

Chemical Analysis

INVESTIGATIONS ON THE POLLUTION OF ISTANBUL STRAIT  
(BOSPHORUS) BY M/S RAB UNION-18 SUNK WITH LIVE SHEEP

CANLI KOYUN YÜKÜ İLE BATAN RAB UNION-18 GEMİSİNİN ISTANBUL  
BOĞAZINI KİRLETMESİNE AİT ARAŞTIRMA

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Hypoxia

**Abstract**

M/S Rab Union-18 that contained twenty thousand live sheep sunk in Istanbul Strait (Bosphorus) on 14 Nov. 1991. To determine the pollution following this accident, sea water samples were collected from different regions at 20 m depths in the vicinity. The samples were Lyophilised and then subjected to chemical and biotoxicity-analysis.

In thin-layer chromatographic analysis, the deterioration products were detected by p-dimethylaminobenzaldehyde and ninhydrine reagents.

Biotoxicity was investigated by *Artemia salina* Leach Bioethality Assay. The samples in which protein degradation was detected did not demonstrate any sign of biotoxicity.

Due to hypoxia some marine organisms such as; *Phycis* sp, *Gobius* spp. *Mullus surmuletus*, *M. barbatus*, *Rapana venosa*, *Mytilus galloprovincialis* ve *Crangon crangon* were totally

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dead. Furthermore dissolved O<sub>2</sub> level was measured 2 mg/L and water transparency value was found 0.5 meter.

## Introduction

The Istanbul strait is an important waterway between Europe and Asia. This strait through which approx. 40000 vessels a year pass in transit are getting remarkably polluted by marine traffic. The total tonnage of vessels passing is 227 million GRT a year. The total cargo transported annually is 42 million tons of which more than 16 million tons are crude oil. Under the Montreux Treaty, commercial vessels, whatever flag, have freedom of passage day and night regardless of their cargo. On the other hand, there is no land based control system for the maritime traffic through the straits and the Marmara Sea thus creating great risk in case of heavy traffic, poor sight, strong waves and vessel failures.

The huge dimensions of marine vessels and subsequent increase in the amount of their cargo have introduced new approaches to avert pollution risk due to sea accidents. The sea, the air and the living species are under great threat because of sea accidents involving large vessels, particularly tankers.

The most unfortunate example was the collision and sinking of a Lebanon flag vessel "Rab Union-18" carrying alive sheep, near Fatih Bridge in 1991 (Fig.1). This vessel sank with 20.000 sheep and its own oil at 32 meters depth and has not yet hauled up onto shore.

Many accidents mostly involving petrol tankers have happened in Istanbul Strait up to date. However, the sinking of M/S Rab Union-18, after its collision with Madonna Lily, involving so many sheep has created great anxiety in public opinion. It was claimed that decay of the bodies of the animals would cause a high degree of pollution in this important water-way.

In the meantime a paper was published on this subject which involved only theoretical calculations but not any experimental studies (Karpuzcu *et al.*, 1992).

This paper presents the results of the chemical and biological analysis of the water samples collected in the vicinity of the sunken ship from 30 jan. 1992 until 8 june 1993 and some other findings on the accident.

## Materials

Sea water samples were collected ship heading and stern light side and 350 m from the ship wreck to Black Sea side by dry suit diving method. All the samples were collected at a depth of 20 m.

*Artemia salina* eggs were purchased from the market.

Silicagel G and solvents used were Merck products.

## Methods

### Chemical Analysis

1 L sea water was collected from each side. Sea water samples were divided into 100 ml portions and lyophilised. 100 mg lyophilisate was dissolved in 0.5 ml distilled water and applied on thin-layer chromatography. Solvent system : n-Butanol:acetic acid : water (3:1:1); Adsorbent:Silicagel G; Reagents:Ninhydrine (in 0.5 % acetone) (Güven and Güneri, 1969) and p-dimethylaminobenzaldehyde (p-DMBA) (in ethanol:hydrochloric acid : 95:5) (Macek, 1972). The plates were heated at 110 °C for 5 min. after spraying with ninhydrine and for 10 min. after spraying with p-DMBA.

