



Environmental Microbiology

Second Edition



Environmental Microbiology

Second Edition

Raina M. Maier

Department of Soil, Water and Environmental Science
University of Arizona
Tucson, Arizona

Ian L. Pepper

Department of Soil, Water and Environmental Science
University of Arizona
Tucson, Arizona

Charles P. Gerba

Department of Soil, Water and Environmental Science
University of Arizona
Tucson, Arizona



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD
PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



ACADEMIC
PRESS

Cover photos:

(left) Adenovirus, copyright Russell Kightley Media, with permission. (middle) A fluorescent in situ hybridization (FISH) image of bacterial colonization of a *Buchloe dactyloides* (buffalograss) root grown in mine tailings with 5% compost. The universal probe EUB338-mix labeled with Cy5 was used to label the bacteria and the image was taken with a Zeiss confocal scanning laser microscope. Image courtesy Sadie L. Iverson, University of Arizona, Tucson, AZ. (right) *Prosopis juliflora* (mesquite) root colonized by the mycorrhizal fungus *Glomus intraradices*, showing spores and hyphae. The sample was stained with trypan blue and imaged with at 40X. Image courtesy Fernando A. Solis-Dominguez

Academic Press is an imprint of Elsevier
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

Copyright © 2009, Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone: (+44) 1865 843830, fax: (+44) 1865 853333, E-mail: permissions@elsevier.com. You may also complete your request online via the Elsevier homepage (<http://elsevier.com>), by selecting "Support & Contact" then "Copyright and Permission" and then "Obtaining Permissions."

Library of Congress Cataloging-in-Publication Data

Application Submitted

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

ISBN: 978-0-12-370519-8

For information on all Academic Press publications
visit our Web site at www.elsevierdirect.com

Typeset by Charon Tec Ltd., A Macmillan Company. (www.macmillansolutions.com)

Printed in China

08 09 10 9 8 7 6 5 4 3 2 1

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER BOOK AID Sabre Foundation
International

It takes a village—thanks to my family, especially Mom, Dad and my daughter Claire.

Raina M. Maier

This book is dedicated to microbes—they're everywhere and I believe they are smarter than we think.

Ian L. Pepper

This book is dedicated to my wife and sons Peter and Phillip for all their support.

Charles P. Gerba



Contents

Preface	xv
Acknowledgments	xvii
The Authors	xix
Contributing Authors	xxi

Part I Review of Basic Microbiological Concepts

1. Introduction to Environmental Microbiology

Raina M. Maier, Ian L. Pepper and Charles P. Gerba

1.1 Introduction	3
1.2 An Historical Perspective	3
1.3 Modern Environmental Microbiology	6
1.4 Purpose and Organization of This Text	7

2. Microorganisms

*Timberley M. Roane, Kelly A. Reynolds, Raina M. Maier
and Ian L. Pepper*

2.1 Classification of Organisms	10
2.2 Eubacteria	10
2.2.1 Cell Envelope	11
2.2.2 Cytoplasm	14
2.2.3 Glycocalyx	16
2.2.4 Appendages	16
2.2.5 Endospores	16
2.2.6 Information Transfer	17
2.2.7 Metabolism	18
2.3 The Archaea	20
2.3.1 Archaean Habitats	21
2.3.2 Archaean Function	21
2.4 Fungi	22
2.4.1 Fungal Structure	22
2.4.2 Fungal Diversity	23
2.4.3 Ecological Considerations	24
2.5 Slime Molds	25
2.6 Protozoa	25
2.6.1 Structure and Function	25
2.6.2 Physiological and Ecological Considerations	26

2.7 Algae	26
2.7.1 Cell Structure	27
2.7.2 Physiological and Ecological Considerations	27
2.8 Viruses	28
2.8.1 Infective Nature of Viruses	29
2.8.2 Procaryotic Viruses	30
2.8.3 Eucaryotic Viruses	32
2.9 Other Biological Entities	33
2.9.1 Viroids	33
2.9.2 Prions	33
References	34

3. Bacterial Growth

Raina M. Maier

3.1 Growth in Pure Culture in a Flask	38
3.1.1 The Lag Phase	38
3.1.2 The Exponential Phase	39
3.1.3 The Stationary Phase	40
3.1.4 The Death Phase	41
3.1.5 Effect of Substrate Concentration on Growth	42
3.2 Continuous Culture	44
3.3 Growth in the Environment	46
3.3.1 The Lag Phase	47
3.3.2 The Exponential Phase	47
3.3.3 The Stationary and Death Phases	47
3.4 Mass Balance of Growth	49
3.4.1 Aerobic Conditions	50
3.4.2 Anaerobic Conditions	52
Questions and Problems	53
References and Recommended Readings	54

