MICROBIAL CONTAMINATION OF MOLLUSKS IN BUTRINTI LAGOON

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Abstract

Food borne disease in our days remains an important issue for public health because they cause infection to consumers and important economic damage to take in consideration.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 Europian Community, Directive 91/492. Escherichia coli (E. coli) is a bacterium found in the faeces of all warm-blooded animals, including man. 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 faecal pollution (human sewage or animal waste) they have been exposed to in the harvesting area. This in turn determines what, if any, treatment shellfish require before they are eaten. EU food law stipulates monitoring, classification and resulting treatments. 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 132 samples. Samples were taken from three stations: North, South and West. Our results show an average value at a range 900 *E.coli* /100 g, and classify production area of bivalve mollusks as B area (14.84% belong to A area and B area 85.16%). Such results, make this lagoon to be classified in a quite polluted production area, which means that before putting them into the market these mollusks must be depurated, which is an obligation and a very important process that minimizes *E.coli* values.The analitical results obtained during the microbiological control of the samples taken in this study also indicate that West station of the lagoon is the most polluted.

Key words: bivalve mollusks, *E.coli*, B area.

1. Introduction

Food borne disease in our days remains an important issue for public health, because they cause infection to consumers and important economic damage to take in consideration [11].

Taking in cosideration the high risk, different international organizations such as WHO, FAO, [6] and also statistic agencies of different countries, draw up global strategies of food safety, and publish evidence for import-export products in relation with unprocessed and proccesed foods [7].The study of *E.coli* in bivalve mussels is chosen as a study, not only for the importance on consumer's health, but also for the exportation of bivalve mussels in European Community [2].

In Albania there is no evidence about the prevalence of this pathogen caused from the consumption of bivalve mollusks[10].Current studies are partial and with very limited data related with the incidence of pathogenes in bivalve mollusks.

The incidence determination and the quantitative evaluation of *Escherichia coli* in bivalve mollusks (*Mytilus galloprovincialis*) in Butrinti Lagoon, represents the aim of this study.

The greatest potential for microbiological contamination during the production of bivalve mollusks is considered their habitat [8].

The level of contamination with bacteria and viruses in the growth zone is considered as crucial for the classification of the final product and determines the needs of their further processing [3].The study of pathogen *E.coli* is related with the importance that represents this microorganism in the determination of freshness and hygienic parameters in bivalve mollusks [9].

Based on the levels of *E.coli* in bivalve mollusks is performed the classification of production areas as a very important economic criteria for their export to EU countries [16].

The studies have shown that consumption of bivalve shellfish contaminated with *E. coli* pathogen

causes toxinfections and intoxications to the customer with a prevalence of 40-60%[5].

According to the European Community under Regulation 854/2004, from a microbiological point of view, the production areas are classified into four categories [6].

2. Material 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 132 samples. Samples were taken from three stations of Butrinti lagoon: North, South and West.

For analysis it is used the operative international standard method, ISO 16649- 3 [14]. This Technical Specification specifies a horizontal method for the enumeration of β-glucuronidase –positive Escherichia coli.ISO 6887-3[15] is used to prepare and test samples of successive decimal dilution . While ISO 7218 [1], 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 [1]. 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 the preparation of test samples were homogenized 10 g of mollusks and 90 ml Buffered Peptone Water (BPW).

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 ± 1o C for 24 ± 2 h . After incubation MMGM tubes were checked for the production of acid and gas in Durham tubes [5].

Positive samples were inoculated in the second selective medium Glucoronic Tryptone Bile agar

(TBX) which is a chromogenic medium and later were incubated in 44 ± 1 ° C for 21 ± 3 h. The presence of green to blue colonies as a result of the action of β-glucuronidase enzyme on the TBX medium indicates the presence of *Escherichia coli* in MMGM tubes (figure 1).

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.

For the statistical analysis it is used a analytical computer program: STATA 10.

3. Results and Discussion

From the study conducted on 132 samples of bivalve mollusks, at three stations: North, South and West were obtained the results that are presented in the table 2.

In table 1, from a microbiological point of view, is shown the percentage according to the classification areas based on Regulation 854/2004 of the European Community and on the three stations in the study.