### Biotoxicity Analysis

*Artemia salina* Leach Assay was used for assessment of Biotoxicity. This method was recommended for determination of cytotoxicity (Meyer, 1982) and marine pollution (Vanhaecke 1981, Vanhaecke and Persoone 1982) as well. A specially designed container which consisted of two unequal parts was used in the experiment. *Artemia salina* eggs were placed in the smaller and darkened compartment and hatched in 3.4 % sodium chloride solution. The phototropic nauplii passing through 2 mm holes to the enlightened larger compartment were collected with the aid of pipette and ten nauplii were transferred to each well (1 ml) of a 24-well plate (Greiner, Germany). The lyophilisate was added to each well at a final concentration of 20 mg/ml. This concentration was chosen because approx. 2 g of lyophilisate was obtained from each 100 ml sea-water sample. Survival was calculated as  $\text{live nauplii/total nauplii} \times \text{well}^{-1}$ .

### Water Analyses

Dissolved O<sub>2</sub> analyses measured with Winkler method, water transparency measured with Secchi disk in the first day of incident area from the shore to the 34 meter depth. The samples were fixed 4.1 % formalin for species determination. Sediment samples were also collected for the H<sub>2</sub>S and CH<sub>4</sub> analyses.

### Survival control of Marine Organisms

Survival control was made on the samples of fish and invertebrates collected.

