Research Journal of Animal, Veterinary and Fishery Sciences _____ ISSN 2320 – 6535
Vol. **1(11),** 5-12, December **(2013)** *Res. J. Animal, Veterinary and Fishery Sci.*

Investigation on the Efficacy of the Traditional Depuration Method of Oysters (C*rassostrea gasar*) used in Lakeside Villages of Southern Benin

René G. Dègnon[1*], Euloge S. Adjou[1], Serges Wongla[2], Edwige Dahouenon-Ahoussi[1], Dominique C.K. Sohounhloué[1]
[1]Laboratory of Research and Study in Applied Chemistry Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O.B: 2009 Cotonou, BÉNIN
[2]Department of Control and Monitoring of Fishery Products and Industries, Direction of Fisheries, Ministry of Agriculture, Livestock and Fisheries, 04 P.O.B: 0903, Cotonou, BENIN

Available online at: www.isca.in, www.isca.me
Received 7th November 2013, revised 20th November 2013, accepted 23rd December 2013

Abstract

Amongst fisheries products, oysters have an important role in the provision of need protein for populace. In Benin, there is beginning to be awareness in oyster as a protein resource. The present study aims to investigate the efficacy of the traditional purge method of oysters (Crassostrea gasar) used in lakeside villages of southern Benin. Thus, four lakeside villages characterized by high activity of collection and parking of oysters were chosen. There are Azizakouè, Djondji, Dègouè and Meko. In each lakeside village, a semi-structured survey was conducted and five parking stations were selected for the study. This survey was also coupled with the sampling of oysters before their parking. After three months of parking, sampling was again conducted to assess the efficacy of the parking on the quality of oysters. Results indicated that, before parking, microbial load of coliforms in oyster samples was high (24.10^6 - 3.10^9 ufc/g) with the presence of E. coli and Salmonella spp. However Vibrio spp was not detected in the analysed samples. Chemical pollution characterised by heavy metals such as copper and lead were also detected. After 3 months of parking, the results of microbiological analyzes indicated that the treatment was more effective in Azizakouè where the average microbial load has significantly decreased (p<5%) and at Djondji where the value rose from 10^7 cfu/g to 10^6 cfu/g. However, the results obtained on stations of Meko and Dégouè indicated that the treatment was ineffective. Results of chemical analyses after the parking of oysters, indicated that, this technique had reduced the lead content (15.96% in samples of Azizakouè, 60.30% in samples of Dégouè, 36.88% in samples of Djondji and 61.65% in samples of Meko), but was ineffective on copper content of oysters.

Keywords: Oysters, parking method, microbiological quality, heavy metal.

Introduction

In recent years, rapid population growth was recorded in most countries of the world, especially in African countries, including Benin. This growth was accompanied by a strong urbanization, intensification of industrial activities and the operation of new farmland. All these changes have led to a huge increase in both quantity and variety of both chemical and organic pollutant emissions that reach streams and have adverse effects on aquatic resources and on their consumers. Fishing has an economic and social development place in many African countries. However, several investigations on Benin's rivers indicate that large quantities of pollutants have been transported to lake due to the traffic of petroleum product from Nigeria. Sources of metal contamination include industrial and medical waste[1], pesticides, petroleum products[2], household products, as well as urban and pharmaceutical waste[3]. Domestic and household sources of metal contamination generally occur as a result of corrosion of metal plumbing fittings, galvanised roofs and wire fences [zinc (Zn), cadmium (Cd)], and healthcare products, such as selenium (Se) containing shampoos or Zinc (Zn) containing baby creams[4]. Silver paint containing aluminium (Al), saucepans and utensils[5], are also possible sources of contamination. According to World Health Organisation [6], metals occur in less than 1% of the earth's crust, with trace amounts generally found in the environment and when these concentrations exceed a stipulated limit, they may become toxic to the surrounding environment. The contamination of fresh waters has become a matter of concern over the last few decades[7,8]; and heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms[9], such as oysters (C*rassostrea gasar*).

The genus *Crassostrea* comprises several species that can be found growing on different regions of the coastal areas. The mangrove oyster, *Crassostrea gasar*, constitutes an important source of income in many communities along the Beninan coast. This species is geographically also distributed along the coast of Central West Africa[10], and South America, from French Guiana to Southern Brazil[11]. As seafoods, they are excellent sources of protein but are highly perishable. Their short shelf life poses serious practical problems of their storage and distribution[12]. The raw consummation of Oysters (ingested whole) observed in some parts of the world leading to the transmission of pathogenic organisms[13]. Thus various bacteria that are aetiologic agents of shellfish-related food infection such as

salmonellosis, shigellosis, Vibrio and Hepatitis A virus had been isolated from oysters[13]. Additionally, septicaemia had been reported among consumers of raw oysters from the Gulf of Mexico[14]. In fact, these bivalves in searching of their food, filter a large amount of water they absorb and retain in their organism a considerable amount of waste of any kind in the water (protozoa, bacteria, plankton, or other debris organic suspended in water). To overcome this problem, lake populations who engage in the marketing of oysters, resort to traditional treatment techniques by parking oysters collected for some time before they are marketed. Then the present study aims to investigate the effectiveness of this traditional method in the purification of oysters.

