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Determination of radionuclides in *Mytilus galloprovicialis* by Alpha and Gamma-Spectroscopy

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Abstract

The natural radionuclides ^{238}U, ^{234}Th, ^{40}K and the main man made ^{137}Cs, have been studied in Mytilus galloprovincialis (Lamarck, 1819) sampled in the Thermaikos gulf – North Aegean Sea, considered as a bioindicator for radiological assessment in the Mediterranean. The ratio $^{234}U/^{238}U$ has also been determined.

In terms of ^{137}Cs, the activity concentrations in seawater from the studied area have been measured as well, and the concentration factors of ^{137}Cs in Mytilus galloprovincialis are given as a parameter of the organism response to radioactive pollution.

Keywords: *Mytilus galloprovincialis;* Bioindicators; Radiological quality; Marine Radioactivity.

Introduction

Evaluation of marine radioactivity levels is based not only on direct measurements of the abiotic components, but also on measurements of the abundance and availability of radionuclides in selected marine organisms. The bioaccumulation of isotopic contaminants by tissues and organs of marine organisms has been studied worldwide and led to the adoption of the bioindicator concept for environmental quality assessment (CANTILLO, 1998). In addition, by using biomonitors, which are consumed by humans, a radiological risk could be assessed early and support the countermeasures for public protection warning. Several approaches have been reported for bioindicator selection based primarily on the concentration factor of the organism selected, sensitivity, habitat, abundance, availability, status in the trophic chain, etc.

In terms of marine radioecology, several studies have been carried out mainly through international scientific frames such as CRC programmes and GIRMED of IAEA, RTD programmes and MARINAMED of EU (AARKROG *et al.*, 1994) and oriented research

studies on a national level. Moreover, a large quantity of data has been derived from projects such as third party services for radiation protection purposes. Thus, background knowledge has been established on the dispersion and behavior of radionuclides in the marine environment and a 'radiation archive' is now available concerning the inventory of artificial and natural radionuclides (selected) in the abiotic components and organisms of the Eastern Mediterranean. Published and unpublished data of ERL/INT-RP/NCMR'D' (FLOROU and KRITIDIS, 1994, FLOROU *et al.*, 2000) are available to study the environmental monitoring using biomonitors as 'organism – sentinels' for radioactive contamination.

Mussels are recognized worldwide as pollution bioindicator organisms (mussel-watch) because they accumulate pollutants in their tissues at elevated levels in relation to pollutant biological availability in the marine environment. Additionally, they are recognized to show an early response to the radioactive contaminants added to ecosystem (FORSTNER & WHITTMAN, 1979).

Mytilus galloprovincialis and other Bivalves as well, are widely cultured in Greece. More than $32x10^6$ kg were produced during the year 2000, whereas a comparative tonnage was imported from other EU and third countries. All fishing goods are being transported in the EU through the market routes according to the data provided by the Ministry of Agriculture (2002).

In the present study the bioaccumulation of alpha (^{238}U) and gamma emitters (^{234}Th, ^{40}K, ^{137}Cs) by *Mytilus galloprovincialis* sampled from the Thermaikos Gulf – North Aegean Sea is examined by using alpha and gamma spectroscopy after appropriate physical and radiochemical treatment procedures. The scope of this study is to evaluate the radiological regime of the Thermaikos gulf through the 'mussel watch' consideration.

Materials and Methods

1. Gamma-spectroscopy

Physical Treatment: Mytilus galloprovincialis samples were collected monthly from two aquaculture farms located at the area of the Thermaikos gulf – North Aegean Sea (Fig. 1) during the period February 1999 -October 2002 (November to April has been defined as the cold period and May to October as the warm one). Forty-one composite samples were analyzed in the work frame of the project. The sampling area is located near the wide area of the Loudias river outflow (a river with a 93 500 ha drainage area). This area consequently receives agricultural, domestic and industrial discharges carried by the river (CATSIKI *et al.*, 2001).

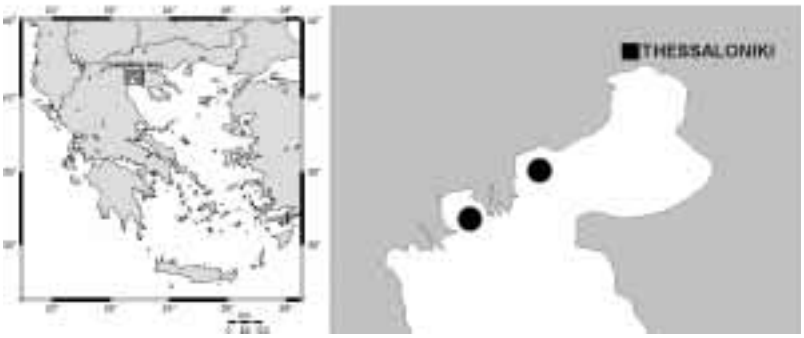

Fig. 1: Location of sampling stations in Thermaikos Gulf.