Part II Microbial Environments

4. Earth Environments

Raina M. Maier and Ian L. Pepper

4.1 Earth's Living Skin	57
4.2 Physicochemical Characteristics of the Earth Environment	58
4.2.1 Earth Environments	58

4.2.2	The Solid Phase	59	Questions and Problems	101
4.2.3	The Liquid Phase	67	References and Recommended Readings	101
4.2.4	Soil Atmosphere	69		
4.3	Soil as a Microbial Environment	70	6. Aquatic Environments	
4.3.1	Biotic Stresses	70	<i>Todd R. Sandrin, Scot E. Dowd, David C. Herman and Raina M. Maier</i>	
4.3.2	Abiotic Stresses	70	6.1 Introduction	103
4.4	Microorganisms in Surface Soils	71	6.2 Microbial Habitats in the Aquatic Environment	103
4.4.1	Bacteria	71	6.2.1 Planktonic Environment	103
4.4.2	Actinomycetes	71	6.2.2 Benthic Habitat	105
4.4.3	Fungi	73	6.2.3 Microbial Mats	107
4.4.4	Algae	74	6.2.4 Biofilms	107
4.4.5	Protozoa	74	6.3 Aquatic Environments	110
4.5	Distribution of Microorganisms in Soil	75	6.3.1 Freshwater Environments	110
4.6	Microorganisms in Subsurface Environments	76	6.3.2 Brackish Water	115
4.6.1	Microorganisms in Shallow Subsurface Environments	77	6.3.3 Marine Water	115
4.6.2	Microorganisms in Deep Subsurface Environments	77	6.3.4 Subterranean Water	117
	Questions and Problems	81	6.4 Aquatic Microbes in the News	117
	References and Recommended Reading	81	6.4.1 Shotgun Sequencing Reveals Secrets about the Sargasso Sea	117
			6.4.2 Aquatic Microbes: Food for the Future	119
			Questions and Problems	120
			References and Recommended Readings	120
5. Aeromicrobiology			7. Extreme Environments	
<i>Ian L. Pepper and Scot E. Dowd</i>			<i>Raina M. Maier</i>	
5.1	Introduction	83	7.1 Low Temperature Environments	124
5.2	Important Airborne Pathogens	83	7.1.1 McMurdo Dry Valleys, Antarctica	124
5.3	Important Airborne Toxins	84	7.2 High Temperature Environments	126
5.4	Aerosols	85	7.2.1 Geothermal Hot Springs	126
5.5	Nature of Bioaerosols	87	7.3 Desiccation and UV Stress	128
5.6	Aeromicrobiological Pathway	87	7.3.1 The Atacama Desert, Chile	128
5.6.1	Launching	88	7.4 Environments Based on Chemoautotrophy	130
5.6.2	Transport	88	7.4.1 Deep-Sea Hydrothermal Vents	130
5.6.3	Deposition	89	7.5 Acidic Environments	132
5.7	Microbial Survival in the Air	91	7.5.1 Acid Mine Drainage	132
5.7.1	Relative Humidity	91	Questions and Problems	133
5.7.2	Temperature	92	References	134
5.7.3	Radiation	92		
5.7.4	Oxygen, OAFs, and Ions	92		
5.8	Extramural Aeromicrobiology	92		
5.8.1	Agriculture	92		
5.8.2	Waste Disposal	94		
5.9	Intramural Aeromicrobiology	95		
5.9.1	Buildings	95		
5.9.2	Public Health	96		
5.9.3	Hospitals and Laboratories	96		
5.10	Bioaerosol Control	97		
5.10.1	Ventilation	97		
5.10.2	Filtration	97		
5.10.3	Biocidal Control	97		
5.10.4	Isolation	98		
5.11	Biosafety in the Laboratory	98		
5.11.1	Biological Safety Cabinets	98		
5.11.2	Biosafety Laboratories	100		
			Part III	
			Detection, Enumeration, and Identification	
			8. Environmental Sample Collection and Processing	
			<i>Ian L. Pepper, Charles P. Gerba and Raina M. Maier</i>	
			8.1 Soils and Sediments	137
			8.1.1 Sampling Strategies and Methods for Surface Soils	138