Material and Methods

Site description: The coastal lagoon has about 60 km of length and is situate between *Togbin* and *Grand-Popo*. From *Grand-Popo* to *Djondji*, this lagoon is called *Lagoon of Grand Popo*, and from *Djondji to Togbin*, it is called *Lagoon of Ouidah*. The hydrology of the lagoon is characterized by both flows of salt water from the sea between the periods of December to June and freshwater from *Mono* river, between July and November. Then it is alternately sweet and salty. Indeed, during the rainy season, fresh water from the Mono river invaded the lagoon and the salinity is low for a few weeks depending on the extent of flooding. The climate is equatorial with a succession of four seasons: two rainy seasons and two dry seasons. The rainfall is about 1200 mm/year; average temperatures are around 27°C; insulation approaching 1700 hours /year, while the relative humidity varies from a minimum of 78% to a maximum of 95%[15]. For the present study, four lakeside villages characterized by high activity of collection and parking of oysters were chosen. There are *Azizakouè*, *Djondji*, *Dègouè* and *Meko* (figure 1).

Survey and sampling: In each lakeside village, a semi-structured survey was conducted. The collected information is relative to the collection and parking sites and the parking time. The questionnaire was administered to a group of 6 collectors of oysters in each lakeside village. Then, five parking point were selected for the study. At each study site, oysters collected are sampled before parking. After three months of parking, sampling was again conducted to assess the efficacy of the parking on the quality of oysters. Each sample consists of ten units of oysters collected aseptically. The sampling was performed under aseptic conditions: sterile latex gloves were used to protect hands during the sampling; oyster's samples was collected and packaged in sterile bags in a portable cooler. The entire sampling equipment is sterilized beforehand with alcohol at 90°.

