# THE ESTIMATION OF SEA WATER QUALITY AT THE MONTENEGRIN COAST FOR MUSSELS FARMING

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# PROCENA KVALITETA MORSKE VODE CRNOGORSKOG PRIMORJA ZA UZGOJ ŠKOLJKI

# Abstrakt

Cilj ovoga rada bio je da se odredi kvalitet morske vode na Crnogorskog primorju vezano za uzgoj Mediteranske skoljke *Mytilus galloprovincialis* ili dagnje. Analizirana morska vrsta može se koristiti i kao bioindikatori zagađenja morske sredine. Uzorkovanje vode i divljih skoljki obavljeno je u periodu od jeseni 2005. do proleća 2007. godine, u četiri sezone, jesen i proleće, na četiri lokacije: u blizini Bar i Rt Đeran, na obali otvorenog mora, i u Bokokotorskom zalivu na lokacijama Sveta Stasija i Herceg Novi. Morske voda i škojlke uzorkovane su istovremeno na svakoj od ispitivanih lokacija u sve četiri sezone. U uzorcima morske vode određivani su fizičko-hemijski parametri, merenjem nutrienata (NO<sub>3</sub><sup>-</sup> i PO<sub>4</sub><sup>-</sup>), T, saliniteta i rastvorenog O<sub>2</sub>, a u uzorcima dagnji teški metali: Hg, Cd, Pb, As, Cu i Zn.

Na osnovu izmerenih vrednosti nutrienata, temperature, saliniteta i rastvorenog kiseonika morske vode crnogorskog priobalja u četiri ispitivane sezone na četiri lokacije, može se zaključiti da je morska voda crnogorskog primorja optimalna za komercijani uzgoj školjki, jer se prvenstveno optimalni uslovi temperature i saliniteta morske vode nalaze u intervalu od 15 °C – 25 °C za temperaturu, i 20 ‰ – 35 ‰ za salinitet. Kiseonik je bio je najveći na lokaciji Sveta Stasija a bez velikih oscilacija na lokaciji Rt Đeran, u sve četiri ispitivane sezone. Sadržaj fosfata bio je veći u prolećnim uzorcima vode u odnosu na jesenje, dok je u slučaju nitrata bilo suprotno, njihov sadržaja bio je manji u prolećnim, a veći u jesenjim uzorcima morske vode. Kada su u pitanju koncentracije teških metali u dagnji sa četiri lokacije u sve četiri ispitivane sezone, u odnosu na kriterijume po Airas-u (2003) koji morsku vodu u odnosu na koncentracije teških metala u školjki deli na pet klasa, od klase I, koja je nezagađena ili malo zagađena, do klase V koja je jako zagađena, razmatran je kvalitet morske vode na crnogorskom priobalju. Na osnovu ovog istraživanja, morska voda na lokaciji Sveta Stasija i Rt Đeran je I klase u odnosu na izmerenu koncentraju Hg, Cd, As, Cu and Zn u školjkama sa ovih lokacija u svim sezonama, međutim u odnosu na koncentraciju Pb u istim, morska voda je II klase ili umereno zagađena. Na osnovu izmerene koncentracije Hg u školjkama sa lokacija H. Novi i Bar, morska voda je zagađena na ovim lokacijama i pripada III klasi, dok u odnosu na izmerene koncentracije preostalih elemenata u školjkama sa ovih lokacija, morska voda je II klase ili umereno zagađena u sve četiri

U odnosu moguće izvore zagađenja morske vode crnogorskog priobalja, posebno u Bokokotorskom zalivu gde su hirološki i biološki uslovi za njeno gajenje najpovoljniji, a u odnosu i na izmerene koncentracije teških metala u školjkama (Hg, Cd, Pb, As, Cu i Zn), školjke moraju biti prečišćene pre njihove komercijalne upotrebe kao hrane.

Ključne reči: morska voda, fizičko-hemijski parametri, dagnja, tragovi metala, klase voda

## INTRODUCTION

The fact is that water and lands in the world today represent limited life resources which are more and more degrade and disturbed, mostly by anthropogenic influence. The investigation of Adriatic is more marked along the Italian coast than along the eastern coast of Adriatic (Joksimovic et al., 2011). Southeast part of Adriatic coast is very interesting for researches because Montenegrin and Albanian coast, i.e., sea and marine environment in those countries, are little investigated on possible pollutants (Babi et al., 1998; Celo et al., 1999; Cullaj et al., 2006, Jovic et al., 2011, Joksimovic et al., 2011, Stankovic et al., 2011). In common with the other coastline areas, the Montenegrin coast-line is also under a great impact of anthropogenic factors and the activities on the shore. The Montenegrin coastal area receives a heavy influx of sewage, industrial effluents, domestic and agricultural wastes, all of which contain varying hazardous chemicals and can cause deleterious effects on an aquatic organism. Additionally, fishing and recreational activities in the coastal area further pollute the Montenegrin coastal waters.

