

## STUDY OF MULLET FISH (*MUGIL CEPHALUS*) CONTAMINATION (BY TOTAL FLORE AND *STAPHYLOCOCCUS AUREUS*) IN THE GULF OF ANNABA / EAST OF ALGERIA

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### Résumé

Dans la wilaya d'Annaba, la pêche du mullet représente 36% des poissons pélagiques vendus sur le marché local [1]. En fait, peu de données étaient disponibles sur la qualité microbiologique du poisson frais destiné pour la consommation humaine. Des Mesures mensuelles du Mulet ont été prises à partir au golf. Les échantillons ont été analysés au cours d'une année d'études. Les résultats de la recherche et le dénombrement de la flore totale et *Staphylococcus aureus* mettre en proéminence le rôle de Oued Seybouss dans le maintien d'un niveau élevé de *Staphylococcus aureus* dans la zone 02. La présence de forte concentration de *Staphylococcus aureus* qui menassent la santé du consommateur en particulier par leur toxine thermostable connus sous le nom staphylococcique.

**Mots clés:** Flore totale, *Staphylococcus aureus*, analyse de la variance, *Mugil cephalus*, golfe de Annaba.

### Abstract

In the province of Annaba, mullet fishing represents 36% of pelagic fish sold at the local market [1]. In fact few data were available as well as the the microbiologic quality of this fresh fish distrebuted for the human consumption. This fish frequents lake mouths mostly brakish water. Monthly measuremnets have been undertaken starting from a Mullet fish captured at the golf and to be destrebuted for human consumption. The samples have been analysed during a year of study. The results of the research and the enumeration of Total flora and *Staphylococcus aureus* bring into prominece the role of Seybouss lake in maintaining a high level of *Staphylococcus aureus* in area 02. The highly concentrated presence of *Staphylococcus aureus* menace the health of the consumer especially by thermostable toxins known as staphylococciques.

**Keywords:** Total flora, *Staphylococcus aureus*, variance analysis, *Mugil cephalus*, gulf of Annaba.

### ملخص

في محافظة عنابة، البوري يمثل 36% من أسماك السطح التي تباع في السوق المحلية [1]. في الواقع البيانات المتاحة قليلة حول النوعية الميكروبيولوجية لهذه الأسماك الطازجة الموجهة للاستهلاك البشري. هذه الأسماك تتردد في الغالب على أماكن وجود المياه القليلة الملوحة. قمن بلخذ عينات بصفة شهرية من سمك البوري المصطاد على مستوى خليج عنابة و الموجه للاستهلاك البشري. أجريه التحاليل للعينات خلال عام من الدراسة. نتائج البحوث والتعداد للبكتريا من نوع *Staphylococcus aureus* و flore totale تبين دور واد سييوس في الحفاظ على مستوى عالي من البكتريا *Staphylococcus aureus* في المنطقة 02. كذلك وجود خطر كبير راجع للتركيز العالية على صحة المستهلك خاصة من قبل السموم المعروفة باسم staphylococciques و المقاومة للحرارة.

**الكلمات المفتاحية:** *Staphylococcus aureus*، flore totale، تحليل التباين، *Mugil cephalus*، خليج عنابة.

*Mugil cephalus* is a type of fish that often frequents coastal ecosystems. Estuarine or lagoon, mullet fish spread broadly in those areas and covers both temperate and tropico-equatorial regions that have a major economic importance both for aquaculture and animal nutrition in terms of flour or fish oil.

North Africa has an availability of brackish water, the *Mugil cephalus* is very popular, and its economic importance is great because it is centralized as the subject of specialized fishery.

In Algeria, *M. Cephalus* is distributed over the entire coastline especially lagoon systems that pour into the Mediterranean Sea.

The Gulf of Annaba is located in eastern Algeria, it is bordered by alignments of virtual references, It is spread over more than eighty kilometers from Cap de Garde in the West to Cape Rosa in the east.

Several valley drainings are linked with the Gulf of Annaba such as Seybouss River that extends from the province of Annaba and Mafrag River that ranges from the province of El Tarf.

Many pathogens represent a threat to human health and the marine ecosystem [2], [3].

In order to estimate the risk of microbiological contamination of Total flora also called Total mesophilic aerobic flora and *Staphylococcus aureus* that affects the consumers' health. We studied the availability followed by the counting methodology of these bacteria Total flora and *Staphylococcus aureus* in *M. Cephalus* caught in East Mediterranean.