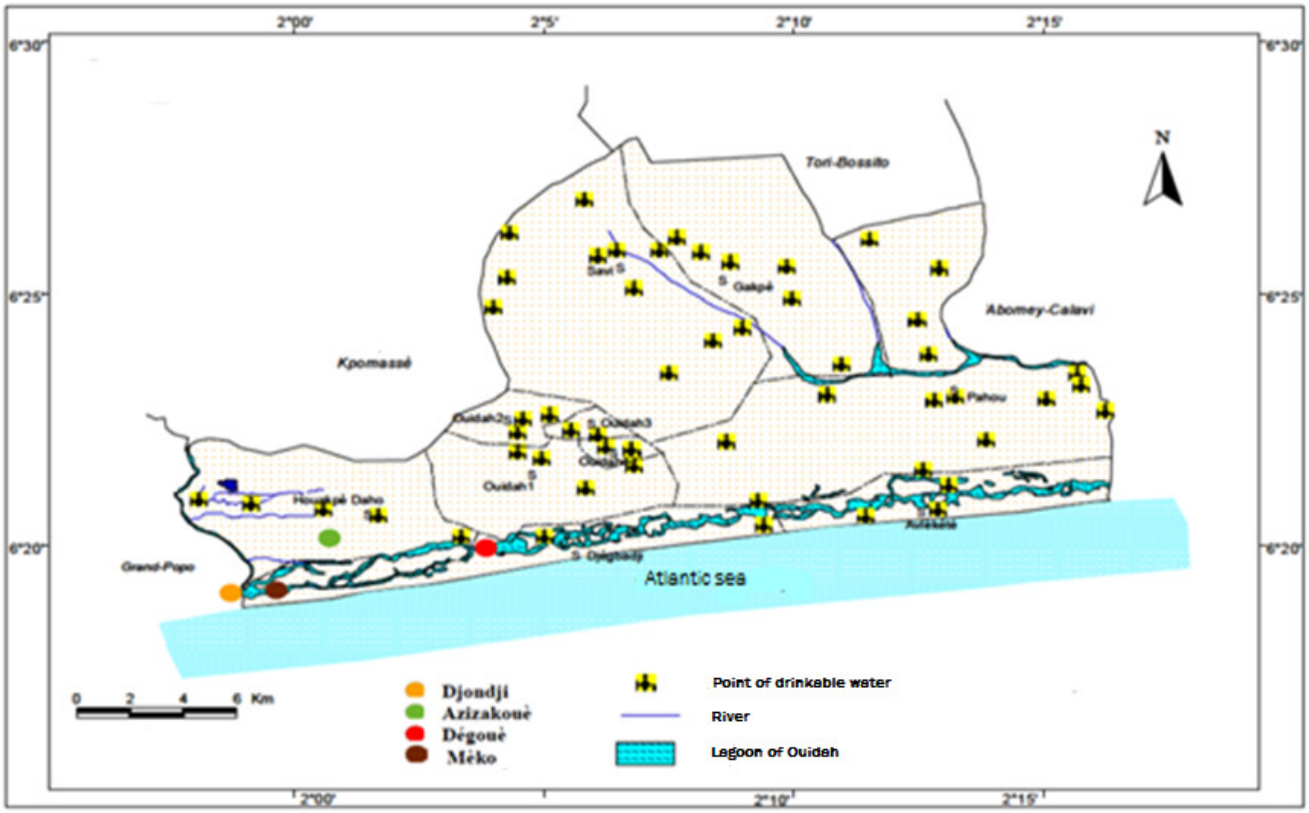

Figure-1
Map of the coastal lagoon of Ouidah and villages of oyster sampling

Microbiological analysis: For the microbiological analyzes, the five oysters collected in each parking point are mixed, and content (flesh and intervalvar water) carefully milled. To 25 g of each milled sample, 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using Eosine Methylene Blue (EMB) medium. The method used for detection of *Salmonella spp*. is that specified by the standard NF V 08-052. The isolation of fungi from samples was performed using dilution plating method. 10 g of each smoked fish sample were added separately to 90 ml of sterile water containing, 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1} dilution. Further, 10-fold serial dilutions up to 10–4 were made. 1 ml volume of each dilution were separately placed in Petri dishes, over which, 10 to 15 ml of potato dextrose agar amended with 60 µg/ml chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days. Fungal isolates from PDAC were sub-cultured on malt extract agar (MEA), and identification was carried out by using a taxonomic schemes primarily based on morphological characters, using the methods described by Singh et *al*.[16]. The detection of *Vibrio spp.* was performed according to the recommendations of the 8th Edition of the Handbook of Analytical Bacteriology FDA (Food and Drug Administration)[17]. Enrichment is made with alkaline peptone water (3% NaCl, pH 8.6) for 24 hours at 37°C. Positivity enrichment results in a bacterial veil in interface air / medium and instant reaction of Indole from Kovacs reagent. Isolation on TCBS (Thiosulfate Citrate Bile salts Sucrose) of an aliquot taken from below the surface of the enrichment broth followed by incubation for 36 hours at 37°C is then performed. The presence of yellow colonies, flat, circular, with raised edge and center, surrounded by a yellow halo (fermentation of sucrose) indicates the presence of *Vibrio spp*. The number of colonies was tracked, using a colony counter. The number of microbial expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution. All media used for microbiological analysis were prepared as indicated by the manufacturer.

Chemical analyses: Aliquots were put in Petri dishes to dry at 120°C until reaching a constant weight. The separated organs were placed into digestion flasks and ultrapure concentrated nitric acid and hydrogen peroxide (1:1 v/v) was added. The digestion flasks were then heated to 130°C until all the materials were dissolved. Digest was diluted with double distilled water appropriately. The elements Cu and Pb were assayed using Atomic Absorption Spectrophotometer (Shimadzu AA 6200).

Statistical Analysis: The analyses were performed as described by Ritterhoff and Zauke (1997). The results presented are the averages of five repeats for each particular sampling point at the different sampling sites, and data analyzed are mean ± SE subjected to one-way anova. Means are separated by the Tukey's multiple range tests when anova was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).

Results and Discussion

Survey: The results of semi-structured surveys realized in the investigated lakeside villages, indicated that the harvesting of oysters is generally makes four times a week. In each village, more than twenty fishers are engaged in the collecting of oysters. This activity is more profitable during the dry season than during the rainy season due to periods of flooding of the lagoon. The average time of the parking varied and is between three months and 36 months. The sale of the oyster is a family activity for most fisher and is either fresh or in processed form. The trade of oyster is considered as a secondary activity. Markets flows are local or sub-regional (Togo, Nigeria). Despite the economical importance of this activity, fishers have received no training related to basic concepts in health, nor modern techniques of oyster treatment. This duration of purge technique can be from two to three months, sometimes years. These mollusks are then returned from time to time to prevent them from eating mud. Results collected from the survey indicated that, the fundamental objective of the parking of oysters is to removal of contaminants by the upturn of filtration and also the multiplication of oysters.

