity is mainly due to the content of free cyanide ($HCN + CN^-$) (Pablo *et al.*, 1997).

Sodium / potassium cyanide is a extremely poisonous. The lethal dose for a man is about 0.1g. Acute toxicity of cyanides is summarized by Casarett and Doull (1986).

The permissible limit of cyanide is maximum 5 µg/L in seawater (ANZECSS 1992).

Various methods are utilized to determine cyanide in seawater and fish. Cyanide may occur as cyanide ions or cyanide complexes with metals.

Cyanide is not stable and degrades in waste water treatment and hydrolysis product is HCN, which is very toxic. The cyanide must be decomposed before analysis. Distillation technique might be sufficient for such a decomposition in acid medium.

The cyanide was analysed directly in the samples or following a pre-treatment as spectrophotometric (Fullana–Barcelo *et al.*, 1995; Ma and Lui, 1983; Do- Nascimento and Schwedt 1993; Ohno 1989, Li, 1987; Csikai and Barnardt 1983), by AAS (Baliza and Soledae, 1982).Some problems arise in the determination of cyanide in seawater .The sample should be preserved at pH 12 and must be kept at low temperature and away of sunlight. Sulfide content interfere with cyanide determination. Nitril and isonitriles interfere with cyanide determination (Rubio *et al.*, 1990). The spectrophotometric method principally consists of colour formed after addition of reagent in pre-treated.The reaction stages applied in this work are :

$NaCN+H_2SO_4$----- $HCN+ Na_2SO_4$
HCN+NaOH----------NaCN
NaCN+Phosphate buffer pH 3.1---HCN
HCN+Chloramine T----CNCl
CNCl+Pyridine/Barbituric acid -------- Polymethine dye

In this paper the findings of cyanide content are reported in seawater, fish and mussel of the adjacent Turkish coasts in March - July 2000 following the Baia accident.

Material

Sampling stations (Fig 1) and dates are indicated below:
Seawater :
The Black Sea ;
Iğneada (İ1, İ2, İ3)(100m away from the coast and sampled with an interval of about 500m on the coastal current) 7 March 2000
Kilyos (KT1, KT2, KT3) 6 March 2000
Rumeli Feneri (RF1, RF2, RF3) 6 March 2000
(K2) 9 March 2000
(K1, K2,K3) 5 July 2000 and 9 Aug 2000
Istanbul Strait
Seawater :
Poyraz-Garipce (K0) 6 March 2000
Garipçe (G1, G2) 6 March 2000

Çalı Burnu-Filburnu (F1,F2,F3,F4,F5, F6, F7,F8, F9, F10) 6 March 2000
Fish :
The Black Sea
Iğneada (İ1, İ2, İ3) 7 March 2000 (Talbot, Gray mullet)
Istanbul Strait
Sarıyer (S) 3 April 2000 (Surmullet, small blue fish, silverside, common braem (Aegean Sea))
Mussel (*Mytilus galloprovincialis*)
The Black Sea
Iğneada (İ1, İ2, İ3) 7 March 2000
Istanbul Strait
Rumeli Feneri (RF1) 6 March 2000
Talbot and gray mullet were brought directly from a fisherman soon after fishing in Iğneada and surmullet, small bluefish, common braem, silverside from the fishmarket in Sarıyer and mussel from Rumeli feneri and Garipçe.All the samples were stored in a freezer (-15°C) until transported ice to the laboratory and stored –22 °C before analyses.

Apparatus: Flask round bottom three necks with conical ground glass joints connected refrigerator and tubes ground glass joint.
Air conditioner (Aerostyle, Evans Electroselenium, London).
Reagents
Cadmium nitrate tetrahydrate (Merck), 3% solution in distilled water .
Phosphate buffer (pH 3.1); 138 g of $NaH_2PO_4.H_2O$ in 900 ml distilled water, adding 70 ml acetic acid (Baker) and diluting with distilled water to 1000 ml.
Barbituric acid reagent: 3g Barbituric acid (Merck) were wetted with distilled water and add 15 ml pyridine the volum was adjusted to 50 ml.
Potassium cyanide (Merck): Standard solution; 100 μg/ml in distilled water, diluted 0.01-15 μg/ml
Sodium hydroxide (Merck): 0.2M solution
Chloramine T (Merck): Solution 1%.in distilled water
Pyridine (Merck)