Composite samples of at least 80 - 200 specimens of a similar size (the length variation was as 2.3 – 8.5cm with regard to the sampling period) were prepared by adding the soft parts. The samples were dried using the ashing procedure for caesium and natural radionuclide measurement (IAEA, 1970). The ashing factor varied as 7.1 – 14.6% of the fresh weight (defined as the percentage yield of the ash to fresh weight of sample tissue). A quantity of 15 – 45g of the ashed sample was put in the appropriate cup and brought for gamma measurement.

Measurement: The samples were measured for 70000 sec in a high-resolution gamma spectrometry system, with an HpGe detector of 20% relative efficiency and computerized multi-channel analyzer of 4000 channel in a total spectrum area of 2000 keV. ORTEC software was used for the analyses of the obtained spectra. The relative statistical error (1σ) does not exceed 18%. The efficiency was determined by a ^{226}Ra standard source of 240 Bq. The calibration correction was made by a ^{60}Co point standard source of 3.7×10^4 Bq, in the 1173.2 keV and 1332.5 keV peaks. Additional calibration in the range below 150 keV has been made by use of ^{238}U standard and the IAEA reference material RGU-1, as well.

2. Alpha-spectroscopy

Six composite samples representing the warm and cold periods of the bulk of samples described above, were radiochemically analyzed for uranium isolation as follows:

Samples pretreatment: A quantity of 10g mussels are dry ashed at 550°C; then ^{232}U is added as a yield tracer. The ashed sample is digested to dryness twice with nitric acid. The wet ashed sample is then dissolved in 8M HCl. The yield tracer used was the ^{232}U standard solution (NIST SRM 4324A).

Uranium determination and measurement: Uranium was separated from 8M HCl by anion exchange on Bio-Rad AG1-X4 and eluted with 0.1M HCl (FISENNE, 1990). The dried eluent is dissolved in 1M ammonium sulphate after addition of two drops of sulphuric acid, adjusted to pH 2.5 with ammonium hydroxide and the source for the alpha-spectrometric measurement is prepared by the electrodeposition of uranium on to stainless steel discs. The stainless steel discs were measured by alpha-spectrometry. The minimum detectable activity was 0.27 mBq ^{238}U/kg (for a counting time of 4400 min), corresponding to 0.022μg U(nat)/kg. The equipment used was a fully automated and integrated alpha spectroscopic system (Alpha Analyst, Canberra), consisting of 4 Passivated Implanted Planar Silicon (PIPS) detectors with 450mm^2 active area. The detectors counting efficiency is 23%. The addition of the yield tracer ^{232}U permitted to calculate the final chemical yield, which resulted for uranium between 40-75%.

Results

The summarized distribution pattern of the gamma emitters (^{234}Th, ^{40}K, ^{137}Cs) are illustrated in Figures 2, 3 and 4 respectively, whereas the evolution of ^{137}Cs concentrations since 1984 is illustrated in Table 1. The ^{238}U activity concentrations and the values of the ^{234}U/^{238}U ratio as well, are given in Table 2. The ^{238}U spectrum of sample No 3 is given in Figure 5, as indicative for the measurement procedure for the spectra analysis.

Discussion

The repeated measurements of the samples over time according to radionuclide half-life, showed the selective bioaccumulation of ^{234}Th, compared to its parent ^{238}U. In terms of the measured concentrations, 39% were in the range 2.0 – 4.0 Bq kg^{-1} fresh weight, whereas a maximum of 9.1 ± 0.9 Bq kg^{-1} fresh weight was observed during the warm period (Figure 2).

A percentage of 61 of the concentrations of ^{40}K range from the detection limit 1.0 to 25 Bq kg^{-1} fresh weight, whereas the maximum concentration of 80.9 ± 2.0 Bq kg^{-1} fresh weight

Table 1
^{137}Cs activity concentrations (Bq kg^{-1} fresh weight) in *Mytilus galloprovincialis* from Aegean Sea - Eastern Mediterranean.