Effect of parking on the microbiological quality of oysters: Tables 1 and 2 showed the results of microbiological analyses performed on oyster's samples before parking and after parking. The analysis of the results showed that, before parking, microbial load of coliforms in oysters sample is very high (24.10^6 - 3.10^9 ufc/g). After 3 months of parking, the results of microbiological analyzes indicated that the treatment was more effective in Azizakouè where the average microbial load has decreased from 30.10^8 cfu /g to 15.10^8 ufc/g and Djondji where the value rose from 10^7 cfu/g to 10^6 cfu/g. However, the results obtained on sites of Meko indicated that treatment was ineffective with a residual flora of 510^8 cfu/g for coliforms. The results obtained in samples collected from the parking sites of Dégouè showed that the microbial load significantly increased (24.10^8 cfu /g to 3.10^9 cfu/ g). Also the evolution of the microbial flora of other germs, including fecal coliforms and *E. coli*, indicated that, in some village the parking technology of oysters was effective in reducing the microbial flora, unlike other village. For the potential pathogens germs, including *Salmonella spp*. and *Vibrio spp*., results indicated that only samples collected at the parking sites of Azizakouè village were contaminated with *Salmonella spp.* before it parking. Unfortunately, the parking technique of oysters has no effect on this pathogenic flora, and they are still in the samples after their parking. For *Vibrio spp*., it was not detected in any of the oyster samples analyzed.

Table-1
Microflora of oysters before parking

Village	Parkage sites	Total flora (UFC/g)	Total coliforms (UFC/g)	Fecal coliforms (UFC/g)	*Escherichia coli* (UFC/g)	*Vibrio spp.* (UFC/g)	*Salmonella spp.* (UFC/g)
Djondji	1	$>3.10^{11}$	7.10^{7}	$46.\ 10^{5}$	$<10^{6}$	Absence	Absence
	2	$>3.10^{11}$	67.10^{6}	$5.\ 10^{6}$	$<10^{6}$		
	3	$>3.10^{11}$	7.10^{7}	$5.\ 10^{6}$	$<10^{6}$		
	4	$>3.10^{11}$	71.10^{6}	$5.\ 10^{6}$	$<10^{6}$		
	5	$>3.10^{11}$	72.10^{6}	$54.\ 10^{5}$	$<10^{6}$		
Average		**$>3.10^{11}$**	**7.10^{7}**	**$5.\ 10^{6}$**	**$<10^{6}$**	**Absence**	**Absence**
Azizakouè	1	$>3.10^{11}$	$>3.10^{9}$	$57.\ 10^{6}$	12.10^{6}	Absence	Presence
	2	$>3.10^{11}$	$>3.10^{9}$	$59.\ 10^{6}$	19.10^{7}		
	3	$>3.10^{11}$	$>3.10^{9}$	$61.\ 10^{6}$	17.10^{6}		
	4	$>3.10^{11}$	$>3.10^{9}$	$63.\ 10^{6}$	18.10^{6}		
	5	$>3.10^{11}$	$>3.10^{9}$	65.10^{6}	$19.\ 10^{6}$		
Average		**$>3.10^{11}$**	**$>3.10^{9}$**	**$61.\ 10^{6}$**	**17.10^{6}**	**Absence**	**Presence**
Meko	1	$>3.10^{11}$	$>3.10^{8}$	33.10^{7}	$68.\ 10^{4}$	Absence	Absence
	2	$>3.10^{11}$	$>3.10^{8}$	45.10^{7}	$7.\ 10^{5}$		
	3	$>3.10^{11}$	$>3.10^{8}$	4.10^{8}	71.10^{4}		
	4	$>3.10^{11}$	$>3.10^{8}$	42.10^{7}	73.10^{4}		
	5	$>3.10^{11}$	$>3.10^{8}$	4.10^{8}	73.10^{4}		
Average		**$>3.10^{11}$**	**$>3.10^{8}$**	**4.10^{8}**	**71.10^{4}**	**Absence**	**Absence**
Dègouè	1	$>3.10^{11}$	$22.\ 10^{6}$	$22.\ 10^{5}$	$<10^{6}$	Absence	Absence
	2	$>3.10^{11}$	25.10^{6}	$22.\ 10^{5}$	$<10^{6}$		
	3	$>3.10^{11}$	24.10^{6}	3.10^{6}	$<10^{6}$		
	4	$>3.10^{11}$	28.10^{6}	$37.\ 10^{5}$	$<10^{6}$		
	5	$>3.10^{11}$	21.10^{6}	$39.\ 10^{5}$	$<10^{6}$		
Average		**$>3.10^{11}$**	**24.10^{6}**	**$3.\ 10^{6}$**	**$<10^{6}$**	**Absence**	**Absence**