As noted in the figure 2 during the two years of study, B area prevails in microbiological classification of bivalve mollusks from Butrinti lagoon (14.84% belong to A area and B area 85.16%). Such results, make this lagoon to be classified in a quite polluted production area, which means that before putting them into the market these mollusks must be depurated, which is a very important process that minimizes *E.coli* values [13].

In the figures 3 and 4 are shown by months the average values for *E. coli* for the two years period, always based on the classification of A and B areas.

It is noted that, despite months during the two years prevails over significantly the B area which highlights again the fact that depuration should be a mandatory process before issuing the shellfish market.

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

Classification of production areas	Microbiological standard s for 100g of meat and liquor	Treatment required
A	≤230 *E. coli* /100g meat and liquor	May go direct for human consumption
B	230 - 4 600 *E. coli*/100g meat and liquor	Must be depurated, heat treated or relayed to meet Class A requirements
C	4 600 - 46 000 *E. coli* /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 *E. coli* /100g meat and liquor	Harvesting prohibited

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

Year	North	South	West	Total
2009	20	20	20	60
2010	24	24	24	72
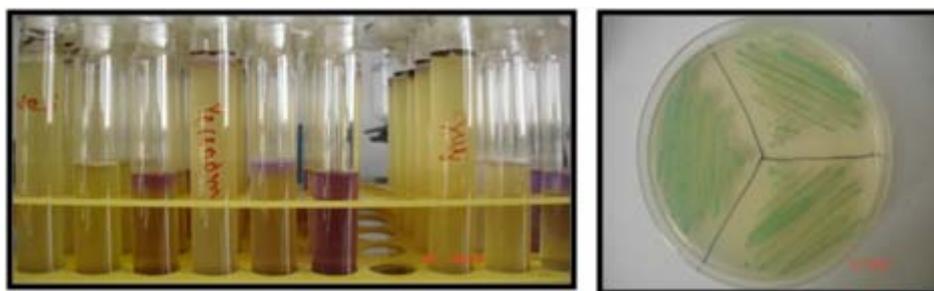
Figure 1: Change of color in MMGM tubes as a result of acid production (in yellow) and typical colonies of *E.coli* in TBX medium.
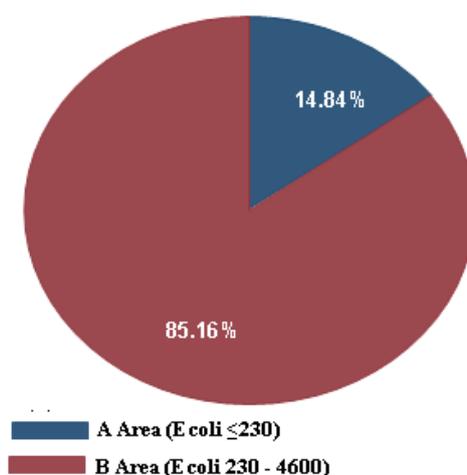
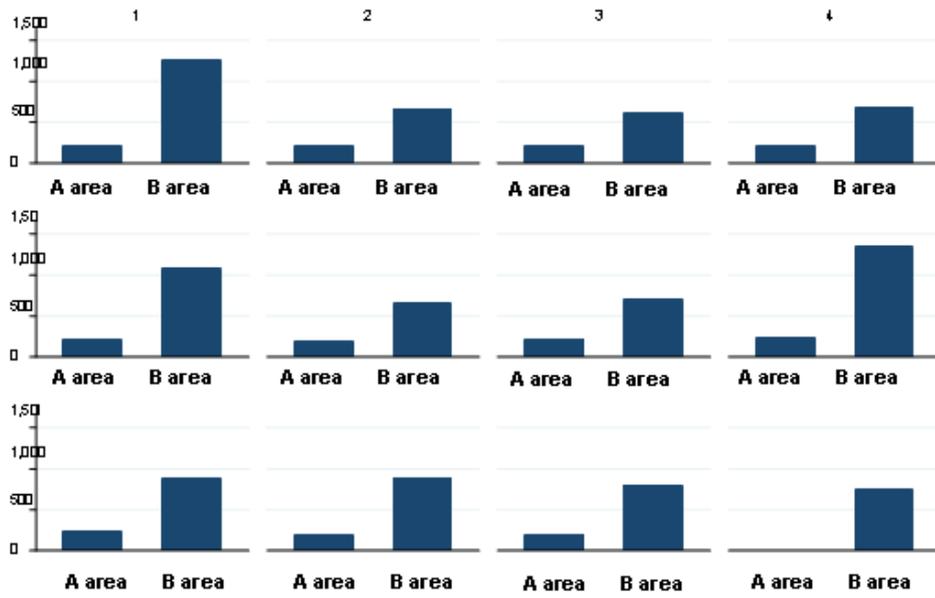**Figure 2:** A and B area in bivalve mollusks classification of Butrinti Lagoon.

