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Emerging Micro-Pollutants in the Environment: Occurrence, Fate, and Distribution

In Emerging Micro-Pollutants in the Environment: Occurrence, Fate, and Distribution; Mitchell, et al.;
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Emerging Micro-Pollutants in the Environment: Occurrence, Fate, and Distribution

Sudarshan Kurwadkar, Editor
California State University − Fullerton, Fullerton, California

Xiaoqi (Jackie) Zhang, Editor
University of Massachusetts − Lowell, Lowell, Massachusetts

David Ramirez, Editor
Texas A&M University − Kingsville, Kingsville, Texas

Forrest L. Mitchell, Editor
Texas A&M AgriLife Research, Texas A&M University System, Stephenville, Texas

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Foreword

The ACS Symposium Series was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from the ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

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Opening Remarks

The importance and legitimacy of understanding emerging contaminants and their implications to human health and the environment cannot be understated. As technology advances, society benefits from the products produced, techniques innovated, and opportunities created; however, there is a flip side. There is a cost associated with today's technology when the complete life cycle is considered. Consider for a moment, nature's tree. No waste is produced in the production of the root system or its leaves. At the end of the tree's lifetime, no part is non-degradable creating lasting contamination for generations to come. Today's state of science and technology is not like the tree. How do persistent organic pollutants generated during the manufacture of man-made products affect the environment and subsequently, human health? How do the novel characteristics of the nanomaterials that provide so much benefit to society interact with environmental media and the physiology of humans? Advances in medicine produce pharmaceuticals saving literally countless lives, but what of the evolutionary adaptation by microorganisms creating antibiotic resistant-super bugs? Additionally, poor disposal practices and excretion via urinary pathways introduces pharmaceuticals to wastewater treatment facilities only to be minimally treated before discharge to natural waterways. What are the ecological consequences of these actions? Consider also the growing demand of the ever scarcer resource of drinking water. Direct water reuse is currently employed in Singapore and many other locations; indirect water reuse is growing in acceptance in the United States and will only increase in the future. How are the trace organic contaminants discharged in the wastewater effluent, (and eventually taken in as drinking water plant influent) affecting human health? What are the appropriate technologies to employ for treatment? Answers to these difficult questions require an economic and pragmatic balance. Cleaner water can be obtained but at what cost? What are the truly negative consequences of emerging contaminants and what are the positive gains afforded by implementing technology A versus technology B (versus a do-nothing approach)? Answers to these questions will require a host of actions to include public debate, appropriate regulatory action and demonstration and validation of viable treatment technologies. Increasing our knowledge of the distribution, transport, fate, and toxicological effects to the environment and human health will assist in all of these capacities. This book addresses these subjects and will be of great utility to researchers, regulators, keen members of the public, and those in the teaching profession. I hope you find it as interesting and useful as I did.

David M. Kempisty, Lt Col, Ph.D., USAF
Assistant Professor
Director, Environmental Engineering and Science Program
Air Force Institute of Technology

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Dedication

This book is dedicated to my caring and loving wife Nanda (I wish she could read!) and children Siddhartha, Gaya, Sachet and Syon (I wish they could understand what the book is all about!)

Sudarshan Kurwadkar

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Preface

Over the last decade, majority of the research focus has shifted from the legacy contaminants to the emerging contaminants of concern. These emerging contaminants are not of recent origin, but their analytical quantification, exposure routes and pathways and consequent human health and ecological effects are now becoming known. The Industrial Revolution introduced new materials hitherto unknown to humanity, optimized industrial processes, and greatly improved our living standards. The net consequences of this progress is the generation and discharge of new waste streams in the environment and as such term 'emerging contaminants' will remain in vogue as we continue to understand the nature, persistence, stability and related human health and ecological risks due to these new contaminants. At this point in time, we are deliberating on emerging contaminants such as pharmaceuticals and personal care products, insecticides, poly- and perfluorinated compounds and engineered nanomaterials. Within few years, there may be more lurking just behind, about whom we will soon learn. Recently, the United States Environmental Protection Agency has declared the perfluorinated compounds (PFCs) as emerging contaminants of concern. The PFCs have been in use since 1950's. It is only now becoming known that they are persistent, bio-accumulative and have serious human health consequences.

Detection of emerging contaminants in various environmental matrices including air, water, soil, and sediments has been reported in various journals and scientific reports with increasing frequency. Yet, little information exists in terms of remediation, human health and ecological risk characterization and promulgation of new regulation to deal with the problems associated with emerging contaminants. Over the past decade, advances in science and technology have brought before us uncertainty about the likelihood of our very survival, be it through the onslaught of antibiotic resistance organisms or through the annihilation of our food web through the extinction of pollinators. It should be noted that, it takes more than a decade-long rigorous scientific endeavor to develop an antibiotic, followed by equally rigorous trials before these antibiotics are released into the market for therapeutic usages. Yet, through improper use of antibiotics (therapeutic vs. sub-therapeutic) coupled with poorly managed waste storage, treatment and disposal facilities, we render these efforts less useful through the growth of antibiotic resistant microbes. It doesn't take the microorganisms very long to become resistant to antibiotics and worst yet, genetically transfer it to the next generation of superbugs. Recent discovery of a bacteria containing *New Delhi Metallo 1* (NDM-1) enzyme illustrates this concern. It is now being confirmed that the bacteria carrying NDM-1 enzyme is practically resistant to most powerful group of antibiotics rendering major

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infections extremely difficult to treat. This may sound alarmist but not farfetched simply because use of antibiotics both in developed and undeveloped countries is not regulated or drug resistance is being monitored.

It is not just the antibiotics, other emerging contaminants such as insecticides have also brought renewed discussion with the European Union banning the neonicotinoid class of insecticides in agriculture operations. As the insecticides fall under the current definition of micro-pollutants and so are discussed in this volume. From the success of DDT in typhus outbreak prevention during the Second World War to the environmental damage suddenly thrust into public awareness by Rachel Carson two decades later, insecticides are a part of modern society. There is no doubt that many advances in agricultural applications, human and animal disease prevention and structural building integrity would not have been made without the modern chemistry of insecticides, but there is equally no doubt about the consequences of their misuse and overuse. As with release of genetically modified organisms into the environment for pest control, there are many emotional and passionate positions taken by the public and by commercial entities on insecticide use. One current debate rages about the neonicotinoid insecticides used in many countries for agricultural pest management and parasite control on animals. Conflicting opinions about the possible adverse impact on non-target species, such as the role of neonicotinoid in honeybee colony collapse disorder, need further investigation before we ban these compounds for agriculture operations. Currently, the regulatory agencies have renewed their focus to better understand the environmental impact of insecticides as they seek re-registration for their continued use in agriculture operations.

In addition to environmental impact of pharmaceuticals and insecticides, engineered nanomaterials also emerged as emerging contaminants of concern. Rapid advances in technology have offered us avenues to harness material properties at nano-scale, where they exhibit distinctly different physical and chemical characteristics than their macro versions. The engineered nanomaterials are covered in this volume simply because they have become an integral part of our lifestyle with more than 1,600 consumer products including pharmaceuticals and personal care products containing engineered nanomaterials. These materials have found applications in wide variety of human endeavors ranging from drug delivery and disease diagnostics to commercial and agricultural operations. The fast paced development of nanotechnology has outpaced our existing understanding of their human health and ecological consequences. This situation is further compounded by the absence of environmental regulatory framework to determine whether nanomaterial consequences outweigh their benefits. With nearly 60% of nano-based human consumer products (30% medical/pharmaceuticals and 29% chemicals and advanced materials) it is disconcerting whether routine exposure to these materials has any unforeseen adverse human health and ecological consequences.

Our understanding of issues concerning emerging micro-pollutants continue to evolve through scientific investigation, analytical sophistication and dissemination. This book is an effort to communicate current knowledge and identify future direction so that we will be better able to manage and mitigate adverse environmental and human health consequences due to emerging

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contaminants. The first chapter of the book provides a general introduction to the world of emerging contaminants, and the need for further research in this area (Chapter 1). The book is divided in two distinct sections to better present this understanding. The first section of the book, "Occurrence, Fate and Detection of Emerging Micro-pollutants" includes a review chapter on antibiotics prepared by an accomplished researcher (Chapter 2). The chapter provides a rich discussion on a variety of antibiotics used in human and agriculture operations followed by the emergence of antibiotic resistant micro-organisms and common methods for detecting and monitoring the resistance. Analytical quantification of emerging micro-pollutants is crucial to understand exposure levels not only from regulatory standpoint but also to develop toxicological profiles through dose-response toxicological studies. The chapter on analytical determination of pharmaceuticals and personal care products (Chapter 3) using state-of-the art analytical instruments such as LC/MS and GC/MS is presented by a renowned analytical chemist.

The remaining three book chapters in this section, deal with the fate, transport and removal of emerging micro-pollutants. These book chapters are prepared by researchers who are currently working towards developing new insights into the environmental mobility and the removal of emerging micro-pollutants. A chapter on transport of emerging contaminants such as hormones in the sub-surface environment provides a new insight into the colloid facilitated transport and how soil particle size plays an important role in carrying these pollutants to the soil environment (Chapter 4). A chapter that augments this understanding is added to include mathematical modeling approach to generalize the transport mechanism (Chapter 5). This chapter though does not deal with the hormones; it covers an ionizable antibiotic and an insecticide. This chapter provides a modeling approach to generalize the transport mechanism by uniquely combining speciation chemistry of antibiotics and how it influences the mobility of antibiotics in the sub-surface environment. In addition to this a modeling protocol for studying the persistence of insecticides under abiotic degradation (hydrolysis) conditions is also included. While these book chapters deal with the fate and transport, a complimentary chapter deals with the removal of antibiotics from drinking water sources (Chapter 6). This book chapter provides a comprehensive understanding of various modeling approaches and a comparative analysis of removal of antibiotics using laboratory developed activated carbon and commercially available activated carbon.

The second section of the book, "Toxicity and Analytical Determination of Emerging Micro-Pollutants" includes a chapter that specifically deals with recognizing the need for research on implications of engineered nanomaterials in the environment. The book chapters in the second section are written by well-known and acknowledged authorities in this area. First book chapter in this section takes a critical look at the existing understanding and recognizes the knowledge gaps on implications of nanomaterials in sustainable wastewater reuse (Chapter 7). Two follow-up chapters provides an in-depth understanding of the toxic response elicited by the mixture of metal oxide nanoparticles and long single-walled carbon nanotubes in the conventional activated sludge wastewater treatment process (Chapter 8 & 9). These three book chapters

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provide comprehensive understanding of nanomaterials and their environmental implications. The next chapter in this section provides a perspective on assessment of anthropogenic perchlorate in the environment. This chapter is written by well-known arctic environmental scientists that seek to establish and reconstruct the records of environmental pollutants through ice core records. This interesting book chapter seeks to differentiate between the naturally occurring perchlorate from the one originating solely from anthropogenic activities (Chapter 10). The last book chapter in this section provides an advanced analytical technique for determination of emerging pollutants in the environment (Chapter 11). The author is an analytical chemist and a recognized research scientist and presents a cogent case for differential mobility spectrometry and how it can be used for low-level detection of emerging micro-pollutants in the environment. This chapter includes two case studies dealing with separation of fungicidal traizoles and rapid detection of napthenic acids that elucidates the application of differential mobility spectrometry for detection of emerging micro-pollutants.

The book covers an important aspect of emerging micro-pollutants with regard to their environmental occurrence, analytical quantification, fate, transport (surface and sub-surface), persistence and removal mechanism. It is our understanding that the book covers majority of the emerging contaminants and their environmental implications and future directions. It is hoped that this book will provide an important source of information to researchers, academicians and environmental regulatory agencies. Together we can make this environment sustainable for generations to come.

Sudarshan Kurwadkar
California State University − Fullerton
Fullerton, California

Xiaoqi (Jackie) Zhang
University of Massachusetts − Lowell
Lowell, Massachusetts

David Ramirez
Texas A&M University − Kingsville
Kingsville, Texas

Forrest L. Mitchell
Texas A&M AgriLife Research, Texas A&M University System
Stephenville, Texas

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Editors' Biographies

Sudarshan Kurwadkar

Dr. Sudarshan Kurwadkar is an Assistant Professor in the Civil and Environmental Engineering Department at California State University, Fullerton. He is a Board Certified Environmental Engineer and a licensed Professional Engineer. Prior to his academic career, he worked in the Division of Environmental Quality with the Missouri Department of Natural Resources. His research interests are in the broadly defined area of fate and transport of emerging contaminants in aquatic and terrestrial environment. This book is the result of his continued research collaboration and mentorship of his co-editors. In his spare time he enjoys outdoor activities such as fishing, camping and kayaking.

Xiaoqi (Jackie) Zhang

Dr. Jackie Zhang is a Professor of Environmental Engineering in the Department of Civil & Environmental Engineering at the University of Massachusetts Lowell (Lowell, MA). Over the years, she has been working with using biofilms for wastewater treatment, nutrients removal from confined animal feeding operations and septic tanks, stormwater management, evaluating the impacts of carbon nanotubes on wastewater treatment, and renewable energy generation.

David Ramirez

Dr. David Ramirez is an Associate Professor of the Department of Environmental Engineering at Texas A&M University-Kingsville (TAMUK). He is the Director of Environmental Technology at the TAMUK's Eagle Ford Center for Research, Education and Outreach. He was also the Director of the Center for Research Excellence in Science and Technology – Research on Environmental Sustainability in Semi-Arid Coastal Areas funded by the National Science Foundation (NSF). He is a recipient of the prestigious 2009 NSF's CAREER award to study the atmospheric transformations and interactions of emerging nanoparticles with hazardous air pollutants. He has led research studies on atmospheric particulate matter pollution characterization and human exposure in Texas cities. He is the author and co-author of over 100 publications and presentations in technical meetings. He has extensive experience in the development of air quality and control technologies for the capture and recovery of organic vapors from waste gaseous streams.

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Forrest L. Mitchell

Dr. Forrest Mitchell is a research project leader for Texas A&M AgriLife Research and professor in the Department of Entomology at Texas A&M University. He is based at the Texas A&M AgriLife Research and Extension Center in Stephenville, TX where he conducts work on insect transmitted plant diseases and diseases of insects. It was during a study of leafhopper vectors of *Xylella fastidiosa* that he became involved with neonicotinoid insecticides used in vector control. In collaboration with Dr. Kurwadkar, that work led to being part of the current volume. When not involved with economic entomology, Dr. Mitchell enjoys working with dragonflies and native bees.

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Chapter 1

Introduction

Sudarshan Kurwadkar,[1,*] Xiaoqi (Jackie) Zhang,[2] Forrest Mitchell,[3] and David Ramirez[4]

[1]Department of Civil and Environmental Engineering, California State University − Fullerton, 800 N. State College Blvd., Fullerton, California 92831, United States
[2]Department of Civil and Environmental Engineering, University of Massachusetts – Lowell, Lowell, Massachusetts 01854, United States
[3]Texas A&M AgriLife Research, Texas A&M University System, 1229 N. US Highway 281, Stephenville, Texas 76401, United States
[4]Department of Environmental Engineering, Texas A&M University − Kingsville, MSC 213, 700 University Blvd., Kingsville, Texas 78363, United States
[*]E-mail: skurwadkar@fullerton.edu

The occurrence, distribution and fate of emerging micro-pollutants (EMs) is of particular concern due to their toxicity and potential risks to human health and ecology. Research on EMs is multidisciplinary in nature primarily due to the convergence of several disciplines such as chemistry, chemical engineering, environmental chemistry, biology, biochemistry, environmental sciences and engineering, agricultural engineering, microbiology, molecular biology, and biomedical engineering. Not surprisingly, the environmental occurrence, distribution and fate of EMs has become a hotly debated topic in the scientific community. Occurrence of EMs in various environmental matrices such as air, soil, surface water and groundwater has been widely reported in various scientific journals. The continued occurrence of EMs could have severe implications to human health and ecology. This chapter introduces the reader, the sources, pathways, fate and distribution of EMs in the environment.

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1. Sources and Fate of Pharmaceuticals in the Environment

1.1. Sources and Occurrence of Antibiotics in the Environment

According to the United States Environmental Protectin Agency (USEPA) pharmaceuticals refer to prescription and over the counter therapeutic drugs and veterinary drugs (*1*). In recent years, occurrence of ABs (ABs) in the environment has been reported in various scientific journals, periodicals and national newspapers with increasing frequency. Most recently, the Associated Press investigation revealed that, nearly 46 million Americans are supplied with water that has been tested positive for trace concentrations of pharmaceuticals including ABs (*2*). Although, potential human health risks due to occurrence of AB's in the environment are yet to be quantified; there are recognized potential human health and ecological consequences (*3*). Varieties of anthropogenic sources through which ABs enter the environment include human therapeutic usages through municipal wastewater; residual concentration of AB's resulting from ABs used in intensive animal agriculture operations such as concentrated animal feed operations facilities, large scale dairy operations, aquaculture industry etc. Animal agriculture introduces ABs into the environment through direct discharge of animal waste containing partially metabolized ABs, surface run-off from land application of manure containing trace ABs and direct grazing of ABs fed animals (Figure 1). Not surprisingly, in the United States, low levels of various veterinary pharmaceuticals (including ABs) have been detected in soil, groundwater, and surface water.