Table-2
Microflora of oysters after parking

Village	Parkage sites	Total flora (UFC/g)	Total coliforms (UFC/g)	Fecal coliforms (UFC/g)	*Escherichia coli* (UFC/g)	*Vibrio spp.* (UFC/g)	*Salmonella spp.* (UFC/g)
Djondji	1	$>3.10^9$	8.10^7	$<10^6$	$<10^6$	Absence	Absence
	2	$>3.10^9$	9.10^7	$<10^6$	$<10^6$		
	3	$>3.10^9$	10^6	$<10^6$	$<10^6$		
	4	$>3.10^9$	14.10^7	$<10^6$	$<10^6$		
	5	$>3.10^9$	9.10^7	$<10^6$	$<10^6$		
Average		**$>3.10^9$**	**8.10^7**	**$<10^6$**	**$<10^6$**	**Absence**	**Absence**
Azizakouè	1	$>3.10^{11}$	13.10^9	46.10^7	$<10^6$	Absence	Presence
	2	$>3.10^{11}$	15.10^9	5.10^6	$<10^6$		
	3	$>3.10^{11}$	15.10^9	5.10^6	$<10^6$		
	4	$>3.10^{11}$	14.10^9	5.10^6	$<10^6$		
	5	$>3.10^{11}$	18.10^9	54.10^7	$<10^6$		
Average		**$>3.10^{11}$**	**15.10^9**	**5.10^6**	**$<10^6$**	**Absence**	**Presence**
Meko	1	$>3.10^{11}$	27.10^9	48.10^8	$<10^4$	Absent	Absence
	2	$>3.10^{11}$	24.10^9	43.10^8	$<10^4$		
	3	$>3.10^{11}$	25.10^9	45.10^8	$<10^4$		
	4	$>3.10^{11}$	25.10^9	5.10^7	$<10^4$		
	5	$>3.10^{11}$	24.10^9	39.10^8	$<10^4$		
Average		**$>3.10^{11}$**	**25.10^9**	**45.10^8**	**$<10^4$**	**Absent**	**Absence**
Dègouè	1	$>3.10^{11}$	$>3.10^9$	1.10^7	$<10^6$	Absent	Absence
	2	$>3.10^{11}$	$>3.10^9$	12.10^8	$<10^6$		
	3	$>3.10^{11}$	$>3.10^9$	14.10^8	$<10^6$		
	4	$>3.10^{11}$	$>3.10^9$	15.10^8	$<10^6$		
	5	$>3.10^{11}$	$>3.10^9$	19.10^8	$<10^6$		
Average		**$>3.10^{11}$**	**$>3.10^9$**	**14.10^8**	**$<10^6$**	**Absent**	**Absence**

Effect of parking on the content of heavy metals: Figures 2 and 3 showed the results of evolution levels of heavy metals, particularly copper and lead, in oysters before parking and after parking. The analysis showed that the content of heavy metals in oyster before parking varied from one village to another. This content is between 8.01 mg / kg to 17.39 mg / kg for copper and between 0.78 mg / kg and 2.07 mg / kg for lead. These results indicated the high level of heavy metals in the lagoon water and also the bioaccumulation potential of heavy metals in oyster's organs. Results obtained after the parking of oysters, indicated that, this technique has a very significant role in the reduction of lead content in oyster. Indeed, the rate of lead content down to 15.96% in sample of Azizakouè, 60.30% in sample of Dégouè, 36.88% in sample of Djondji and 61.65% in sample of Meko (figure 1). However, results obtained during the evaluation of the parking effect on copper content indicated that the parking technology is effective in only Mèko and Djondji (figure 2).

Discussion: Amongst fisheries products, oysters play an important role in the provision of need protein for the populace. They are cultivated in large numbers in countries. In Benin, there is beginning to be awareness in oyster as a protein resource and employment for artisanal fishermen. The high level of microbial contamination of oyster indicated however a potential health risks for the consumer. These results could be attributed to the frequent contamination of water with sewage in addition to the endogenous microflora of the oysters. *Crassostrea gasar* is a filter feeder and selects out microorganisms from the surrounding water, concentrating such within its body. It uses the same principles to cleanse itself of such organisms when placed in clean or purified water[18]. The level of bacteria load of the surrounding water therefore, to a large extent determines that of oysters. Ukwade[19], in a microbiological studies of oyster from water source of Lagos lagoon, reported that oysters were discovered to be contaminated with coliform and *E.coli,* and the analysis of water revealed a positive correlation in microbial counts of oysters and the surrounding water.

