

## MICROBIAL CONTAMINATION OF MYTILUS GALLOPROVINCIALIS IN BUTRINTI LAGOON: PUBLIC HEALTH RISK AND CONTROL STRATEGES KONTAMINIMI MIKROBIAL I MYTILUS GALLOPROVINCIALIS NE LAGUNEN E BUTRINTIT: RREZIKU I SHENDETIT PUBLIK DHE STRATEGJITE E KONTROLLIT

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### PËRMBLEDHJE

Kontrolli mikrobiologjik i molusqeve dhe habitatit te tyre konsiderohet nje process i rendesishem i lidhur me sigurine ushqimore. Escherichia coli eshte nje bakter i gjetur ne fecet e te gjithe kafsheve me gjak te ngrohte, perfshire ketu edhe njeriun. Ne Europe dhe kudo, perdoret si tregues i cilesise higjenike te molusqeve. Niveli i E. coli ne molusqe tregon nivelin e ndotjes fekale ne te cilen ato jane ekspozuar dhe percakton trajtimin qe kerkohet perpara konsumit te tyre. Ky studim eshte kryer ne molusqe (Mytilus galloprovincialis) ne Lagunen e Butrintit nga tre stacione: Veri, Jug, Perendim, gjate janar 2009 - dhjetor 2010. Rezultatet tregojne se gjate 2010 vlerat e E.coli ne molusqe jane shume me te larta se ne vitin 2009. Gjithashtu veme re se vlerat e tre stacioneve jane me te larta ne Perendim. Klasifikimi i molusqeve ne zone B dhe C tregon se eshte domosdoshmerisht e nevojshme te kryhet depurimi.

**Fjalet kyce:** molusqe bivalve, E.coli, zona B, depurimi,  $\beta$ -glucuronidase-pozitive

### SUMMARY

Microbiological control of bivalve mollusks and their habitat is considered an important process related to food safety. Escherichia coli is a bacterium found in the feces of all warm-blooded animals, including man. In Europe and elsewhere it is used as an indicator of the sanitary quality of shellfish. The level of E. coli in shellfish shows how much faecal pollution they have been exposed to and determines what treatment shellfish require before they are consumed. This study was conducted on shellfish (Mytilus galloprovincialis) taken from Butrinti lagoon in three stations: North, South and West, during January 2009 - December 2010. The results show that during 2010 the values of E.coli in shellfish are much higher than in 2009. We also notice that the values are higher in the west station. Classification of shellfish in B and C production area means that it is necessarily to perform the depuration process.

**Key words:** bivalve mollusks, E.coli, B area, depuration,  $\beta$ -glucuronidase-positive

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### INTRODUCTION

Human habitation has had a dramatic effect on the condition of the nation's coastal habitats and resources. A primary concern in shellfish growing areas is contamination from human sewage and animal wastes and the related health risks associated with the consumption of

contaminated shellfish[1]. Main sources of fecal pollution include municipal sewage systems, on-site sewage systems, storm water runoff, marinas and boaters, farm animals, pets and wildlife. As is the case with other coastal habitats, the condition and classification of shellfish growing areas generally correlate with human population

densities and land uses in the adjacent shorelines and uplands[4]. Population growth and development are rapidly changing the landscape of Butrinti Lagoon and, in turn, are placing greater pressure on shellfish harvesting [7]. When left unchecked, the process of urbanization can leave coastal areas permanently unfit for the harvest and consumption of shellfish [8].

The purpose of this study is to perform the classification of shellfish (*Mytillus galloprovincialis*) [10] production areas in Butrinti lagoon (North, South and West station).

to in the harvesting area [5]. This determines what, if any, treatment shellfish require before they are consumed. Based on the levels of *E.coli* in bivalve mollusks [11] is performed the classification of production areas as a very important economic criteria for their export to EU countries. According to the European Community under Regulation 854/2004, from a microbiological point of view, the production areas are classified into four categories: A, B, C and D (Table 1)

**MATERIALS AND METHODS**

This study was conducted on samples of bivalve mollusks (*Mytilus galloprovincialis*) taken from Butrinti lagoon during the period January 2009 - December 2010, where it is analyzed a total of 147 samples (Table 2). Samples were taken from three stations of Butrinti lagoon: North, South and West. (Figure 1).