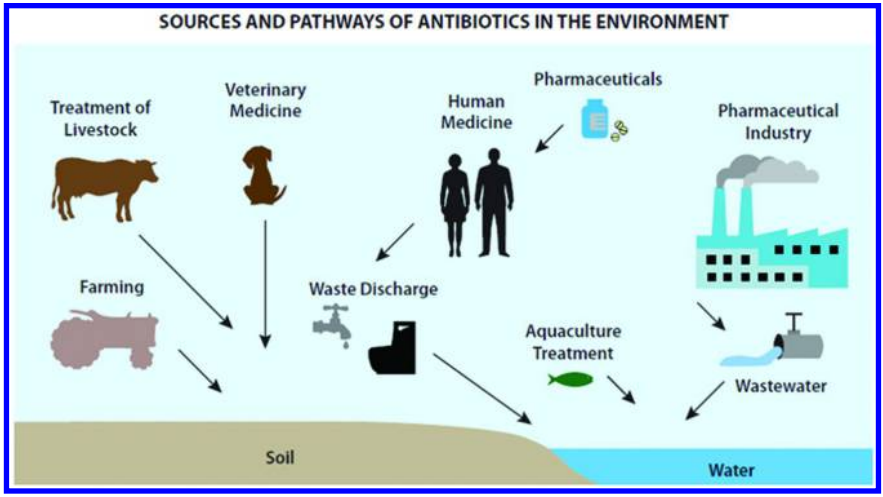

Figure 1. Sources and pathways of pharmaceuticals and personal care products in the environment

1.2. Antibiotics Classes and Usages

The major classes of ABs primarily used in animal agriculture are tetracyclines, sulfonamides, aminoglycosides, β-lactams, macrolides,

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lincosomides and ionophoric monensin (trade name Rumensin). ABs are used in animal agriculture for therapeutic, prophylactic and sub-therapeutic purposes (*4*). Therapeutic usages are intended to cure the disease (treating opportunistic infections) whereas sub-therapeutic usages are intended to improve feed efficiency and accelerate animal growth, thereby making animal products available to consumers in a faster and cheaper way (*3*). Prophylactic usages of ABs are preventive in nature and typically administered prior to the onset of the disease. Therapeutic and prophylactic usages are justifiable and as such are prudent ways of using the ABs for the purpose for which they are manufactured. Unfortunately, majority of ABs used in intensive animal agriculture are for sub-therapeutic purposes. For example, monensin, alone accounts for approximately 13 % of total sub-therapeutic livestock ABs usages in the United States and has been widely detected in the environment (*5*).

Use of ABs in intensive animal agriculture is so pervasive that the feed itself nowadays comes pre-mixed with the ABs. Case in point, monensin, an ionophoric antibiotic approved by the United States Food and Drug Administration (USFDA) is routinely used in dairy industry mostly for sub-therapeutic purposes for improving feed efficiency and weight gain (*6*). Similarly, tylosin (macrolide class of ABs) is also used for weight gain and to improve feed efficiency. Use of tylosin is far too common in concentrated animal feed operations facilities such as large swine and cattle farms. The use of ABs has become such an integral part of animal agriculture practices that ABs' importance for therapeutic purposes has rather diminished. Increased emphasis on using ABs has also diminished the importance of good hygienic environment for the animals, as a result, animals are often reared in a confined and crowded environment. Although sub-therapeutic use of ABs is apparently noble, it may have potential environmental repercussions.

1.3. Antibiotics in the Environment, Human Health, and Ecological Concerns

Considering the prevailing use/misuse of ABs in intensive animal agriculture, it warrants an investigation about their occurrence in the environment. Past research studies have demonstrated that often the animals do not metabolize the ABs completely, thereby excreting part of the unmetabolized ABs through feces and urines. It is not clear how much of the ABs administered for different purposes such as therapeutic, sub-therapeutic and prophylactic gets excreted in the environment. Researcher have, reported that once the ABs are administered, nearly 90 % are excreted back into the environment in partially metabolized form (*7*). Although, the excreted amount of ABs depends on the molecular structure, dosage level, age of the animal and the route of administration (feed additive versus injections) (*3*, *8*), it is not clear how animals metabolize the ABs when they are sick as oppose to when they are not.

Once the ABs are released into the environment, some of the ABs such as sulfonamides are relatively stable and do not degrade under biotic or abiotic degradation conditions. Consequently ABs could be found in water, wastewater, soil, sediment and manure samples. Presence of various ABs in surface water, sediments, municipal wastewater, animal waste lagoons, and groundwater

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underlying lagoons was confirmed by several researchers (*9–11*). Environmental occurrence and behavior of monensin in intensive bovine production system in Argentina is confirmed (*12*). Other researchers have confirmed the presence of monensin in river water and aquatic sediments in Colorado, they attributed the detection of monensin to the feedlots in the watershed (*13*). In Southern Ontario, monensin has been detected in nearly 75 % of stream samples with concentration ranging from 0.006 to 1.2 $\mu g L^{-1}$ (*14*).

Occurrence of ABs in the environment is a cause of concern due to potential human health and ecological consequences particularly the emergence of antibiotic resistance micro-organisms. It should be noted that excluding monensin, some of the classes of ABs are also common to human therapy. For example, tylosin an antibiotic of macrolide class is routinely used as a feed additive in animal agriculture, its equivalent in human therapy is erythromycin. If the microbes become resistant to tylosin there is a good possibility that, they will be resistant to erythromycin antibiotic as well. Growth of antibiotic resistant microorganisms is an acknowledged adverse impact on the environment. Direct consequences on human health due to ingestion of low level of ABs have not been well understood yet, however, several studies have documented an indirect evidence of how occurrence of ABs in the environment is impacting human health. For example, studies have documented that, in human therapy, often times, the first line of ABs were rendered ineffective due to the microbes being resistant to it. Continued occurrence of ABs in the environment might perturb microbial ecology and might as well pose human health risks. Prudent usages coupled with regulatory oversight will certainly help in combating environmental pollution due to ABs and may curb the growth of ABs resistant microbes. This is a serious concern because ABs are our last defense mechanism to fight off various infectitious diseases and annihilation of ABs will have severe human health repercussions.

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2. Sources and Fate of Neonicotinoid Insecticides

2.1. Use of Insecticides – A Historical Perspective

Released for pest control in the 1994, the first generation neonicotinoid insecticide imidacloprid remains in wide use (*15*). Large scale seed treatments of field crops such as corn, horticultural applications in fruit pest management, the toxic ingredient in baits used for pest fly control in large animal operations, homeowner sprays for lawn insects and flea treatments for dogs and cats are a few of the applications. Second generation neonicotinoids as exemplified by thiamethoxam (introduced in 2001) and the third generation exemplified by dinotefuran (introduced in 2005) differ in their increased water solubility from the first generation. Along with low mammalian toxicity, aqueous solubility and therefore translocation within the plant that appeals to agricultural producers. Although these neonicotinoids can greatly reduce vector density and disease outbreak in vineyards, its persistence in the environment due to this and other uses as well as potential human health and ecological risks have largely been ignored.

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Since most neonicotinoids are fairly stable in the environment, it is possible that their occurrence may pose a threat to both aquatic and terrestrial organisms.

2.2. Environmental Occurrence of Insecticides

As discussed earlier, insecticides have been used historically and have served the humanity well. The environmental implications of some of these insecticides is now becoming known. This is due to the development of sophisticated analytical instruments that can detect low levels of insecticides in the various environmental matrices and also due to the increased emphasis on environmental monitoring (*15*). Agriculture application is one of the major pathways of occurrence of insecticides in the environment (Figure 2). Unlike most formulations of carbamates, organophosphates and pyrethroids, neonicotinoids do not necessarily require topical application for effectiveness. Seed treatments are effective at not only protecting the germinating seed, but the young plant as well. Applications made in irrigation water through drip lines effectively protect grape from leafhopper and froghopper pests that feed directly in the plant xylem veins and do not chew leaves. Foliar applications in cotton are not only topically active, but translocate through the leaves and protect against both chewing and probing insects.

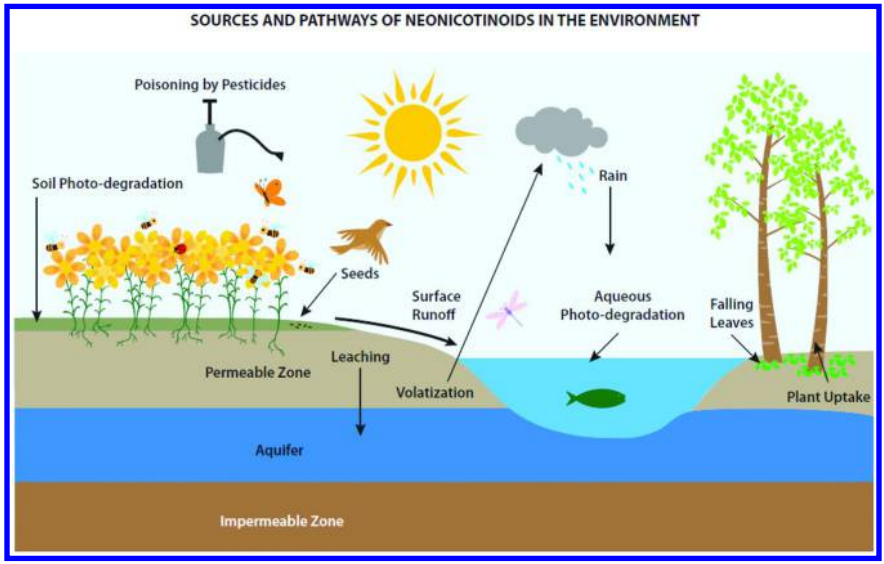

Figure 2. Sources, pathways and fate of occurrence of neonicotinoids in the environment

Different applications methods results in occurrence of neonicotinoids in a variety of environmental matrices such as soil, air, surface water and groundwater. For example, it was reported that surface runoff from turf treated with granular formulation has twice as high concentration of imidacloprid compared to the

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wettable powder (*16*). Furthermore, the chemical properties of neonicotinoids are favorable for their occurrence in terrestrial and aquatic ecosystem through runoff, leaching and drainage from agricultural fields (*17*, *18*). Various pathways through which insecticides enter the aqueous and soil environment and their fate is governed by factors such as sub-surface transport, retention and transformation processes (Figure 2) (*19*). Studies have reported that neonicotinoids can persists in soils for more than a year and nearly 80 to 98 % of residual neonicotinoids eventually enters surface water (through runoff) and groundwater (leaching) (*20*). There is great public concern about the environmental transport of pesticides, particularly their persistence and toxicity to non-target species.

2.3. Environmental Occurrence of Insecticides and Ecological Impact – A Divided Opinion

Neonicotinoids are fairly stable towards abiotic and biotic degradation processes. For example, they are stable under photolytic and hydrolytic conditions. Abiotic degradation mechanisms especially sorption, hydrolysis and photolysis, determines their bioavailability, degradation, volatilization, leaching potential and transport to subsurface and surface environment (*21*). Persistence coupled with the toxicity associated with the application of neonicotinoids requires broader understanding of their application processes. Majority of the neonicotinoids are applied either as granules into the soil or as seed-dressing during crop planting (*20*). Pest managers reasoned that since neonicotinoids are encased in the plant, non-target poisoning of beneficial insects would be reduced. Lady beetles, lacewings and other predators that do not chew leaves or imbibe sap would be protected. Neonicotinoids can escape the plant however, through routes such as guttation, flower nectar and pollen. The latter two have been indicted as routes of transmission to pollinators, especially honey bees that make use of both resources. Neonicotinoids, especially imidacloprid, are claimed to be associated with massive honey bee mortality, collectively referred to as colony collapse disorder (CCD). Research has shown that sub-lethal doses of imidacloprid cause behavioral changes in colonial bees and result in reduced colony vigor and reproduction. Yet critics point out that these studies are artificial and require experimental poisoning of the bee colonies since the insecticide levels needed to produce results are not found in hives. This is particularly true because honeybees housed under laboratory conditions and exposed to high concentrations do exhibit toxic response. But then the concentrations are unrealistically high and highly unlikely to be found in the field conditions. Lack of exposure studies at environmentally relevant concentrations have lead the researchers to conclude that, crop-applied neonicotinoids may not be a major risk factor for honeybee colonies (*22*).

Some researchers maintained that with the level of concentrations that were detected in seed treatment in corn, soybean and cotton, there is a possibility that honeybee may be exposed to neonicotinoids; however, the authors were not certain whether that level of concentration (1 to 6 ng/g) pose serious risk to honeybees (*23*). While there is conflicting information about the role of neonicotinoids in CCD in honeybees, toxic response exhibited by other non-target

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species need to be considered. Some researchers maintained that concentration of neonicotinoids in the post application scenario will indeed affect the non-target species. For example, seed coated with 1-17 mg/kg of neonicotinoids could result in 5 to 10 μgL^{-1} concentration in the sap which is sufficient to control the sucking and chewing insects. While this is beneficial and effective, it also exposes non-target species such as bees, butterflies, moths, and hoverflies (*20*). Studies have reported that neonicotinoid concentrations in global surface water exceeds the exposure threshold and may pose both short-and long-term impact to aquatic invertebrate species (*24*).

3. Nanoparticles in the Environment: Sources, Pathways, Occurrence and Toxicity

3.1. Overview of Environmental Occurrence of Nanoparticles

Widespread application of nanotechnology in a variety of industrial applications has spawned new waste streams, which could potentially impact human health and the ecology. Two major sources that contributes to the environmental occurrence of nanoparticle include myriad anthropogenic activities and natural events. Naturally occurring nanoparticles have existed in the environment for millions of years and human beings to some extent must have evolved and adapted to live in their presence (*25*, *26*). Not much is known about the impact on human health or ecology from exposure to engineered nanomaterial (ENMs) introduced in the environment due to anthropogenic activities. The fact is that ENMs are used in practically every aspect of human endeavors including but not limited to, industrial application, agricultural applications, biomedicine, pharmaceuticals and personal care products, simply because they offer an easy way to manipulate their physico-chemical properties (*27*). It is disconcerting to know that ENMs have become an integral part of our existence yet very little is known about their adverse environmental impact, primarily because of the lack of analytical methods to detect and to quantify the ENMs (*28*). More so, at nano scale between 1 and 100 nm (1 nm = 10^{-9} m) most ENMs have enhanced reactivity, higher mobility (depending on surface coating) in the environment and consequently greater potential for exposure through different pathways (*29*).

Various sources contributes to the environmental occurrence of ENMs ranging from waste products from industrial processes to accidental releases. These includes combustion processes, industrial emissions, atmospheric deposition, sorption and transport to aquatic system (*30*). Figure 3, shows the various routes through which ENMs enter the environment. It should be noted that vast majority of consumer products contains ENMs and disposal of these consumer products after their use serve as an additional input of ENMs in the environment. Furthermore, ENMs enter the environment their release into the ambient air through manufacturing and handling of nanoparticles. Given the widespread use and documented occurrences of ENMs in the environment, it is essential that we understand the toxicity associated with these materials. The ENMs are classified into five major groups this chapter includes the toxicity associated with carbon nanomaterials only.

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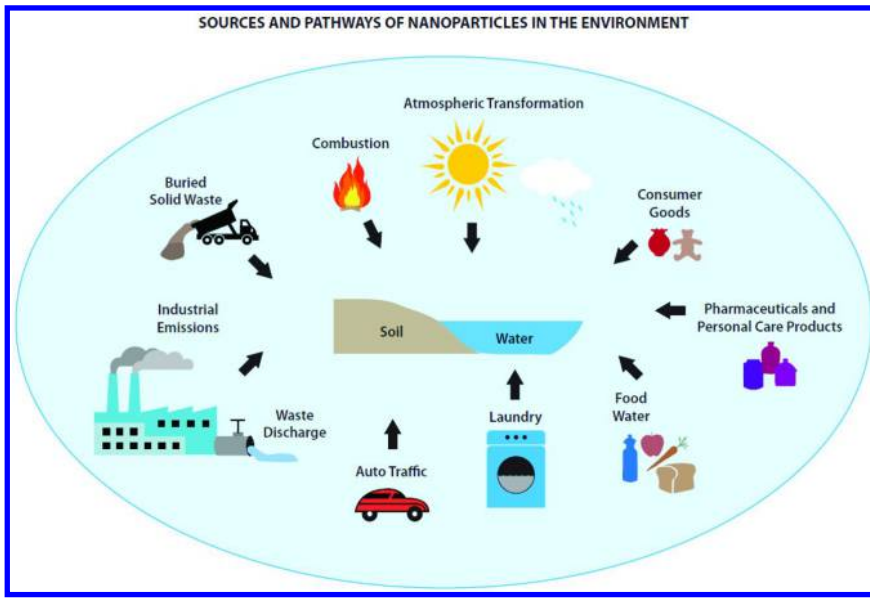

Figure 3. Sources and pathways of environmental occurrence of nanomaterials

Carbon nanotubes (CNTs) are a group of nanoparticles that are considered a novel material with growing commercial application due to their unique properties. Discovered in the early 1990's, CNTs are tubular, graphite sheets consisting of sp^2 carbon bonds typically with diameters of 1.4 nm and lengths in the microns (*31*). The large surface area to volume ratio, tensile strength, and electrical properties make CNTs ideal components of composites, sensors and probes, and energy storage devices, such as fuel cells. In the United States alone, $1.5 billion has been invested into nanotechnology in 2008 (*32*). As a result of the increasing development of CNT application and production, there arises an increasing concern regarding the implications of CNTs to biological processes and systems.

3.2. Human Health and Ecological Risks Due to Nanoparticles

Great strides have been made in understanding the human health implications of nanomaterials (*33*, *34*), the potential ecological risks (e.g., wildlife such as largemouth bass) (*35*) and impacts of nanoparticle released to the environment. Animal inhalation and aspiration studies have demonstrated that exposure to single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) results in acute pulmonary inflammation and long term chronic effects such as thickening of the bronchial wall, fibrosis and granulomas (*36*). Microbial toxicity of CNTs has been demonstrated under pure culture conditions. An in vitro assay showed that *staphylococcus aureus* and *staphylococcus warneri* bacteria could not grow over the CNTs films (*37*). Antimicrobial activity of SWCNTs was observed after *escherichia coli* were exposed to SWCNTs (*38*,

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39), In another study, Bottini et al. (*40*) reported that 80 % of the cells exposed to oxidized CNTs at the concentration of 400 μgL^{-1} were killed. The impact of CNTs on a mixed-cultured environmental system such as an activated sludge wastewater treatment process has also been examined and it has been reported that CNTs can pose toxicity towards the microbial communities in the form of respiratory inhibition (*41*, *42*).