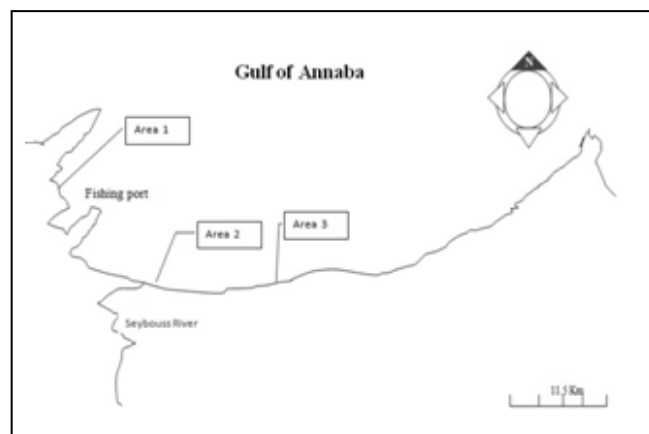
## MATERIALS AND METHODS

### Presentation of the study area

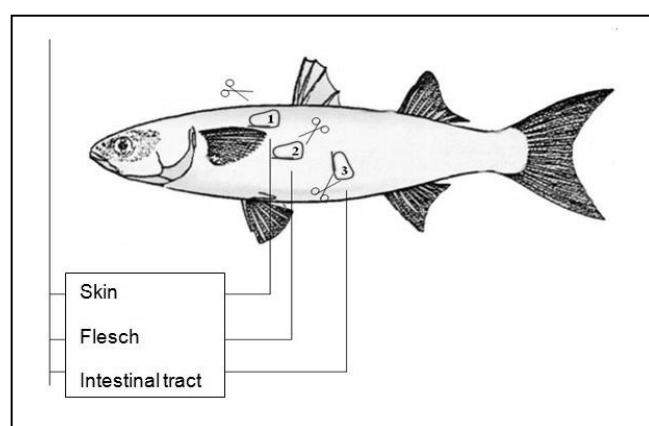
The Gulf of Annaba is located in eastern Algeria, it is divided between two province "Annaba and El Tarf" (Figure 1) three areas are selected in this study, two At Annaba: (area 01 represented by grounding beach "La Caroube" and area 02 represented by the area of grounding beach "Sidi Salem"), and one at El Tarf (represented by the Large beach "El-Batah") [4]

### Period and sampling method

We take aseptically and randomly 45 individuals a month for one academic year (October 2009 - September 2010). Three abstraction levels are selected, listed from outside to inside as a result of the skin, flesh and digestive tract (Figure 2).



**Figure 1:** Localization of study areas in the gulf of Annaba [5].



**Figure 2:** Sampling level in the Mullet fish (*Mugil cephalus*).

### Method for microbiological analysis

According to [6] (Total flora) and to [7] (*Staphylococcus aureus*):

Concerning Total flora (aerobic germs obtained at 30° C). The are put at the bottom a petri dish in PCA (*Plate Count Agar*) medium, poured into two petri dishes with 01ml of premium substance obtained from mixing the flesh, skin and digestive track separately with nutritional medium.

Under the same conditions, seeding dilutions and incubation boxes at 30 ° C, aerobically for 72 hours. From the number of colonies obtained in Petri dishes, we calculate the number of microorganisms per gram of sample.

For the detection and enumeration of *S. aureus* the method used involves seeding the surface of selective medium which is the Baird Parker, cast in two series of boxes, under the same conditions of seeding dilutions obtained from the premium substance and incubation boxes 37 ° C for 24 to 48 h.

The operation is repeated with dilution 10<sup>2</sup> and the following dilutions up to 10<sup>5</sup>. then we calculate the number of *S. aureus* per gram of sample, from the number of typical colonies obtained in boxes chosen at dilution giving a significant result, and confirmed by the coagulase test.

**Method of statistical analysis**

the test is done either by comparing the value of Fobs with a theoretical value F1-α extracted from the table of Fisher F for a significance level α = 0.05 or 0.01 or 0.001 for K1 and K2 and degrees of release, or by comparing the value of the probability P always with the different values of α = 5% or 1% or 0.1%.

According to this hypothesis of equal means is rejected at level α = 0.05, 0.01, or 0.001, is said conventionally that difference is significant, highly significant or very highly significant. It usually marks the differences of one, two or three asterisks [8].

**RESULTS**

Fish consumption with poor quality directly affects the consumer’s health that includes different cooking methods and diets, the mullet fish is consumed with a minimum of cooking especially smoked [9].