8.1.2	Sampling Strategies and Methods for the Subsurface	139	10.2	Culture Media for Bacteria	176
8.1.3	Sample Processing and Storage	141	10.2.1	General Media Used for Culturing Bacteria	176
8.2	Water	144	10.2.2	New Approaches to Enhanced Cultivation of Soil Bacteria	183
8.2.1	Sampling Strategies and Methods for Water	144	10.3	Cultural Methods for Fungi	184
8.2.2	Processing Water Samples for Virus Analysis	145	10.4	Cultural Methods for Algae and Cyanobacteria	185
8.2.3	Processing Water Samples for Detection of Bacteria	147	10.5	Cell Culture–Based Detection Methods for Viruses	186
8.2.4	Processing Water Samples for Detection of Protozoan Parasites	148		Questions and Problems	188
8.3	Air	149		References and Recommended Readings	188
8.3.1	Sampling Devices for the Collection of Air Samples	149			
8.4	Detection of Microorganisms on Fomites	153			
	Questions and Problems	154			
	References and Recommended Readings	154			
9.	Microscopic Techniques		11.	Physiological Methods	
	<i>Timberley M. Roane, Ian L. Pepper and Raina M. Maier</i>			<i>Todd R. Sandrin, David C. Herman and Raina M. Maier</i>	
9.1	History of Microscopy	157	11.1	Introduction	191
9.2	Theory of Microscopy	157	11.2	Measuring Microbial Activity in Pure Culture	192
9.3	Visible Light Microscopy	159	11.2.1	Substrate Utilization	192
9.3.1	Types of Light Microscopy	159	11.2.2	Terminal Electron Acceptors	195
9.3.2	Sample Preparation	162	11.2.3	Cell Mass	196
9.4	Fluorescence Microscopy	163	11.2.4	Carbon Dioxide Evolution	197
9.4.1	Direct Counts	164	11.3	Choosing the Appropriate Activity Measurement for Environmental Samples	198
9.4.2	Fluorescent Immunolabeling	165	11.4	Carbon Respiration	198
9.4.3	Fluorescent <i>In Situ</i> Hybridization	165	11.4.1	Measurement of Respiratory Gases, CO ₂ and O ₂ , in Laboratory and Field Studies	199
9.4.4	Confocal Laser Scanning Microscopy	167	11.4.2	The Application of Respiration Measurements in Environmental Microbiology	202
9.4.5	Flow Cytometry	167	11.4.3	Tracer Studies to Determine Heterotrophic Potential	207
9.5	Electron Microscopy	167	11.4.4	Anaerobic Respiration as an Indicator of Microbial Activity	209
9.5.1	Scanning Electron Microscopy	167	11.5	Incorporation of Radiolabelled Tracers into Cellular Macromolecules	209
9.5.2	Transmission Electron Microscopy	169	11.5.1	Incorporation of Thymidine into DNA	210
9.5.3	Elemental Analysis	170	11.5.2	Incorporation of Leucine into Protein	210
9.6	Scanning Probe Microscopy	171	11.6	Adenylate Energy Charge	211
9.6.1	Atomic Force Microscopy	171	11.7	Enzyme Assays	212
9.7	Imaging	171	11.7.1	Dehydrogenase Assay	212
	Questions and Problems	171	11.8	Stable Isotope Probing	215
	References	172	11.9	Functional Genomics and Proteomics-Based Approaches	215
			11.9.1	Functional Genomics	215
			11.9.2	Proteomics	217
				Questions and Problems	219
				References and Recommended Readings	221
10.	Cultural Methods				
	<i>Ian L. Pepper and Charles P. Gerba</i>				
10.1	Cultural Methods for Isolation and Enumeration of Bacteria	173			
10.1.1	Enumeration and Isolation Techniques	173			
10.1.2	Plating Methods	174			
10.1.3	Most Probable Number Technique	175			

12. Immunological Methods

Scot E. Dowd, Marilyn J. Halonen, and Raina M. Maier

12.1 Introduction	225
12.2 What Is an Antibody?	226
12.2.1 Antibody Diversity	227
12.2.2 Antibody Specificity	227
12.2.3 Antibody Affinity	227
12.2.4 Polyclonal and Monoclonal Antibodies	228
12.2.5 Antiglobulins	228
12.3 Immunoassays	230
12.3.1 Fluorescent Immunolabeling	232
12.3.2 Enzyme-Linked Immunosorbent Assays	233
12.3.3 Competitive ELISA	234
12.3.4 Immunomagnetic Separation Assays	235
12.3.5 Western Immunoblotting Assays	236
12.3.6 Immunoaffinity Chromatography Assays	237
12.3.7 Immunocytochemical Assays	238
12.3.8 Immunoprecipitation Assays	239
Questions and Problems	240
References and Recommended Readings	241

13. Nucleic Acid–Based Methods of Analysis

Deborah T. Newby, Elizabeth M. Marlowe and Raina M. Maier

13.1 Structure and Complementarity of Nucleic Acids	243
13.2 Obtaining Microbial Nucleic Acids from the Environment	245
13.2.1 Extraction of Nucleic Acids from Environmental Samples	245
13.3 Gene Probes and Probing	246
13.3.1 Colony Hybridization or Lifts	248
13.3.2 Southern and Northern Hybridizations	248
13.3.3 Fluorescent <i>In Situ</i> Hybridization (FISH)	251
13.3.4 Microarrays	251
13.3.5 Phyloarrays	254
13.4 Polymerase Chain Reaction	254
13.4.1 The Steps of PCR	254
13.4.2 Design of Primers	258
13.4.3 PCR Detection of Specific and Universal Genes	258
13.4.4 RT-PCR	259
13.4.5 ICC-PCR	260
13.4.6 Seminested, Nested, and Multiplex PCR	261

