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Determination of the Levels of Indicator Bacteria, *Salmonella* spp. and Heavy Metals in Sea Snails (*Rapana venosa*) from the Northern Marmara Sea, Turkey

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Abstract

Sea snail (*Rapana venosa*) samples were collected from the northern coast of the Marmara Sea and analyzed to determine bacteriological pollution in the form of Faecal coliform, *Esherichia coli*, and *Salmonella* spp. as well as to measure levels of the heavy metals, Zn, Pb, Cu, Cd, As using by atomic absorption spectrophotometer. Samples were collected through the June 2000 - November 2001 from the shore and from depths of 10 – 12 meters of the northern Marmara Sea. Within the total of 75 groups of *R. venosa* samples which consisted of at least 6 individuals in each group, the highest bacteriological values, 11 x 10^3 cfu/g *Esherichia coli*, >24 x 10^3 Faecal coliform, were determined in the August sampling period. *Salmonella* spp. was determined in 2 groups of samples. Within the total of 55 groups of *R. venosa* samples, consisting of at least 3 individuals in each group, heavy metal levels ranged between Zn: 18.0–52.0 ppm, Pb: 0.52–1.25 ppm, Cu: 21.6–49.3 ppm, Cd: ND - 0.08 ppm. Arsenic was found below detection limit. No temporal variation was observed in the heavy metal concentrations.

Key Words: Heavy metals, Pollution, Bioaccumulation, Marmara Sea, *Esherichia coli*, Faecal coliform, *Rapana venosa*.

Introduction

The Sea of Marmara, an inner sea, is of great economical importance in the Turkish fishing industry. The sea is under the influence of chemical and biological pollution due to the fact that the inland is heavily populated with respect to dwelling, industrial activity, and marine transportation (Topcuoğlu *et al*., 1990a; Topcuoğlu *et al*., 1990b; 1995; 2000; Erentürk *et al*., 1990; Kut *et al*., 1990; 2000; Esen *et al*., 1999). Moreover, the metal levels in the Marmara Sea have been increased from Black Sea by opposite water current between Black Sea and the Aegean Sea (Topcuoğlu, 2000).

Due to the difficulties in determination of toxic elements in sea water, bivalve species are accepted as an indicator due to their high accumulation abilities (Walne, 1974). The elements that exceed the normal concentrations due to the influence of environmental pollutants are collected in the species that are at the top of the food chain. Amounts of metal residue that pass through the food chain in inhabitants of the land may rise to a hundred fold, whereas in aquatic media, biomagnifications may rise to a thousand fold (Hammond, 1971; Huginin and Bradley, 1975). This situation leads to the necessity of monitoring studies in aquatic media (Tort *et al*., 1987; Villarreal *et al*., 1986; Johnson, 1988).

Providing quality safety of *R. venosa* from their catch to the marketing to the consumers has great importance terms of human health as well as economical and ecological points. In this study the level of heavy metals and bacteriological pollution in the samples of sea snails (*R. venosa*), the indicator organism chosen, collected from the northern Marmara Sea has been determined and comparative results were evaluated.

Materials and Methods

Rapana venosa samples were collected by diving from Florya-Ambarlı seashore, (Marmara Sea, Turkey) and with the help of divers and immediately transported to the laboratory during the period between June 2000 and November 2001 (Figure 1).

Kruskal- Wallis nonparametric Anova test was used for statistical analysis.

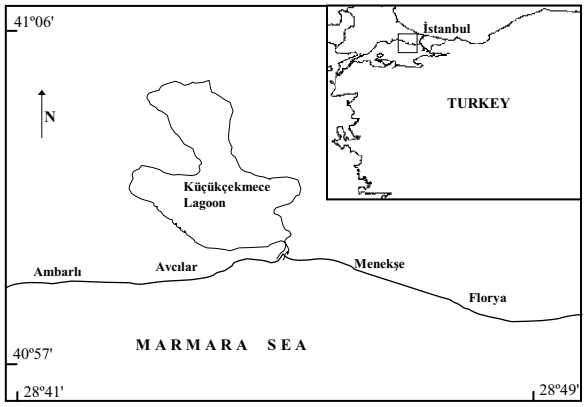

Figure1. Study Area, Ambarlı-Avcılar-Menekse-Florya Seashore, (Northern Marmara Sea, Turkey)

Bacteriological Methods

Samples for the microbiological analyses were transferred to the laboratory in a cold chain under aseptic conditions to avoid the possibility of bacterial contamination. In the analyses of coliform and *E. coli*, at least 6 individuals were accepted as a group and a total of 10 grams were taken from 6 individuals to form a sample group. A total of 75 groups *R. venosa* were examined. The diluted homogenous solutions of samples were prepared with 0.1% buffered peptone water; 25:225 (w/v) for the *Salmonella* spp. analyses and 10:100 (w/v) for the *Esherichia coli* and coliform analyses.