The Mediterranean mussel *M. galloprovincialis* (L.) is widely distributed in the coastal waters of Montenegro and started to cultivate on farms for the market, while wild ones are still hand-collected for personal consumption. In light of this, cultivation of mussels along the Montenegrin coast has been increasing, 150 tones/per year in last decade (FAO, 2007b), particularly in the Boka Kotor Bay due to the good natural conditions, but it is still underdeveloped. The contribution of aquacultured mussels in Montenegro is insignificant, but there is potential for its development. Hence, data

about basic physical-chemical factors (T, salinity, oxygen concentration and nutrients content) and trace metal concentrations in marine environment and their accumulation levels in mussels is essential to assess quality of sea water for mussels farming. This can also provide information about metal contents in marine organisms actively consumed by humans.

This study, initiated from fall 2005–spring 2007, is related to wild mussels because of their hand-collection for human consumption and the expansion of mussel aquaculture in Montenegro, especially in area of the Bay after the year 2000. The aim of this work was to determine the sea water characteristics and the trace metals levels of Zn, Cu, Pb, Cd, As and Hg in the soft tissue of wild mussels *M. galloprovincialis* collected on the Montenegrin costal area to estimate the sea water quality for mussels farming in this part of the Adriatic.

#### MATERIAL AND METHODS

Research presented in this paper includes four sites in the coastal water of Montenegro where sea water and wild mussels were sampled in the same time at the same place. Localities were chosen based on the intensity of pollutions influence from the land and the specific characteristics of locality: in the Boka Bay, sites Sveta Stasija, and Nerceg Novi, and at the open coastal area of Montenegro, port Bar and Rt Djeran, Fig. 1.



**Figure 1**. Sampling sites of the mussel *M. galloprovincialis* from the SE Adriatic, Montenegro

Samples of sea water were taken directly from the surface in the polyethylene bottles 0.5 L volume and physical-chemical factors (nutrients, T,  $O_2$  and salinity) were measured at the sampling locality with MultiLine 4 labs. The mussel samples were collected seasonally from each site in each season; 25–30 mussels similar in length were selected,

cleaned and rinsed with deionized water. All samples were measured before freeze-drying at -40 °C for 48 hours, and after, homogenized to a fine powder and stored at -10 °C until analyses. The average water content of the soft mussels tissues were: 81.8 % (spring) and 79.8 % (fall). Samplings were conducted for two years from 2005 to 2007, in spring and fall seasons.

Five replicates per site were analyzed for trace metals: approximately 0.5 g of the soft tissue was digested with a mixture of concentrated  $HNO_3$  (65 % Merck, Suprapur) and  $H_2O_2$  (30 % Merck, Suprapur) in a High Microwave Digestion System (CEM COR-PORATION, MDS-2100). The digested samples were diluted to 25 ml with Milli Q water and stored in polyethylene bottles. The Fe, Mn, Zn, Cu, Ni and Co concentrations were determined by using F-AAS (PerkinElmer, AAnalyst 200) with an air–acetylene flame, but Pb and Cd by GF-AAS (PerkinElmer, 4100ZL, with Zeeman background correction) technique. HG/CV AAS technique was used for analyses of As and Hg (PerkinElmer, AAnalyst 200). The accuracy of the applied analytical procedure for the determination of heavy metals in mussels was tested using SRM 2976 (Mussel homogenate; NIST) material.

## **RESULTS AND DISCUSSION**

The information related to surface water samples at the investigated sites is given in Table 1. The nutrients,  $NO_3^-$  and  $PO_4^-$ , are essential compounds for phytoplankton growth. Low nutrients in water leads to low phytoplankton density and therefore low feed level for mussels. The content of  $NO_3^-$  and  $PO_4^-$  was the highest in the spring 2006 in spring, with lowest  $NO_3^-$  range at harbor Bar (1.9 – 4.9 mg/L), but the  $PO_4^-$  content was always little be higher in spring seasons at the all locations and measured  $PO_4^-$  values were between 0.17 - 0.35 mg/L. Dissolved oxygen is needed for mussels respiration and its range was between 6.1 - 8.5 mg/L with the best oxygen water conditions at Sv. Stasija and H. Novi locations, Tab.1. Low dissolved oxygen levels can cause decline in mussels feeding and growth. Considering the temperature and salinity, the coastal water of Montenegro for mussels growth is optimal, since the mussel *M. galloprovincialis* grows to optimum size in sea water of temperature 15 °C – 25 °C and salinity between 20 ‰ – 35 ‰ (Braby and Somero, 2006).