13.4.7 PCR Fingerprinting	263
13.4.8 Real-time PCR	263
13.4.9 Advantages and Disadvantages of PCR	266
13.5 Recombinant DNA Techniques	266
13.5.1 Cloning	266
13.5.2 Metagenomics	268
13.5.3 Sequence Analysis	270
13.5.4 Comparative Genomics	270
13.6 Restriction Fragment Length Polymorphism Analysis	271
13.6.1 Theory and Concept	271
13.6.2 RFLP Analysis of Whole Genomes	271
13.6.3 RFLP Analysis of PCR Sequences	272
13.6.4 Fluorescent Fragment Length Polymorphism Techniques	272
13.6.5 Pulsed Field Gel Electrophoresis	274
13.6.6 Advantages and Disadvantages of RFLP and PFGE Analyses	275
13.7 Denaturing/Temperature Gradient Gel Electrophoresis	275
13.7.1 Theory and Concept	275
13.7.2 Advantages and Disadvantages of DGGE/TGGE	276
13.8 Plasmid Analysis	277
13.8.1 Theory and Concept	277
13.8.2 Advantages and Disadvantages of Plasmid Analyses	277
13.9 Reporter Genes	277
13.9.1 Theory and Concept	277
13.9.2 Specific Reporter Gene Systems	278
13.9.3 Advantages and Disadvantages of Reporter Genes	280
Questions and Problems	281
References and Recommended Readings	281

Part IV Microbial Communication, Activities, and Interactions with Environment and Nutrient Cycling

14. Biogeochemical Cycling

Raina M. Maier

14.1 Introduction	287
14.1.1 Biogeochemical Cycles	287
14.1.2 Gaia Hypothesis	287
14.2 Carbon Cycle	289
14.2.1 Carbon Reservoirs	289
14.2.2 Carbon Fixation and Energy Flow	290
14.2.3 Carbon Respiration	290

14.3 Nitrogen Cycle	299	16.2.3 Quorum Sensing and Cross-Talk	339
14.3.1 Nitrogen Reservoirs	300	16.3 Signaling in Gram-Positive Bacteria	340
14.3.2 Nitrogen Fixation	300	16.3.1 γ -Butyrolactones	341
14.3.3 Ammonia Assimilation (Immobilization) and Ammonification (Mineralization)	302	16.3.2 Peptide Signaling	341
14.3.4 Nitrification	305	16.4 Other Types of Signaling	342
14.3.5 Nitrate Reduction	306	16.4.1 Autoinducers-2 and -3	342
14.4 Sulfur Cycle	309	16.4.2 Eavesdropping on the Party Line	342
14.4.1 Sulfur Reservoirs	310	16.4.3 Bacterial Communication Interference	343
14.4.2 Assimilatory Sulfate Reduction and Sulfur Mineralization	311	16.4.4 Interkingdom Communication	344
14.4.3 Sulfur Oxidation	311	16.4.5 Host–Bacterial Communication	345
14.4.4 Sulfur Reduction	313	16.5 Summary and Core Concepts	345
14.5 Iron Cycle	314	Questions and Problems	345
14.5.1 Iron Reservoirs	314	References and Recommended Readings	345
14.5.2 Iron in Soils and Sediments	314		
14.5.3 Iron in Marine Environments	315	17. Bacterial Communities in Natural Ecosystems	
14.5.4 Iron Oxidation	316	<i>Raina M. Maier and Ian L. Pepper</i>	
14.5.5 Iron Reduction	317	17.1 Bacterial Communities	347
Questions and Problems	317	17.2 Bacterial Diversity in Natural Systems	348
References and Recommended Readings	318	17.2.1 What is a Species?	348
		17.2.2 Diversity in Soil	348
15. Consequences of Biogeochemical Cycles Gone Wild		17.2.3 Diversity in the Ocean	349
<i>David C. Herman and Raina M. Maier</i>		17.3 Functional Diversity and the Resilience of Bacterial Communities	350
15.1 Introduction	319	17.3.1 Soil Bacterial Communities	350
15.2 Microbially Influenced Corrosion	320	17.3.2 Soil–Plant–Microbe Interactions	351
15.2.1 Metal Corrosion	320	17.4 Microbial Diversity and Natural Products	353
15.2.2 Microbially Induced Concrete Corrosion	322	Questions and Problems	355
15.3 Acid Mine Drainage and Metal Recovery	323	References and Recommended Readings	355
15.3.1 Acid Mine Drainage	323		
15.3.2 Metal Recovery	325	18. Global Change and Microbial Infectious Disease	
15.3.3 Desulfurization of Coal	326	<i>Ian L. Pepper and Charles P. Gerba</i>	
15.4 Biomethylation of Metals and Metalloids	326	18.1 Environmental Human Pathogenic Microbes	357
15.5 Nitrous Oxide and Earth's Atmosphere	327	18.1.1 Indigenous Pathogens of Soilborne Origin	359
15.6 Nitrate Contamination of Groundwater	329	18.1.2 Water-Based and Airborne Human Pathogenic Microbes	360
15.7 Composting	330	18.2 Routes of Exposure	360
Questions and Problems	332	18.2.1 What We Breathe	360
References and Recommended Readings	332	18.2.2 What We Eat	360
		18.2.3 What We Drink	361
16. Microbial Communication: Bacteria– Bacteria and Bacteria–Host		18.3 Environmental Change and Microbial Infectious Diseases	362
<i>Leland S. Pierson III, Raina M. Maier, and Ian L. Pepper</i>		18.3.1 Global Climate Change and Microbial Infectious Disease	362
16.1 Introduction	335	18.3.2 Urbanization and Deforestation	363
16.2 Communication via Quorum Sensing in Gram-Negative Bacteria	336	Questions and Problems	363
16.2.1 N-Acyl Homoserine Lactones (AHLs)	336	References and Recommended Readings	363
16.2.2 Quorum Sensing in <i>Agrobacterium tumefaciens</i> , a Ubiquitous Plant Pathogen	337		