3.3. Toxicity and Physicochemical Properties of Nanoparticles

Physicochemical properties of nanoparticles have been recognized as important factors that can greatly affect nanoparticles toxicity. Comparative studies have shown that SWCNTs exhibited higher toxicity compared to MWCNTs. Jia et al. (*43*) reported the following order of toxicity on a per-mass basis in alveolar macrophages: SWCNTs > MWCNTs > quartz > fullerenes. Kang and coworkers (*44*) also reported that the degree of toxicity was dependent on the type of nanomaterial (SWCNTs were more toxic than MWCNTs) and bacterial species (*Bacillus subtilis* was less sensitive than Gram-negative species). Oxidized CNTs were found to be more toxic than un-oxidized, pristine CNTs (*45*). Long carboxylic functionalized SWCNTs exhibited a higher microbial respiratory inhibition than the short and non-functionalized SWCNTs (*42*).

3.4. Toxicity Mechanisms

Several potential mechanisms of the toxicity imposed by CNTs have been reported. However, the exact causes of microbial toxicity due to interactions with CNTs is still in debate. It could be a single factor, such as cell penetration or oxidative stress via contact, or a combination of many effects. Several studies have modeled and examined cell penetration and destruction by nanotubes. Liu et al. (*46*) described them as “nano darts”, using their small size to puncture and destabilize the outer membrane wall of cells, leading them to lysis and death. Oxidative stress has also been reported to occur, with a possible result of cell death. Interactions between the cells and the surface of the nanotubes can oxidize molecules vital to the cells (*47*). Nanotubes have also been shown to have the capacity to enter and oxidize DNA, disrupting the protein chains and resulting in cell failure during mitosis (*48*).

3.5. Toxicity of Catalytic Metal Residues

The toxicity effects of catalytic metal residues in unpurified CNTs have been examined, but the findings are limited and inconclusive. When Fe was used as a catalyst to synthesize SWCNTs, greater oxidative stress and permeation to the human keratinocyte cells was observed (*49*). In contrast, Bello et al. (*47*) found no significant associations between soluble metal content and biological oxidative damage using human blood serum. Kang et al. (*50*) found that Fe content in MWCNTs did not correlate to bacterial cell membrane damage. The presence of extracellular polymeric substances has been found to mitigate the respiratory inhibition caused by CNTs (*31*).

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3.6. Dispersion and Level of Toxicity

The level of dispersion had a very strong correlation with the level of toxicity observed. The physical properties of CNTs are known to hinder efforts to disperse them in fluids. When placed in a polar solution such as water, the individual pristine nanotubes will aggregate together to form larger clumps, many of which will settle out. This aggregation lowers overall contact with cells and reduce any impact of cell penetration (*46*). Dispersing these raw carbon nanotubes is often assisted with the addition of chemicals and surfactants (*51–54*) or natural organic matter (*53*).

4. Other Emerging Micro-Pollutants in the Environment

4.1. Hormones and Endocrine Disrupting Compounds

According to the National Institute of Environmental Health Sciences endocrine disruption compounds (EDC) could be of natural or anthropogenic origin that may essentially mimic or interfere with the functioning of hormones in the body (*55*). Municipal wastewater as well as wastewater generated in the concentrated animal feeding operations are two of the most common sources of hormones (both synthetic and natural) and EDCs in the environment. Municipal wastewater treatment plant and the traditional anaerobic lagoons (used in animal agriculture operations) are not designed to treat low levels of hormone or endocrine disrupting compounds; consequently they release them directly into the nation's water bodies (*56*). A comprehensive study of source water, finished drinking water and distribution system (tap) water from 19 U. S water utilities that serves 28 million people revealed that, among other organic compounds (including pharmaceuticals) the water contained various steroid hormones and EDCs such as estrone, 17 β-estradiol, and atrazine (*57*). The occurrence of EDCs in the water resources is a cause of concern because researchers believe that exposure to EDCs can cause adverse ecological impact. For example, it has been shown that exposure to EDCs leads to an impairment of reproductive system of fish and increased vulnerability to infection causing general decline in fish population (*58*, *59*). In general, low level exposure to hormones and EDCs has wide range of human health and ecological implications.

4.2. Perfluorinated Compounds in the Environment

Perfluorinated compounds (PFCs) are widely used in variety of consumer and industrial products since 1950's (*60*). They are essentially made up of long carbon chain to which fluorine is attached. The most commonly used PFCs are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). These compounds are widely used in a variety of industrial, household and consumer products. Because PFCs are inert, non-wetting, slippery, non-toxic, non-stick, fire resistant, and high temperature resistant, they have been used in practically every aspect of human endeavors ranging from cookware to firefighting foams (*60*). In fact, these are the same properties that made PFCs so versatile had

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made them environmentally undesirable. Because of their chemical properties, PFCs are persistent (resistant to biodegradation), bioaccumulative and also undergo biomagnification (*61*). According to the USEPA release statement EPA-820-F-13-005 (*62*), majority of the population in the industrialized world has been exposed to PFCs throughout their life, consequently elevated concentrations of PFOS and PFOA has been detected in large number of blood serum samples. Exposure to PFCs and elevated concentration in blood stream have adverse health effects including immunotoxicity, decreased sperm count, low birth weight, thyroid disease and high cholesterol (PFOA only) (*62*). Because of their adverse effect to human health and ecology, the USEPA has decided to phase out the PFCs by the end of year 2015 (*62*).

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4.3. Polybrominated Diphenyl Ethers in the Environment

Polybrominated diphenyl ethers (PBDEs) (also known as brominated flame retardants) have been in use since 1970's however, most recently they are included in the list of emerging contaminants of concern. These are extensively used halogenated compounds and are recalcitrant, bioaccumulative and environmentally persistent (*63*, *64*). Lot of consumer goods such as electrical equipment, construction materials, coatings, textiles and polyurethane foam involves use of PBDEs. They have been known to cause tumors and neurodevelopmental toxicity, endocrine disruption and thyroid hormone imbalance (*63*, *64*). In children, they have been known to cause subtle and measurable developmental problems (*64*). This is particularly worrisome considering the fact that breast milk samples (n = 47) collected from milk banks in Austin and Dallas TX showed presence of PBDEs (13 different PBDEs congeners) with concentrations ranging from 6.2 to 419 ng/g lipids (*65*). Although the PBDEs have been used as a flame retardants and have served their purpose well in saving human life and property, their potential toxicity to children and infants need to be further investigated to better understand the casual relationship between exposure to PBDE and developmental toxicity in children.

5. Summary

Occurrence, distribution, fate, and human health and ecological impacts due to exposure to EMs is currently being discussed in various scientific community. The EMs pose a serious challenge, because little information is currently available about persistence, toxicity, exposure frequency and duration. This challenge is further compounded by the fact that we do not know whether the metabolites are more toxic than the parent compound. Currently various EMs such as pharmaceuticals, insecticides, PFCs and PBDEs are being researched to better understand risk to human health and ecology. Some of the EMs such as pharmaceuticals, PFCs and PBDEs are persistent and bioaccumulative and may interfere with human endocrine disrupting system. Both PFC and PBDE have been used since very long time yet, their adverse effect on human health and ecology is now becoming known. Various federal agencies such as the

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USEPA and USFDA are currently working on developing regulations to minimize environmental impact of pharmaceuticals, insecticides, nanomaterials, PFCs, and PBDEs. In fact, both PFCs and PBDEs are now being phased out and are being replaced by environmentally benign substitutes. The USFDA is proposing to restrict the use of pharmaceuticals in animal agriculture only for therapeutic purposes. This efforts will likely to run into trouble simply because a lot of animal agriculture facilities relies on sub-therapeutic use of pharmaceuticals.

On the other hand, neonicotinoid class of insecticides even though banned in Europe due to their potential toxicity to honey bees and other non-target species, these insecticides are continued to be used in the United States. The scientific community is divided on the toxicity associated with neonicotinoids. Review of the existing studies suggests conflicting reports on the possible adverse effect to honeybees due to exposure to neonicotinoids. Even though, recent studies indicate that neonicotinoid toxicity is not a factor in CCD. Many studies have sought to pin a single factor to CCD, but it is becoming apparent that the disorder presents a syndrome of multiple causes of which pesticides, not just neonicotinoids, are one. While other studies indicated that neonicotinoids can directly or indirectly affect terrestrial and aquatic vertebrate wildlife that obviously warrants further consideration. As it stands, social experiments such as the ban on imidacloprid, thiamethoxam and acetamiprid in four major crops that was recently instituted by the European Union will, if nothing else, provide data on the consequences of such decision and if it was well-founded.

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Chapter 2

Antibiotics in the Environment: A Review

Sudha Goel*

Civil Engineering Department, IIT Kharagpur, Kharagpur 721302, India
***E-mail: sudhagoel@civil.iitkgp.ernet.in**

A major group of micropollutants that are of growing concern is antibiotics. Antibiotics are chemotherapeutic compounds used in animal husbandry and for human health reasons for preventing or treating infections, as growth promoters and sometimes as food preservatives. Partial or incomplete metabolism and inefficient removal during wastewater treatment have paved the way for antibiotics to enter all parts of the environment including water, sediment, soil, etc. via wastewater discharges and agricultural runoff. Selective pressure due to widespread overuse of antibiotics has resulted in the emergence and spread of antibiotic-resistant pathogens. Some bacteria are resistant to more than one antibiotic and are termed multiple antibiotic-resistant (MAR) bacteria. This chapter provides an overview about the presence of MAR bacteria in various parts of the environment, analytical methods, concentrations of antibiotics in the environment and treatment processes for their removal from wastewaters.

Introduction

Antimicrobials including antibiotics are now recognized as a major group of emerging micropollutants. Antimicrobials are defined as compounds or substances that kill or inhibit the growth of microbes. Antibiotics are a sub-set of antimicrobials and the term was used for anti-bacterial compounds only. However, the term is now often used synonymously with antimicrobials in recent literature (*1*). An example of an antibiotic with more than anti-bacterial uses is clindamycin that is used against protozoa to combat malaria and toxoplasmosis.

Antibiotics have been used for therapeutic purposes for both humans and animals since the discovery of penicillin in 1928. They have been used as

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growth promoters in animal husbandry since 1946 (*2*, *3*) and for prevention of diseases in plants and animals (*4*). These compounds have also been used as food preservatives (legally under the Food, Drug and Cosmetic Act) since 1955 in the USA (*5*).

Since the discovery of antibiotics in 1928, their production and use has grown exponentially world-wide. Estimated annual antibiotic production in the world ranges from 100,000 to 200,000 tons (*1*). Total global production in the last 50 years is estimated to be 1 million tons (*6*). Consumption patterns, however, vary from country to country and for different uses. Currently, India is the third largest producer of antibiotics in the world with an estimated annual production ranging from 2588 metric tons in 2005-2006 to 2472 metric tons in 2007-2008 (*7*). Estimates of antibiotics use for veterinary versus human purposes is 2:1 (*8*, *9*).

Antibiotics can be of natural or anthropogenic origin. Most antibiotics produced and released into the market eventually find their way into the environment. The fate and transport of antibiotics in the environment is shown schematically in Figure 1. All antibiotic uses: therapeutic, growth promotion or food and agriculture use, lead to the release of these substances into soil, air, water, wastewater and sediment. Antibiotics excreted by humans and animals, and released during animal slaughter are collected as wastewater. Surface runoff from animal husbandry, agricultural and food processing facilities may enter stormwater or wastewater collection systems from where they are discharged directly into surface waters, or on land or are treated in wastewater treatment plants.

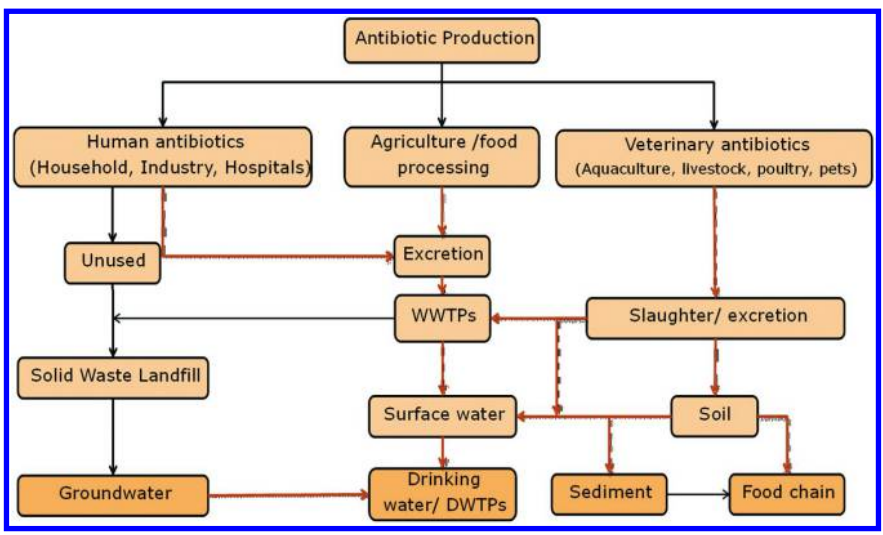

Figure 1. Sources and pathways of occurrence of antibiotics in the environment (see color insert)

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Unused antibiotics end up in landfills from where they may be released in leachate which can contaminate surface water, groundwater and soil. Contaminated water resources could potentially lead to contamination of drinking water sources. Occurrence of antibiotics in environment could consequently lead to the widespread prevalence of antibiotic-resistant bacteria including pathogens. Chronic exposure to these compounds and/or multiple antibiotic-resistant (MAR) bacteria in various parts of the environment poses a serious challenge for human and veterinary therapy.

Discharges from conventional wastewater treatment plants containing residual concentrations of antibiotics and the presence of MAR bacteria or antibiotic-resistant genes (ARGs) is now well-documented in treated wastewaters as detailed in the following sections. Where wastewaters are discharged without treatment or after partial treatment, the levels of antibiotic residuals are expected to be much higher since conventional wastewater treatment does not target the removal of such compounds. Hospital effluents are another major source of antibiotics in the environment (*10*). In one study, the concentrations of ciprofloxacin in hospital effluent were found to be one order of magnitude higher than those in raw sewage and several orders of magnitude higher than in river water (*11*).

In several parts of the world, water reuse strategies are being used or explored. The presence of antibiotics or MAR bacteria or ARGs in treated drinking water is a potential health hazard. This has even more serious implications for 'wastewater to drinking water' treatment (*12*). Of the two case studies that are known, no impacts have been reported in Namibia where wastewater to drinking water treatment has been practiced since 1968. The second case is Singapore where the same degree of treatment has been in effect since 1998. No documentation or published literature regarding the presence of antibiotics in their wastewater or in their treated drinking water was found.

Two of the earliest studies to document the presence of various groups of micropollutants including antibiotics in receiving water bodies were conducted between 1996 to 1998 in Germany (*13*) and from 1999 to 2000 in the USA (*14*). Antibiotics were the median group of compounds found in US streams in the latter study, their total concentrations were much lower than most other groups of micropollutants but the number of compounds detected in this group was the highest. Research interest and activity in this area increased at the same time with an increasing number of publications after 2003 (*15*).

Classes of Antibiotics

Antibiotics are classified based on their structural and chemical properties. These classes of antibiotics are summarized with their applications as well as side effects in Table 1 (*1*, *16–19*).

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Table 1. Antibiotic types, their applications and side effects

Class - Group	*Some examples*	*Applications*	*Side effects*
Aminoglycosides	Neomycin, Streptomycin, Tobramycin, Paramomycin	For infections from aerobic bacteria and Gram-negative bacteria	Affects kidneys, liver, ear functions
Glycopeptides	Teicoplanin, Vancomycin	For infections from Gram-positive bacteria	
Macrolides	Azithromycin, Clarithromycin, Erythromycin, Telithromycin, Spectinimycin, Roxithromycin, Tylosin	For streptococcal infections, respiratory infections, feed additive for animals	Gastrointestinal discomfort
β-lactams Penicillins	Amoxicillin, Ampicillin, Carbenicillin, Cloxacillin, Penicillin, Meticillin	For wide range of infections, streptococcal infections	Rashes and fever, allergic reactions
β-lactams Carbapenems	Ertapenem, Meropenem, Doripenem	For infections from both Gram-positive and Gram-negative bacteria	
β-lactams Cephalosporins	Cefalotin, Cefamandole, Cefoxitin, Cefeprime, Cefalexin, Cefprozil, Cefuroxime	For infections from Gram-negative bacteria	Rashes and fever, cross-allergic reactions to β-lactams (Penicillin)
Polypeptides	Bacitracin, Colistin, Polymyxin B	For eye, ear and bladder infections	
Fluoroquinolones	Ciprofloxacin, Enoxacin, Ofloxacin, Levofloxacin	For urinary tract infections, skin infections, respiratory infections	Nausea, diarrhea, vomiting, stomach pain, arthropathies in young
Sulfonamides	Sulfamethizole, Sulfanilamide, Sulfamethazine, Sulfamethoxazole, Sulfapyridine, Sulfadiazine, Sulfathiazole, Sulfamethoxine	For urinary tract infections	Affects kidneys

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Table 1. (Continued). Antibiotic types, their applications and side effects

Class - Group	*Some examples*	*Applications*	*Side effects*
Tetracyclines	Tetracyclines, Chlortetracyclines, Oxytetracyclines	For infections of respiratory tract, urinary tract	Hepatotoxic
Phenicol	Chloramphenicol	Veterinary use	Anaemia
Lincosamides	Lincomycin	Veterinary use	Gastrointestinal problems

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Antibiotics and How They Work

Antibiotics are classified as bacteriostatic or bactericidal. Bacteriostatic antibiotics prevent bacterial reproduction while bactericidal antibiotics destroy bacteria. Different antibiotics work by inhibiting various cell functions or damaging different cell organelles. Some of the major mechanisms are (*6*):

a) Inhibition of nucleic acid synthesis: e.g., Rifampicin, Chloroquine
b) Inhibition of protein synthesis: e.g., Tetracyclines, Chloramphenicol
c) Action on cell membranes: e.g., Polyenes, Polymyxin
d) Interference with enzyme systems: e.g., Sulphamethoxazole
e) Action on cell walls: e.g., Penicillin, Vancomycin.