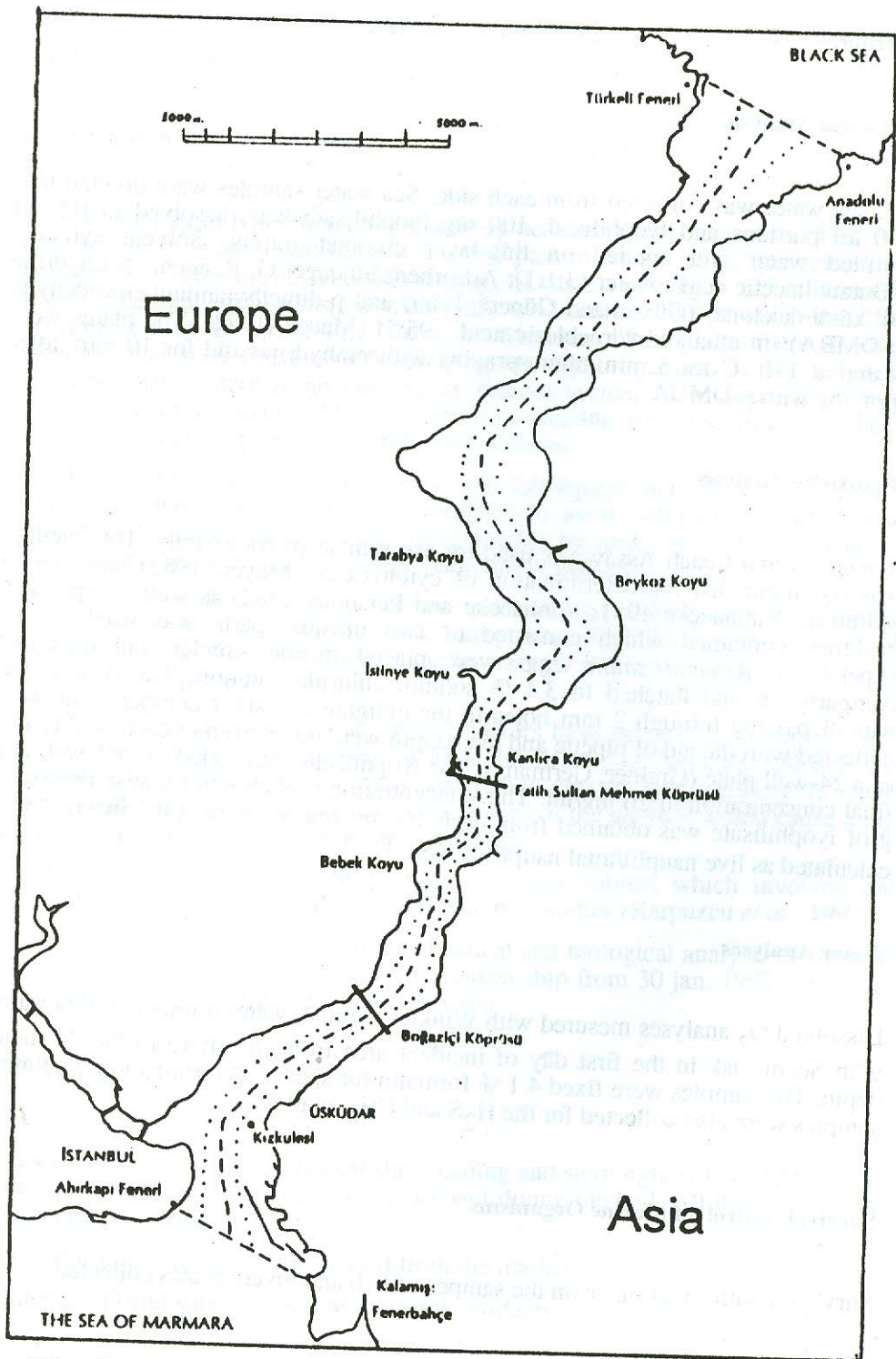


Fig.1 Map of Istanbul strait  
x Ship wreck

Table 1. The results of chemical and toxicity tests of sea water samples

Sample		Reagent		Survival
No	Date	N	PDABA	%
1	30.01.1992	-	-	100 ± 0
2	"	-	-	97 ± 6
3	"	-	-	97 ± 6
4	"	-	-	93 ± 6
5	"	-	-	100 ± 0
6	"	-	-	100 ± 0
7	"	-	-	100 ± 0
8	"	-	-	100 ± 0
9	"	-	-	97 ± 6
10	"	-	-	100 ± 0
K	"			90 ± 17
1	02.03.1992	-	+	100 ± 0
2	"	-	+	100 ± 0
3	"	-	+	100 ± 0
4	"	-	+	97 ± 6
5	"	-	+	100 ± 0
6	"	-	+	100 ± 0
7	"	-	+	97 ± 6
8	"	-	+	100 ± 0
9	"	-	+	100 ± 0
K	"		+	97 ± 6
1	01.04.1992	-	+	100 ± 0
2	"	-	+	98 ± 5
3	"	-	+	100 ± 0
K	"		+	93 ± 12
1	07.05.1992	+	+	100 ± 0
2	"	+	+	98 ± 5
3	"	+	+	93 ± 6
K	"			93 ± 6

Sample		Reagent		Survival
No	Date	N	PDABA	%
1	16.06.1992	+	+	98 ± 5
K	"	+	+	95 ± 6
1	24.09.1992	+	+	100 ± 0
2	"	+	+	93 ± 6
3	"	+	+	97 ± 6
K	"			87 ± 6
1	06.10.1992	+	+	90 ± 10
2	"	+	+	100 ± 0
3	"	+	+	97 ± 6
K	"			87 ± 6
1	04.11.1992	+	+	97 ± 6
2	"	+	+	100 ± 0
K	"			88 ± 12
1	18.11.1992	+	+	97 ± 6
2	"	+	+	97 ± 6
K	"			88 ± 12
1	07.04.1993	-	+	97 ± 6
2	"	-	+	97 ± 6
K	"			83 ± 6
1	28.04.1993	-	+	90 ± 10
2	"	-	+	100 ± 0
K	"			87 ± 6
1	08.06.1993	-	+	93 ± 6
2	"	-	+	97 ± 6
K	"			87 ± 6

30.1.1992-1. 4.1992: 350 m from the ship wreck to Black Sea side (1,4,7,10)

From the near of the slip (2,5,8)

From the far of the slip (3,6,9)

7. 5.1992 : Upside of the slip 15 m (1,2) ; Front of port side 15 m (3)

16. 6.1992 : Discharged water from the wreck (1)

24. 9.1992 : Discharged water from the wreck 5m (1),15m (2) and 25m (3)

6. 10.1992 : Discharged water from the wreck 22m (1),30m (2) and 33m (3)

4. 11.1992 - 8. 11.1992 : Fore side of the wreck (1), Stern side of the wreck (2)

7. 4.1993 - 28. 4.1993 : Fore side of the wreck (1), Stern side of the wreck (2)

8. 6.1993 : Bottom (1), Upside (2)

## Result and Discussion

The analytical results of sea-water samples collected between 30 Jan. 1991 and 8 June 1993 are given and summarized in Table 1.