19. Microbial Transport

Deborah T. Newby, Ian L. Pepper and Raina M. Maier

19.1 Factors Affecting Microbial Transport	365
19.1.1 Microbial Filtration	366
19.1.2 Physiological State	366
19.1.3 Microbial Adhesion—The Influence of Cell Surface Properties	367
19.1.4 Impact of pH on Microbial Transport	371
19.1.5 Impact of Ionic Strength on Transport	371
19.1.6 Cellular Appendages	372
19.1.7 Hydrogeological Factors	373
19.1.8 Persistence and Activity of Introduced Microbes	375
19.2 Factors Affecting Transport of DNA	375
19.3 Novel Approaches to Facilitate Microbial Transport	376
19.3.1 Ultramicrobacteria	376
19.3.2 Surfactants	376
19.3.3 Gene Transfer	377
19.4 Microbial Transport Studies	377
19.4.1 Column Studies	377
19.4.2 Field Studies	378
19.4.3 Tracers	379
19.5 Models for Microbial Transport	380
19.5.1 Advection–Dispersion Models	380
19.5.2 Filtration Models	381
Questions and Problems	381
References and Recommended Readings	382

Part V

Remediation of Organic and Metal Pollutants

20. Microorganisms and Organic Pollutants

Raina M. Maier

20.1 Introduction	387
20.2 Environmental Law	388
20.3 The Overall Process of Biodegradation	390
20.4 Contaminant Structure, Toxicity, and Biodegradability	393
20.4.1 Genetic Potential	393
20.4.2 Toxicity	394
20.4.3 Bioavailability	394
20.4.4 Contaminant Structure	396
20.5 Environmental Factors Affecting Biodegradation	397
20.5.1 Redox Conditions	397
20.5.2 Organic Matter Content	398
20.5.3 Nitrogen	398
20.5.4 Other Environmental Factors	398
20.6 Biodegradation of Organic Pollutants	399

20.6.1 Pollutant Sources and Types	399
20.6.2 Aliphatics	402
20.7 Bioremediation	414
20.7.1 Addition of Oxygen or Other Gases	416
20.7.2 Nutrient Addition	417
20.7.3 Sequential Anaerobic-Aerobic Degradation	418
20.7.4 Addition of Surfactants	418
20.7.5 Addition of Microorganisms or DNA	418
Questions and Problems	419
References and Recommended Readings	419