Why Do Bacteria Become Resistant to Antibiotics?

Exposure to antibiotics results in spontaneous mutation of the genetic material (*6*). Even though these mutations are relatively rare (10^{-7} to 10^{-8} for an individual base pair), they are easily propagated in large bacterial populations (*6*). The resistance genes that result from these mutations can be transferred or propagated by vertical or horizontal gene transfer. Vertical gene transfer is propagation of the mutation from a parent cell to its progeny while horizontal gene transfer can occur in three different ways (*6*):

a) Transformation: bacteria pick up plasmids from the external environment,
b) Transduction: virus-mediated transfer of DNA,
c) Conduction: transfer of DNA by cell-to-cell contact.

Dose-Response Relationships and Antibiotics

Models used to describe the relationship between doses or antibiotic concentrations in environmental samples and bacterial response to them can be assumed to be linear, with threshold levels or hormetic (*20*). Linear models assume that there is no safe dose while models with a threshold value are based

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on evaluating a 'no observed adverse effects level' (NOAEL). Hormesis is a relatively new concept that has not yet been incorporated into regulations unlike the other two models. Low doses of toxins, i.e., below the NOAEL, are assumed to have some beneficial effects. There is sufficient statistical evidence to show that hormesis effects were apparent in a large number of toxicological studies including those with antibiotics like penicillin (*21*, *22*).

Detection and Monitoring

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MAR bacteria or ARGs have been detected in all parts of the environment, i.e., in all environmental matrices. From an analytical perspective, detecting these bacteria or genes is relatively easy in comparison to measuring antibiotic concentrations in different environmental samples. Since antibiotic concentrations in the environment are generally in the ng/L or lower range and a large number of methods and instruments are now available, developing and testing appropriate analytical methods is a major research challenge at this time (*23*) .

MAR Bacteria or ARGs and Their Prevalence in the Environment

MAR bacteria or ARGs have been detected in different environmental samples. The disc diffusion method (*24*) is very popular in India for detecting MAR bacteria in all types of environmental samples. The presence of MAR bacteria in a sample may indicate the presence of antibiotics in the environment and therefore, is an indirect method. The results are at best qualitative since no quantification of antibiotic concentration is generally done. The materials and specific media required for doing these tests are easily available. However, it is time-consuming due to the long incubation times and is highly labor intensive. Resource consumption in terms of media, chemicals and disks is also very high.

A summary of published studies conducted in India is provided in Table 2. Most of these studies were done for aquatic samples with a few studies with air, soil and sediments. A total of 23 studies in India were accessed at the time of this review; the earliest study was published in 1995. All these studies were based on isolation and determination of MAR bacteria using the disk diffusion method (*24*). Of these 23 studies, two studies were conducted with soil and two studies with air samples while 19 studies were conducted with water samples. Of these 19 studies included were 2 studies with sediments – 3 in marine areas, 1 in mangroves, 1 in a standing surface water body, 2 with wastewaters and the remaining 13 studies with river water. All except one of the 13 riverine studies were in the Ganga river basin.

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Table 2. Summary of studies for assessing the prevalence of MAR bacteria in environmental samples in India

River (Ref. No.)	*Location*	*Number of sites, seasons*	*Media*	*Number of antibiotics tested*	*Bacterial isolates [No. of isolates]*	*ARI value*
Ganga (*25*)	Kannauj, Fatehgarh	2, 4 seasons	Water	15	*E. coli [90]*	*Not reported*
Gomti (*26*)	Lucknow	4	Water	15	*E. coli [4]*	*Not reported*
Mahananda (*27*)	Siliguri, WB	3: 2 sewage sites; one river site	Wastewater and river water	7	*Various Gram negative sp. [20]*	*Not reported*
Ganga (*28*)	Gaumukh to Rishikesh	20	Water	10	*E. coli [14]*	*0.007 to 0.05*
Ganga (*29*)	Kanpur	5	Water	15	*E. coli [75]*	*Not reported*
Godavari (*30*)	Nanded	6	Water	8	*E. coli, C. freundii, C. diversus, E. aerogens, Klebsiella [60]*	*Upstream = 0.15; Downstream = 0.43*
Gomti (*31*)	Lucknow	6	Water	11	*E. coli [90]*	*Not reported*
Gomti (*32*)	Lucknow water distribution system, Aishbagh	6	Drinking Water	15	*E. coli [81]*	*Not reported*
Ganga (*33*)	Kanpur	5	Water	13	*Enterococci [85]*	*Not reported*
Saryu (*34*)	Faizabad	3	Water	15	*E. coli [42]*	*Not reported*
Yamuna (*35*)	Mathura, Delhi	2 sites; 3 seasons	Water	19	*Pseudomonas [144]*	*Not reported*

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Table 2. (Continued). Summary of studies for assessing the prevalence of MAR bacteria in environmental samples in India

River (Ref. No.)	*Location*	*Number of sites, seasons*	*Media*	*Number of antibiotics tested*	*Bacterial isolates [No. of isolates]*	*ARI value*
Mangroves (*36*)	Muthupettai	6	Water	10	*Heterotrophic bacteria [680]*	*0.33 to 0.48*
Marine (*37*)	Coast of Puri	6	Water + sediment	12	*E. coli, S. aureus, E. faecalis, P. aeruginosa, P. mirabilis, Vibrio, Klebsiella species [38]*	*0.066 to 0.083*
Marine (*38*)	Coast of Chennai	3	Water + sediment	10	*E. coli, Vibrio sp., Salmonella sp. and Enterococcus sp. [960]*	*Seawater: 0.29 to 0.316; Sediment: 0.306 tp 0.343*
Coastal and non-coastal water (*39*)	Kalimela, Malkangiri and Brahmgiri, Puri, Odisha	2	children stools, cow dung and drinking water	10	*E. coli [696]*	*Not reported*
Byramangala tank (*40*)	Bengaluru	4 sites	Water	25	Various species, [*not reported*]	*Not reported*
Hooghly (*41*)	Kolkata	11 sites; 3 seasons	Water	12	Various Gram negative sp. [163]	*0.08 to 0.63*
Ganga (*42*)	Uttarakhand	32	Water	12	*Staphylococcus* sp. [128]	*Not reported*
Sewage treatment plant (*43*)	Karnataka	3	Sewage	12	*E. coli [209]*	*Not reported*

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River (Ref. No.)	*Location*	*Number of sites, seasons*	*Media*	*Number of antibiotics tested*	*Bacterial isolates [No. of isolates]*	*ARI value*
Not applicable (*44*)	Gwalior Fair	6 (fair) and 3 (residential)	Air	13	*S. aureus, coagulase-negative staphylococci, Enterococcus species, Bacillus species, Escherichia coli, and Pseudomonas species [300]*	*Not reported*
Not applicable (*45*)	Akola	10 Hospitals	Air	10	*Klebsiella pneumonie (25.76 %), Staphylococcus aureus (21.74%), Pseudomonas aeruginosa (23.23 %), E. coli (10.77 %), E. faecalis (9.08 %), Proteus mirabilis (2.87 %) and Proteus vulgaris (6.5 %). [2014]*	*Not reported*
Not applicable (*46*)	Hyderabad	57 samples; urban and agricultural soils	Soil	16	*Actinomycetes [14], Staphylococci [126], Bacillus [57], Pseudomonads [37], Enterobacteriaceae [30]*	*Not reported*
Not applicable (*47*)	Aligarh, UP	Not reported; agricultural soil	Soil	7	*Pseudomonas sp. [40], Azotobacter [12], Rhizobium [12]*	*Not reported*

The prevalence of antibiotic-resistant bacteria can be quantified in terms of MAR values or Antibiotic Resistance Index (ARI) = *A/NY* where A is the total number of resistant determinants recorded in a population of size N for the specified location and Y is the total number of antibiotics tested in the sensitivity test. ARI values have a threshold of 0.2 below which little or no exposure to antibiotics and subsequent resistance is implied while higher values indicate greater exposure and greater resistance. MAR values were calculated in only 6 out of these 23 published studies that are summarized in Table 2. Please note that different species were tested in only 6 out of the 23 studies (not the same six studies!). In some of these studies, the number of isolates were not mentioned. Seasonal effects were examined in 3 studies.

Two studies were conducted with water and sediment samples from marine environments. ARI values reported for samples collected from the coast of Chennai were far greater than for samples off the coast of Puri (*37*, *38*). None of the samples in the Puri study exceeded the threshold value of 0.2 and there was a clear decrease in ARI values with increase in distance from the coast towards the sea. Bacteria were most resistant to ampicillin in the Puri study while they were most resistant to vancomycin and penicillin in the Chennai study. Both studies showed that bacterial resistance was lowest to chloramphenicol.

Different bacterial species (163 isolates) and 12 antibiotics were examined for antibiotic resistance from three different aquatic environments (*41*). The results for water samples from River Hooghly in Kolkata (a highly polluted area) were compared to relatively unpolluted water samples in Kharagpur, i.e., from River Kangsabati and groundwater. The highest ARI values (0.57 to 0.63) were found downstream of Kolkata in the post-monsoon sampling and are attributed to municipal and industrial wastewater discharges including those from slaughter houses in the south of the city. In this study, bacteria were most resistant to furazolidone and ampicillin in all three seasons and least resistant to chloramphenicol. In general, ARI values were greater where urbanization, population and pollutant discharges were greater (*28*, *37*, *41*, *48*).

ARGs have been detected in various environmental samples and genes for resistance to tetracycline, aminoglycosides, quinolones and β-lactams were detected in sewage treatment plant effluent, lagoon, groundwater, river, sea and lake water, soil, sediments, and even treated drinking water (*49*, *50*). In general, resistance to antibiotics emerges soon after these compounds are released into the environment (*12*).

Measuring Antibiotic Concentrations in Environmental Samples

Water

A recent literature survey covering 236 published reports from 41 countries showed that water contamination by pharmaceuticals including antibiotics was extensive due to widespread consumption and subsequent disposal to rivers (*15*). Of the 61 most studied pharmaceuticals, 25 were antibiotics (Figure 2). The relative frequency of detection and median concentration of antibiotics

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were highest in Asia compared to Europe, N. America and global values. The maximum concentration reported for an antibiotic was 6.5 mg/L for ciprofloxacin while sulfapyridine had the highest mean detection frequency in these studies. Median concentrations of most antibiotics were in the ng/L range, but at least four of the antibiotics listed here had median concentrations >1 µg/L. Sixteen of these antibiotics had maximum concentrations >1 µg/L.

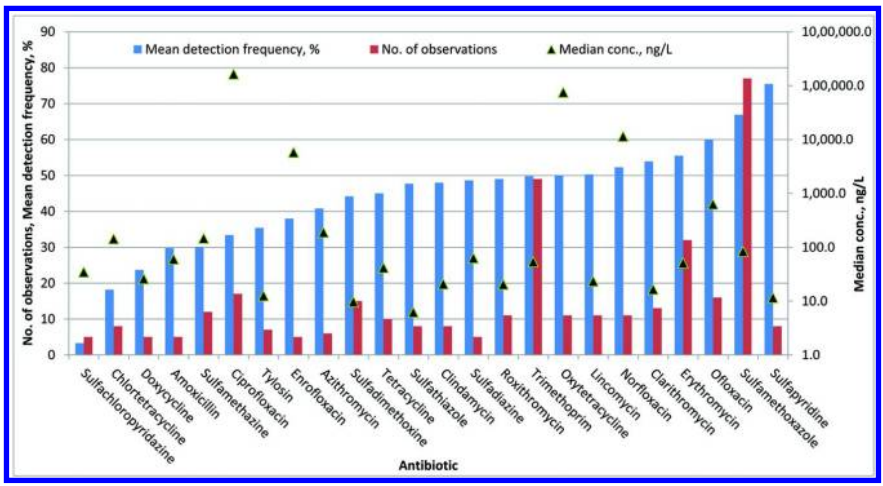

Figure 2. Antibiotics detected in river samples in different countries (based on data from (15)). (see color insert)

Soil

There is sufficient evidence to show that soil microbes serve as an ancient reservoir of antibiotic-resistant genes that remain available for exchange with clinical pathogens thereby maintaining and disseminating resistance in the environment (*51*). Different classes of antibiotics have been detected in soil ranging from 0.5 to 900 µg/kg (*19*). Highest concentrations were reported for tetracycline and can be attributed to its ability to persist and accumulate in the environment. Three antibiotics – trimethoprim, sulfadiazine and triclosan were found most frequently in soil studies from various countries with detectable concentrations ranging from 0.64 to 60.1 µg/kg (*52*). Antibiotics tend to sorb to soils to varying extents depending on several factors including pH, nature of soil and the structural and chemical properties of the antibiotic compound. Several studies have been published describing the sorption behavior of various classes of antibiotics on different types of soils.

Most antibiotics have well-defined pH values at which they exist as cations, zwitterions or anions. Since most soils carry a net negative surface charge under ambient conditions, these compounds are highly sorptive as cations and become

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more mobile, i.e., dissolve into the aqueous phase as zwitterions and anions. Their sorption coefficients have been measured for various soil-compound combinations. Sulfonamides were observed to be more mobile at pH > 7.5 with three different loamy soils and at high pH values, their mobility approximated that of a conservative bromide tracer (*53*, *54*). In another study with 30 different soils and ciprofloxacin (a popular use fluoroquinolone), the partitioning of the compound between solid and aqueous phase was found to be dependent mainly on pH and the cation exchange capacity of the soil (*55*).

Analytical Methods

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Over the last two decades or more, a large number of analytical methods have been developed and tested for measuring antibiotics. These methods include thin layer chromatography and bioluminescence screening and more expensive and sensitive methods like HPLC, LC-MS, LC-MS-MS and LC-ESI-MS-MS. Many different detectors like UV fluorescence and photodiode arrays (PDA) are available and have been tried successfully with HPLC for antibiotic quantification in environmental samples. Detection limits for HPLC/MS/MS ranged from 0.5 ng/L for clarithromycin to 30 ng/L for vancomycin (*19*).

Sample extraction and concentration of antibiotics for detection is necessary in all cases for separating antibiotics from the background material and a large number of methods such as lyophilization, liquid-liquid extraction and solid phase extraction have been employed (*23*). Various modifications of each of these methods has been employed and currently the most popular method is the use of hydrophilic-lipophilic balanced cartridges for solid phase extraction (SPE). This step remains the greatest challenge in measuring antibiotic concentrations due to issues of sensitivity, recovery, detectability of specific compounds with individual methods, and range of concentrations detected. A detailed review of analytical methods is available (*23*). Concentration factors range from 100-1000 and recoveries of analytes vary widely from study to study resulting in lack of comparability of results which is a major concern. Results of direct injection versus SPE using LC-ESI-MS-MS were compared with freshwater and seawater samples (*56*). Concentrations measured by direct injection were only slightly underestimated compared to SPE.

Removal during Conventional Wastewater Treatment

Antibiotics can be removed to some extent during conventional wastewater treatment. Different classes of antibiotics tend to be removed to different extents mainly due to differences in their structural and chemical properties and the extent to which they can be biodegraded. The most recent review of antibiotics removal during conventional wastewater treatment plants (WWTPs) covered 14 countries/regions and found overall removals ranging from 12.5 to 100% (*57*).

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One example of antibiotics removal during conventional wastewater treatment is provided in Table 3 (*58*). Overall antibiotics removals were 89% but lincosamides (11%) and sulfonamides (25%) were removed to the least extent during conventional wastewater treatment in comparison to all the other classes of antibiotics that were monitored. In this study, physico-chemical processes like microfiltration and reverse osmosis were used to achieve additional removals for all classes of compounds including the more recalcitrant ones resulting in overall removals of 94%.

Table 3. Removal of antibiotics after conventional wastewater treatment and after advanced treatment (based on (*58*)).

Antibiotic group	*Influent conc., ng/L*	*Conventionally treated effluent conc., ng/L*	*% removal in conventional treatment plant*	*Influent conc to MF/RO plant., ng/L*	*MF/RO effluent conc., ng/L*	*% removal in MF/RO plant*
Beta-lactams	5340	30	99	125	0	100
Quinolones	3980	680	83	640	60	91
Lincosamides	62	55	11	11	1	91
Macrolides	Not quantified			180	16	91
Tetracyclines	0	0	10	0	100	
Polyether ionophores	10	2	81	45	0	100
Sulfonamides	362	270	25	295	0	100
Other	340	50	85	80	5	94
Overall	10093	1087	89	1386	82	94

Significant differences in removal efficiencies have been noted during conventional wastewater treatment in different parts of the world. Reasons for differences in removal efficiencies are not always clear and can be attributed to the lack of controlled studies that are essential for defining the factors affecting their degradation.

Sorption of antibiotics to sludge and subsequent removal is a major mechanism for their removal in conventional wastewater treatment plants, rather than biodegradation alone during biological treatment. Antibiotics removal in full-scale wastewater treatment plants (WWTPs) can therefore be attributed to sorption and biodegradation along with various other mechanisms. Antibiotic removal efficiencies in WWTPs are summarized in Table 4 based on various studies (*59*).