Time period y	^{137}Cs Bq kg^{-1} fresh weight	Remarks
1984 – 85*	0.68 ± 0.10	
1986 – 87*	7.10 ± 1.80	Excluded maximum observed 50 ± 7 (3 samples from one station)
1988 – 95*	0.46 ± 0.28	
1999 – 2002	0.92 ± 0.68	Mean Value ± Standard deviation of 41 samples from Thermaikos gulf (present study)
LLD	0.1	Measurement in ashed soft tissue transformed into wet weight

**Published data (FLOROU, 1996)*
LLD: Low Limit of Detection

Table 2
^{238}U concentration in Mytilus galloprovincialis (Bq kg^{-1} fresh weight).

No	Sampling period	^{238}U concentration			^{234}U/^{238}U
		Bq/kg	µg/kg	Error % *	Bq/Bq
1	Warm Period 2000	0,40	32,12	3,37	1.43
2	Warm Period 2002	0,60	48,27	3,96	1.10
3	Cold Period 1999	0,24	19,25	1,48	1.22
4	Cold Period 2000	0,22	17,61	1,37	1.27
5	Cold Period 2001	0,37	30,05	2,07	1.48
6	Cold Period 2002	0,10	8,12	0,43	1.09

**Expanded uncertainty at 95% confidence level.*

was observed during the cold period (Figure 3). The reported concentrations of ^{40}K in seawater for the Aegean Sea vary 9.8 to 11.8 Bq l^{-1} (FLOROU, 1992). Thus, *Mytilus galloprovincialis* seems to bioaccumulate ^{40}K several times its concentration in seawater in general, although the isotopic abundance of ^{40}K amounts only 0.01% of the stable isotopes ^{39}K and ^{41}K.

As it is shown in Figure 4, 45% of ^{137}Cs levels in *Mytilus galloprovincialis* range near the detection limit. The overall data range from the detection limit 0.2 to 2.5 Bq kg^{-1} fresh weight, with maximum values 2.4 ± 0.1 and 1.9 ± 0.6 Bq kg^{-1} fresh weight were observed during the cold and warm period respectively.

As it is shown in Table 1, concentrations up to 50 ± 7 Bq kg^{-1} wet weight of ^{137}Cs were detected in *Mytilus galloprovincialis* sampled in Evoikos Gulf in the Central Aegean Sea a few days after the Chernobyl nuclear accident, whereas the respective mean value concerning the Aegean and Ionian Sea was 7.10 ± 1.80 Bq kg^{-1} wet weight (maximum value excluded from the mean), which was more than one order of magnitude higher compared the pre-accident levels. The levels of ^{137}Cs in *Mytilus galloprovincialis* were, more or less, back to the pre-accident ones observed in the Eastern Mediterranean since 1988 (FLOROU, 1996). Nevertheless, the Black Sea water influence into the North Aegean Sea has also an impact on the fluctuations of the concentrations in *Mytilus galloprovincialis* (FLOROU, 2002).

It is noteworthy that, according to the recent data of ^{137}Cs concentrations in seawater from the studied area (supporting measures, ERL, 2003) and the measurements of the

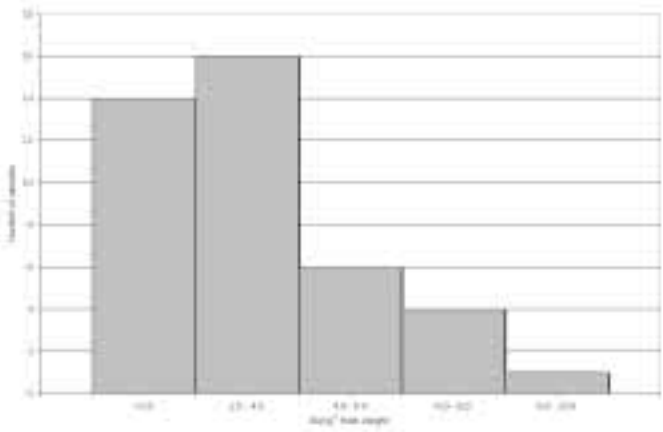

Fig. 2: Distribution of ^{234}Th in *Mytilus galloprovincialis* (Bq kg^{-1} fresh weight).