21. Microorganisms and Metal Pollutants

Timberley M. Roane, Christopher Rensing, Ian L. Pepper and Raina M. Maier

21.1 Metals in the Environment	421
21.2 Cause for Concern	422
21.3 Metals Defined	422
21.3.1 The Essential Metals	423
21.3.2 The Toxic Metals	423
21.3.3 The Nontoxic Nonessential Metals	423
21.4 Metal Sources	424
21.4.1 Anthropogenic Sources	424
21.4.2 Natural Sources	424
21.5 Metal Solubility, Bioavailability and Speciation	425
21.5.1 Metal Chemistry	426
21.5.2 Cation-Exchange Capacity	427
21.5.3 Redox Potential	427
21.5.4 pH	427
21.6 Metal Toxicity Effects on the Microbial Cell	427
21.7 Mechanisms of Microbial Metal Resistance and Detoxification	429
21.7.1 General Mechanisms of Metal Resistance	430
21.7.2 Metal-Dependent Mechanisms of Resistance	430
21.8 Methods for Studying Metal–Microbial Interactions	432
21.8.1 Culture Medium	432
21.8.2 Measurement of Total, Soluble, and Bioavailable Metal	433
21.9 Microbial Metal Transformations	434
21.9.1 Oxidation–Reduction	434
21.9.2 Methylation	435
21.10 Physicochemical Methods of Metal Remediation	435
21.11 Microbial Approaches in the Remediation of Metal-Contaminated Soils and Sediments	437
21.12 Microbial Approaches in the Remediation of Metal-Contaminated Aquatic Systems	438

Questions and Problems	439
References and Recommended Readings	440

Questions and Problems	498
References and Recommended Readings	498

Part VI Water- and Foodborne Pathogens

22. Environmentally Transmitted Pathogens

Charles P. Gerba

22.1 Environmentally Transmitted Pathogens	445
22.2 Bacteria	447
22.2.1 <i>Salmonella</i>	447
22.2.2 <i>Escherichia coli</i> and <i>Shigella</i>	448
22.2.3 <i>Campylobacter</i>	449
22.2.4 <i>Yersinia</i>	450
22.2.5 <i>Vibrio</i>	451
22.2.6 <i>Helicobacter</i>	452
22.2.7 <i>Legionella</i>	453
22.2.8 Opportunistic Bacterial Pathogens	454
22.2.9 Blue-Green Algae	455
22.3 Parasitology	457
22.3.1 Protozoa	458
22.3.2 Nematodes	465
22.3.3 Cestodes (<i>Taenia saginata</i>)	467
22.3.4 Trematodes (<i>Schistosoma mansoni</i>)	467
22.4 Viruses	469
22.4.1 Enteric Viruses	469
22.4.2 Respiratory Viruses	475
22.5 Fate and Transport of Pathogens in the Environment	479
Questions and Problems	480
References and Recommended Readings	481

23. Indicator Microorganisms

Charles P. Gerba

23.1 The Concept of Indicator Organisms	485
23.2 Total Coliforms	486
23.2.1 The Most Probable Number (MPN) Test	487
23.2.2 The Membrane Filter (MF) Test	487
23.2.3 The Presence–Absence (P–A) Test	487
23.3 Fecal Coliforms and <i>Escherichia coli</i>	490
23.4 Fecal Streptococci	490
23.5 <i>Clostridium Perfringens</i>	491
23.6 Heterotrophic Plate Count	491
23.7 Bacteriophage	492
23.8 Other Potential Indicator Organisms	493
23.9 Standards and Criteria for Indicators	494
23.10 Microbial Source Tracking	496

Part VII Wastewater Treatment and Disinfection

24. Wastewater Treatment and Biosolids Reuse

Charles P. Gerba and Ian L. Pepper

24.1 The Nature of Wastewater (Sewage)	503
24.2 Modern Wastewater Treatment	506
24.2.1 Primary Treatment	506
24.2.2 Secondary Treatment	506
24.2.3 Tertiary Treatment	511
24.2.4 Removal of Pathogens by Sewage Treatment Processes	511
24.2.5 Removal of Organics and Inorganics by Sewage Treatment Processes	513
24.3 Oxidation Ponds	513
24.4 Septic Tanks	515
24.5 Land Application of Wastewater	516
24.6 Wetlands and Aquaculture Systems	518
24.7 Sludge Processing	521
24.7.1 Stabilization Technologies	521
24.7.2 Sludge Processing to Produce Class A Biosolids	522
24.8 Land Application of Biosolids and Animal Wastes: An Historical Perspective and Current Outlook	523
24.8.1 Class A versus Class B Biosolids	523
24.9 Methods of Land Application of Biosolids	524
24.10 Pathogens of Concern in Class B Biosolids	524
24.10.1 Other Biological Concerns with Biosolids	525
24.10.2 Risks from Pathogens in Biosolids	527
24.11 Pathogens in Animal Manures	528
Questions and Problems	528
References and Additional Readings	529

25. Drinking Water Treatment

Charles P. Gerba

25.1 Water Treatment Processes	531
25.2 Water Treatment Requirements	533
25.3 Water Distribution Systems	534
25.4 Organic Carbon and Microbial Growth in Distribution Systems	536