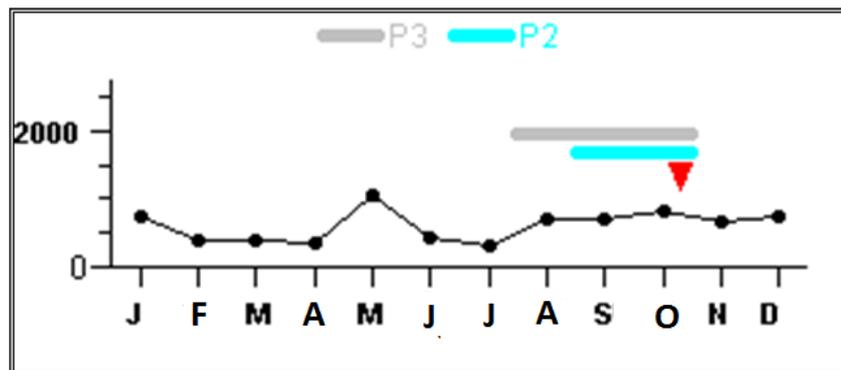**Figure 3:** Mean values of *E. coli* according to A and B areas.

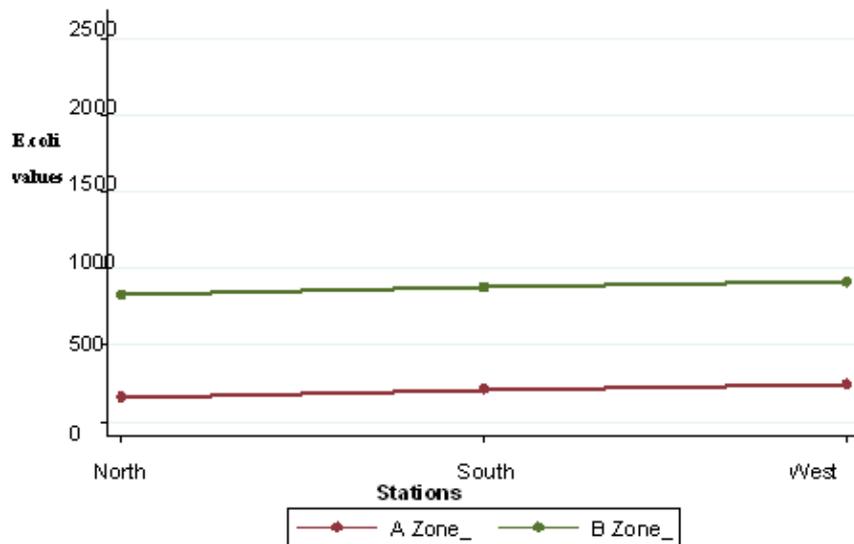**Figure 4:** Mean values of *E. coli* during the years 2009-2010.

Figure 5: Mean values of E. coli during 2 years according to station N, S, W.

By statistical analysis (analytical computer program STATA 10):

Peak date = October 24th

Chi-square = 142.39 (DF = 2} P = 0.000

2-month peak: September to October

21.3% of events

Test statistic = 10.32 P < 0.005

3-month peak: August to October

30.8% of events

Test statistic = 10.92 P < 0.005

Results obtained show that in May are noticed the maximum values of *E. coli*, but since these results are taken from analysis of two samples on the same date, it is considered as an event not very significant because these values do not persist in time. This phenomenon can be explained as a result of deficiencies in sampling and inadequate conditions of transport. Statistical analysis conducted shows that the 2-month peak values of *E. coli* were in September and October with 21.3% of cases. Months August-September and October represent 30.8% of cases, of *E.coli* peak values received during the 2 year analysis.

Analytical results obtained during the bacteriological control of the samples in the study show that many factors affect the level of pollution of water, constituting a major source of contamination of bivalve mollusks [12]

As a very populated area (at the time of peak values obtained: in particular tourism) the possibility of fecal contamination remains one of the main reasons [7] for the results obtained in this season of the year.