30 Jan 1991 : The samples responded to p-DMBA but not to ninhydrine. It seemed that as the fleshe had not started to decay probably due to low water temperature

7 May 1992-18 November 1992 : The samples were gaves pink colour with ninhydrine and yellow colour p-DMBA, which indicated that decay was intensive. Ninhydrine reagent is specific for detection of protein and the related degradation product. It may be concluded that the decay of the animal bodies began approximately 5 months after the accident

7 April 1993-8 June 1993 : No spot with ninhydrine but yellow coloured spot with p-DMBA are formed between 2 March 1992 and 8 June 1993. Many compounds contained i.e amine groups, indol derivatives, etc. react with the p-DMAB. Thus positive reaction with p-DMAB depends on end products that contains amiine groups.

Brine shrimps have been utilised in various bioassay systems as toxicants in marine environments (Vanhaecke *et al*, 1981, Vanhaecke and Persoone 1982) Survival rates of the control and the test groups were not significantly different at 20 mg/ml concentration. In generally the samples cannot be considered as biotoxic since 1 mg/ml is the highest toxic level for any substance to be tested in this assay-system.

Dissolved O<sub>2</sub> values have decreased to 2 mg/L. Furthermore, water turbudity has increased and transparency value decreased to 0.5 meter. Red and green algae population existing in the area when the vessel sank died totally. As a result of this, mass mortality has been determined in economically valuable fish population like, *Gobius* spp. *Mullus surmuletus*, *Mullus barbatus*, *Physis* sp. living in benthic zone. Fish stocks also declined in the incident area. Mass mortality has also been observed in *Rapana venosa* and *Mytilus galloprovincialis* and their fishing amounts decreased. *Crangon crangon*, known as shore shrimp has totaly disappeared the sudden changes in food web has decreased biological diversity. Benthic live is still mortal in incident area.

In conclusion, this investigation showed that in the test period the degradation of drown sheep continued and some marine organisms lost or died oxygen content decreased, turbidity increased in the accident area.

## Özet

Istanbul Boğazı'nda 14 Kasım 1991 tarihinde Madonna Lily gemisi ile çarpışarak batan Rab-Union 18 adlı gemi 20.000 canlı koyun içermesi nedeni ile kamuoyunda kirlilik açısından büyük kaygı uyandırmıştır. Geminin içinde çürüyen koyunların İstanbul boğazını önemli derecede kirlettiği öne sürülmüştür. Bu çalışmada kontrol grubu için geminin Karadeniz yönünde 350 m uzağından kirlenmemiş bölgede ve kimyasal kirlilik saptanması için geminin pupa feneri ve pruva istikametinden (geminin içindeki kirli suların çıktığı bölgeler) 20 metre derinlikten alınan su örnekleri üzerinde çalışıldı. Bu örnekler liyofilize edildi ve ince-tabaka kromatografisi ile kimyasal analizleri ve "*Artemia salina*" testi ile de

biyotoksitesisi araştırıldı. Ninhidrin reaktifine kazadan 5 ay sonra (1.4.1992) cevap alınmıştır. 2.3.1992 ve çalışmanın bittiği 8.6.1993 tarihleri arasında ise p-DMBA reaktifine cevap veren leke saptanmıştır. Buna karşı alınan bütün örneklerde biyotoksik etkiye rastlanmamıştır. Ayrıca bu çalışmalarda deniz suyunda çözünmüş oksijen değerinin 2 mg/L, görünürlüğünün ise 0.5 m'ye düştüğü tespit edilmiştir. Alman sediment örneğinde CH<sub>4</sub> ve H<sub>2</sub>S tayini yapılmıştır. Deniz organizmalarından; *Phycis* sp, *Gobius* spp. *Mullus surmuletus*, *M. barbatus*, *Rapana venosa*, *Mytilus galloprovincialis* ve *Crangon crangon* türlerinde toplu ölümler saptanmıştır.

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