**Analysis and statistical results of ANOVA per zone**

Table 01 show that there are differences ranging from high to very high significance between the skin, flesh and digestive track of Total flora while there is a significant difference in three levels of sampling of *S. aureus* in the area 01.

**Table 1 :** Results of variance analysis between the levels of samples, the average number for the Total flora and *S. aureus* of the zone 01.

Bacteria	level	dof	Sa	F	P <sub>rob</sub>	Obs
<i>S.aureus</i>	skin	11	322626	1,380	0,246	ns
	flesh	11	1873	0,710	0,717	ns
	TD	11	9439	1,740	0,125	ns
Total flora	skin	11	2,219E11	4,930	0,001	***
	flesh	11	2,637E13	10,560	0,000	***
	TD	11	2,203E15	4,870	0,010	**

ns = non-significance (P>α =0,05), \* = significance (P ≤ α =0,05), \*\* = highly significant (P ≤ α =0,01), \*\*\* = very high significance (P ≤ α =0,001), dof= degree of freedom, CM= square average , F = F-test (Fisher test) of observation and variety, P<sub>rob</sub>= probability

The values of the probability P for the analysis of variance (Table 02) clearly show that there are no significant differences between sampling levels for skin and digestive tract for *S. aureus* when we observed differences in high to very high significance at all three levels of sampling for the Total flora and in the flesh for *S. aureus*.

**Table 2:** Results of variance analysis between the levels of samples, the average number for the Total flora and *S. aureus* of the zone 02.

Bacteria	level	dof	Sa	F	P <sub>rob</sub>	Obs
<i>S. aureus</i>	skin	11	124942702	0,960	0,509	ns
	flesh	11	3114015	2,910	0,014	**
	TD	11	49436641	1,410	0,232	ns
Total flora	skin	11	2,460E13	1082,15	0,000	***
	flesh	11	1,470E13	3,270	0,007	**
	TD	11	4,520E17	16,300	0,000	***

The results for Zone 03 (Table 03) show that there are no significant differences between the flesh and the digestive tract of *M.cephalus* concerning *Staphylococcus aureus* however, the skin for *Staphylococcus aureus*, flesh and the digestive tract for Total flora range from high to very high significant differences.

**Table 3 :** Results of variance analysis between the levels of samples, the average number for the Total flora and *S. aureus* of the zone 03

Bacteria	level	dof	Sa	F	P <sub>rob</sub>	OBS
<i>S. aureus</i>	skin	11	199672	7,570	0,000	***
	flesh	11	2323	0,400	0,943	ns
	TD	11	2033	0,750	0,686	ns
Total flora	skin	11	3,974E10	1,770	0,118	ns
	flesh	11	13246364	2,860	0,015	**
	TD	11	2,147E11	4,720	0,001	***

**Analysis and statistical results of ANOVA per bacteria**

**Results by level of withdrawals per month for the Total flora in the area 01**

The test analysis of variance (Table 04) indicates no significant differences between levels of monthly average number of samples for the months of December, January, July and September however, we distinguish differences from high to very highly significant differences relatively to the remaining months for Total flora in zone 01.

**Results by level of withdrawals per month for the Total flora in the area 02**

The comparison between intake levels of monthly average using the method of analysis of variance (Table 05) indicates that there is no significant difference five months of the year, while for the remaining months; there are significant differences to very highly significant at all three levels of sampling.

**Table 4:** Results of variance analysis comparison between sampling levels of the average monthly number of Total flora for the area 01

Month	Dof	Sa	F	P <sub>rob</sub>	OBS
October	2	43560000	4,750	0,058	*
November	2	4,761E09	5,290	0,047	*
December	2	360000	2	0,216	ns
January	2	360000	2	0,216	ns
February	2	92160000	512	0,000	***
March	2	9210000	521	0,000	***
April	2	4,244E15	4,720	0,059	*
May	2	4,830E15	5,370	0,0046	**
June	2	9,990E12	0,520	0,617	ns
July	2	43560000	4,750	0,058	*
August	2	43560000	4,750	0,058	*
September	2	1,368E11	1,370	0,324	ns

**Table 5:** Results of variance analysis comparison between sampling levels of the average monthly number of Total flora for the area 02

Month	Dof	Sa	F	P <sub>rob</sub>	OBS
October	2	1.296E9	1.410	0.314	ns
November	2	9.216E9	512	0.000	***
December	2	4.625E9	5.080	0.051	*
January	2	9.575E9	1053.310	0.000	***
February	2	1.139E11	1.050	0.408	ns
March	2	1.307E11	1.420	0.312	ns
April	2	9.861E17	1.1E5	0.000	***
May	2	9.920E17	1.1E5	0.000	***
June	2	1.111E17	1	0.421	ns
July	2	45630000	5.020	0.052	*
August	2	11970000	1.300	0.339	ns
September	2	630000	7	0.027	*

#### Results by level of withdrawals per month for the Total flora in the area 03

The value of the probability P of the test analysis of variance showed in (Table 06) that there are significant differences for the month of January, May and June only, while for the other months of the year for the Total flora of the area 03, there are no significant differences.