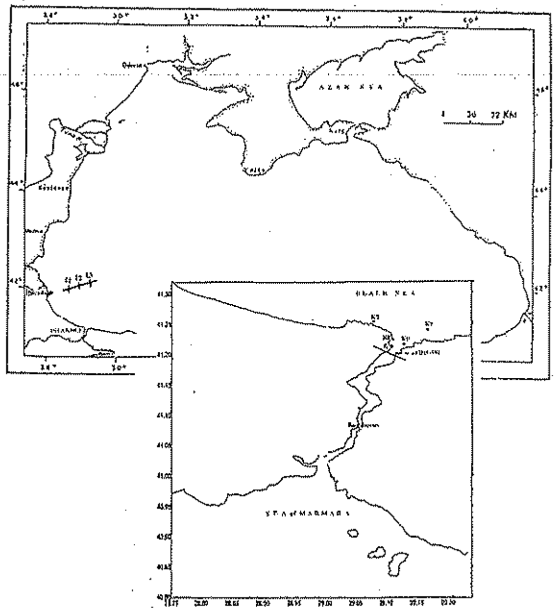


Figure 1. Sampling stations

Methods

The methods of Csikai and Barnard (1983) modified by us was used for the spectrophotometric determination of cyanide in seawater, fish and mussel.After the sampling 5 ml of 5%NaOH solution was added to 1L seawater for preservation of cyanide ions (except the samples to be lyophilized).

Calibration curves of potassium cyanide was plotted in two manner:

0.01–0.15 μg/ml of Potassium cyanide solution were added to :

a) distilled water
b) b) 18‰ NaCl,

add 50 ml 0.2 M sodium hydroxide, 15 ml phosphate buffer (pH 3.1) and 2 ml of chloramine T solution and mix. 1–2 min.

Add 5 ml pyridine-barbituric acid reagent, the volume was adjusted to 100 ml and mixed throughly. After at least 8 min, the absorbance was read at 580 nm against blank as 0.2M NaOH.

1-Determination of cyanide in seawater

1.1-1L seawater sample was taken from the Black Sea, İğneada,Kilyos and Rumeli Feneri, İstanbul Strait; Çalıburnu-Filburnu, and Garipçe, added 5 ml 5% NaOH solution. 50 ml of this sample was transferred into a flask, round bottom three necks with conical ground glass. Sulfuric acid was added from one of the necks by a separatory funnel. The air flow was given from the other neck and third neck of the flask is attached to refrigerator connected to the tubes containing a)cadmium nitrate, and b) 0.2M NaOH.The flask is heated. The liberated HCN gas from cyanide ions first passed through a tube containing cadmium nitrate solution, then connected to a second tube (the absorber tube) containing 0.2 M sodium hydroxide.This distillation continues for 20 min, then the absorber tube content was transferred to 100 ml volumetric flask and continued as described in calibration curve plotting technique.The determination by using the curve plotted with 18‰ NaCl solution.

1.2-1L seawater sample was lyophilized. The residue was dissolved in 50 ml distilled water then transferred into the flask round bottom three necks and continue the method as described above.The cyanide amount was determined by using the curve plotted with distilled water.

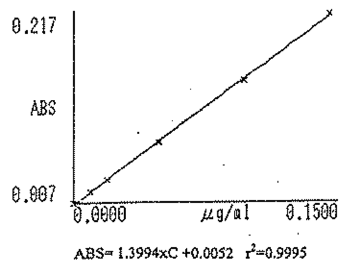
2-Determination of cyanide in fish and mussel samples

2.1- 150 g fish flesh and 50 g gut sample were mixed with 300ml distilled water for ½ h then filtered. The filtrate was mixed with 25 ml DCM to remove oil part. The water phase was centrifuged and lower layer taken and filtered then analysed as mentioned above. The determination was made by using the curve plotted with distilled water .

2.2- 50 g mussel samples were grounded then mixed in 50ml distilled water for ½ h. The filtrate was mixed with 25 ml DCM to remove oil part and the water phase analysed as mentioned above.

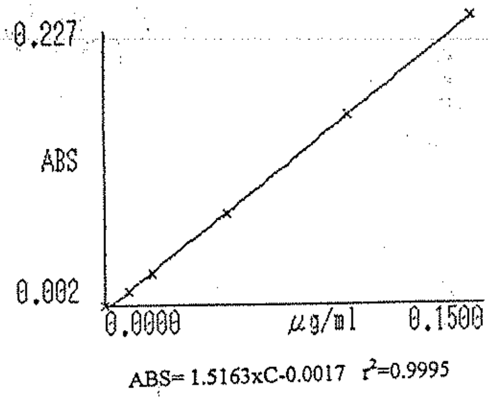
Results

The standard curves of potassium cyanide in distilled water and 18‰ NaCl are shown in Fig. 2-3 respectively. The calibration graph was linear from 1–150 μg/ml.