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Table 4. Removal of antibiotics in full-scale wastewater treatment plants

Antibiotic (Ref. No.)	*Removal efficiency, %*	*Description*
4-Epitetracycline (*60*)	Median: from 31 to 95	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Amoxycillin (*61*)	75 (winter, 4 WWTPs); 100 (summer, 3 WWTPs)	24 h composite samples
Azithromycin (*62*)	-26 to 55	24 h composite samples; 2 WWTPs
(*60*)	Median: from -78 to 82	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Chloramphenicol (*63*)	>93	Grab samples from 2 WWTPs
(*64*)	45	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
Ciprofloxacin (*61*)	60 (winter); 63 (summer)	24 h composite samples
(*65*)	86 (activated sludge process), 79 (denitrifying process) and 96 (oxidation ditch)	24 h composite samples from 12 WWTPs
(*60*)	Median: from -582 to 98	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Clarithromycin (*61*)	0 (winter, 4 WWTPs); 0 (summer, 3 WWTPs)	24 h composite samples
(*62*)	-45 to 20 5.6, 14*	24 h composite samples; 2 WWTPs; fixed bed reactors*
(*66*)	54	4 samplings
(*60*)	Median: -210 to 49	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Cloxacillin (*60*)	Median: -500 to 36	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Doxycycline (*60*)	Median: 0 to 95	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs

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Table 4. (Continued). Removal of antibiotics in full-scale wastewater treatment plants

Antibiotic (Ref. No.)	*Removal efficiency, %*	*Description*
Enrofloxacin (*60*)	Median: 0 to 49	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Erythromycin (*61*)	0 (winter, 4 WWTPs); 0 (summer, 3 WWTPs)	24 h composite samples
(*62*)	-22 to 6	24 h composite samples; 2 WWTPs
(*62*)	-13, 7	24 h composite samples; 2 WWTPs; fixed bed reactors*
(*67*)	23.8	24 h composite samples
(*66*)	25	4 samplings
(*64*)	26	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
(*60*)	Median: -18 to 69	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Lincomycin (*61*)	0 (winter, 4 WWTPs); 0 (summer, 3 WWTPs)	24 h composite samples
(*60*)	Median: -14 to 34	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Norfloxacin (*64*)	66	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
(*60*)	Median: 0 to 87	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Ofloxacin (*61*)	43, 57	24 h composite samples
(*63*)	>84	Grab samples from 2 WWTPs
(*67*)	23.8	24 h composite samples
(*65*)	75 to 88	24 h composite samples from 12 WWTPs
(*64*)	57	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
(*60*)	Median: -53 to 99	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs

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Table 4. (Continued). Removal of antibiotics in full-scale wastewater treatment plants

Antibiotic (Ref. No.)	*Removal efficiency, %*	*Description*
Penicillin V (*60*)	Median: 0 to 91	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Roxithromycin (*68*)	-80 to 27	24 h composite samples from 3 WWTPs
(*62*)	-18 to 38 4, 35	24 h composite samples; 2 WWTPs; fixed bed reactors*
(*69*)	-8 to 61	24 h composite samples; 4 WWTPs
(*66*)	33	4 samplings
(*64*)	48	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
(*60*)	Median: -304 to 0	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Spiramycin (*61*)	0 (winter, 4 WWTPs); 0 (summer, 3 WWTPs)	24 h composite samples
Sulfadiazine (*63*)	>97	Grab samples from 2 WWTPs
(*64*)	50	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
Sulfamethazine (*60*)	Median: -96 to 0	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Sulfadimidine (*64*)	50	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
Sulfamethoxazole (*70*, *71*)	57, 57	24 h composite samples
(*72*)	50	Grab samples for sludge
(*61*)	17 (winter, 4 WWTPs); 71 (summer, 3 WWTPs)	24 h composite samples
(*68*)	-280 to 66	24 h composite samples from 3 WWTPs
(*62*)	-138 to 60 -61, 29	24 h composite samples; 2 WWTPs; fixed bed reactors*
(*69*)	33, 62	24 h composite samples; 4 WWTPs
(*63*)	>98	Grab samples from 2 WWTPs
(*67*)	55.6	24 h composite samples

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Table 4. (Continued). Removal of antibiotics in full-scale wastewater treatment plants

Antibiotic (Ref. No.)	*Removal efficiency, %*	*Description*
(*66*)	24	4 samplings
(*64*)	0 to 64	Grab samples from 2 WWTPs; 24 h composite samples from 2 WWTPs
(*60*)	Median: 0 to 94	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Sulfapyridine (*62*)	-107 to 72	24 h composite samples; 2 WWTPs; fixed bed reactors*
Tetracycline (*60*)	Median: 0 to 95	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs
Trimethoprim (*73*, *74*)	<1 to 50	24 h composite samples
(*62*)	-40 to 20 17, 12*	24 h composite samples; 2 WWTPs; fixed bed reactors*
(*60*)	Median: 0.1 to 91	24 h composite samples for influent and effluent and grab samples for biosolids; 6 WWTPs

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Based on these studies (summarized in Table 4), it was clear that some antibiotics were not removed at all during conventional wastewater treatment. Negative numbers for removal efficiencies have been reported in several studies and imply that more compounds were in solution after treatment rather than before treatment. This is attributed to dissolution of the compounds from solid matrices like bile and feces into aquatic samples and analytical issues (*60*). Lincomycin (a lincosamide) could not be removed during conventional wastewater treatment in one study (*61*) and in two other studies, its removals were very poor (*58*, *60*). Macrolides like clarithromycin, erythromycin, and spiramycin were not removed during conventional wastewater treatment (*61*). However, removals of azithromycin, another macrolide, varied greatly in two different studies (*60*, *62*). Sulfonamides, on the other hand, were removed to varying extents in different studies and no generalizations about their removals during conventional treatment are possible at this time. Chlorine- (e.g., chloramphenicol) and fluorine-containing compounds (e.g., fluoroquinolones) were relatively easy to remove in conventional WWTPs. Removals of trimethoprim also varied greatly in different studies and no generalizations are possible.

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Removal of Antibiotics by Physico-Chemical Treatment

A review of physico-chemical methods for removing antibiotics from different environmental samples included publications between 2000 and 2010 (*75*). Most of these studies were conducted with high concentrations of antibiotics (generally a single compound) in the mg/L range (72 compounds) while a few studies were conducted in the μg/L (23 compounds) or ng/L range (4 compounds). Of the 120 water samples used in these studies, 59 were conducted with distilled water, 13 with distilled deionized water and 6 with ultrapure water. Results of these studies are not comparable with other matrices such as synthetic wastewater, wastewater treatment plant effluent, wastewater from pharmaceutical companies and industrial wastewater. Natural water samples were also studied in a few cases with ground water, lake, river and sea water. While controlled laboratory studies with water of varying degrees of purity are important for understanding the mechanisms of removal and determining maximum removal efficiencies under optimum conditions, these studies are not directly applicable under field conditions. Since treatment methods for antibiotic removal are to be applied to either wastewater treatment plant effluents or drinking water sources, it is important to conduct studies with real samples for greater relevance.

Ozonation was the most studied (or reported) physico-chemical method used for antibiotic removal followed by photolysis, semiconductor photocatalysis and Fenton and photo-Fenton reactions. Adsorption (in conjunction with other processes like ozonation, chlorination or coagulation) and membrane filtration were also frequently studied processes and provided >90% removals of antibiotics. Average maximum removal efficiency reported for ozonation alone was 75% and ranged from 34% with penicillin to 100% in studies with macrolides and tetracyclines. High removals of sulfonamides, cephalosporins, etc. were also obtained with ozonation.

Removal of Antibiotics by Biological Treatment

Most antibiotics tested were not biodegraded under aerobic conditions and few were degraded under anaerobic conditions (*1*). Very few studies were found in the literature where antibiotics removal could be attributed to biodegradation only (*59*). This is understandable given the anti-bacterial nature of these compounds. Only five biodegradation studies with seven different antibiotics were summarized in this review. Tetracycline (was not degraded after biological treatment), ceftriaxone (<1% removal efficiency), azithromycin (0.4 and 0.5% removal efficiencies under aerobic and anaerobic conditions, respectively) and trimethoprim (<1%) were the most recalcitrant antibiotics. On the other hand, sulfamethoxazole (99% under both, mesophilic and thermophilic conditions, in a pilot-scale anaerobic digester) and roxithromycin (85 and 95%, same conditions) were the most biodegradable antibiotics. Benzylpenicillin was only moderately biodegradable with 25% removal.

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Chapter 3

Emerging Micro-Pollutants Pharmaceuticals and Personal Care Products (PPCPs) Contamination Concerns in Aquatic Organisms - LC/MS and GC/MS Analysis

M. Abdul Mottaleb,[1,*] Mohammed J. Meziani,[1] M. Abdul Matin,[2] M. Musavvir Arafat,[1] and Mohammad A. Wahab[3]

[1]Center for Innovation and Entrepreneurship, Department of Natural Sciences, Northwest Missouri State University, Maryville, Missouri 64468, United States
[2]Department of Chemistry, Sungkyunkwan University, Suwon, 440-746, South Korea
[3]Australian Institute for Bioengineering and Nanotechnology, Corner of College and Cooper Roads, The University of Queensland, St Lucia, Brisbane Qld 4072, Australia
[*]E-mail: mmottaleb@yahoo.com or mmottal@nwmissouri.edu; Voice: +1 660 562 0820; Fax: +1 660 562 1188

Occurrence of pharmaceuticals and personal care products (PPCPs) and other organic wastewater contaminants are reported in various environmental matrices. Non-regulated emerging contaminants PPCPs enter into aquatic systems through sewage/wastewater treatment plants after consumption and use by humans and animals. High-end analytical tools such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are invariably used to examine PPCPs at sub-ng/g levels from air, waters, sediments, effluents, aquatic organisms and humans. Studies have indicated that some PPCPs are persistent and have bioaccumulation potential leading to ecological effects and sexual abnormalities in fish. Investigations reveal that PPCPs can biologically transform to breakdown products forming adducts suited for monitoring biomarkers of exposure. Several researchers have identified that PPCPs can impair swimming

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behavior in fathead minnow (*Pimephale promelas*) and interfere with thyroid axis in the zebra fish (*Danio rerio*). Thus, the presence of PPCPs in the environment justifies attention of regulatory authorities and health agencies to address the emerging concern issues requiring further investigation on their eco-toxicological effects and balances.

1. Introduction

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Today one of the most important environmental issues is the exposure of man-made chemicals to aquatic organisms and wildlife causing interference of reproduction and development (*1*, *2*). These chemicals hazards the human daily life through environmental processing systems (*3*). Pharmaceuticals are manufactured chemicals commonly employed for the treatment of human and domestic animals ailment. Personal care products (PCPs) are chemical substances that are used for individual's cosmetic purposes. Tons of those chemicals are produced annually worldwide (*4*). After consumption, these compounds are loaded or excreted into ecosystems via urine, feces or residues as either parent compounds or their metabolites. Wastewater treatment plants are not commonly designed to eliminate the pharmaceuticals and personal care products (PPCPs) because they are non-regulated water pollutants continuously contaminating our environment every day (*5*). Many people are unaware that a new environmental health concern about PPCPs has emerged among scientists around the world. These compounds will be all around us as long as human and livestock co-exist. They are termed as micro-pollutants because they are found in trace concentration levels in the range of nanograms to low micrograms per liter (or kg) in different environmental matrices. Nowadays regulatory authorities, health agencies, and professional organizations all over the globe are greatly concerned about the presence, occurrence, fate of the non-regulated PPCPs contaminants in human and environment (*6*).

Until recently, the U.S. Environmental Protection Agency (EPA) and other agencies have been primarily concerned about monitoring and regulating a relatively small number of so-called "priority pollutants" in air, water, and soil of the USA. However, the greatly escalating use of PPCPs has resulted in the manufacture of thousands of new complex chemicals that enter the environment in large quantities, especially from the wastewater/sewage treatment plants. Ultimate fates of most of the PPCPs emerging contaminants are in aquatic environment, and thus these compounds are appeared in ecosystems and frequently detected in different environmental compartments and organisms at different concentration levels (*7*). This chapter will discuss the sources, occurrences and fate of emerging contaminants emphasizing PPCPs and their detection and analysis by liquid chromatography - mass spectrometry (LC-MS) and gas chromatography -mass spectrometry (GC-MS).

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2. Emerging Contaminants

New analytical techniques have allowed scientists to characterize the chemical compounds in the environment at extremely low level on the order of sub-ng/g. These chemicals are frequently detected in water and had not been identified previously at such low levels. These are often termed as "emerging contaminants" (ECs) because adequate data associated with the presence, frequency of occurrence, fate, mechanisms and sources are not available to determine their risk to human health and the ecosystem (*5*). ECs are used daily in homes, farms, businesses and industry as detergents, fragrances, prescription and non-prescription drugs, disinfectants, and pesticides etc. Some ECs have been commonly found in water resources around the world and across the USA (*8–10*).

Several studies have reported that many ECs present in municipal wastewater effluent can act as endocrine disruptors at concentrations capable of inducing feminization of fish (*11*, *12*). The feminization has been linked to exposure to compounds that mimic estrogen activity. However, it has also been determined that thousands of compounds have the potential to interact with components of the endocrine system, altering the natural action of the hormone (*13*, *14*). More conditions have been reported in both freshwater and marine fish species (*8*, *12*, *15–17*). The occurrence of some ECs correlates with ecological effects and sexual abnormalities in fish (*18–20*). In another study, complex mixtures of ECs at environmentally relevant concentrations were reported to inhibit the growth of human embryonic cells (*21*, *22*). There are other evidences that some ECs are persistent in the environment and survive through conventional water treatment that cause development issues to aquatic organism (*22*). The U.S. EPA is working to improve its understanding of a number of ECs, particularly PPCPs among others.

3. PPCPs and Its Sources

PPCPs are very large group of chemicals with variety of structures, conformations and functional groups. These include prescription, over-the-counter (OTC) and veterinary drugs, fragrances, cosmetics, sun-screens, diagnostic agents, nutraceuticals, biopharmaceuticals, and growth enhancing constituents used in livestock operations. PPCPs enter the environmental system through effluent discharge from wastewater/ sewage treatment plants, inappropriate disposal of expired or unused drugs, shower drain, runoffs from agricultural soils receiving bio-solid amendments/animal farms, residues from drug manufacturing companies, nursing homes and hospital facilities. These huge pools of substances ultimately get mixed in water bodies reaching the resourceful river streams where aquatic organisms and wildlife live. Many activities of human and other organisms depend on the river systems. These compounds have been frequently detected in different environmental matrices such as air, waters, sediments, sewage sludge, humans and fish (*23–39*). PPCPs are easily dissolved in aquatic environment and do not evaporate at normal temperature and pressures. Moreover some PPCPs appear to show low biodegradation rate and high lipophilicity, and have high bioaccumulation potential as environmental

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contaminants (*6*). Figure 1 illustrates a simplified schematic diagram that shows a route of PPCPs transportation from personal usages to waters and aquatic organisms.

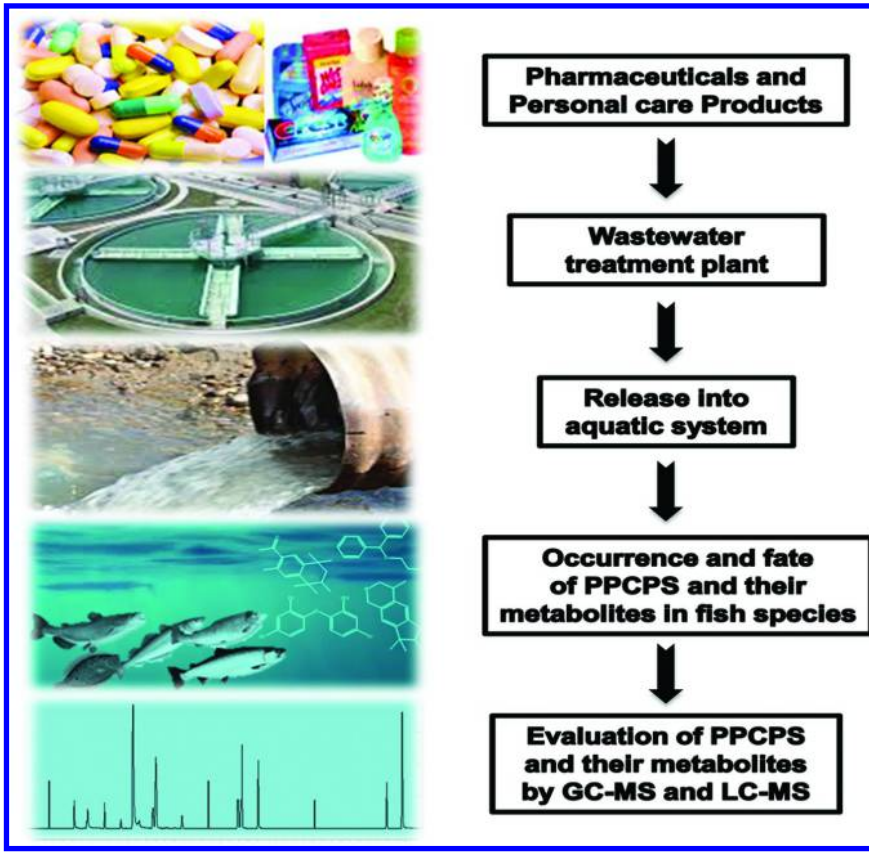

Figure 1. A schematic diagram of the occurrence and fate of PPCPS and their metabolites in fish species and their analysis with GC-MS and LC-MS.

4. Occurrences of PPCPs in Environment and Aquatic Organisms

PPCPs are used to ensure the treatment and protection of public and animals health from uncomfortable situation. Commercial and domestic use and discharge of these compounds into municipal sewage have contributed to their occurrence in aquatic environment and organisms. PPCPs and their metabolites have been detected in aquatic and terrestrial organisms (*23*), surface water (*24*), air (*25*), sewage effluent (*26*), lake Michigan water and sediments (*27*), industrial sewage sludge (*28*), municipal effluents (*29*), marine sediments (*30*), marine mammals (*31*), effluent-dominated river water fish (*32*, *33*), Pecan Creek fish (*34*), and German fish specimen bank (*35*), fish-eating birds and fish (*36*), receiving

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marine waters and marine bivalves (*37*). PPCPs and metabolites have also been identified in human milk (*38*), and human blood (*39*). More importantly, series of studies have indicated that PPCPs are not only accumulated but also subsequently metabolized to reactive intermediates that form covalent bound protein adducts in human (*40*) and aquatic organisms such as fish (*41*, *42*).