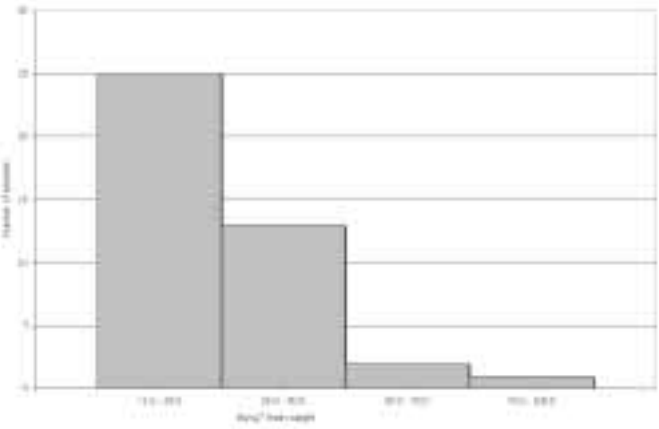

Fig. 3: Distribution of ^{40}K in *Mytilus galloprovincialis* (Bq kg^{-1} fresh weight).

present study (measured concentrations in seawater 3.4 – 17.7 Bq m^{-3}), the concentration factors of ^{137}Cs for *Mytilus galloprovincialis*, show a wide range 40 – 400. As no apparent variation has been recorded in the ^{137}Cs concentrations in seawater, the estimated wide range of the concentration factors should be attributed to biological parameter of the organism (e.g. stage of the life cycle). KRITIDIS & FLOROU, (1994) has reported similar evidence in terms of freshwater environment.

The principal uranium isotopes (^{238}U and ^{234}U) resulted in radioactive equilibrium (Fig. 5): Therefore, ^{234}U concentration is the same as that of ^{238}U. The ^{235}U activity is negligible because of the low activity of the total uranium in the sample concerning the detection activity (^{235}U < 20 μBq/g fresh weight). The range of ^{234}U/^{238}U values 1.09 to 1.43 (Table 2) is a strong evidence of the natural origin of ^{238}U measured in *Mytillus galloprovincialis* (instead of the possible presence of Depleted Uranium due to the Kossovo conflict).

The values for ^{238}U are found between 0.1 - 0.6 Bq kg^{-1} (8-50 μg kg^{-1} fresh weight). The minimum value was reported in the cold period

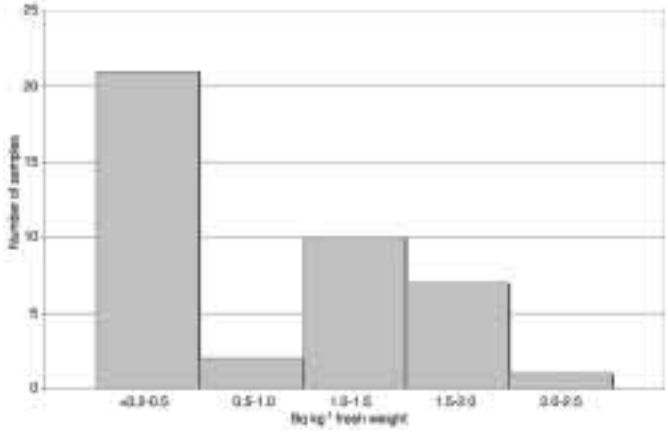

Fig. 4: Distribution of ^{137}Cs in *Mytilus galloprovincialis* (Bq kg^{-1} fresh weight).

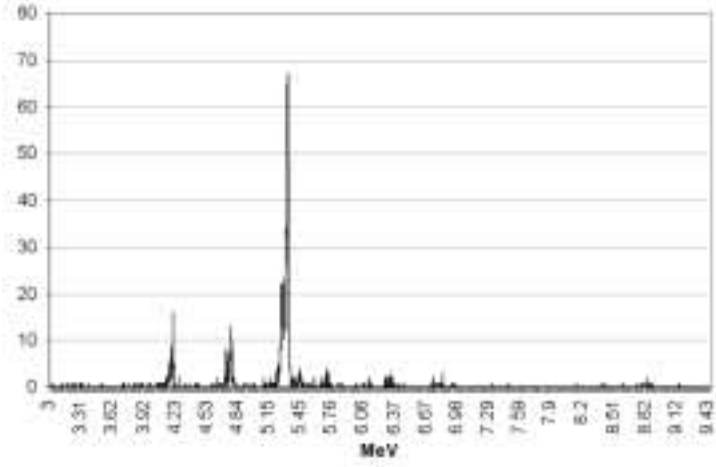

Fig. 5: Alpha-spectrum of uranium separated from the sample Nr. 3.

and the maximum one in the warm period (Table 2). Although, it seems that the concentrations measured in the composite samples from the warm period are higher than the respective ones of the cold period, the limited number of samples avoids justification. Nevertheless, *Mytilus galloprovincialis* seems to bioaccumulate ^{238}U, even the radionuclide present low biological mobility (WHICKER and SCHULTZ, 1982) and there is a little evidence of ^{238}U bioaccumulation in the literature (CIESM, 2002).