The analyses of coliform and *E. coli* were carried out in accordance with the Most Probable Number (MPN) technique: Sample dilutions of 10^{-1}, 10^{-2}, and 10^{-3} with buffered peptone water were transferred to three series of test tubes each containing 10 ml of Modified Lauryl Sulphate Triptose Broth (Harrigan, 1998; FDA, 1998). Following 24–48 hours of incubation at 37ºC, positive tubes were transferred to tubes containing Brilliant Green Bile Broth (BGLB) and incubated for 24 – 48 hours at 37ºC. The numbers of test tubes giving positive results with the BGLB were noted. Samples from the positive BGLB tubes were transferred into EC broth and incubated for 24 hours at 44.5±0.2ºC. Contents of the positive tubes were transferred into eosin methylene blue agar medium and then following into plate count agar medium. Indol, methyl red, Voges – Proskauer and citrate (IMVIC) tests (FDA, 1998) were conducted. The number of tubes that gave positive results to the BGLB and EC broth were noted and the amount of coliform and *E. coli* in the samples were calculated according to the MPN table. Results were presented as MPN/100g (FAO, 1992; FDA, 1998; Harrigan, 1998).

Chemical Methods

Those samples to be analyzed for heavy metals were transferred to the laboratory in polyethylene bags. The samples were kept away from metallic materials to avoid contamination. The flesh of the snails was separated from the shell and the edible parts were used for the analyses. Two parallel groups of samples were classified into 55 groups; each group consisted of 3 individual snails and were weighed as 5 grams. Samples were digested with conc. HNO_3: $HCIO_4$ (5:1) (Extra pure Merck) and filtered. All samples were prepared for the analysis in accordance with FAO Technical Report No: 158 (Bernhard, 1976).

Flame technique was used on the Atomic Absorption Spectrophotometer (AAS) for the Cu and Zn analyses. The Graphite Furnace (AAS) was used for the Pb and Cd analyses. Measurements were carried out by Varian 880 Atomic Absorption Spectrophotometer Graphite Furnace (AAS-G) (Anon., 1989; 1990; 1997).

Results were presented as (ppm-dry weight) minimum, maximum and arithmetic mean values. Intercalibration homogenate samples (IAEA) were used as a quality control for the analytical methodology. The reference material (MA-A-2) was analyzed in triplicate for Cu, Zn, Cd, Pb. The values obtained for the analysis were within the specified tolerances for each metal. The detection limits for heavy metals were: As, 0.006; Cd, 0.00015; Pb, 0.0004; Cu, 0.15; Zn, 0.08 ppm.

Results

The monthly data with respect to concentrations of Zn, Pb, Cu, Cd, and as have been summarized in Table 1. The total of 55 groups of *R. venosa* samples were collected between June 2000 and November 20001.

The lowest levels of Zn were found 20.5 ppm as average in samples collected in June 2001, whereas the highest levels of Zn were found 41.5 ppm in the samples collected in July 2001. The levels of Zn ranged between 32.5 ppm and 37.5 ppm in the other months. The lowest average concentrations of Pb, 0.78 ppm, and Cu, 29.4 ppm, were found in the

Table 1. Concentrations of Zn, Pb, Cu, Cd, As in samples of *R. venosa* collected on the northern seashore of the Marmara Sea, Turkey. Dry weight µg/g (2000-2001)

Heavy metals	June Min- Max Mean n:9	July Min- Max Mean n:11	August Min- Max Mean n:10	September Min- Max Mean n:8	October Min- Max Mean n:7	November Min- Max Mean n:10
Zn	18.0-24.0 20.5	32.0-52.0 41.5	21.0-45.0 32.5	24.0-51.0 37.0	29.0-47.0 37.5	23.0-49.0 35.5
Pb	0.55-1.02 0.78	0.52-1.11 0.81	0.57-1.20 0.88	0.59-1.25 0.92	0.54-1.07 0.80	0.57-1.23 0.90
Cu	21.6-37.2 29.4	24.0-39.1 31.5	28.2-44.2 36.2	29.3-49.3 39.3	23.0-47.5 35.2	21.9-44.0 32.9
Cd	ND-0.08 0.04	ND-0.07 0.03	ND-0.05 0.02	ND-0.07 0.03	ND-0.08 0.04	ND-0.04 0.02

ND: Not detectable; n: number of samples

samples collected in June. Maximum levels of Pb, 0.92 ppm, and Cu, 39.3 ppm, were found in the samples collected in September. Concentrations of Cd <0.00015 ppm were noted as ND. Average concentrations of Cd were ranged between 0.02–0.04 ppm throughout the sampling periods. The levels of Arsenic were measured below detection limit (<0.006 ppm) in the sampling area for all of the 55 groups of samples.