Studies illustrated that many PPCPs are environmentally persistent, bioactive, and have bioaccumulation potential (*43–46*). For example PPCPs, triclosan (TCS), an antimicrobial agent, has been widely used in dental care products, disinfectants, hand soaps, footwear, skin care creams and textiles. TCS and its methyl metabolites were detected in surface waters (*47*), biosolids (*48*), fish (*49*) and algae (*50*). Still, the fate and chemistry of TCS are not fully understood. TCS is quite stable for hydrolysis; however its photolysis was identified as one of the major pathways of degradation in surface waters (*51*). Other research groups have shown that TCS in surface water may be toxic to certain algae species. Orvos et al. (*52*) found no observed-effect concentration (72-h growth) at 500 ng/L for algae *Scenedesmus subspicatus* while Wilson (*53*) reported that TCS may cause significant increase in Synedra algae and a substantial reduction of the rare genus Chlamydomonas algae at 15 ng/L and 150 ng/L. Levy et al. (*54*) demonstrated that TCS can block bacterial lipid biosynthesis inhibiting the enzyme enoyl-acyl carrier protein reductase, which leads to a possible development of bacterial resistance to TCS. Recent studies have shown that TCS impaired swimming behavior and altered expression of excitation-contraction coupling proteins in fathead minnow (*Pimephales promelas*) (*55*) and interfered with thyroid axis in the zebra fish (*Danio rerio*) (*56*).

5. PPCPs Detection and Characterization Methods

Due to the advent of high capability state-of-art instruments, PPCPs and other water contaminants have been detected at sub-ng/g levels. In particular, liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) techniques have made huge improvement for the analysis of PPCPs from environmental specimens. Pharmaceuticals and their metabolites are polar compounds and get easily dissolved in water or polar solvents, which is special advantage for LC-MS analysis. The LC-MS method employs either isocratic or gradient elution profile to separate and identify the complex composites/mixtures of samples using different polarities (polar or medium polar or mixed polar) mobile phases with analytical column such as C18. The LC - tandem mass spectrometry (LC-MS/MS) with positive- and or negative modes of operations using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are able to detect PPCPs up to sub-ng/g. The presence of unknown compounds are ascertained and characterized by comparing with mass signals and retention of properties of pure standards. On the other hand, PCPs are relatively non-polar and are favorable to get dissolved and extracted in relatively non-polar organic solvents. After cleaned up, the samples are analyzed by GC-MS/MS or - selected ion monitoring (SIM) modes with electron ionization (EI) or negative ion chemical ionization (NICI) based on sample nature. The

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GC-MS/MS or GC-SIM-MS allow for detecting PPCPs up to sub-ng/g levels. The presence of unknown compounds are characterized by establishing over 80% to 99% agreement with standard compounds of the respective samples (*34*, *57*). Examples of some analyses performed by LC-MS and GC-MS for different environmental samples are illustrated below.

5.1. PPCPs Analysis by LC-MS

Numerous research groups reported the identification and analysis of emerging PPCPs contaminants (*33*, *36*, *58*). Our research group (*33*, *58*) developed LC-MS/MS methods for the analysis of pharmaceuticals and metabolites from environmental fish samples. A gradient mixture of water and methanol solvents consisting of 0.1% (v/v) formic acid in water and 100% methanol was pumped through a Agilent Technologies extended-C18 (dimension; length 15 cm x i.d 2.1 mm (5 μm packing materials size, 80 Å) analytical column to achieve the separation of the complex mixture of PPCPs. Additional operating parameters included injection volume, 10 μL; column temperature, 30 °C; flow rate, 350 μL/min. Column effluents were monitored by MS/MS using a Varian model 1200L triple-quadrupole mass analyzer equipped with an electrospray interface (ESI). Table 1 shows the structures with functional groups, physicochemical properties, therapeutic use and mass spectrometry-operating parameters for individual compound of the target PPCPs compounds that are commonly used in human and animal applications.

A nonlinear gradient elution profile consisting of 0.1% (v/v) formic acid and methanol resulted in near base line resolution of the majority of analytes in approximately 50 min (Table 2). At the end of each run, the column was equilibrated for 15 min using isocratic elution of 93% formic acid and 3% methanol mobile phase followed by the next injection. Figure 2 displays chromatographic separation of 25 target pharmaceuticals and metabolites, 5 surrogates and 2 internal standards that were spiked to the clean fish tissues by LC-MS/MS using electrospray ionization (ESI) positive (+) and negative (-) modes.

Employing the extraction protocol and LC-MS/MS method (*58*), all target compounds listed in Table 1 were analyzed from environmental fish samples that were grabbed from the Pecan Creek, Denton, Texas, approximately 650 m downstream from the effluent discharge. The presence and characterization of the target analytes were confirmed based on comparison of retention time and relative intensities of fragment ions that were observed from spiked and environmental fish specimens. Four pharmaceutical compounds were detected over method detection limits (MDLs). Figure 3 displays a typical LC-MS/MS ion chromatogram for identification of diphenhydramine, diltiazem, carbamazepine, norfluoxetine drugs. In the case of norfluoxetine one fragment ion (296 >134) was produced providing enough intensity to be observed under the MS/MS condition (Table 1). A comparison of MDLs, limit of detection (LOD) and limit of quantitation (LOQ) of 25 target compounds are shown in Table 3. It can be seen that MDLs are approximately 3 to 10 times higher than LOD for majority of the target analytes reported in the table.

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Table 1. Analyte-dependent mass spectrometry parameters for target compounds.

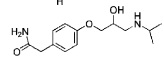
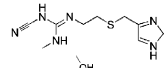
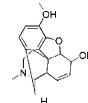
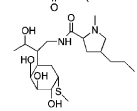
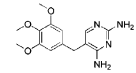
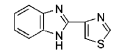
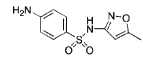
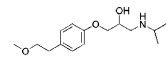
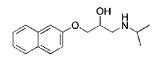
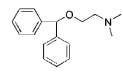
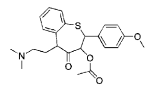
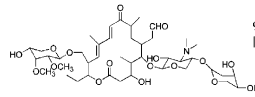
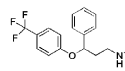
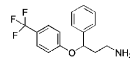
Compound	Use	Structure	Precursor Ion	Collision Energy (eV)	Product Ion	pKa[a]
ESI POSITIVE ANALYTES						
Acetaminophen	analgesic		152 $[M + H]^+$	-11.0	110	9.86
Atenolol	anti-hypertension		267 $[M + H]^+$	-21.5	145	9.16
Cimetidine	anti-acid reflux		253 $[M + H]^+$	-13.5	159	7.07
Codeine	analgesic		300 $[M + H]^+$	-38.0	215	8.25
1,7-dimethylxanthine	caffeine metabolite		181 $[M + H]^+$	-15.5	124	8.50
Lincomycin	antibiotic		407 $[M + H]^+$	-15.5	359	8.78
Trimethoprim	antibiotic		291 $[M + H]^+$	-17.5	261	7.20
Thiabendazole	antibiotic		202 $[M + H]^+$	-23.0	175	
Caffeine	stimulant		195 $[M + H]^+$	-16.0	138	
Sulfamethoxazole	antibiotic		254 $[M + H]^+$	-13.0	156	5.81
Mtoprolol	anti-hypertension		268 $[M + H]^+$	-15.5	191	9.17
Propranolol	anti-hypertension		260 $[M + H]^+$	-11.0	116	9.14
Diphenhydramine	Antihistamine		256 $[M + H]^+$	-11.5	167	8.76
ESI POSITIVE ANALYTES						
Diltiazem	anti-hypertension		415 $[M + H]^+$	-22.0	178	8.94
Carbamazepine	anti-seizure		237 $[M + H]^+$	-13.5	194	
Tylosin	antibiotic		916 $[M + H]^+$	-31.5	174	7.39
Fluoxetine	antidepressant		310 $[M + H]^+$	-6.0	148	10.1
Norfluoxetine	fluoxetine metabolite		296 $[M + H]^+$	-4.5	134	9.05

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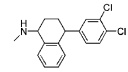
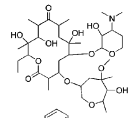
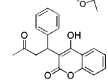
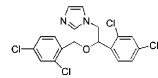
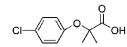
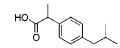
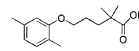
Table 1. (Continued). Analyte-dependent mass spectrometry parameters for target compounds.

Compound	Use	Structure	Precursor Ion	Collision Energy (eV)	Product Ion	pKa[a]
ESI POSITIVE ANALYTES						
Sertraline	antidepressant		306 $[M + H]^+$	-11.0	275	9.47
Erythromycin	antibiotic		716 $[M + H - H_2O]^+$	-18.0	558	8.16
Warfarin	anti-coagulant		309 $[M + H]^+$	-14.0	163	4.50
Miconazole	antibiotic		417 $[M + H]^+$	-27.5	161	6.67
ESI NEGATIVE ANALYTES						
Clofibric Acid	antilipemic		213 $[M - H]^-$	15.4	127	3.18
Ibuprofen	analgesic		205 $[M - H]^-$	7.0	161	4.41
Gemfibrozil	antilipemic		249 $[M - H]^-$	13.0	121	4.75

[a] Calculated values obtained from the SciFinder database (© 2006 American Chemical Society).

(*SOURCE: Reproduced with permission from reference 58, Copyright© American Chemical Society*).

Table 2. Time-scheduled gradient elution program. (*SOURCE: Reproduced with permission from reference (58), Copyright© American Chemical Society*).

Time (min)	*Mobile phase composition (%)*	
	0.1% formic acid	*Methanol*
0	93	7
2	93	7
7	85	5
12	85	5
21	52	48
28	52	48
34	41	59
45	2	98
50	2	98
51	93	7
65	93	7

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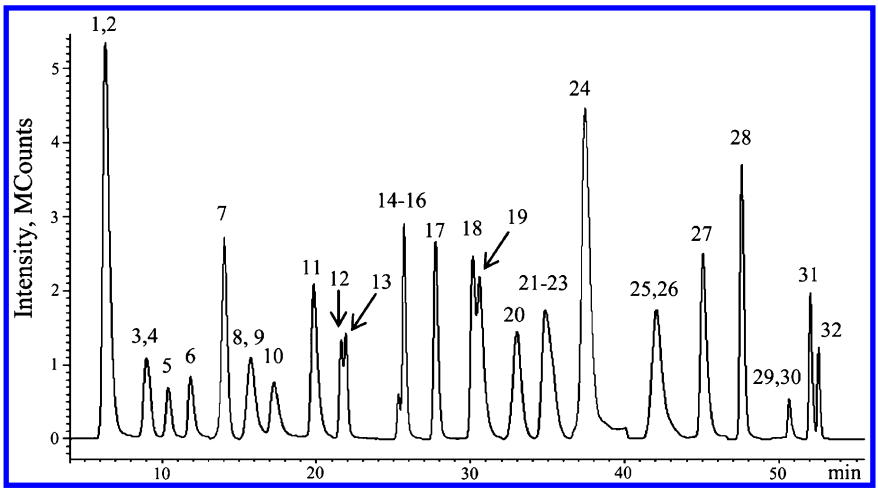

Figure 2. LC-MS/MS total ion chromatogram resulting from analysis of clean tissue spiked with a mixture of pharmaceutical standards. Peak identifications are as follows: (1) acetaminophen-d_4, (2) acetaminophen, (3) atenolol, (4) cimetidine, (5) codeine, (6) 1,7-dimethylxanthine, (7) lincomycin, (8) trimethoprim, (9) thiabendazole, (10) caffeine, (11) sulfamethoxazole, (12) 7-aminoflunitrazepam-d_7 (+IS), (13) metoprolol, (14) propranolol, (15) diphenhydramine-d_3, (16) diphenhydramine, (17) diltiazem, (18) carbamazepine-d_{10}, (19) carbamazepine, (20) tylosin, (21) fluoxetine-d_6, (22) fluoxetine, (23) norfluoxetine, (24) sertraline, (25) erythromycin, (26) clofibric acid, (27) warfarin, (28) miconazole, (29) ibuprofen-13C_3, (30) ibuprofen, (31) meclofenamic acid (-IS), and (32) gemfibrozil. (Reproduced with permission from reference (58), Copyright© American Chemical Society).

In LC-ESI-MS/MS analyses, matrix influence is a critical factor that essentially needs consideration. These matrix effects come from co-extracted matrix constituents that can affect analyte ionization using either ESI positive or negative modes (*36*, *58*). An approach, developed by our group (*58*), was used to measure the matrix influence for extraction solvents that promoted recoveries. In the protocol, aliquot amount of clean muscle tissue was extracted, centrifuged, and reconstituted in 0.1% formic acid. Sample extracts were then spiked with a known amount of each analyte prior to analysis. Aqueous formic acid (0.1% v/v) was also spiked with the same concentration of target compounds and analyzed as a matrix-free reference sample. Concentrations of analytes derived from an internal standard based calibration curve that were prepared using standards solution containing 0.1% formic acid are tabulated in Table 4. This table clearly demonstrates the matrix influence on analysis of PPCPs by LC-MS/MS. To overcome the matrix effect in analyzing the samples that influence mass signal generation, matrix-match calibration curve was proven to be an excellent method that minimized the matrix effect in quantitation of analytes from the

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environmental samples such as fish (*58*). The concentrations of target compounds detected in Pecan Creek environmental fish samples were quantified based on matrix-match calibration curve and results are presented in Table 5. The highest concentration of norfluoxetine was reported in the range of 3.49 - 5.14 ng/g.

5.2. PPCPs Analysis by GC-MS

PPCPs have been detected from environmental and biological matrices at sub-ng/g level by GC-MS. Based on the sample nature and complexity, specimens are prepared for GC-MS analysis. At least one extraction and one clean-up steps are required in preparation of samples for GC-MS examination. As fish samples are complex matrices containing lipids, fat etc., a wide range of extraction and clean-up techniques are available to handle the samples prior to analysis (*34*, *57*, *59–62*). Extraction methodologies include Soxhlet extraction, microwave assisted extraction, ultrasound-solid liquid extraction, and pressurized liquid extraction (PLE) and clean-up approaches are silica gel, florisil, and/or gel permeation chromatography (GPC) (*34*, *57*, *59–62*). A simplified schematic diagram of fish sample extraction is shown in Figure 4 and the extraction protocol is used to prepare the grocery market fish fillet specimens for GC-MS analysis (*57*).

The GC-MS technique analyzes the PPCPs from biological samples using selected ion monitoring (SIM) or MS/MS modes. Our research group developed GC-SIM-MS and GC-MS/MS methods (*34*, *35*) for PPCPs analysis from fish and other environmental samples following U.S. EPA protocols. For example, Mottaleb et al. (*34*) detected the presences of PCPs in environmental fish collected from Pecan Creek and Clear Creek streams, Denton, TX, USA. Clear Creek was not impacted by effluent discharges and is routinely used as a local reference stream by the City of Denton, Texas Watershed Protection program. On the other hand, Pecan Creek receives almost all annual effluent discharge from the Pecan Creek Water Reclamation Plant. Effluent-dominated streams are likely worse-case scenarios for investigating environmental exposures to PPCPs. Because these streams receive limited upstream dilution, wastewater contaminants and resident organisms may receive continuous life cycle exposures. Table 6 displays the personal care products that include UV filters, synthetic musks, alkylphenols, antimicrobial agents and insect repellent are extensively used in daily life and chosen as target compounds to analyze them in the sampled fish by GC-MS (*34*). Figure 5 represents a GC-SIM-MS total ion chromatogram for standard calibration level showing the separation compounds that were targeted in the environmental fish collected from the Pecan Creek, Denton, Texas.

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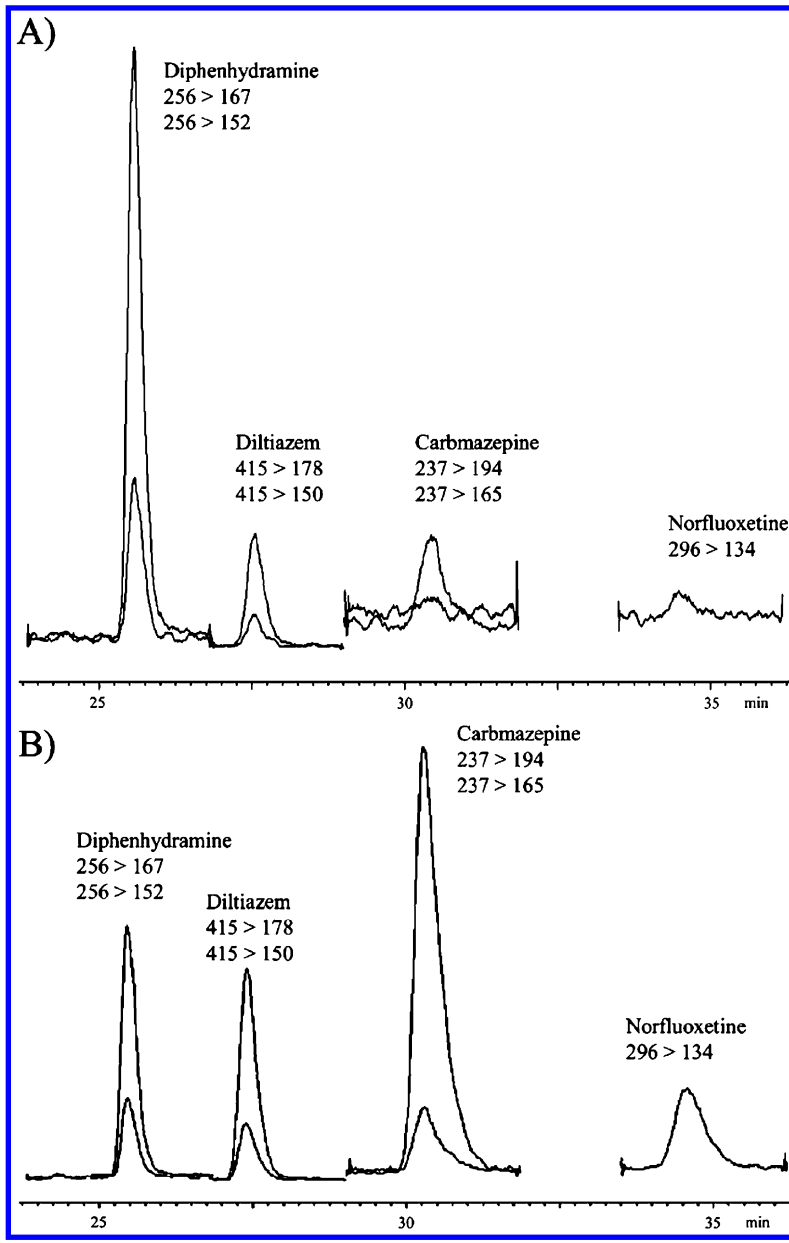

Figure 3. LC-MS/MS reconstituted ion chromatograms displaying analyte-specific quantitation and qualifier ions monitored for (A) a tissue extract from a fish (Lepomis sp.) collected in Pecan Creek and (B) an extract from clean' tissue spiked with known amounts of diphenhydramine (1.6 ng/g), diltiazem (2.4 ng/g), carbamazepine (16 ng/g), and norfluoxetine (80 ng/g). The higher m/z fragment is more intense in all cases. (Reproduced with permission from reference (58), Copyright© American Chemical Society).