Using the published ^{238}U values for sea water from the North Aegean Sea in conjunction with the respective values in *Mytilus galloprovincialis* (FLOROU, 1992) of the present study, the concentration factors of ^{238}U range between 6 to 15, which is conformed with the statements mentioned above for low biological mobility of the radioisotope considered.

Conclusion

Alpha and gamma-spectroscopy applied in mussels (*Mytilus galloprovincialis*) sampled from the Thermaikos Gulf – North Aegean Sea, showed selective bioaccumulations of ^{234}Th, ^{40}K, ^{137}Cs among other gamma emitters. Uranium although of low biological mobility, seems to be accumulated by the organism. The distributions of the radionuclides show the greatest percentage shifted to the lowest levels of the concentrations measured.

References

AARKROG, A., ANGELOPOULOS, A., CALMET, D., DELFANTI, R., FLOROU, H., RISICA, S. & ROMERO, L., (alphabetical order), 1994. Radioactivity in the Mediterranean Waters in: Seminar on the Radiological Exposure of the population of the European Community from Radioactivity in the Mediterranean Sea. CEC, ENEA, Rome 17-19 May 1994. Report of Working Group 2, Project 'MARINAMED'. EUR 15564 pp: 75-115.

CANTILLO, A. Y., 1998. Comparison of results of Mussel Watch programs of the United States and France with worldwide Mussel Watch studies. Mar. Poll. Bull., 36(9): 712-717.

CATSIKI, V. A., FLOROU, H., STROGYLOUDI, E. & CHALOULOU, CH., 2001. The use of Mytilus galloprovincialis as a bioindicator for the detection of heavy metals and caesium-137 in an estuarine ecosystem (Thermaikos gulf, N. Aegean Sea). Proc.: Interreg Meeting, North Aegean System Functioning and Inter-Regional Pollution, N.C.M.R., Kavala 28 – 30 May, 2001.

CIESM, 2002. Mediterranean Mussel Watch: Designing a regional program for detecting radionuclides and trace contaminants. Marseille – France, 18 – 20 April 2002. CIESM WORKSHOP SERIES No 15, ISSN 1563-2727 133p.

ERL, 2003. Radiocaesium measurements in marine environment. Data of the ERL achieves. Environmental Radioactivity Laboratory, INT-RP, NSCR 'D', Attiki.

FISENNE, I. M., 1990. 'Uranium in biological and environmental materials', Environmental Measurements Laboratory, U.S. DOE, Section 4, HASL-300, 28th Edition.

FLOROU, H., 1992. Behavior and dispersion of long lived radionuclides in the marine ecosystem of Greek environment. Ph. D, Thesis. University of Athens, Athens 1992 253p.

FLOROU, H., 1996. ^{137}Cs in abiotic component and biota from the Aegean and Ionian Sea - Greece. Chemistry and Ecology Vol.12 No 4 pp: 253-258.

FLOROU H., 2002. The use of bioindicators in environmental quality assessment. Proc.: Stakeholders' Conference on Approaches to the Management of Environmental radioactivity. European Commission, Luxembourg 2 –3 – December 2002.

FLOROU, H. & KRITIDIS, P., 1994. The dispersion of ^{137}Cs in the Aegean Sea. Radiochimica Acta 66/67 pp: 415-417.

FLOROU, H., KRITIDIS, P., POLIKARPOV, G., G., EGOROV, V., DELFANTI, R. & PAPUCI, C., 2000 Title: Dispersion of caesium-137 in the Eastern Mediterranean and the Black Sea: the time evolution in relation to the sources and pathways. Progress Report, IAEA, R. C. No 11086, IAEA 11/2000 5p.

FORSTNER, U. & WITTMAN, G.T.W., 1979. Metal pollution in the aquatic environment. Springer-Verlag Berlin, Heidelberg, New York, 1979.

IAEA, 1970. Reference methods for marine radioactivity studies. TRS No 118. IAEA, Vienna 1970 284p.

KRITIDIS, P. & FLOROU, H., 1995. Environmental study of radioactive caesium in Greek Lake fish after the Chernobyl accident. J. Environ. Radioactivity Vol. 28 No 3 pp: 285-293.

MINISTRY OF AGRICULTURE, 2002. Data on aquaculture units in the coastal areas of the Greek peninsula. General Direction of Fisheries, Direction of Aquacultures and Inland Waters, Ministry of Agriculture, Athens, Greece.

WHICKER, W. & SCHULTZ, V., 1982. Radioecology Nuclear Energy and the Environment. Vol. I, CRC Press Inc, Boca Raton, Florida, 1982.