ABS=1.3994xC+0.0052 r^2:0.9995

Figure 2. Standard curve of potasium cyanide in distilled water.



$ABS=0.4406xC+0.0013$ r^2:0.9961

Figure 3. Standard curve of potassium cyanide in 18‰ NaCl solution.

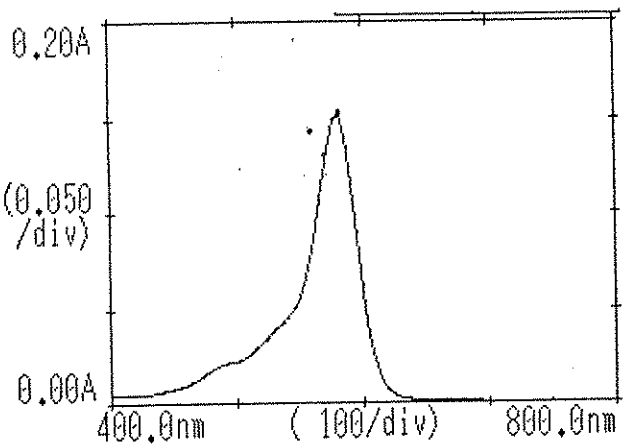


Fig.4. Absorbance curve for cyanogen chloride- pyridine -barbiturate

The absorbance curve for the pyridine-barbiturate system with cyanide as cyanogen chloride is shown in Fig. 5. It was concordant given by Csikai and Barnard (1983).
The results of cyanide in seawater, fish and mussel are given in Table 1-6. Cyanide levels were expressed as µg/L in seawater and as .µg/g in fish or mussel.

1-Seawater

Table 1. Potassium cyanide level in İğneada, the Black Sea, according to the standard curve plotted with 18‰ NaCl (µg/L).

Sampling stations	1	2
İ1	0.34	3.06
İ2	2.04	4.38
İ3	1.11	-

1)Seawater sample, 2) 3µgKCN added to seawater sample

Table 2. Potassium cyanide level in Kilyos and Rumeli Feneri stations according to the standard curve plotted with 18‰ NaCl (µg/L).

Sampling stations	1	2
KT1	1.56	4.19
KT2	0.81	4.56
KT3	0.59	3.59
RF1	0.50	3.60
RF2	0.29	3.22
RF3	0.25	3.17

1)Seawater sample, 2)3µgKCN added to seawater sample

Table 3. Potassium cyanide level according to the standard curve plotted with 18‰ NaCl in the Black seawater (µg/L).

Sampling stations		1	2
K1	(9/8/2000)	0.13	-
	(6/3/2000)	3.01	5.87
K2	(14/6/2000)	1:14	-
	(9/8/2000)	0.38	-
K3	(9/8/2000)	0.21	-

1)Seawater sample, 2) 3µgKCN added to seawater sample

Csikai and Bernard method for the analysis of cyanide in waste water was applied to the seawater our with slight modification. In evaluating the procedure which was used, potassium cyanide was added to seawater samples (Table 1-6). The recovery of cyanide was complete.

Tablo 4 Potassium cyanide level according to standard curve plotted with 18‰ NaCl in Istanbul Strait (µg/L).

Sampling stations		1	2
K0	(6/3/2000)	1.25	3.36
	(5/7/2000)	1.91	-

1)Seawater sample, 2)3µgKCN added to seawater sample

Table 5. Potassium cyanide level according to standard curve plotted with 18‰ NaCl in Garipçe stations (µg/L).

Sampling stations (6/3/2000)	1	2
G1	0.34	3.17
G2	0.59	3.72

1)Seawater sample, 2) 3µgKCN added to seawater sample

Table 6. Potassium cyanide level according to standard curve plotted with 18‰ NaCl Çalı burnu- Fil burnu stations (µg/L).