No.	Location	Sampling Date	PO <sub>4</sub> (mg/L)	NO <sub>3</sub> (mg/L)	Disolved O <sub>2</sub> (mg/L)	Temperature T(° C )	Salinity (‰)
1.	Herceg Novi, in Boka Kotor Bay	fall 2005 spring 2006 fall 2006 spring 2007	0.16 0.35 0.12 0.20	2.1 5.8 1.6 6.7	7.8 6.6 7.4 6.1	19.9 24.5 19.6 22.4	29.6 26.8 36.3 32.6
2.	Sveta Stasija, in Boka Kotor Bay	fall 2005 spring 2006 fall 2006 spring 2007	0.18 0.34 0.33 0.17	1.8 6.0 1.0 1.1	7.7 8.2 8.5 8.1	19.2 26.0 18.8 18.1	32.3 15.9 30.8 16.2
3.	Bar, at the open coastal area	fall 2005 spring 2006 fall 2006 spring	0.16 0.33 0.13 0.30	2.6 4.9 2.2 1.9	7.2 7.3 6.8 7.3	19.8 24.0 20.9 20.5	35.5 29.3 36.7 35.2
4.	Rt Djeran, at the open coastal area	2007 fall 2005 spring 2006 fall 2006 spring 2007	0.20 0.32 0.10 0.17	2.2 3.9 1.0 1.8	7.0 7.1 7.0 6.9	19.0 24.8 18.4 20.2	18.6 28.6 36.2 30.5

 Table 1. Sampling data of the sea water and the Mediterranean mussel *M. gallopro-* 

 vincialis
 collected from the four locations of the SE Adriatic coast, Montenegro

Nos. follow those indicated in Fig.1.

Wild or aqua cultured mussels are usually collected for human consumption at the coastal area of Montenegro, especially in the Boka Kotorska Bay. The mussel soft tissues are typically eaten whole and after harvesting for human consumption they were not depurated on the possible pollutants. Coastal waters of Bokakotorska Bay, in regard to open sea, are most exposed to anthropogenic eutrophication (Joksimovic, 2010) with waste originated from the human activities on the coastlines land and sea water (Jovic et al., 2011). The Montenegrin sea water quality was determined on the basis of the Airas (2003) criteria which connects the concentration levels of trace metals in mussels with five different water quality classes: from class I, unpolluted, to class V, highly polluted. The concentrations of Hg, Cd, Pb, As, Cu and Zn in the investigated mussel samples are shown in Table 2.

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No	Location	N	WB	Hg (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
1	<b>H. Novi,</b> B. Kotor Bay	5	Dry	<b>0.59</b> (0.15-1.0)	<b>2.41</b> (1.7-2.9)	<b>7.0</b> (3.5 – 10.1)	<b>13.3</b> (8.5-17.8)	<b>8.0</b> (4.6-11.5)	<b>175.5</b> (151.9-190)
2	<b>Sv. Stasija</b> B. Kotor Bay	5	Dry	<b>0.27</b> (0.03-0.9)	<b>2.02</b> (1.4 -2.9)	<b>9.8</b> (8.1 -11.4)	<b>4.42</b> (1.9-7.4)	<b>7.5</b> (5.9-8.9)	<b>133.5</b> (82.0-205)
3	Bar at the open coastal area	5	Dry	<b>0.49</b> (0.25-1.0)	<b>2.13</b> (1.0-3.53)	<b>12.9</b> (8.5- 16.3)	<b>5.82</b> (2.7-8.0)	<b>14.5</b> (12.6-17.2)	<b>205.9</b> (101-300)
4	<b>Rt Djeran</b> at the open coastal area	5	Dry	<b>0.07</b> (0.030.14)	<b>1.73</b> (1.0-2.3)	<b>4.7</b> (1.30- 7.9)	<b>8.9</b> (4.2-20.5)	<b>9.8</b> (7.4-12.4)	<b>188.3</b> (118.1-345)

**Table 2.** The concentrations (mean, range min–max, mg/kg) of mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), copper (Cu) and zinc (Zn) in the soft tissues of mussels collected at the Montenegrin coastal area from the fall 2005 – spring 2007

Nos. correspond to locations indicated in Fig.1.

