

# **EFFECT OF CERTAIN TYPES OF BACTERIA IN WATER ENVIRONMENT ON DISTRIBUTION OF SOME VECTOR SNAILS**

By

**Ahmed Mohamed Abd Allah Azzam**

Assistant Researcher, Theoder Bilharz Research Institute

B. Sc. Microbiology Chemistry, Ain Shams University, ٢٠٠١

M. Sc. of Environmental sciences, Institute of Environmental  
Studies & Researches, Ain Shams University, ٢٠٠٨

**A Thesis Submitted in Partial fulfillment  
of  
The Requirement for Doctor of Philosophy  
in  
Environmental Science**

**Department of Basic Science**  
Institute of Environmental Studies & Researches Ain  
Shams University

٢٠١٢

**APPROVAL SHEET**

**EFFECT OF CERTAIN TYPES OF BACTERIA IN  
WATER ENVIRONMENT ON DISTRIBUTION OF  
SOME VECTOR SNAILS**

By

**Ahmed Mohamed Abd Allah Azzam**

Assistant Researcher, Theodor Bilharz Research Institute  
B. Sc. Microbiology Chemistry, Ain Shams University, ٢٠٠١  
M. Sc. of Environmental sciences, Institute of Environmental  
Studies & Researches, ٢٠٠٨

This Thesis Towards a Doctor of Philosophy in Environmental Science  
has been Approved by:

**Prof. Dr.\ Mohamed Khaled Ibrahim**

Prof. of Bacteriology, Head of Microbiology Department,  
Faculty of Science, Ain Shams University

**Prof. Dr.\ Mansour Galal Ibrahim**

Prof. of Aquatic Ecology, Zoology Department,  
Faculty of Science, Monofiya University

**Prof. Dr.\ Abd El-Halim Abdou Saad**

Prof. of Aquatic Ecology, Zoology Department,  
Faculty of Science, Ain Shams University

**Prof. Dr.\ Bayaummy Bayaummy Mostafa**

Prof. of Environmental Researches, Head of  
Environmental Researches Department, Theodor Bilharz  
Research Institute

٢٠١٢

# EFFECT OF CERTAIN TYPES OF BACTERIA IN WATER ENVIRONMENT ON DISTRIBUTION OF SOME VECTOR SNAILS

By

**Ahmed Mohamed Abd Allah Azzam**

Assistant Researcher, Theodor Bilharz Research Institute  
B. Sc. Microbiology Chemistry, Ain Shams University, ٢٠٠١  
M. Sc. of Environmental sciences, Institute of Environmental  
Studies & Researches, ٢٠٠٨

Under the supervision of:

**Prof. Abd El-Halim Abdou Saad**

Prof. of Aquatic Ecology, Zoology Department,  
Faculty of Science, Ain Shams University

**Prof. Bayaummy Bayaummy Mostafa**

Prof. of Environmental Researches, Head of  
Environmental Researches Department, Theodor  
Bilharz Research Institute

**Prof. Samah Saad El-Din Abou El-Magd**

Prof. of Clinical Microbiology, Clinical  
Microbiology Department, Theodor Bilharz  
Research Institute

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا بِمَا عَلَّمْتَنَا  
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة - آية رقم ٣٢

## ***ACKNOWLEDGMENT***

First of all, I strongly owe my thanks to God for lighting the way and directing me across the route to every success I reached and may reach.

I would like to express my deep gratitude to **Dr. Abd El-Halim Abdou Saad Prof.** of Aquatic Ecology, Zoology Department, Faculty of Science, Ain Shams University, for valuable help, advice and guidance during the course of this work and for revising the manuscript of thesis.

I am greatly indebted and profoundly grateful to **Dr. Bayaumi Bayaumi Mostafa Prof.** of environmental research, Head of Environmental Research Department, Theodor Bilharz Research Institute, for suggesting and planning the point of research and the valuable and unlimited help in the laboratory and field work.

I feel deep gratitude to **Dr. Samah Saad El-Din Abou El-Magd Prof.** of Clinical Microbiology, Theodor Bilharz Research Institute, for encouragement, fruitful discussion; unlimited help in the laboratory work and continuous help during writing of this thesis.