#### Results by level of withdrawals per month for *Staphylococcus aureus* at the area 01

A comparison of harvest levels in the average number of monthly *S. aureus* using analysis of variance (Table 07) shows that there are no significant differences during the course of the year, that is to say that whatever the month, the number of bacteria remains constant (during the course of our study) in fish caught in area 01.

**Table 6:** Results of variance analysis comparison between sampling levels of the average monthly number of Total flora for the area 03

Month	Dof	Sa	F	P <sub>rob</sub>	OBS
October	2	11970000	1.190	0.368	ns
November	2	270000	1.500	0.296	ns
December	2	89910000	8.750	4.058	ns
January	2	4.782E9	5.310	0.047	*
February	2	360000	2	0.216	ns
March	2	10890000	1.070	0.400	ns
April	2	1.452E11	1.600	0.278	ns
May	2	4.782E11	5.310	0.047	*
June	2	4.865E11	5.410	0.045	*
July	2	15210000	1.690	0.262	ns
August	2	43560000	4.750	2.058	ns
September	2	39960000	4.400	0.067	ns

**Table 7:** Results of variance analysis comparison between sampling levels of monthly average number of *S. aureus* for the area 01

Month	Dof	Sa	F	P <sub>rob</sub>	OBS
October	2	890178	3.17	0.115	ns
November	2	303333	3.17	0.115	ns
December	2	1111	0.50	0.630	ns
January	2	3211	1.16	0.375	ns
February	2	3333	1.50	0.296	ns
March	2	71111	0.77	0.503	ns
April	2	4444	0.80	0.492	ns
May	2	1111	0.50	0.630	ns
June	2	1111	0.50	0.630	ns
July	2	1111	0.50	0.630	ns
August	2	399011	0.99	0.426	ns
September	2	111111	1.37	0.324	ns

#### Results by level of withdrawals per month for *Staphylococcus aureus* at the area 02

The results obtained at the area 02 (Table 08) show that there are significant to highly significant differences for the months of December, January, February, June and September for the bacterium *S. aureus*, while there is no significant difference during the remaining months.

#### Results by level of withdrawals per month for *Staphylococcus aureus* at the area 03 (Table 09)

Area 03 provides insignificant results for most months of the year except the months of October and December where differences are significant to highly significant.

**Table 8:** Results of variance analysis comparison between sampling levels of monthly average number of *S. aureus* for the area 02

Month	Dof	Sa	F	P <sub>rob</sub>	OBS
October	2	5444444	0,500	0,630	ns
November	2	7444444	0,790	0,497	ns
December	2	38523333	5,270	0,048	*
January	2	2403333	21,420	0,002	**
February	2	490000	5,250	0,048	*
March	2	781111	2,930	0,130	ns
April	2	171444444	0,660	0,552	ns
May	2	270467778	0,840	0,476	ns
June	2	64111111	9,020	0,016	**
July	2	32444444	3,440	0,101	ns
August	2	24111111	0,600	0,581	ns
September	2	86760000	964	0,000	***

**Table 9:** Results of variance analysis comparison between sampling levels of monthly average number of *S. aureus* for the area 03

Month	Dof	Sa	F	P <sub>rob</sub>	OBS
October	2	467778	11,380	0,009	**
November	2	7233	1,120	0,386	ns
December	2	381111	7	0,027	*
January	2	1111	0,500	0,630	ns
February	2	1111	0,500	0,630	ns
March	2	3333	0,600	0,579	ns
April	2	1111	0,500	0,630	ns
May	2	7778	1,750	0,252	ns
June	2	1111	0,500	0,630	ns
July	2	1111	1	0,422	ns
August	2	1111	0,170	0,850	ns
September	2	4444	0,360	0,709	ns

**DISCUSSION**

Eating, in a general sense is a fundamental factor that affects the consumer health. Its effects can be noted on both the quantity and quality of animal products. Although this idea is easily accepted by consumers, knowing the negative effects that follow the consumption of a mediocre fish (contaminated or toxic. In our study we noted many bacteria evolution in terms of number in fresh *M. cephalus*. The peak of 10<sup>7</sup> calculated of Total flora in the area 01 does not make the fish uneatable by human consumption because acceptance limits are not exceeded.