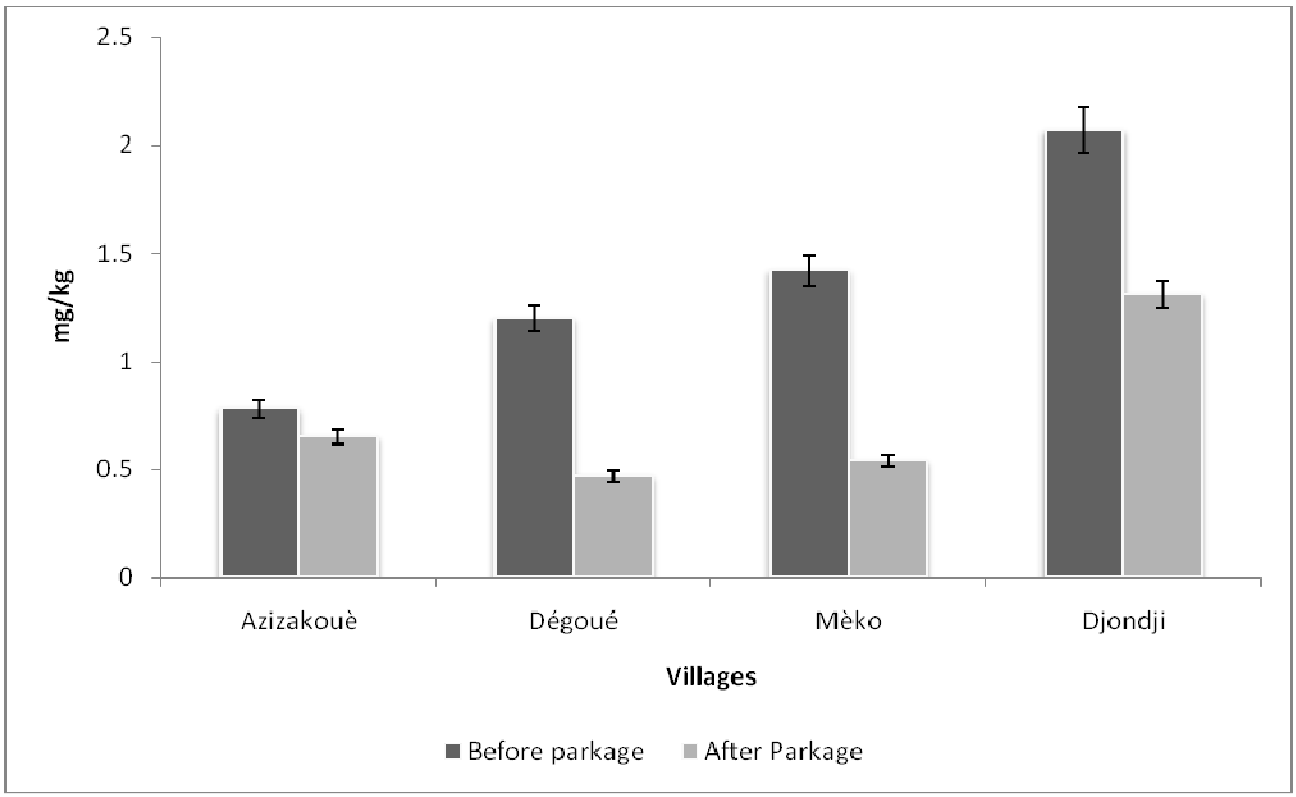

Figure-2
Lead (Pb) content of oyster samples

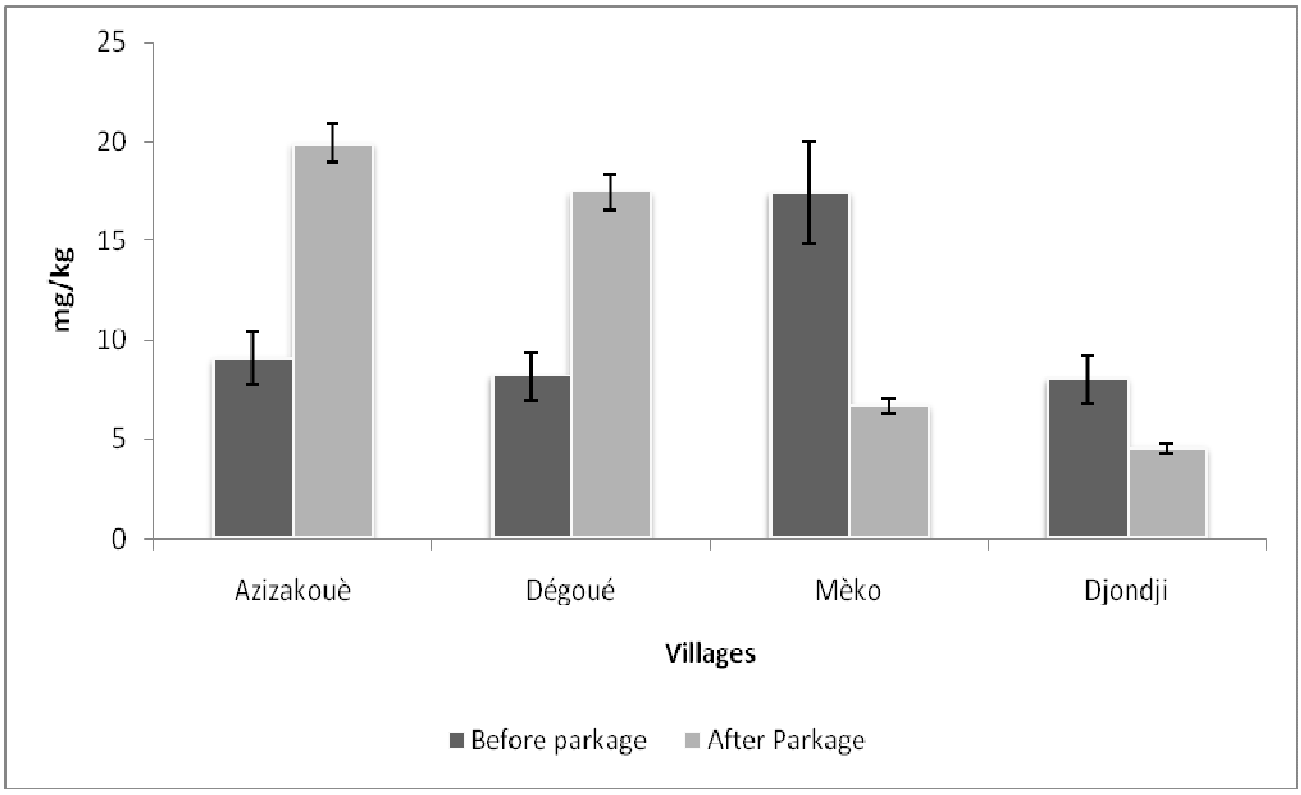

Figure-3
Copper (Cu) content in oyster samples

The presence of *Salmonella spp* in the oyster revealed the possible presence of other pathogens as indicative of the high faecal counts. This is also reported by Ukwade[19]. However, the significant public health problems associated with shellfish arise from their ability to concentrate viruses and bacteria from the surrounding waters[20]. In this study, all oyster samples analysed were not contaminated with *Vibrio spp* and were satisfactory in relation to the requirement of the standard. The results of the evaluation of the microbial flora of oysters after parking showed that this method effectively reduces the microbial contamination. However, the reduction rates are significant in the lakeside villages of Azizakoue and Djondji, then indicates an efficiency of 50%. The non-effectiveness of this method of parking observed in the villages of Meko and Dégoué could be related to the fact that in these areas, the circulatory flow of water in the lagoon is low due to the relief, which does not allow the relatively rapid turnover of the water in which bathes the oysters. According to Dore and Lees[21], if the treatment is done properly, bacteria are generally reduced to undetectable levels, while viruses are reduced to a much lower rate because they are stronger and more persistent than bacteria.

There has been a general global increase in industrial activity over the past few decades, resulting in a significant application of metals in the various processes; in turn causing a great escalation of metals in the environment. Although, some metals are essential to human health and may be found naturally at low concentrations in the body, these may act as toxicants when humans or animals are exposed to high concentrations; which cause wide-ranging toxicological effects in these organisms as the metals