I am also deep thankful to all staff members and team of Environmental Research Department, Theodor Bilharz Research Institute, for their great help in this work.

Finally, I can not find suitable words to express my deepest thanks to my father, mother and my wife for their help and support during the preparation of this work.

## ABSTRACT

The present study revealed the effect of certain bacterial species in water bodies on distribution and natural infection of vector snails of *Schistosoma mansoni*, *S. haematobium* and *Fasciola* spp.. Field study carried out in seven Egyptian governorates (Giza, Cairo, Qaluobia, Sharkia, Ismailia, Beheira and Damietta) during successive four seasons starting from spring ٢٠٠٩ to winter ٢٠١٠.

The total coliform bacterial counts (TC), *Enterococcus faecalis*, *Clostridium perfringens* and presence of certain Gram-negative bacilli bacteria were tested in ٣٢٨ water samples.

Only Cairo and Ismailia governorates showed the accepted mean values of TC counts by ٣٦٣ and ٣٧٨ MPN/١٠٠ ml respectively, but Beheira, Sharkia, Damietta, Qaluobia and Giza recorded the unaccepted ones.

Most of collected water samples (٦٧,٤%) were found positive for *E. faecalis* bacteria and Beheira was the most polluted governorate for TC count (٧٦٦/١٠٠ ml) and sewage pollution (٨٨,٥%). All collected water samples recorded negative results for the presence of *C. perfringens* bacteria.

The number of isolations of Gram-negative bacilli bacteria isolated through study period was ٥٥٤. Isolations were identified for seven bacterial genera including *Escherichia coli* (٥٩,٢%), *Proteus* spp. (١٥,٩%), *Enterobacter aerogenes* (١٢,٨%), *Klebsiella pneumoniae* (٤,٣%), *Pseudomonas aeruginosa* (٢,٩%), *Citrobacter* spp. (٢,٧%) and *Vibrio* spp. (٢,٢%). These bacterial species causes many diseases and the crops

irrigated by water contaminated with these bacteria considered a source of serious diseases.

In the field study, the vector snails *Lymnaea natalensis* were found highly distributed than *Biomphalaria alexandrina* and *Bulinus truncates*. These snails and infected ones recorded the highest distribution percentages by ٥١,٦% and ٥٩,٣% respectively in polluted water with total coliform range ١٠٠ - ٣٠٠ MPN/١٠٠ ml. On the other hand, water contaminated with *P. aeruginosa* and *Vibrio* spp. bacteria was found to be more repellent to snails.

Laboratory study declared that *P. aeruginosa*, *Vibrio* spp., *E. aerogenes*, *Bacillus thuringiensis*, *E. faecalis* and *K. pneumoniae* have effect on mortality rate, egg-laying capacity and protein content of lab *B. alexandrina*, *B. truncates* and *L. natalensis* snails. The percentage of infection and the rate of cercarial production of *B. alexandrina* snails infected by *S. mansoni* miracidia in the presence of these bacterial species were affected.

The effect of bacterial species that were isolated from water on the efficiency of Bayluscide and copper sulfate showed that these bacteria have very low effect on CuSO<sub>4</sub>, but it was found to be capable of degraded Bayluscide especially *E. faecalis* and *P. aeruginosa* bacteria.

# **CONTENTS**

	<b>Page</b>
<b>I. INTRODUCTION</b>	١
<b>II. REVIEW OF LITERATURE</b>	٥
١. Bacterial pollution in River Nile and some water bodies in Egypt	٥
٢. Bacterial indicators for water pollution	٨
٣. Effect of some environmental factors on water quality and bacterial content	٩
٤. Distribution of vector snails of <i>Schistosoma mansoni</i> , <i>S. haematobium</i> and <i>Fasciola</i> spp. in some Egyptian governorates	١٣
٥. Effect of certain bacterial species on some vector snails	١٦
٦. Effect of different bacterial species on Bayluscide and copper sulfate molluscicides	١٨
<b>III. MATERIALS AND METHODS</b>	٢١
<b>١. Field studies</b>	٢١
١. ١. Samples collection	٢٥
١. ٢. Detection of physicochemical characters of water	٢٥
<b>٢. Laboratory studies</b>	٢٦
٢. ١. Bacteriological analysis	٢٦
٢. ١. ١. Detection of total coliforms in water samples	٢٦
٢. ١. ٢. Identification of some Gram-negative bacilli bacteria present in water samples	٢٩
٢. ١. ٣. Detection of <i>Enterococcus faecalis</i> in water samples	٢٩
٢. ١. ٤. Detection of <i>Clostridium perfringens</i> in water samples	٣٠
٢. ١. ٥. Gram stain	٣٠
٢. ٢. Detection of natural trematodes larval infection of snails	٣١