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Table 3. Investigated linear range, LOD, LOQ, and MDL for target analytes in fish muscle tissue[a]. (*SOURCE Reproduced with permission from reference (58), Copyright© American Chemical Society*).

Analyte	*Linear range[a] (ng/g)*	*LOD[b] (ng/g)*	*LOQ[c] (ng/g)*	*MDL[d] (ng/g)*
Acetaminophen	3.12 - 400	0.30	0.99	4.40
Atenolol	1.25 - 160	0.48	1.62	1.48
Cimetidine	0.625 - 80	0.24	0.81	1.04
Codeine	4.60 - 600	1.07	3.55	6.11
1,7-dimethylxanthine	0.625 - 80	0.17	0.58	1.02
Lincomycin	3.12 - 400	0.63	2.09	5.53
Trimethoprim	1.25 - 160	0.79	2.63	2.15
Thiabendazole	1.25 - 160	0.14	0.47	2.63
Caffeine	3.12 - 400	0.34	1.15	3.93
Sulfamethoxazole	1.25 - 160	0.23	0.76	2.29
Metoprolol	1.25 - 160	0.25	0.85	2.50
Propranolol	0.625 - 80	0.01	0.03	1.07
Diphenhydramine	0.0625 - 8	0.01	0.03	0.05
Diltiazem	0.09 - 12	0.04	0.13	0.12
Carbamazepine	0.625 - 80	0.03	0.12	0.54
Tylosin	3.12 - 400	1.18	3.93	5.02
Fluoxetine	4.69 - 600	0.76	2.54	6.73
Norfluoxetine	3.12 - 400	0.32	1.08	2.90
Sertraline	3.12 - 400	0.21	0.71	3.57
Erythromycin	3.12 - 400	0.85	2.84	6.42
Clofabric acid	1.25 - 160	0.10	0.32	2.69
Warfarin	0.625 - 80	0.09	0.29	0.86
Miconazole	3.12 - 400	0.39	1.32	10.8
Ibuprofen	25 - 3200	3.14	10.4	45.9
Gemfibrozil	3.12 - 400	0.25	0.85	6.68

[a] Clean tissues employed in the determination of these parameters were extracted using a 1:1 mixture of 0.1 M acetic acid (pH 4) and methanol. [b] LOD, calculated as3 times the standard deviation in the background signal observed for the replicate analysis of a tissue blank. [c] LOQ, calculated as 10 times the standard deviation in the background signal observed for the replicate analysis of a tissue blank. [d] MDL, determined by multiplying the one-sided Student's t–statistics at 99% confidence limit times the standard deviation observed for eight replicate analyses of a matrix spike (spiking level ≤ 10 x MDL).

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**Table 4. Observed matrix effects for extracted tissues samples[a]. (*SOURCE: Reproduced with permission from reference (58), Copyright© American Chemical Society).*

Analyte	*Retention time, min*	*Conc in HCOOH, ng/mL*	*PO4-MeOH, pH 6, ng/g*	*HAc-MeOH, pH 4, ng/g*	*HAc-MeCN, pH 4, ng/g*	*TFA-MeOH, pH 2.4, ng/g*	*MeOH-MeCN, ng/g*
Acetaminophen	6.4	220	186(-15%)	194(-12%)	197(-11%)	154(-30%)	195(-11%)
Atenolol	9.1	110	141(+29%)	117(+7%)	118(+8%)	98(-11%)	102(-7%)
Cimetidine	8.9	60	51(-16%)	57(-5%)	63(+5%)	53(-11%)	53(-11%)
Codeine	10.4	330	330(-0%)	335(+2%)	331(-0%)	299(-9%)	326(-1%)
1,7-dimethylxanthine	11.8	40	41(+3%)	43(+4%)	43(+7%)	38(-4%)	39(-2%)
Lincomycin	14.0	220	239(+9%)	228(+4%)	231(+5%)	201(-8%)	224(+2%)
Trimethoprim	15.6	90	97(-8%)	87(-3%)	87(-3%)	81(-10%)	80(-11%)
Thiabendazole	15.8	90	83(-8%)	84(-6%)	84(-7%)	81(-10%)	86(-4%)
Caffeine	17.2	210	222(+6%)	218(+2%)	228(+8%)	223(-6%)	228(+8%)
Sulfamethoxazole	19.8	85	66(-23%)	81(-5%)	81(-4%)	75(-12%)	76(11%)
Metoprolol	21.9	85	83(-2%)	78(-9%)	81(-5%)	80(-6%)	72(15%)
Propranolol	25.3	40	32(-19%)	35(-11%)	30(-24%)	31(-23%)	19(-52%)
Diphenhydramine	25.8	4	3.3(-17%)	3.2(-20%)	3.1(-21%)	3.1(-23%)	2.2(-45%)
Diltiazem	27.7	4	6.3(+4%)	5.8(-4%)	5.6(-6%)	5.7(-5%)	4(-34%)
Carbamazepine	30.6	40	31(-22%)	36(-11%)	35(-12%)	35(-12%)	35(-12%)

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Table 4. (Continued). Observed matrix effects for extracted tissues samples[a]

Analyte	*Retention time, min*	*Conc in HCOOH, ng/mL*	*PO4-MeOH, pH 6, ng/g*	*HAc-MeOH, pH 4, ng/g*	*HAc-MeCN, pH 4, ng/g*	*TFA-MeOH, pH 2.4, ng/g*	*MeOH-MeCN, ng/g*
Tylosin	32.9	210	334(+64%)	237(+13%)	248(+18%)	261(+24%)	262(+25%)
Fluoxetine	34.9	220	92(-58%)	141(-36%)	99(-55%)	72(-67%)	91(-58%)
Norfluoxetine	35.2	200	69(-65%)	116(-42%)	87(-56%)	53(-74%)	81(-60%)
Sertraline	37.2	220	47(-78%)	117(-47%)	63(-71%)	31(-86%)	93(-58%)
Erythromycin	42.0	200	510(+155%)	1000(+400%)	2422(+1111%)	1476(+638%)	1934(+867%)
Clofabric acid	42.4	70	165(+136%)	38(-46%)	53(-25%)	238(+240%)	42(-39%)
Warfarin	45.0	40	20(-49%)	31(-22%)	29(-27%)	18(-55%)	25(-38%)
Miconazole	47.5	400	43(-89%)	87(-78%)	73(-82%)	19(-95%)	196(-51%)
Ibuprofen	50.6	1600	4045(+152%)	1271(-20%)	1289(-19%)	5491(+243%)	859(-46%)
Gemfibrozil	52.5	200	307(+54%)	129(-35%)	119(-41%)	275(+38%)	110(-45%)

[a] Reported concentrations represent calculated values for each analyte in spiked matrix. Values in parenthesis represent in percent differences in concentration relative to observed for HCOOH. Negative sign indicates suppression of the analyte response. Solvent notations are as follows: PO4, phosphate buffer; MeOH, methanol; Hac, 0.1 M acetic acid buffer; MeCN, acetonitrile; TFA, 0.1 M trifluoroacetic acid buffer.

Table 5. Concentrations of analytes (ng/g of wet weight) detected muscle tissues from fish collected in Pecan Creek, Denton County, TX. *(SOURCE: Reproduced with permission from reference (58), Copyright© American Chemical Society*).

Analyte	*Range (n=11)*	*Mean (n=11)*
Diphenhydramine	0.66 – 1.32	0.96
Diltiazem	0.11 – 0.27	0.21
Carbamazepine	0.83 – 1.44	1.16
Norfluoxetine	3.49 – 5.14	4.37

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Employing the extraction protocols illustrated in Figure 4, recently we reported the concentration of four frequently observed PPCPs in grocery stores edible fish fillets by GC-SIM-MS (*57*). In this investigation, the presence of the target compounds in fish extracts was confirmed based on similar mass spectral features and retention time with pure standards. Mass spectral features and retention times of the target compounds provided by the fish extracts were used for characterization by comparing with the authentic standards. Representative SIM ion chromatograms shown in Figure 6 (i) are for (A) a standard solution containing, 100 pg/µL of triclosan, an antimicrobial agent and (B) a Whiting (genus *Merlangius*) fillet extract. These chromatograms illustrate an excellent similarity of the retention time of triclosan eluted from the capillary column at 21.28 min with an increment of 0.01 min in the Whiting fish fillet sample. An increment of retention time, up to 0.08 min, was observed for other fish such as Salmon and Tuna fillet extracts (not shown), and peak widths at half-height were about 3 s. The variations of retention behavior were expected because of interferences from the matrix which co-extracted with the target compounds from fish specimens. These small variations in retention time allowed for identification and quantification without any interruption. Individual identities of the compounds extracted from the grocery store fish fillet were characterized based on comparison of the relative ion abundance ratios between the quantification and the qualifier ions mass signals. The presence of target compounds in the fish fillet extracts was confirmed when the difference of the relative abundance ratio was less than or equal to approximately ± 20%, or an agreement of the relative abundance ratio of 80% or over. Figure 6 (ii) compares typical mass spectra derived from the ion chromatogram (Figure 6 (i) for (A) standard triclosan and (B) Whiting fillet extract solution). These spectra display an excellent agreement of the mass spectral features/mass signals with a variation of about ±10%. When similar agreement of ion relative abundance ratio and retention features were observed, the presence, characterization and quantification of other compounds in the different fish samples were established. Table 7 shows the concentrations of the compounds that were characterized and quantified in grocery stores fish species. The values of detected compounds are approximately 1 to 3 orders of

magnitude lower than the fish that were collected from the environmental sites (*33–36*, *58*).

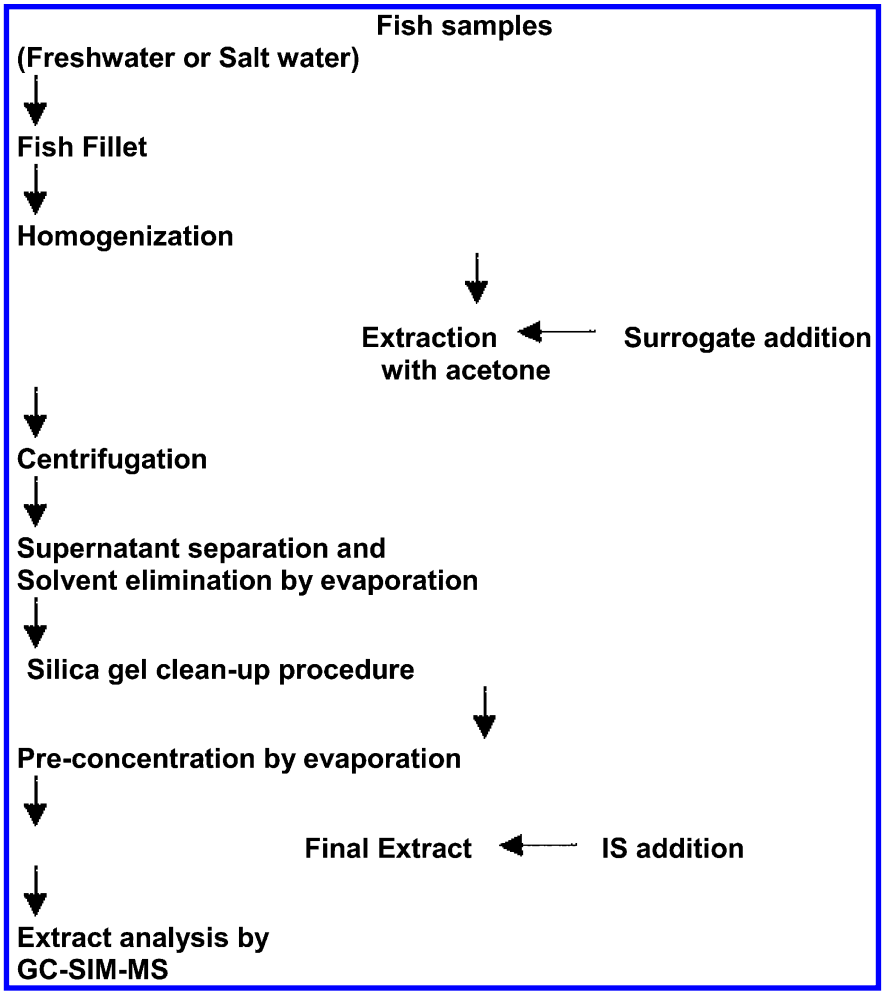

Figure 4. A simplified schematic diagram of the experimental procedure used for extraction and analysis of nitromusks, antimicrobial agent and antihistamine from edible fish fillets. (Reproduced with permission from reference (57), Copyright© Elsevier).

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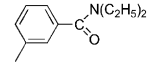
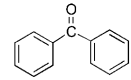
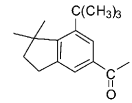
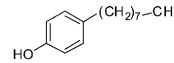
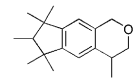
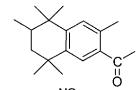
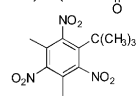
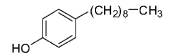
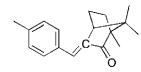
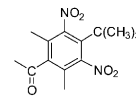
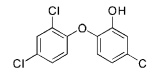
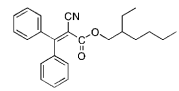
Table 6. Brand and IUPAC names, use, group, retention time, structure, MS/MS and SIM ions for target analytes.

Analyte	Use/group	t_R (min)/ Segment	Chemical Structure	MS/MS transition (m/z), excitation amplitude (V)/storage level 9m/z)	SIM ions
m-Toluamide (N,N-diethyl m-methylbenzamide)	Insect repellent	7.10/1		190>**145**[a], 175, 0.7/83.6	91,**119**[a] 190
Benzophenone (diphenyl ketone)	Sun screen/UVF	7.79/2		182>**153**, 0.8/80.1	77, 105, **182**
Celestolide (4-acetyl-1,1-dimethyl-6-tert-butylindane)	Fragrance/SM	8.74/3		229>**173**, 131, 0.8/100.9	173, **229**, 244
p-Octylphenol	Surfactant metabolite/AP	10.60/5		278>**179**, 0.6/122.6	165, 180, **278**
Galaxolide (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl cyclo-penta[g]-2-benzo pyrane)	Fragrance/SM	11.35/6		243>**213**, 171, 0.8/107.1	213, **243**, 258
Tonalide (7-acetyl-1,1,3,4,4,6-hexamethyltetralin)	Fragrance/SM	11.50/6		243>**187**, 159, 0.8/107.1	201, **243**,258
Musk xylene (1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene)	Fragrance/SM	11.51/6		282>**265**, 248, 0.6/124.3	**282**, 283, 297
p-Nonylphenol	Surfactant metabolite/AP	12.93/7		292>**179**, 0.6/128	149, 179, **292**
4-MBC (4-methylbenzylidine camphor)	Sun screen/UVF	15.94/8		211>**169**, 155, 0.7/92.9	115, 211, **254**
Musk ketone (4-aceto-3,5-dimethyl-2,6-dinitro-tert-butylbenzene)	Fragrance/SM	16.14/8		304>**287**, 214, 0.7/134.1	217, **261**, 366
Triclosan (4-choloro-2-hydroxyphenyl-2,4-dichlorophenyl ether)	Anti-microbial	17.04/9		347>**200**, 310, 0.8/153.1	200, **345**, 362
Octocrylene (2-methylhexyl-2-cyano 3,3-diphenylacrylate)	Sun screen/UVF	24.82/12		250>**248**, 221, 0.8/110.2	177, 249, **361**

[a] Bold print indicates *m/z* used for quantitation. UVF, ultra-violet filter; SM, synthetic musk; AP, alkylphenol. (*SOURCE: Reproduced with permission from reference 34, Copyright© Elsevier).*

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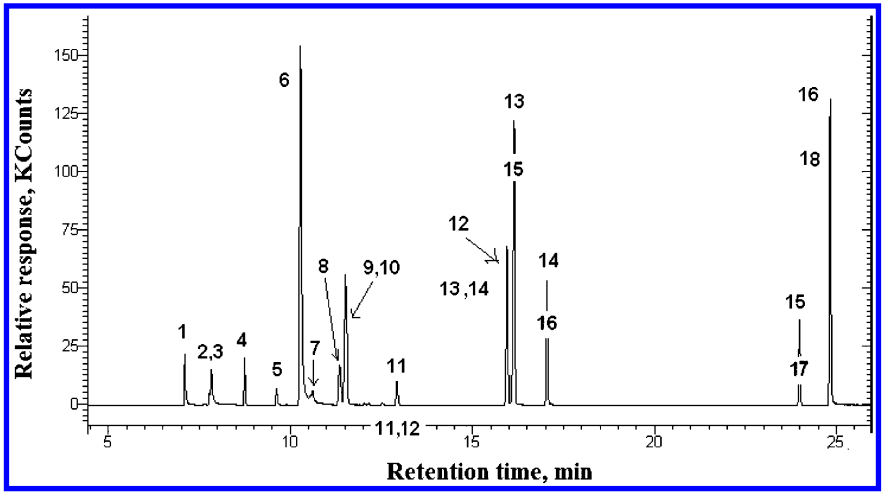

Figure 5. GC-SIM-MS representative total ion chromatogram for a calibration standard. Peak identifications are as follows: (1) m-toluamide, (2) benzophenone, (3) benzophenone-d_{10}, (4) celestolide, (5) pentachloronitrobenzene, (6) phenanthrene-d_{10}, (7) p-n-octylphenol, (8) galaxolide, (9) tonalide, (10) musk xylene, (11) p-n-nonylphenol, (12) [$^{13}C_6$]p-n-nonylphenol, (13) 4-methylbenxylidine camphor, (14) 2,2'-dinitrobiphenyl, (15) musk ketone, (16) triclosan, (17) mirex, and (18) octocrylene. (Reproduced with permission from reference (34), Copyright© Elsevier).

