CONTENTS

Chapter		Page
ACKNOWLEDGMENTi CONTENTii LIST OF SCHEMESiii LIST OF TABLESiv		
I.	INTRODUCTION	1
2.	LITERATURE REVIEW	3
	2.1 Characters of contaminants	3
	2.2 Sources of contamination of molluscs	4
	2.3 Prevalence of contaminants in molluscs	
	2.4 Public health significance of contaminants	14
3.	MATERIALS AND METHODS	19
	Part I: Microbiological examination of molluscs	19
	3.1. Collection of Samples	19
	3.2. Preparation of samples homogenate	19
	3.3. Microbiological examinations	19
	3.3.1. Determination of total halophilic bacteria	19
	3.3.2. Determination of total halophilic mould & yeast	20
	3.3.3. Determination of total coliforms (MPN/g)	20
	3.3.4. Isolation and identification of some pathogens	20
	3.3.4.1. Isolation and identification of Vibrio parahaemolyticus	20
	3.3.4.2. Enumeration and identification of Staphylococcus aureus	23
	3.3.4.3. Detection of faecal coliform: (Eijkmann test)	24
	3.3.4.4. Detection and identification of <i>E-coli</i>	24
	3.3.4.5. Detection and identification of Salmonella	25
	Part II: Effect of some organic acids on total halophilic and tota counts in Om-El Kholoul.	
4.	RESULTS	29
5.	DISCUSSION	35
6.	CONCLUSIONS and RECOMMENDATIONS	42
7.	SUMMARY	44
8.	REFERENCES	45
	ARABIC SUMMARY	

6. CONCLUSION AND RECOMMENDATIONS

In Egypt and many parts of the world molluscus become an important source of marine protein. As they are sedentary, grow and/or cultivated abundance in relatively small areas. The achieved results in the present investigation revealed that most examined Om-Elkholoul samples showed high microbial contamination and unacceptable according to international recommended limits. The lowest contamination was found in squid samples although some samples were unacceptable according to *E.S* (2800/2006). This indicating bad sanitary conditions and/ or faecal contamination under which molluscs were exposed from the water in which they live and catching till reaching the consumers resulting in both public health hazards and economic losses. The natural acidifying and flavoring acids (lemon juce and venigar) have strongly antimicrobial effect specially in foods consumed raw.

The presents study showed that, the dipping of molluscs as Om El-Kholoul in 5% cirtic or acitic acid for ten minutes can reduce the possible hazard of high total halopilic and coliform counts to be within the acceptable limits. However, acetic acid is relatively more efficient than citric acid.

Therefore, the following suggestive hygienic measures of corrective and preventive actions should be applied to control growth of naturally occurring microorganisms on molluscess as well as prevent their contamination with both food poisoning and/ or food intoxication bacteria to be safe for human consumption:

- I- Governmental regulations should be applied to control contamination in harvesting area of the marine environment.
- II- Preventing contamination of molluscans shellfish by removal or reduction of polluting source by:
 - a- Local investigations for monitoring the marine environment to determine the distribution of hazardous substances and their sources.
 - b- Harvesting molluscs must be from unpolluted waters away from sewage or waste water runoff.
 - c- Prevention of sewage drain into sea water or at least applying of physical and/or chemical treatments of these sewags before draining to prohibit the main source of enteric bacteria contamination to molluscs.
 - d- Obligatory application of hygienic training programs including personal hygiene and good hygienic practices for fishermen and molluscs seller as well as continual renewal of their hygienic certificates. Also, the periodical hygienic inspection to fish markets and ensuring implementation of the following:
 - 1- Education and training of personal is essential for efficient control at all stages of shellfish production from the hygienic point of view to aid in prevention of shellfish borne disease.
 - 2- Workers should wear protective clothes and gloves when handled raw molluses and always kept clean.
 - 3- People who handle molluscs must be disease free.
 - 4- Workers with wound or abrasions must never handle molluscs.
 - 5- Harvested molluscs should be refrigerated as soon as possible after harvesting and immersed in an ice during selling to minimize the microbial proliferation.
 - 6- Periodical and perfect hygienic removal of waste.