٢. ٣. Testing of pathogenecity of certain bacterial species on lab vector snails of <i>Schistosoma mansoni</i> , <i>S. haematobium</i> and <i>Fasciola</i> spp.	٣١
	<b>Page</b>
٢. ٣. ١. Preparation of bacterial suspensions	٣١
٢. ٣. ٢. Effect of certain bacterial species on the mortality rate and egg- laying capacity of lab vector snails	٣٣
٢. ٣. ٣. Effect of certain bacterial species on protein content of digestive and hermaphrodite glands of lab vector snails	٣٣
٢. ٣. ٤. Effect of certain bacterial species on the infection rate and cercarial production of <i>Biomphalaria alexandrina</i> snails	٣٥
٢. ٣. ٥. Preparation of <i>Bacillus thuringiensis</i> spore-crystal suspension	٣٦
٢. ٤. Detection of lethal concentration of <i>B. thuringiensis</i> spore-crystal suspension, Bayluscide and copper sulfate for some vector snails	٣٧
٢. ٥. Effect of bacterial species isolated from water on efficiency of Bayluscide and Copper sulfate	٣٨
٢. ٦. Effect of certain bacterial species on <i>Dapdnia magna</i>	٣٩
٢. ٧. Statistical analysis	٣٩
<b>IV. RESULTS</b>	<b>٤٠</b>
<b>١. Field Studies</b>	<b>٤٠</b>
١. ١. Bacterial examination of surface water in some Egyptian governorates	٤٠
١. ١. ١. Total coliforms	٤٠
١. ١. ٢. <i>Enterococcus faecalis</i> bacteria in water samples	٤٦
١. ١. ٣. <i>Clostridium perfringens</i> bacteria in water samples	٤٨
١. ١. ٤. Isolated Gram-negative bacilli bacteria from water samples	٥١
١. ٢. Effect of some environmental factors on water quality and bacterial content of surveyed water bodies	٥٩
١. ٣. Distribution of vector snails of <i>Schistosoma mansoni</i> , <i>S. haematobium</i> and <i>Fasciola</i> spp. in seven surveyed Egyptian governorates	٧٩
١. ٤. Detection of natural larval trematodes infection of collected vector snails of <i>S. mansoni</i> and <i>S. haematobium</i> and <i>Fasciola</i> spp. from surveyed governorates	٨١

١. ٥. Effect of total coliform bacterial count on distribution of vector snails of <i>S. mansoni</i> , <i>S. haematobium</i> and <i>Fasciola</i> spp.	٨٩
	<b>Page</b>
١. ٦. Effect of total coliform bacterial count on natural trematodes infection of collected vector snails of <i>S. mansoni</i> and <i>S. haematobium</i> and <i>Fasciola</i> spp.	٨٩
١. ٧. Effect of isolated bacterial species from water on distribution of vector snails of <i>S. mansoni</i> , <i>S. haematobium</i> and <i>Fasciola</i> spp.	٩٥
١. ٨. Effect of isolated bacterial species from water on natural trematodes infection of collected vector snails of <i>S. mansoni</i> , <i>S. haematobium</i> and <i>Fasciola</i> spp.	٩٨
<b>٢. Laboratory Studies</b>	١٠١
٢. ١. Testing of pathogenecity of certain bacterial species on lab vector snails of <i>Schistosoma mansoni</i> and <i>S. haematobium</i> and <i>Fasciola</i> spp.	١٠١
٢. ١. ١. Effect on mortality rate	١٠١
٢. ١. ٢. Effect on egg-laying capacity	١٠٢
٢. ١. ٣. Effect on protein content of digestive and hermaphrodite glands	١٠٣
٢. ١. ٤. Effect of certain bacterial species on the infection rate and cercarial production of <i>Biomphalaria alexandrina</i> snails	١١٦
٢. ٢. Testing of molluscicidal activity of spore-crystal suspension of <i>Bacillus thuringiensis</i> bacteria	١١٩
٢. ٣. Effect of lethal concentrations of Bayluscide and copper sulfate on lab vector snails	١٢١
٢. ٤. Effect of bacterial species isolated from water on efficiency of Bayluscide and copper sulfate	١٢١
٢. ٥. Effect of certain bacterial species on <i>Daphnia magna</i>	١٢٥
<b>V. DISCUSSION</b>	١٢٨
<b>VI. SUMMARY</b>	١٤٥