tend to accumulate in several tissues. Heavy metals were defined as those with a higher density than 5 g/ml [22]. Results of copper content of oysters ranged from 4.56 mg/kg to 19.90 mg/kg and not exceeding the rate of 30 mg/kg for products consumption. Results obtain after parking of oysters showed the ineffectiveness of treatment on 50% of villages (Azizakouè and Dégouè). These observations coincide with those of Mallet et *al*.[23] who also noted that the copper content increased after the parking of oysters. They concluded that this contamination come from sediments. The evaluation of the heavy metal content after penning indicated a significant reduction in the lead content of samples at all parking stations. These results confirm the effectiveness of this method in reducing chemical pollutants including heavy metals. However, the results of the levels of copper in oyster samples after their parking indicates a decrease in content only on the parking stations of Meko and Djondji. These results show that the parking oysters in view of the reduction of pollutants depends also on the type of pollutant involved in chemical pollution.

Conclusion

Oysters are an important source of animal protein for use as a dietary supplement in diets mainly dominated by vegetable protein or even deficient protein diets. However, results from this study indicated that oysters may be contaminated with pathogenic microorganisms and chemical contaminants, including lead and copper. Purge methods commonly used in lakeside villages could significantly reduce this pollution, but do not fully ensure the safety of oysters. So attention must be paid to this growing sector in Benin to ensure the health of consumers.

Acknowledgments

The authors are grateful to the Food Engineering Technology Department of Polytechnic School of Abomey-Calavi University (UAC) for their financial support. Authors wish to express their gratitude to Nelly Dagnon and Nelly Houeto for the technical assistance.