Inspection carried out, indicates that rural fecal waters flow into the sea without previous treatment or disinfection [4].

Finally, we present in the figure 5 of the values of *E.coli* about three stations in the study. It is seen a growing trend towards the west of the lagoon due to multiple feeds and water flows from rural areas without being disinfected before.

Based in all the figures and tables above, the results indicate that during the two years our values represents an average of about 900 g. E.coli/100.Based on the European Community Regulation 854/2004 these mollusks production areas are classified as B zone and require further treatments such as depuration, relocation to an A area or treatment with an approved method. Among these treatments depuration is a obligatory process and very important to reduce the values of E. coli.

4. Conclusions

- In the analyzed samples, during period 2009-2010, in three stations: north, south and west, in the classification, dominates significantly B area against A area (85.16% B area and 14.84% A area);

- The highest values of *E.coli* are seen in West station, due to multiple feeds and water flows from rural areas without being disinfected before. These values make this station more contaminated than two others;

- The classification of shellfish production in the B area shows that they must necessarily pass through the depuration process, to minimize the *E. coli* values of, before issuing their market;

- Factors affecting the *E. coli* presence to high values are from Bistrica flows as a result of heavy rainfalls, high temperatures during the year, which significantly favor the *E.coli* growth. Also a very significant influence has the presence of residential areas near the Butrinti Lagoon (especially in the West station) and the lack or failure functioning of septic tanks and animal grazing near the lagoon.

5. References

1. Austin B and Austin D: **Methods for the microbiological examination of fish and shellfish.** 2002, 273-293.

2. Bell Ch, Kyriakides A: *E.coli*, a **practical approach to the organism and its control in foods.** 2007.

3. Bijo B.: **Udhëzues i kontrollit hogjeno-sanitar të produkteve ushqimore me origjinë shtazore.** 1997, 27-39.

4. Chinabut S, Somsiri T, Limsuwan C, Lewis S: **Problems associated with shellfish farming,** *Rev. sci. tech. off. int. Epiz*: 2006, **25** (2), 627-635.

5. Çabeli P: **Bakteriologjia-Mykologjia Veterinare**. 2006.

6. FAO Fisheries & Aquaculture Mytilus galloprovincialis. **http://www.fao.org/fishery/culturedspecies/Mytilus_galloprovincialis**

7. Flloko A: **Aquaculture in Albania. Eurofish Magazine** 2002, 2, 49-66.

8. Flloko A: **A short overview of the status of aquaculture in Albania**. *Studies and reviews, interactions between aquaculture and capture fisheries* 2005, (78).

9. Glasoe S, Christy A: **Coastal Urbanization and Microbial Contamination of Shellfish Growing Areas**, Publication No: PSAT 04-09, 2004.

10. Loubersac L, Do Chi T, Fiandrino A, Jouan M, Derolez V, Lemsanni A, Rey-Valette H, Mathe S: **Microbial contamination and management scenarios in a Mediterranean coastal lagoon**. *TWM, Transit. Waters Monogr.* 2007, 1, 107-127, University of Salento – SIBA.

11. Mussel - Wikipedia, the free encyclopedia **http://en.wikipedia.org/wiki/Mussels**

12. Sulaj K, Beli E, Bijo B, Telo D, Shalari Y: **Bacterial monitoring of Albanian water coastline and live bivalve mollusks on 2003.** *Albanian Journal of Agriculture Science,* 2004, **1** (3), 87-92.

13. Technical Committee lSO /TC 34:ISO 7218: **Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations.** *Proceedings of The international Organization for Standardization,* 2007.

14. Technical Committee lSO /TC 34, Food products: **ISO/TS 16649-3 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of P-glucuronidase-positive Escherichia coli - Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-P-Dglucuronide.** *Proceedings of The international Organization for Standardization,* 2005.

15. Technical Committee lSO /TC 34: **ISO 6887-3: Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 3: Specific rules for the preparation of fish and fishery products.** *Proceedings of The international Organization for Standardization,* 2004.

16. Yilmaz Ü, Bülgün B: **Occurrence of Vibrio and Other Pathogenic Bacteria in Mytilus galloprovincialis and Venus gallina Harvested from the Marmara Sea**, *Turk J Vet Anim Sci* 2005, **29**, 409-415.