Sampling stations (6/3/2000)	1	2
FB1	1.47	4.22
FB2	1.31	4.35
FB3	0.99	4.08
FB4	0.09	3.20
FB5	1.39	3.49
FB6	1.59	4.51
FB7	1.74	4.06
FB8	1.47	4.56
FB9	1.29	4.38
FB10	1.96	4.81

1)Seawater sample, 2) 3µgKCN added to seawater sample

It was assayed cyanide in 18 and 22‰ NaCl and also 18‰ NaCL, containing ,NaOH and Cd $(NO_3)_2$.

We found that NaCl concentrations in seawater interferes with the spectrophotometric procedure as indicated by Ohno (1988). It was found that cyanide amount must be determined by curve plotted through NaCl content of the sea area under investigation .

2-Fish and mussel

The cyanide conternt of fish and mussel are listed in Table 7 and 8.

2.1:Fish

Table 7. Cyanide content in fish .

Samples	**Flesh (μg/g)**
Talbot	0.0052 (from Rumannian) 0.032 (from Iğneada) -
Gray mullet	0.0093
Surmullet	0.017
Small blue fish	0.0072
Silversid	0
Common bream	0.013 (in flesh) 0.054 (in gut)

2.2:Mussel

Table 8. Cyanide content of mussel.

Mytilus galloprovincialis	**(μg/g)**
Garipçe	0.028
Rumeli Feneri	0.11

Our findings varied in seawater 0.34-3.01 μg/L in the Black Sea and 0.09-1.47μg/L in the Bosphorus. In the Bulgarian coasts cyanide content was found, on the region of cape Shabla 400m away from the coast <2 μg/L and 1 km. inwards Dobroudzha camping site <2 μg/L, near Balchik the port, < 2 μg/L in Albena resort bridge in northern part of the resort <2 μg/L in 1–2 March 2000. At the other examined stations cyanide was not detected.According to their report, the standard for cyanide in the

Black sea was 50µg/L (Anonymous a).The values for cyanide content in the Black Sea water in Bulgaria varied 0.000 and <0.002mg/L.They are too low when compared with the declared value of 50 µg/L standard for cyanide content in the Black Sea (Anonymous a). This standard is highest than ANZECSS limit. Our max. values found in all examined seawater as 3.01µg/L which is lower than the cyanide limit of ANZECSS(1992). The cyanide content in fish and mussel is considerably very low. The person must be consumed many kg of fish and mussel for toxique effect.

Cyanide content in Turkish coast of the Black Sea, Istanbul Strait and Sea of Marmara has not been recorded before this report. It is thought hereby that a regular investigation in the Black Sea for cyanide content must be carried out.

Özet

Bu çalışmada Romanya da Baia altın madeninde meydana gelen kaza sonucu Tissa nehrine ve oradan da Tuna nehrine akan 22 milyon galon potasyum siyanür çözeltisinin meydana getirdiği kirliliğin, Karadeniz ve Istanbul Boğazı deniz suyu ile balık ve midyeleri üzerine etkisi araştırılmıştır. Deniz suyunun, direkt olarak analizi NaOH ilavesinden sonra veya liyofilizasyon sonucu bakiye üzerinde yapılmıştır. Balık ve midyeler ise su ile ekstraksiyonu takiben analize tabi tutulmuştur. Tespit edilen siyanür miktarı Karadeniz istasyonlarından K2 ve K3 de 0.13-3.01 µg/L, İğneada da 0.11-0.34 µg/L, Kilyos Rumeli fenerinde 0.25-1.56 µg/L, Istanbul Boğazında 0.09-1.79 µg/L bulunmuştur. Bu miktarlar ANZECSS' in deniz suyu için verdiği limit değerin (5 µg/L) altında bulunmuştur.

Balıklar üzerinde yapılan çalışmalarda Kalkan balığında siyanür içeriğinin Romanya örneğinde 0.0052 µg/g, İğneada örneğinde 0.032 µg/g, Kefal balığında 0.0093µg/g , Tekir de ise 0.0017 µg/g, Çinekop ta 0.0072 µg/g, Gümüş balığında 0.00 µg/g ve Çipurada yenen kısımda 0.013 µg/g ve sindirim sisteminde 0.054 µg/g bulunmuştur. Bu miktarlar çok düşük olup insan sağlığı için tehlike oluşturmayacak orandadır. Midyede Garipçe örneğinde 0.028 µg/g, Rumeli Feneri örneğinde 0.056 µg/g bulunmuştur. Bu deniz canlılarındaki siyanür miktarı insan sağlığı için bir tehlike teşkil etmez.

Bu çalışma Türkiye sahillerinde siyanür ile ilgili ilk çalışmadır.

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