References

1. Nwajei G.E., Obi Iyeke G.E. and Okwagi P., Distribution of Selected Trace Metal in Fish Parts from the River Nigeria, *Res. J. Recent Sci.,* **1(1),** 81-84 **(2012)**

2. Dorigo U., Bourrain X., Berard A., Leboulanger C., Seasonal changes in the sensitivity of river microalgae to atrazine and isoproturon along a contamination gradient, *Sci. Tot. Environ.,* **318,** 101-114 **(2004)**

3. Mowat F.S., Bundy K.J., Correlation of field-measured toxicity with chemical concentration and pollutant availability, *Environ. Intern.,* **27**, 479-489 **(2001)**

4. Brooks B.W., Foran C.M., Richards SM, Weston J, Turner PK, Stanley JK, Solomo N, KR, Slattery M, La pont TW Aquatic ecotoxicology of fluoxetine, *Toxicol. Lett.,* 142, 169-183 **(2003)**

5. Alloway B.J., The origins of heavy metals in soils. In: Alloway BJ (ed.) *Heavy Metals in Soil* (2nd edn.) Blackie Academic and Professional, London. England, 57p **(1995)**

6. Friberg L., Nordberg G.F., Kessler E., Vouk V.B., Handbook of the Toxicology of Metals (2nd edn.). Vols 1 and 2. Elsevier Science. Amsterdam Publishers. Amsterdam. Netherlands. 6, **(1986)**

7. WHO., World Health Organisation. Inorganic Mercury (Envi-ronmental Health Criteria). International Program on Chemical Safety118**,** Geneva **(1991)**

8. Vutukuru S.S., Acute effects of Hexavalent chromium on survival. Oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major carp: *Labeo rohita, Int. J. Environ. Res. Public. Health.,* **2(3)**, 456- 462 **(2005)**

9. Velez D., Montoro R., Arsenic speciation in manufactured seafood products: a review*, J food Protect,* **61 (9),** 1240-1245 **(1998)**

10. Farombi E.O., Adelowo O.A., Ajimoko Y.R., Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias*

gariepinus) from Nigeria ogun river*, Int. J. Environ. Res. Public. Health*., **4(2),** 158-165 **(2007)**

11. Lapègue S., Boutet I., Leitão A., Heurtebise S., Garcia P., Thiriot-Quiévreux C., Boudry P., Trans-Atlantic distribution of mangrove oyster species revealed by 16S mt DNA and karyological analyses, *The Biological Bulletin* **202**, 232–242 **(2002)**

12. Lazoski C., Gusmão J., Boudry P., Solé-Cava A.M., Phylogeny and phylogeography of Atlantic oyster species: evolutionary history, limited genetic connectivity and isolation by distance, *Marine Ecology Progress Series, **426**,* 197–212 **(2011).**

13. Frazier W.C., Westhoff D.C., Contamination, preservation and spoilage of fish and other seafood, *Food Microbiology*, 4th Edn. McGraw-Hill Book Company, Singapore, 253 **(2000)**

14. CDC, Surveillance for foodborne disease outbreaks-United States 1998-2002. *Morb-Mortal Wkly Report Surveill Summ*, 55/SS-10, 42 **(2006)**

15. FAO/WHO, Risk assessment of *Vibrio vulnificus* in raw oysters. Interpretative summary and technical report. Food and Agriculture Organization of the United Nations/World Health Organization, Geneva, Switzerland 65 **(2005)**

16. Laleye P., Akele D., Etude participative des dynamiques des populations halieutiques du complexe du lac Ahémé, chenal Aho, lagunes côtière, Gbagan, Bas – Mono et rivière Sazué. Rapport final d'étude, 130 **(2005).**

17. Singh K., Frisvad J.C., Thrane U., Mathu S.B., An illustrated manual on identification of some seed borne Aspergilli, Fusaria, Penicillia and their mycotoxins. Danish Government, Institute of seed pathology for developing countries, Hellerup, Denmark **(1991**)

18. FDA., Food and Drug Administration. In Bacteriological Analytical Manuel 8th ed., *Vibrio cholera, V. parahemoliticus, V. vulniticus and other Vibrio spp*. AOAC International, Arlington, USA **(1995)**

19. Ange C.L., The biology and culture of tropical oysters, *Iclams Studies and Reviews* **13,** 27-42 **(1986)**

20. Ukwade I.M., Microbiological studies of the mangrove oyster Crassostrea gasar (Adanson 1757) from an experimental fish farm of the Lagos lagoon, Nigeria. Nigeria Institute for Oceanography and Marine research Lagos. Niomr Technical Paper N° 55, 10 **(1990)**

21. Adams M.R., Moss M.O., Microbiology of primary food commodities: In, *Food Microbiology*, 3rd Edn. The Royal Society of Chemistry, Cambridge, UK. 122 **(1999)**

22. Dore W.J., Lees D.N., Behaviour of *Escherichia coli* and male specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration, *Applied and Environmental Microbiology* **61,** 2830-2834 (**1995**)

23. Jarup L., Hazards of heavy metal contamination, *Br. Med. Bull.* **68**, 167–182 **(2003)**

24. Mallet A.L., Carver C.E., Hardy M., The effect of floating bag management strategies on biofouling, oyster growth and biodeposition levels, *Aquaculture* **287**, 315-323 **(2009)**