N - Number of observations

On the basis of the Airas (2003) criteria in the present study, the sea water from Rt Djeran and Sveta Stasija is unpolluted or slightly polluted by Hg, Cd, As, Cu and Zn related to their concentrations in mussels from these areas and belongs to the class I, but related to Pb concentrations in mussels, sea water from these areas belongs to the class II or moderately polluted. Related to the content of Hg in mussels from the Herceg Novi and Harbor Bar, these areas are polluted by Hg and sea water belongs to the class III, but related to the concentrations of rest elements in wild mussels, sea water in the area of Herceg Novi and Harbor Bar is class II or moderately polluted.

Comparing the metal levels in mussels from different Adriatic areas (Scancar et al., 2007; Cardellicchio et al., 2008; Stankovic et al., 2011), the obtained data indicates that the metal levels found in the wild *M. galloprovincialis* from the Montenegrin coastal area are similar to the metal levels reported from other Adriatic areas.

### CONCLUSIONS

It is oblivious from water classifications related to the metal concentrations in wild mussels, before their consumption, that they have to be depurated. As this was a pilot study to evaluate the environmental quality of the coastal waters of Montenegro for future mussels farming, the continued monitoring of this sea area is necessary in order to control the water quality and amount of heavy metals in mussels for human consumption.

#### ACKNOWLEDGEMENTS

This research was financed by Ministry of Education and Science, Republic of Serbia, Contract No. III43009 and EAR, Contract No. 04SER02/05/00.

#### REFERENCES

*Airas, S. (2003).* Trace metal concentrations in blue mussels *Mytilus edulis* (L.) in Byfjorden and the coastal areas of Bergen. Master thesis in Marine Biology, Institute for Fisheries and Marine Biology, University of Bergan, Norway, 1-63.

Babi, D., Çelo, V., Çullaj A., Pano, N. (1998). Evaluation of the heavy metals pollution of the sediments of Vlora Bay Adriatic Sea, Albania. *Fresenius Environmental* Bulletin, 7(9-10), 577-584.

*Braby, C.E. and Somero, G.N. (2006).* Following the heart: Temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology*, 209 (13), 2554–2566.

*Cardellicchio, N., Buccolieri, A., Di Leo, A., Giandomenico, S., Spada, L. (2008).* Levels of metals in reared mussels from Taranto Gulf (Ionian Sea, Southern Italy), *Food Chemistry*, 107, 890–896.

Celo, V., Babi, D., Baraj, B., & Cullaj, A. (1999). An assessment of heavy metal pollution on the sediments along the Albanian Coast, *Water, Air and Soil Pollution*, 111(1-4), 235-250.

*Cullaj, A., Lazo, P., Duka, S. (2006).* Heavy metals and metallonine levels in mussel samples from the Albanian sea coast, MAP/Med Pol., Biological Effects Monitoring Programme, MAP Technical Reports Serial No.166, Alessandria, Italy, pp. 141-151.

FAO (2007b). Fisheries and Aquaculture Department, National Aquaculture Sector Overview, Montenegro. http://www.fao.org/fishery/countrysector/naso montenegro/en

*Joksimovic, D. (2010).* Eutrophication in Sea Water of the Montenegrian Coast at Adriatic Sea in 2005-2007. BALWOIS 2010 Conference, 25-29 May 2010, Ohrid, Republic of Macedonia, 2010-155, 1-4.

Joksimovic, D., Tomic, I., Stankovic, R. A., Jovic, M., Stankovic, S. (2011). Trace metal concentrations in Mediterranean blue mussel and surface sediments and evaluation of the mussels quality and possible risks of high human consumption. *Food Chemistry*, in press.

Jovic, M., Stankovic, R.A., Slavkovic Beskoski, L., Tomic, I., Degetto, S., Stankovic, S. (2011). The environmental quality of the coastal water of the Boka Kotorska bay (Montenegro) using mussels as a bioindicator. Journal of Serbian Chemical Society, ISSN 0352-5139, in press.

Scancar, J., Zuliani, T., Turk, T., Milacic, R. (2007). Organotin compounds and selected metals in the marine environment of Northern Adriatic Sea. *Environmental Monitoring and Assessment*, 127 (1-3), 271-282. *Stankovic, S., Jovic, M., Stankovic, R.A., Katsikas, L. (2011).* Pollutant trace elements in the Mediterranean mussel *Mytilus galloprovincialis* as seafood: risks to human health. Book Series: Sustainable Agriculture, in Environmental Chemistry, Volume 2, (E. Lichtfouse, E., Hamelin, M., Navarrete, M., Robert, D. eds.), *Springer*, New York, USA, in press.