Questions and Problems	538
References and Recommended Readings	538

26. Disinfection

Charles P. Gerba

26.1 Thermal Destruction	540
26.2 Kinetics of Disinfection	541
26.3 Factors Affecting Disinfectants	542
26.4 Halogens	545
26.4.1 Chlorine	545
26.4.2 Chloramines	545
26.4.3 Chlorine Dioxide	546
26.4.4 Bromine and Iodine	546
26.5 Ozone	547
26.6 Metal Ions	547
26.7 Ultraviolet Disinfection	548
26.8 Photodynamic Inactivation	550
26.9 Gamma and High-Energy Irradiation	550
Questions and Problems	551
References and Recommended Readings	551

Part VIII Urban Microbiology

27. Domestic and Indoor Microbiology

Charles P. Gerba and Ian L. Pepper

27.1 Household Sources of Pathogens	555
27.1.1 Air	555
27.1.2 Food	556
27.1.3 Water	556
27.2 Fomites: Role in Disease Spread	558
27.2.1 Occurrence of Pathogens on Fomites	559
27.2.2 Persistence of Pathogens on Fomites	559

27.3 Transfer of Pathogens	559
Questions and Problems	562
References and Recommended Readings	562

28. Microorganisms and Bioterrorism

Ian L. Pepper, Christopher Y. Choi and Charles P. Gerba

28.1 Microbial Agents of Concern as Weapons of Bioterrorism	566
28.2 Bioterrorism and Potable Water	568
28.2.1 Real-Time Monitoring in Water Distribution Systems	568
28.2.2 Real-Time Monitoring	569
28.2.3 Contaminant Transport Mechanisms and Water Quality Modeling	569
28.3 Bioterrorism and Agriculture	571
28.3.1 Contamination via Airborne Microbial Agents	571
28.3.2 Foot-and-Mouth Disease	574
28.4 Transmission by Fomites	574
References and Recommended Readings	574

29. Risk Assessment

Charles P. Gerba

29.1 The Concept of Risk Assessment	575
29.2 Elements of Risk Assessment	575
29.3 The Process of Risk Assessment	577
29.3.1 Hazard Identification	577
29.3.2 Exposure Assessment	578
29.3.3 Dose–Response Assessment	579
29.3.4 Risk Characterization	580
29.4 Microbial Risk Assessment	581
Questions and Problems	586
References and Recommended Readings	587

Index	589
-------	-----

Preface

Historically, environmental microbiology can be traced to studies of municipal waste treatment and disposal. In the first Edition of *Environmental Microbiology*, we recognized that this field had expanded to the study of earth, water, and air systems, including the interaction of indigenous microbes with organic and inorganic pollutants, behavior of pathogens introduced into these systems, and the discovery and application of new microbes and their products to benefit human health and welfare. In the intervening years since, there has been a virtual explosion of knowledge on microbial diversity and communities in various environments. As a result, in the second edition of *Environmental Microbiology* we have added chapters on extreme environments, as well as microbial communities and communication among microorganisms. Similarly, in recognition of ever-increasing human population pressures and climate change, we have added chapters on domestic microbiology, bioterrorism, and the impact of global change on microbial infectious disease.

Microbes are everywhere, all over the world and in every imaginable environment. For example in soil, just one gram contains billions of microorganisms and all their associated activities. Imagine the challenge of studying all the major groups of microbes found in each of earth's biomes given the magnitude of their immense diversity. Imagine then the challenge of developing strategies to harness and manipulate their activities. That is what environmental microbiology is about. We invite you to use this text to begin the exciting adventure of understanding microorganisms in their many environments.

This text has eight subject areas presented in a logical progression: (i) foundation chapters to provide an adequate background for the advanced material presented in subsequent chapters; (ii) chapters on microbial environments, including earth, aquatic, and atmospheric; (iii) chapters on detection and quantitation of microbial activity, including cultural, microscopic, physiological, molecular, and immunological approaches; (iv) chapters on microbial

interactions with their environment from element cycling to microbial communication to development and movement of bacterial communities, (v) chapters on microbial remediation of organic and metal pollutants, (vi) chapters on water and food-borne pathogens, (vii) chapters on waste treatment and drinking water, and finally, (viii) chapters on urban issues including domestic and indoor microbiology, bioterrorism, and risk assessment. This textbook is designed for a senior-level undergraduate class or a graduate-level class in environmental microbiology and to serve as a reference for scientists and engineers interested in this field. The overall objectives of the text are to define the important microbes involved in environmental microbiology, the nature of the different environments in which the microbes are situated, and the methodologies used to monitor the microbes and their activities and, finally, to evaluate the effects of these microbes on human activities. This book represents a joint effort led by three authors who have diverse yet complementary backgrounds in environmental microbiology. The authors are close colleagues at the University of Arizona and all have large and active research programs. They have worked together extensively on a variety of practical problems using advanced, interdisciplinary approaches. Examples include microbiology of extreme environments, biotechnology applications of microbial surfactants, molecular detection of emerging pathogens, transport of microbes and DNA through soil, and microbial risk assessment. Their extensive research programs have provided a number of the examples used in this text to illustrate important learning points. Key contributions to this text were also made by eleven colleagues who collaborate with the authors at the University of Arizona. This group has worked closely together, resulting in a textbook that has continuity in depth and style, and that is state-of-the-art at the time of press.