- 7- Perfect cleaning and disinfecting of water cycles with enough water supply inside fishmarkets.
- III- Awareness of hygienic practices for consumers to protect them against the possible health hazard from eating molluses through the following preventive actions.
 - 1- Avoid eating raw shellfish and bivalve shellfish must be treated by:
 - a- Dipping in 5% acetic acid (vinegar) or citric acid (lemon juice) for at least ten minutes.
 - b- Boiling them until the shell open and continue for more five minutes. Molluscs those opened before boiling and/or don't opened by boiling must be not eaten.
 - c- Efficient cooking of squid.
 - 2- Avoiding of cross contamination between cooked and raw shellfish and other sea foods.
 - 3- Personal hygiene and also good hygienic practices must be taken in consideration.

All these corrective and preventive actions aid to prevent possible microbial hazard or at least reduce them to be within acceptable limit in molluscs to be fit for human consumption.

7. SUMMARY

Since molluscan shellfish are subject to the risk of contamination with various pathogens either during their presence in their aquatic environments or after being harvested for marketing.

Ninety random samples of Gandoufly, Om El-Kholoul and squid (30 each) were collected from different retail markets of Alexandria Governorate. The samples were transferred directly in an ice box to laboratory where they were subjected to microbiological examinations.

The examinations revealed that the mean values of total halophilic bacteria in Gandoufly, Om El-Kholoul and squid samples were $6.1 \times 10^4 \pm 1.2 \times 10^2$, $2.8 \times 10^5 \pm 7.4 \times 10^4$ and $6.8 \times 10^4 \pm 9 \times 10^3$ cfu/g respectively. All examined samples harbered halophilic bacteria (100% incidence). 46.7%, 33.3% and 76.7% of the examined molluscs samples have total halophilic count range between $10^4 - 10^5$ /gm, respectively.

The incidence of halophilic mould in Gandoufly, Om El-Kholoul and squid samples was 60%, 76.7% and 66.7% with mean value of $2.4 \times 10 \pm 7$, $2.5 \times 10 \pm 4$ and $7.7 \times 10 \pm 1.3 \times 10$, respectively. Also the incidence of halophilic yeast was 56.7%, 60% and 100% with mean values of $1.57 \times 10^2 \pm 7.6 \times 10$, $1.1 \times 10^2 \pm 2.2 \times 10$ and $1.88 \times 10^2 \pm 2 \times 10$, respectively.

All examined molluscan samples (100%) were positive for coliforms with mean values of $1.8 \times 10^2 \pm 6.1 \times 10$, $1.1 \times 10^3 \pm 4.2 \times 10^2$ and $1.03 \times 10^2 \pm 2.7 \times 10$, respectively.

Vibrio parahaemolyticus was isolated from Gandoufly, Om El-Kholoul and squid samples at an incidence of 30, 23.3 and 10%. While the incidence of each of Staphylococcus aureus was 43.3%, 63.3% and 36.7%, the faecal coliform was 33.3, 56.76 and 13.3% and E.coli was 26.7, 40 and 10%, respectively. Salmonellae could not be isolated from any examined sample.

In the experimental section of this work the effect of some organic acids (citric and acetic) on total halophilic bacteria and *coliform* count in Om El-Kholoul was investigated. The concentration of both acids was 5%. The Om El-Kholoul sample was dipped in each acid for 1, 5 and 10 minutes. The result revealed reduction of both halophilic and coliform counts in the examined samples, but the acetic acid had more bactericidal effect than citric acid, and this effect is directly proportional to time of dipping.

The different sources of contamination, measures suggested to prevent or minimize such contamination, as well as the hygienic and public health significance of isolated microorganisms have been discussed.