

**ISOLATION AND CHARACTERIZATION OF ARSENIC  
TOLERANT MICROORGANISMS FROM TUBE WELL WATER OF  
NAWALPARASI, NEPAL**

**A**

**Dissertation**

**Submitted to the Central Department of Microbiology  
Tribhuvan University**

**In Partial Fulfillment of Requirements for the Award of the Degree of  
Master of Science in Microbiology  
(Environment and Public Health)**

**By**

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**2008**

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## ACKNOWLEDGEMENT

This research work has been supported by many people whose advice and encouragement were critical throughout the period of this work. I would like to express my gratitude to all the people who helped me to complete this dissertation.

Respectfully, I would like to express my sincere gratefulness to my honorable supervisor **Dr. Dwij Raj Bhatta**, Associate Professor, Central Department of Microbiology, T.U.) for his supervision, constructive suggestion, advices, scholastic inspiration and encouraging guidelines throughout the study period

I am really very delighted and want to express my thankfulness to my supervisor **Mr. Binod Lekhak**, Assistant Professor, Central Department of Microbiology, for his kind, valuable and timely guidance, supervision throughout the study period. and his kind guidance in laboratories for thesis writing.

I would like to thank **Prof. Dr. Shital Raj Basnyat**, for his guidance during the initial period of study. Likewise I am also thankful to **Dr. Linda Smith** for her valuable support and continuous encouragement all the way from United States.

I sincerely like to express my appreciation to **Dr. Anjana Singh**, Associate Professor and Head of Department of Microbiology, T.U. for her deep understanding valuable suggestions and kind co-operation during this work.

I acknowledge profound gratitude to all the members of Microbiology Department different libraries, departments and organizations especially TU, ENPHO and Red Cross of Nawalparasi for providing me their insightful help.

Finally I admire and adore my family and friends for their moral support and encouragement.

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## ABSTRACT

The present study was an attempt to identify the microorganisms present in ground water of high arsenic aquifer. A total of 43 samples of ground water from tube wells were randomly collected from Kunwar villages, Nawalparasi, Nepal. The area was high arsenic aquifer where public health has been badly affected by the arsenic in the tube well water. Arsenic concentration ranges from 10 ppb–620 ppb were tested using arsenic testing kits of the collected samples. And other physiochemical parameters were also tested at the sampling site. All together ten samples were transported to the laboratory of Central Department of Microbiology T.U. Kirtipur for microbiological analysis. The samples were analyzed in the laboratory for total coliform count, presence of *E. coli*, heterotrophic count and isolation and identification of arsenic tolerant bacteria. All the samples were found to be contaminated with coliform bacteria as tested by membrane filter technique. The total coliform count in the sample Tkw6 was higher ( $35 \times 10^7$  cfu/100ml. The sample Tkw3 (As 400 ppb) found to have higher *E. coli* that is 40 per 100ml. And total bacterial count of the sample Tkw6 found to be  $85 \times 10^7$  per ml .

Most of the isolated arsenic tolerant microorganisms were pigment producing bacteria. In all the isolates of microorganism 40% were Gram positive cocci, 25% Gram positive rods, 25% Gram negative rods, 5% Gram negative cocci, and 5% yeast and mold. Arsenic tolerant bacteria were isolated in the selective enrichment medium and identified by colony morphology, cell morphology and biochemical tests. Arsenic tolerance level for randomly selected strains were checked and isolates were found to be tolerant up to 100ppm (100,000ppb) arsenic concentration. Isolated arsenic tolerant isolates from groundwater of Nawalparasi were *Micrococcus* spp. *Escherichia coli*, yeast, *Streptococcus faecalis*, *Bacillus* spp. *Staphylococcus* spp. *Pseudomonas* spp. Thus the study was an research to know general flora residing high arsenic aquifer by isolating and characterizing them.

**Key words:** Arsenic, Arsenate respiration, anaerobes, ground water, Hyperthermophiles

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## LIST OF ABBREVIATIONS

A/A:	Acid / Acid
ADB:	Asian Development Bank
APHA:	American Public Health Association
As:	Arsenic
As(V):	Arsenate Pentavalent Spices
As(III):	Arsenite Trivalent Spices
BA:	Blood Agar
CAOs:	Chemoautotrophic arsenite oxidizers
CFU:	Colony Forming Unit
DARPs:	Dissimilatory Arsenate Respiring Prokaryotes
DGGE:	Denaturing Gradient Gel Electrophoresis
DMA:	Dimethylarsonic acid
DNA:	Deoxyribonucleic Acid
DWSS:	Department of Water Supply and Sewerage
ENPHO:	Environment and Public Health Organization
g:	Grams

H <sub>2</sub> S:	Hydrogen Sulphide
HAOs	Heterotrophic Arsenite Oxidizers
IARC:	International Agency for Research on Cancer
LF:	Lactose Fermenting
MA:	MacConkey Agar
MIC:	Minimal Inhibitory Concentration
MF:	Membrane Filter
mg/L:	Miligram Per Litre
ml:	Mililitre
µg:	Microgram
MMA:	Monomethylarsonic Acid
MR:	Methyl Red
NA:	Nutrient Agar
NEWAH:	Nepal Water for Health
NLF:	Non-lactose fermenting
Nm:	Nano meter
No.:	Number
NRC:	National Research Council
NWSC:	Nepal Water Supply Cooperation
NTU:	Nephelometric Turbidity Unit

ppm:	Parts Per Million
ppb:	Parts Per Billion
RNA:	Ribonucleic Acid
rpm:	Revolution Per Minute
SIM:	Sulphide Indole Motility
SPC:	Standard Plate Count
UNICEF:	United Nations International Children Education Fund
VDC:	Village Development Committe
VP:	Voges Proskauer
WECS:	Water and Energy Commission Secretariat
WHO:	World Health Organization

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