Raina Maier, Ian Pepper and Charles Gerba



Acknowledgments

Textbook development: We would like to acknowledge various federal funding agencies that have supported our research throughout the years providing rich and varied perspectives to bring to bear on the topic of Environmental

Microbiology. These include the National Science Foundation, the National Institute of Environmental Health Sciences, the Environmental Protection Agency, the US Department of Agriculture and the Department of Homeland Security.



The Authors

All three authors are professors in the Department of Soil, Water and Environmental Science at the University of Arizona.



Raina M. Maier Ph.D., Rutgers University, 1988. Currently, Professor of Environmental Microbiology and Associate Director of the University of Arizona NIEHS Superfund Basic Research Program. Dr. Maier's research is focused on developing a basic understanding of how to evaluate and control microbial activity

in disturbed and extreme environments ranging from mine tailings to cave environments to the Atacama Desert, Chile. She is known for using an interdisciplinary approach to study the interaction microorganisms with both biotic and the abiotic components of their environment. Dr. Maier has earned an international reputation for her work on microbial surfactants (biosurfactants) a class of fascinating secondary metabolites with possible uses in remediation, biological control, surface coatings, and the cosmetic and pharmaceutical industries

"Environmental microbiology remains one of the relatively unexplored and extremely exciting frontiers of science. So little is yet known about environmental microbes—partially because they quickly become lab rats when taken out of their environment—that the possibilities for new discoveries are limitless."



Ian L. Pepper Ph.D., The Ohio State University, 1975. Currently, Professor of Environmental Microbiology. Dr. Pepper's diverse research interests are reflected in the fact that he is Fellow of The American Association

for the Advancement of Science, The American Academy of Microbiology, the Soil Science Society of America, and the American Society of Agronomy. He is also Director

of the National Science Foundation Water Quality Center at the University of Arizona. Dr. Pepper has been active in the area of soil molecular ecology as well as waste utilization including biosolids and effluent reuse. More recently he pursues research on real-time monitoring of microbial contaminants in potable water, and "smart water distribution systems."

"Microbes are in the air we breathe, the water we drink and the food we eat. In fact there are more microbes within our bodies than mammalian cells. On this basis alone, microbes are fascinating, and when you study environmental microbes, it takes your breath away."



Charles P. Gerba Ph.D., University of Miami, 1973. Currently, Professor of Microbiology. Dr. Gerba is a Fellow of the American Academy of Microbiology. He is recipient of the A. P. Black Award from the American Water Works Association for outstanding contributions to Water Science, and the McKee Award from the Water

Environment Federation for outstanding contributions to groundwater protection. He has an international reputation for his methodologies for pathogen detection in water and food, pathogen occurrence in households, and risk assessment.

"My interest in microbiology was sparked by Paul DeKruif's inspiring tales of the scientific achievements of early microbiologists in the book The Microbe Hunters and my mother's error in giving me a microscope for Christmas instead of the chemistry set I wanted. In my first summer job out of college, I was introduced to environmental microbiology by studying sewage disposal. Later, I examined the fate of viruses in sewage discharged into the ocean. These beginnings led me to an exciting and adventurous career in environmental microbiology where every day brings a new problem to be addressed."



Contributing Authors

Edition 2



Scot E. Dowd Ph.D. Microbiologist, USDA ARS Livestock Issues Research Unit, Lubbock, TX.



Marilyn J. Halonen, Professor of Pharmacology, University of Arizona



Christopher Y. Choi, Professor of Agricultural and Biosystems Engineering, University of Arizona



Elizabeth M. Marlowe Ph.D. Assistant Director of Microbiology-Molecular Testing, Southern California Permanente Medical Group



David C. Herman Ph.D. in Microbiology, University of Arizona



Deborah T. Newby Ph.D. Idaho National Laboratory



Leland S. Pierson III Professor of Plant Pathology, University of Arizona



Timberley M. Roane Associate Professor of Microbiology, University of Colorado, Denver



Christopher Rensing Associate Professor of Microbiology, University of Arizona



Todd R. Sandrin Associate Professor of Microbiology, University of Wisconsin, Oshkosh



Kelly A. Reynolds Associate Professor of Public Health, University of Arizona