The results obtained from zone 2 show us that there is a high concentration of *S aureus* that dominates over Total flora, this can be explained by the physical quality of water (low salinity) influenced by the arrival of a freshwater (Seybouse River) which empties into the sea at the beach grounding of Sidi Salem in one hand and on the other hand the tolerance of *S. aureus* at a reasonable level of salinity that fluctuates between 37.1 and 37.4 ‰ by location and season in the Gulf of Annaba [10].

This is in coordinance with study done by [11] showed in their study the significant relationship between water quality and the fish that habours it.

The high level of bacteria calculated on the mullet fish skin in area 02 can be justified by the fact that the skin represents the first barrier against all external attacks (bacteria) by the secretion of mucus and fixed asphyxiation bacteria and preventing them from moving towards the organs including the flesh.

The values of counted bacteria in zone 3 are different according to the various sampling levels that include flesh, skin and digestive track.

Despite the number of counted *S. aureus* on the skin of *Mugil cephalus* fishing in Area 03, the fish is still acceptable because the maximum acceptable tolerance and limits are not exceeded, this growth may be the result of discharge from Mafrag River into the wide coastline of El Batah.

On the contrary, Area by 03, which is of good bacteriological quality justified by the relatively small number of the Total flora, because it is a wide open coastline, renewable by the effect of wind, currents and beach swells to a height greater than 0.5 m which strikes towards Cape Rosa [12]. Area 03 is thus constantly renewed.

For the bacterium *S. aureus* :

- the test of analysis of variance between levels of withdrawals, the average monthly number tells us that there are no significant differences between the skin, flesh and digestive track throughout the year of study for area01, while only the months of December, January, February, June and September show significant differences between the skin, flesh and digestive tract.
- Peak recorded in the month of September for the area 02. This can be explained by climatic disturbances characterized by heavy rains during the month of September [13] leading to flooding of agricultural land and farming land nearby, thus promoting fertilization of aquatic medium and development of *S. aureus* (temperature, humidity, salinity ..) on the contrary, during the month of June, we recorded increase in the nombre of staph aureus which only lasts during this month and will decrease during July and August under the influence of solar light, [14] noted that it was difficult to prevent the growth of certain bacteria in the concentration of salt herring less than 6‰.

These results coordinate with different studies conducted by many authors that showed the role of solar light in terms of eliminating the bacteria in salt water [15] [16] [17] [18] [19] this cqn be explained by the bactericide effect of ultra violet radiation cause peroxyde ions that will make the cell permiable to inorganic salt that will alter the osmotic pressure [20].

- Concerning area 03 the peak was recorded during the month of October. This peak can be explained by the reduced volume of water that flooded Mafrag river when the rainfall rate increases. Despite the relatively high level of *S. aureus* in area 03, the risk on consumer health is reduced because the toxins secreted by the bacteria are heat labile.

## CONCLUSION

This present study that was taking place over the course of the year helped us show the importance of differentiation of sampling levels ( skin, flesh and digestive tract) in the same species to measure the number of two bacteria (Total flora and *S. aureus*) in *M. cephalus* that is distributed for human consumption. The samples that we retrieved provided us with various evolutions of bacteria depending on each area (area01, 02,03).

The relationship between the sampling levels and bacteria depend on the location (area). The more aquatic system is rich in fertilizers and organic substance, the number of bacteria increases (the fertilizing elements: light, temperature, humidity unlike the protozoa that plays a major role in reducing the number of bacteria.

- Concerning Total flora in area 01 the number of bacteria changes depending on sampling levels. *Staph aureus* has constant values this means that if the value slow the fish can be consumed and if the values of *S. aureus* are high( limits exceeded) the fish is considered toxic.
- In area 02, number of Total flora does not change during the course of a year. However, the number of *S. aureus* stays variable which means it can menace the human consumption safety, even it is cooked the bacteria in the flesh can resist the high temperature.
- In area 03, the fish caught in this area can be distributed for human consumed throughout the year, and depending on the results obtained this area is not polluted

The installation of a water treatment station upstream of the bey is needed to ensure the protection of consumer health and increase the production of mullet fish by the use of the entire bey (area 02 included) .

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