## VII. REFERENCES

١٥٥

## VIII. ARABIC SUMMARY

١٧٣

### LIST OF TABLES

<i>Table No.</i>	<i>Page</i>
١. MPN index and ٩٥% confidence limits for various combinations of positive results when five tubes are used per dilution (١, ١,٠ and ٠,١ ml portion of sample).	٢٨
٢. Means of total coliform bacterial counts of water samples collected from seven governorates during four seasons.	٤٤
٣. Percentage of positive water samples for <i>E. faecalis</i> bacteria collected from seven governorates during four seasons.	٤٩
٤. Positive water samples for <i>E. faecalis</i> bacteria collected from seven governorates during four seasons.	٥٠
٥. Positive isolations for certain Gram-negative bacterial species in seven surveyed governorates.	٥٦
٦. Isolated Gram-Negative bacterial species from water samples during four seasons.	٥٧
٧. Percentage of Gram-negative bacterial isolations during four seasons.	٥٨
٨. Means of temperature of water in different surveyed governorates.	٦١
٩. Total coliform bacterial counts in water samples of different temperatures during four seasons.	٦١
١٠. Number of bacterial species isolations collected from water of different temperatures during four seasons.	٦٢
١١. Means of pH of water in different surveyed governorates.	٦٦
١٢. Total coliform bacterial counts in water samples of different pH during four seasons.	٦٦
١٣. Number of bacterial species isolations collected from water of different pH during four seasons.	٦٧
١٤. Means of electric conductivity of water in different surveyed governorates.	٧١
١٥. Total coliform bacterial counts in water of different electric conductivity during four seasons.	٧١

١٦. Number of bacterial species isolations collected from water of different electric conductivity during four seasons.	٧٢
١٧. Means of total dissolved salts of water in different surveyed governorates.	٧٦

**Table No.**

**Page**

١٨. Total coliform bacterial counts in water samples of different total dissolved salts during four seasons.	٧٦
١٩. Number of bacterial species isolations collected from water of different total dissolved salts during four seasons.	٧٧
٢٠. Total collected snails during four seasons from surveyed governorates.	٨٣
٢١. Distribution ratio (snails/sample) of different vector snails in seven surveyed governorates.	٨٥
٢٢. Percentage of infection of vector snails collected from seven surveyed governorates during four seasons.	٨٦
٢٣. Infected snails collected from surveyed governorates during four seasons.	٨٧
٢٤. Total snails collected from water of different coliform bacteria during four seasons.	٩١
٢٥. Infected vector snails collected from water of different coliform bacteria during four seasons.	٩٣
٢٦. Distribution ratios of vector snails collected from water samples contaminated with certain bacterial species during four seasons.	٩٦
٢٧. Percentage of infection of vector snails collected from water contaminated with certain bacterial species during four seasons.	٩٩
٢٨. Effect of certain bacterial species on mortality rate of <i>B. alexandrina</i> snails.	١٠٥
٢٩. Effect of certain bacterial species on mortality rate of <i>B. truncatus</i> snails.	١٠٦
٣٠. Effect of certain bacterial species on mortality rate of <i>L. natalensis</i> snails.	١٠٧
٣١. Effect of certain bacterial species on egg-laying capacity of <i>B. alexandrina</i> snails.	١٠٩
٣٢. Effect of certain bacterial species on egg-laying capacity of <i>B. truncatus</i> snails.	١١٠
٣٣. Effect of certain bacterial species on egg-laying capacity of <i>L. natalensis</i> snails.	١١١