Classification of production areas	Microbiological standards for 100g of meat and liquor	Treatment required
A	≤230 <i>E. coli</i> /100g meat and liquor	May go direct for human consumption
B	230 - 4 600 <i>E. coli</i> /100g meat and liquor	Must be depurated, heat treated or relayed to meet Class A requirements
C	4 600 - 46 000 <i>E. coli</i> /100g meat and liquor	Must be relayed for 2 months to meet Class A or Class B requirements - may also be heat treated
Prohibited	> 46 000 <i>E. coli</i> /100g meat and liquor	Harvesting prohibited

**Table 1.** Production areas according to the criteria of classification and treatment

Microbiological control of bivalve mollusks and their habitat is considered an important process related to food safety, and is also a duty and directive of European Community, Directive 91/492. *Escherichia coli* (*E. coli*) is a bacterium found in the feces of all warm-blooded animals, including man[6]. In Europe and elsewhere it is used as an indicator of the sanitary quality of bivalve shellfish. The level of *E. coli* in bivalve shellfish shows how much fecal pollution (human sewage or animal waste) they have been exposed

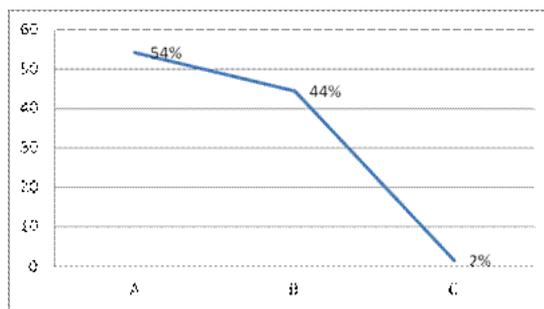


**Figure 1.** Map of Butrinti lagoon: three stations: N, S, W

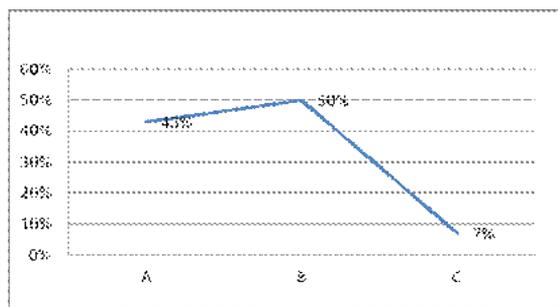
For analysis performance is used the operative international standard method, ISO 16649- 3 [18], which aims to count *Escherichia coli* β - glucuronidase-positive in bivalve mollusks, ISO 6887-3[19] to prepare and test samples of successive decimal dilution. While ISO 7218[17], is used for the interpretation of results. Test samples were prepared using 15-30 representatives (live bivalve mollusks) for each sample. Mollusks analyses were performed using the muscular and also the intravalvular fluid [16]. Each shell was washed with a special brush in

running water, especially in the opening side. Furthermore, they were rinsed with distilled water, placed on a plate and covered with an absorbent paper. For each sample was homogenized 10 g, using Buffered Peptone Water (BPW) in the amount of 90 ml, up to 2 minutes, preparing the initial suspension, as sample for the test. From the initial suspension were prepared subsequent dilution  $10^{-2}$ ,  $10^{-3}$  which were subsequently inoculated in selective enrichment medium, Mineral Modified Glutamate Medium (MMGM), 3 x 5 tubes and were incubated in the thermostat at  $37 \pm 1^\circ \text{C}$  for  $24 \pm 2 \text{ h}$ . After incubation MMGM tubes were checked for the production of acid and gas in Durham tubes [20].