The highest levels of Faecal coliform and *E. coli* within the total of 75 samples analyzed were found in the samples collected during the months of August 2000 and 2001, whereas the lowest levels were found in the months of June 2000 and 2001. In the samples of August 2000, *Salmonella* spp. was found in both groups of samples of Faecal coliform and *E. coli;* however, *Salmonella* spp. was not isolated in the other samples (Table 2).

Discussion

The mean values of contamination found in the 75 samples under bacteriological analysis were between 15x10 and $24x10^3$ and above. It is concluded that the area is under the influence of the waste products of dwellings and naval transportation. *Salmonella* spp., a pathogenic microorganism that causes infections and intoxication, being found in both samples. Disposal of waste waters in this area should be reconsidered. Recreational activities in the area have been effected by pollutants. This situation will lead to further ruin of the bacteriological norms in the sea medium as time proceeds and thus prevent productive utilization.

The samples of sea snails were collected during the fishing season and theirs being ripe enough for consumption through the June - November. In the northern seashore of the Marmara Sea particular locations for sea snails have not been specified. There has been no statistically significant difference in spatial and temporal effects on organisms considering metal distribution. However, according to the findings, samples from near the Küçükçekmece lagoon, which is connected to the Marmara Sea via a 2 kilometre long strait, was different in comparison to the other samples. Bacterial pollution of samples collected from the area near the mouth of the strait as compared to the other samples, was found to be higher. This situation was interpreted to be a reflection of the lagoon, which is in a state of eutrophication due to the decrease of sources of fresh water as well as increase of domestic and industrial activities. Samples of sediment from the Küçükçekmece lagoon were analyzed: concentration of Zn was found to be 122 ppm, whereas concentration of Zn in samples of sediment from the Marmara Sea was found to be 15.2 ppm (Esen *et al*., 1999). Comparing the results of the study summarized in the article herein and the values of heavy metals found in the analyses of sea snails from different areas of the Marmara Sea (Topcuoğlu *et al*., 1994; Topcuoğlu 2000), levels of the some heavy metals Zn (83 ppm), Cd (14.5 ppm), Cu (82 ppm) are higher in those samples of sea snails collected from the Bosphorous. Concentrations of heavy metals in sea snails collected from the Black Sea have been reported to be lower as compared to those found in the Bosphorous (Güven, 1991). These results indicate that the Bosphporus area of the Marmara Sea is under greater influence of local pollution comparing to the Black Sea and the Küçükçekmece Lagoon. The author is continuing studies based on a regional basis within the Marmara Sea. Pollutants are added to the sea by direct discharges of industrial and other wastes by pipeline and dumping sewage sludge and industrial wastes at sea. It was concluded that fluctuations of the

Table 2. Levels of Faecal coliforms*, Esherichia coli* in samples of *R. venosa* collected on the northern seashore of the Marmara Sea, Turkey. MPN/100g (2000 - 2001)

Months	Number of Samples	Faecal coliforms Min-Max Mean	*Esherichia coli* Min-Max Mean
June	15	15x10-15x10^2 8x10^2	7x10-23x10 15x10
July	10	21x10^2-11x10^3 6x10^3	15x10-21x10^2 11x10^2
August	14	46x10^2->24x10^3 14x10^3	2x10^2-11x10^3 5x10^3
September	12	24x10^2-11x10^3 67x10^2	23x10-46x10^2 24x10^2
October	13	15x10^2-46x10^2 3x10^3	2x10^2-15x10^2 8x10^2
November	11	43x10-24x10^2 14x10^2	2x10^2 –93x10 14x10^2
Total	75	15x10->24x10^3	7x10-11x10^3

results in one area with respect to the time factor were caused by changes in local inputs, which changed from time to time. There have no significant differences in the values among the various sampling periods.

Chemical and bacterial pollution effect the optimum productivity in the marine environment. Analytical controls will be able to lead to rational and reasonable utilization of natural resources. The northern coast of the Marmara Sea is effected by domestic and industrial inputs. Despite these influences, levels of heavy metals in sea snails from this area were found to be lower than the sea snails collected from the Bosphorous area. But these areas must be observed and protected for ecological and sanitary purpose. There is a need to continue more detailed, extensive observations and studies and thus minimize the sources of pollution in the area.

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