٣٤. Similarity index of SDS-PAGE of digestive and hermaphrodite glands of treated lab vector snails with certain bacterial species.	١١٣
٣٥. Effect of certain bacterial suspensions on the infection rate and cercarial production of <i>B. alexandrina</i> .	١١٧
<i>Table No.</i>	<i>Page</i>
٣٦. Percentage of mortality of spore-crystal suspension of <i>Bacillus thuringiensis</i> bacteria for lab vector snails and free living stages.	١٢٠
٣٧. Effect of lethal concentrations of Bayluscide and copper sulfate on lab vector snails.	١٢٢
٣٨. Effect of certain bacterial species isolated from water on the efficiency of lethal concentration of Bayluscide for lab vector snails.	١٢٣
٣٩. Effect of certain bacterial species isolated from water on the efficiency of lethal concentration of copper sulfate for lab vector snails.	١٢٤
٤٠. Effect of certain bacterial species on <i>Daphnia magna</i>	١٢٦

## ***LIST OF FIGURES***

<b><i>Fig. No.</i></b>	<b><i>Page</i></b>
١. Map of seven Egyptian governorates of field study.	٢٤
٢. Means of total coliform bacteria of water samples collected from seven governorates during four seasons.	٤٤
٣. Means of total coliform bacterial counts during four seasons in seven governorates.	٤٥
٤. Percentage of positive water samples for <i>E. faecalia</i> bacteria collected from each governorate during four seasons.	٤٩
٥. Number of positive water samples for <i>E. faecalia</i> bacteria collected during four seasons.	٥٠
٦. Percentage of positive isolations for certain Gram-negative bacteria in seven surveyed governorates.	٥٦
٧. Percentage of different Gram-negative bacterial species isolations during four seasons.	٥٨
٨. Number of water samples of different total coliform bacteria collected from water of different temperatures.	٦٣
٩. Number of bacterial species isolations collected from water of different temperatures during four seasons.	٦٣
١٠. Number of water samples of different total coliform bacteria collected from water of different pH.	٦٨
١١. Number of bacterial species isolations collected from water of different pH during four seasons.	٦٨
١٢. Number of water samples of different coliform bacteria collected from water of different electric conductivity.	٧٣
١٣. Number of bacterial species isolations collected from water of different electric conductivity during four seasons.	٧٣
١٤. Number of water samples of different coliform bacteria collected from water of different total dissolved salts.	٧٨
١٥. Number of bacterial species isolations collected from water of different total dissolved salts during four seasons.	٧٨
١٦. Percentage of total vector snails collected during four seasons.	٨٤

١٧. Number of different vector snails collected during four seasons	٨٤
---	----

<i>Fig. No.</i>	<i>Page</i>
١٨. Distribution ratio of different vector snails in surveyed governorates.	٨٥
١٩. Percentage of infection of different vector snails in surveyed governorates.	٨٦
٢٠. Percentage of total infected vector snails collected during four seasons.	٨٨
٢١. Percentage of different infected snail vectors collected during four seasons.	٨٨
٢٢. Percentage of vector snails collected from different coliform bacteria during four seasons.	٩٢
٢٣. Number of different vector snails collected from water of different total coliform bacteria.	٩٢
٢٤. Number of total infected snails collected from water of different coliform bacteria during four seasons.	٩٤
٢٥. Number of different infected vector snails collected from water of different coliform bacteria.	٩٤
٢٦. Distribution ratio of total vector snails collected from water contaminated with certain bacterial species during four seasons.	٩٧
٢٧. Distribution ratio of different vector snails collected from water contaminated with certain bacterial species.	٩٧
٢٨. Percentage of infection of total vector snails collected from water contaminated with certain bacterial species during four seasons.	١٠٠
٢٩. Percentage of infection of different vector snails collected from water contaminated with different bacterial species.	١٠٠
٣٠. Effect of different bacterial species on survival time of lab vector snails.	١٠٨
٣١. Effect of different bacterial species on reduction of egg-laying capacity of lab vector snails.	١١٢
٣٢. Similarity index of SDS-PAGE of digestive and hermaphrodite glands of treated lab vector snails with certain bacterial species.	١١٣
٣٣. SDS-PAGE patterns of digestive and hermaphrodite glands of control and treated <i>B. alexandrina</i> snails with certain bacterial species.	١١٤