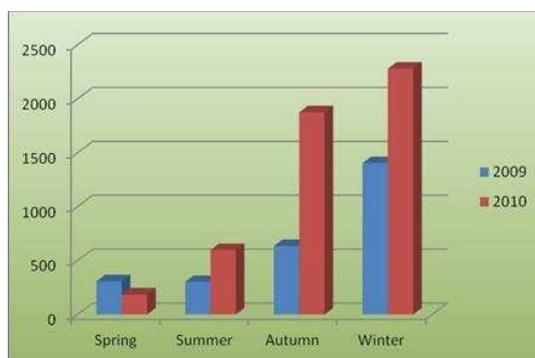
covers a higher percentage than the other two zones (B and C). Although area B is the lowest percentage during this year, this percentage still remains a concern for the consumption of bivalve mollusks of this lagoon [9].



**Graphic 1.** The percentage of A, B and C area during 2009



**Graphic 2.** The percentage of A, B and C area during 2010



**Graphic 3.** Average values of E.coli depending on the seasons

Year	North	South	West	Total
2009	24	24	24	72
2010	25	25	25	75

**Table 2.** Samples of mollusks analyzed during the period January 2009 - December 2010

Positive samples were inoculated in the second selective medium Glucronic Tryptone Bile agar (TBX) which is a chromogenic medium and later were incubated in  $44 \pm 1^\circ \text{C}$  for  $21 \pm 3 \text{ h}$ . The presence of green to blue colonies as a result of the action of  $\beta$ -glucuronidase enzyme on the TBX medium indicates the presence of *Escherichia coli* in MMGM tubes.

On the basis of positive tubes for each dilution, the Most Probable Number (MPN) acquired is calculated on the basis of a chart of the methodology presented in ISO 7218.

**RESULTS AND DISCUSSIONS**

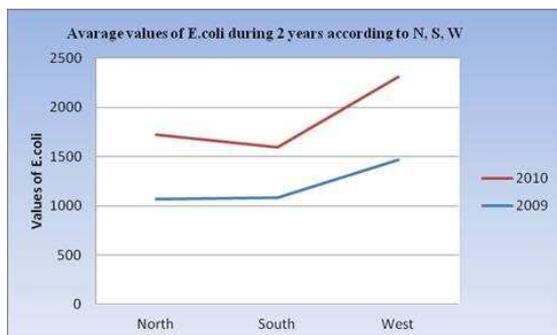
From the study conducted on 147 samples of bivalve mollusks, at three stations: North, South and West were obtained the results that are presented in the charts below.

In graphics 1 and 2 are presented the percentage of mollusks production areas (A, B, C) as in 2009 as well as in 2010. We notice that in 2009, Area A

In 2010, is distinguished that mollusks production areas have increased compared to 2009. Also is quite interesting the fact that C area during this

year holds a value of 7%, which in contrast to other years appears significantly increased. Mollusks which are classified as C area production, according to EC regulations must perform depuration as an obligatory process for a very long period of time [15].

In the graphic 3 are presented average values for E.coli but separated two years depending on the seasons. The average values, in autumn and winter are higher, and clearly prevail over the other two seasons. This shows that the factors which affect the values of E. coli are present and exercise greater action or for a longer time compared with two other seasons [13]. In the same graphic we see that during 2010, the average values of E. coli in the summer, autumn and winter have increased almost two fold compared to the average values of these seasons in 2009.



**Graphic 4.** Average values during two years

In the graphic 4 are combined the average values during two years (2009 and 2010), according to the lagoon stations. We notice that during the two years the value curves have a correlation between them. The values of E. coli in bivalve mollusks grow toward the West station, which makes it the most polluted station of Butrint lagoon. This may come as a result of multiple feeds and water flows from rural areas without being disinfected before.

Classification of shellfish in the area B and C of production means that they must necessarily pass the depuration process, to minimize the values of E. coli, before issuing their market. Factors which affect the high values of E. coli, in

the Butrinti lagoon, may be municipal sewage and private activities, industrial wastewater sources, rain precipitation and river flows from Bistrica. [12]

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