6. Effect and Biotransformation of PPCPs in Aquatic Organisms

The effects of PPCPs are different from conventional pollutants because drugs are purposefully designed to interact with cellular receptors at low concentrations and to cause specific biological effects. Unintended adverse effects can also occur from interaction with non-target receptors. Environmental toxicology focuses on acute effects of exposure rather than chronic effects. Effects on aquatic life are a major concern because aquatic organisms receive more exposure risks than those for human, and are exposed on a continual and multi-generational basis with higher concentrations of PPCPs in untreated water. The risks posed to aquatic organisms are largely unknown because of trace level concentration of the PPCPs. Some of the known potential impacts on organisms include delayed development in fish, delayed metamorphosis in frogs, and a variety of reactions including altered behavior and reproduction (*1*, *2*). Recent studies have indicated that many of pharmaceuticals and metabolites are environmentally persistent, bioactive, and have potential for bioaccumulation (*43*, *44*). Acute aquatic toxicities of drugs and metabolites were examined on marine bacterium (*Vibrio fischeri*), a freshwater invertebrate (*Daphnia magna*), and the Japanese medaka fish (*Oryzias*

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latipes) by Kim et al. (2007) (*63*). They demonstrated that *Daphnia* was the most susceptible among the tested organisms. Correa and Hoffmann (*64*) studied the variation of magnitude of response effect of drugs d-amphetamine, sodium pentobarbital, diazepam, β-carboline, and saline before and after inducing into of knife-fish (*Gymnotus carapo*). They concluded a reduction of the degree of alertness by the barbiturate and a decrease in emotionality and/or stress by the benzodiazepine with the novelty response. Brandao et al. (*65*) evaluated the biochemical and behavioral effects of neuro-active anticonvulsant drugs (diazepam, carbamazepine, and phenytoin) on pumpkin-seed sunfish (*Lepomis gibbosus*) and showed behavioral changes of sunfish through oxidative stress parameters such as glutathione reductase, glutathione S-transferases, catalase and lipid peroxidation.

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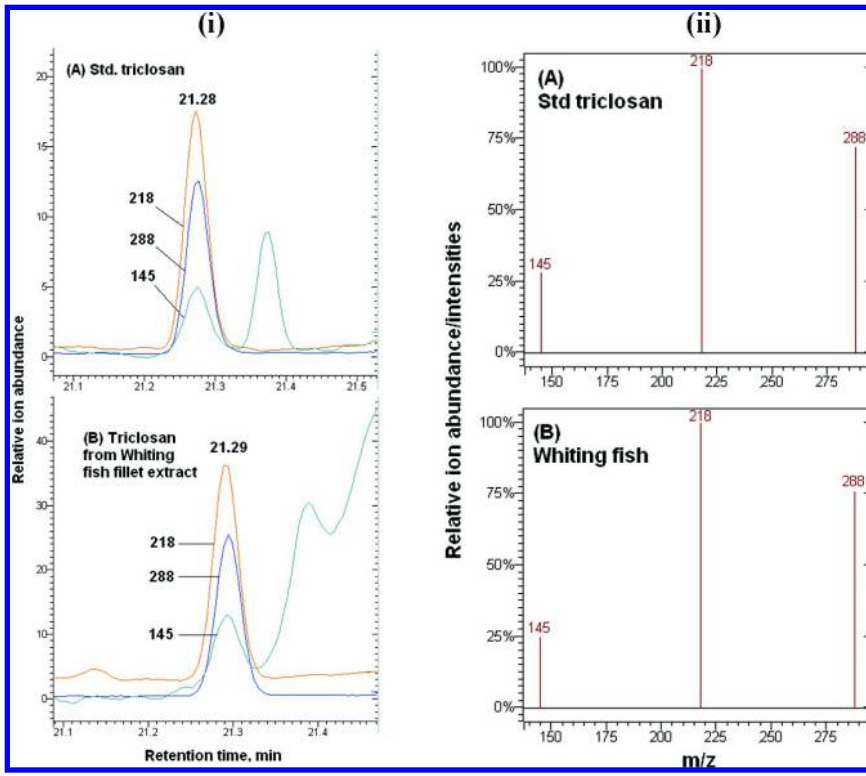

Figure 6. (i) Representative GC-SIM-MS ion chromatograms for (A) a standard solution containing, 100 pg/µl of triclosan and (B) a Whiting (genus Merlangius) fillet extract (left). (ii) Typical GC-SIM-MS mass spectra for (A) standard triclosan and (B) Whiting fillet extract solution derived by selecting the data file from Figure 6 (i) (A), retention time 21.28 min and Figure 6 (i) (B), retention time 21.29 min, respectively (right). (Reproduced with permission from reference (57), Copyright© Elsevier).

Table 7. Concentration of target analytes in fish fillets received from local grocery stores, Maryville, Missouri, USA and their comparison with environmental fish samples. (*SOURCE: Reproduced with permission from reference (57), Copyright© Elsevier*).

Name of fish	*Concentration of analytes in edible fish fillets (ng g^{-1})*				
	HHCB	***AHTN***	***DPH***	***MK***[a]	***TCS***
Tilapia	0.876	0.813	0.679	nd	4.122
Catfish	0.276	0.429	0.939	nd	2.086
Swai	0.336	0.190	0.189	nd	1.782
Flounder	0.892	0.904	1.182	nd	7.472
Salmon	0.250	0.068	1.037	nd	0.942
Whiting	0.263	0.431	0.503	nd	3.699
Pollock	0.163	0.304	0.692	nd	1.011
Yellow fin Tuna	0.343	0.269	0.811	nd	2.292

[a] nd – not detected.

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The ultimate fate of PPCPs is formation of metabolites in biological species that may show potential impacts on organisms or could be used as a biomarker of environmental exposure. Mottaleb and coworkers (*41*, *66*, *67*) investigated the biotransformation and toxicokinetics of PPCPs especially nitromusks fragrance ingredients musk xylene (MX) and musk ketone (MK) using trout fish as model. The fish were exposed with nitromusks compounds. Details of fish exposure, extraction, and analysis of breakdown product or metabolites have been reported (*41*, *66*, *67*). Previously we demonstrated the formation of amine (cysteine - hemoglobin) adducts through enzymatic biotransformation reaction between MX/MK and trout (*41*, *68*). The reduction of a nitro-group in MX and MK led to the formation of amine adducts of hemoglobin that could be suitable as a biochemical endpoint useful for exposure monitoring and assessment of potential hazards resulting from MX and MK compounds (*41*, *66–68*).

Recently our group (*42*) also reported that the bound metabolites obtained from liver proteins may be used as indicators of internal exposure to chemical carcinogens. Table 8 illustrates the trout exposed with different dosages with MX and MK compounds over a period of 7 days. The metabolites of nitromusks or other related nitroarenes, bound to the cysteine sulfhydryl group (– SH) of proteins in liver as biomarkers of exposure, could potentially be used to assess continuous exposure over a longer time range, and thus, may be better suited for risk assessment than quantitation of urinary metabolites (*69*). The biological transformation processes of MX and MK to their corresponding amine metabolites, with cysteine containing proteins in the liver results in adducts formation are shown in Figure 7. Nitroarenes are enzymatically reduced to nitroso reactive intermediates, nitrosoarenes, capable of covalently binding with the – SH

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group of cysteine amino acids in proteins to form an acid/base labile sulfonamide adducts that hydrolyzes to aromatic amines in the presence of aqueous base (*70*). The aromatic amines were considered to be good dosimeters for the target tissue (*71*).

Fish exposure protocols, liver samples collection, homogenization and composite preparation together with bound metabolites extraction procedure have been reported in our earlier report (*42*). Briefly, bound metabolites or adducts were extracted from exposed liver composite taking about 0.35-0.60 g sample into a 20 mL glass vial, where 10 mL of 0.5% sodium dodecyl sulfate, 1 mL of 10 N sodium hydroxide and 400 ng (100 µL of 4 ng/µL) of surrogate naphthalene-d8 were added. The mixture was then extracted for 1 h at 50 rpm in room temperature. Following extraction, samples were rinsed into a 50 mL centrifuge tube using 2 mL nanopure water and centrifuged at 13,000 rpm for 1 h at 22 °C. The supernatant was decanted into a 50 mL glass tube and then extracted 4 times with 10 mL n-hexane at room temperature, while stirring for 5 min. The extraction was performed slowly at 7 rpm to minimize the formation of the emulsion that developed during stirring. Sealed tubes were sonicated for approximately 2 min to break the emulsion. Developed pressure inside the tube was released by opening the cap. The tubes were placed in a refrigerator for about an hour to freeze the sample. A clear hexane layer was obtained as an extract on the top of the aqueous layer in the tube. The extract was transferred into a conical flask by disposable Pasteur pipette. The extract was then passed through a column containing granular anhydrous Na_2SO_4 to remove the residual water. Prior to passing the extract, about 8 mL of n-hexane was run through the drying column to activate the Na_2SO_4 granules in the column. The solvent was evaporated almost to dryness, under a gentle stream of nitrogen at room temperature. Subsequently, the sample was reconstituted in 180 µL of n-hexane where 50 ng (25 µL of 2 ng/µL) IS (phenanthrene-d10) was added. The solution was sealed in GC vials and analyzed by GC-SIM-MS.

Spike recovery study and limit of detection (LOD) measurements of 2-amino-MX, 4-amino-MX and 2-amino MK metabolites were accomplished and were valued as 95 - 114% with relative standard deviation < 10% with recovery measurements range ofd 0.91 - 3.8 ng/g, respectively. The amino metabolites formed as cysteine-protein adducts in trout liver were quantified against internal standard based calibration curve. Table 9 illustrates the concentration of metabolites observed in the trout liver. For the toxicokinetic study, a single dose of 0.03 mg/g MX or MK over the period of 1 day, 3 days, and 7 days was applied to trout fish with appropriate controls. Table 8 details the exposure schedule. Figure 8 displays the graphical representation of the toxicokinetics assessment of the 2-AMX, 2-AMK and 4-AMX metabolites. This plot evaluated the natural logarithm (ln) of the concentration of the metabolites versus sampling times for the dosing level of 0.03 mg/g. The time dependence of the adduct development for each of the metabolites indicated a maximum formation at 1 day (24 h) after exposure. As expected, the kinetics of the elimination of the protein adducts showed the negative slope linear relationship curve. We estimated the half-lives of 2 - 9 days for the metabolites in the trout liver based on assumption of first-order kinetics.

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Table 8. ***In vivo*** **trout exposure dosing schedule with nitro musk compounds and salmon oil vehicle. (*SOURCE: Reproduced with permission from reference (42), Copyright© Elsevier*).**

Exposure time, Day	*MX exposure*					*MK exposure*				
	MX concMX conc. (mg/mL)	*Fish wet weight, (g)*	*Liver wet weigh, (g)*	*MX dose /trout, (mg)*	*Average dosing level, (mg/g)*	*MK conc. (mg/mL)*	*Fish wet weigh, (g)*	*Liver wet weigh, (g)*	*MK dose /trout, (mg)*	*Average dosing level, (mg/g)*
1- Day	10	202	0.75	2.0	0.01	10	257	0.88	2.6	0.01
		256	0.87	2.5			237	0.77	2.4	
		165	0.50	1.6			222[a]	NC	2.2	
	30	180	0.75	5.4	0.03	30	199	0.70	6.0	0.03
		256	0.92	7.5			230	0.76	6.9	
		280[b]	1.41	8.4			212	0.69	6.3	
	100	236	0.76	24.0	0.10	100	272	1.26	27.0	0.10
		264	0.92	26.0			271	1.26	27.0	
		204	0.74	20.0			197	0.78	20.0	
	300	250	0.88	75.0	0.30	300	190	0.70	57.0	0.30
		310	1.52	90.0			270	1.18	81.0	
		227	0.71	69.0			250	1.00	75.0	
	Control	206	0.76	0.20 mL, exposed with salmon oil only						
		304	1.52	0.30 mL, exposed with salmon oil only						
		184	0.70	0.18 mL, exposed with salmon oil only						

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Exposure time, Day	*MX exposure*					*MK exposure*				
	MX concMX conc. (mg/mL)	*Fish wet weight, (g)*	*Liver wet weigh, (g)*	*MX dose /trout, (mg)*	*Average dosing level, (mg/g)*	*MK conc. (mg/mL)*	*Fish wet weigh, (g)*	*Liver wet weigh, (g)*	*MK dose /trout, (mg)*	*Average dosing level, (mg/g)*
3-Days	30	208	0.79	6.3	0.03	30	278	1.22	8.4	0.03
		244	0.81	7.2			156	0.46	4.5	
		193	0.71	6.0			196	0.68	6.0	
	Control	253	0.89	0.25 mL, exposed with salmon oil only						
		272	1.23	0.27 mL, exposed with salmon oil only						
		233	0.75	0.23 mL, exposed with salmon oil only						
7-Days	30	212[b]	0.71	6.3	0.03	30	121	0.30	3.6	0.03
		230	0.76	6.9			241	0.88	7.2	
		204	0.74	6.0			167	0.53	5.1	
	Control	273	1.12	0.27 mL, exposed with salmon oil only						
		305	1.45	0.30 mL, exposed with salmon oil only						
		250	0.89	0.25 mL, exposed with salmon oil only						

[a] Trout was found dead and the liver sample was not collected (NC). [b] Trout was found sick (not equilibrium condition) and the collected liver specimen was not used in this study for composite preparation. All control liver samples were mixed together to make one control composite specimen. The collected MX and or MK exposed liver samples were composited mixing three liver for each dosing level with exception of sick or dead fish liver.

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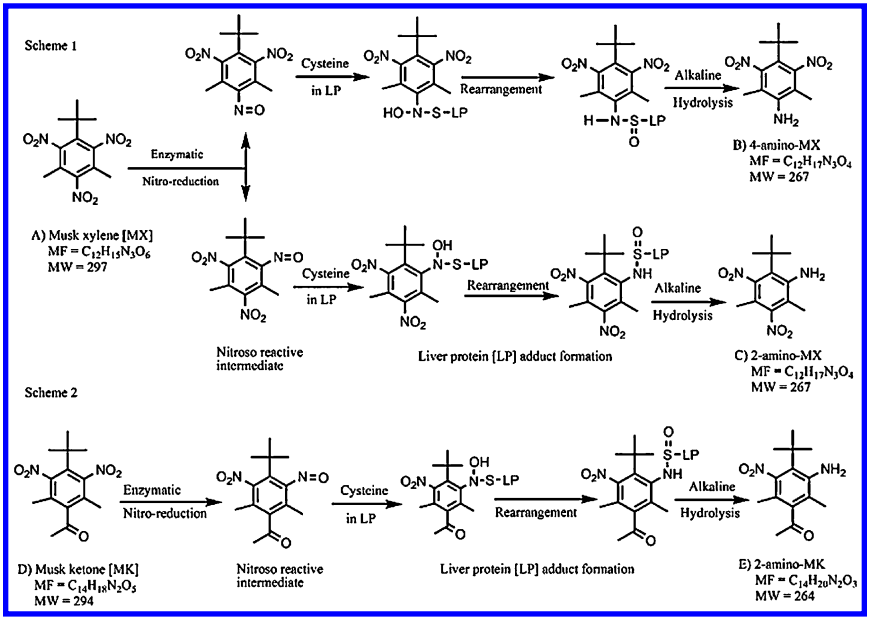

Figure 7. A possible biological transformation pathway of nitro musks (MX or MK) to corresponding metabolite amino compounds illustrating formation of nitroso adduct with cysteine containing proteins in the fish liver. Scheme 1: musk xylene (A) to 4-amino-musk xylene (B) and to 2-amino-musk xylene (C). Scheme 2: musk ketone (D) to 2-amino-musk ketone (E). (Reproduced with permission from reference (42), Copyright© Elsevier).

Table 10 illustrated the individual values of the elimination rate constants and the half-lives of the 2-AMX, 2-AMK and 4-AMX metabolites in the fish liver. A variation of rate constant of the metabolites suggested that the toxicokinetics are more complex than a simple first-order reaction because additional internal biological processes transpire in the living organisms. Nitromusks (MX and or MK) were identified as inducers of hepatic cytochrome P450 2B enzymes and P450 1A1 and 1A2 isoenzymes (*72*, *73*) and are non-genotoxic (*74*, *75*). Although the nongenotoxic carcinogenesis is not fully understood, it is believed that a non-genotoxic mechanism, such as increased cell proliferation, might be responsible for the increase in the liver tumors (*76*). In our investigation (*42*), the MX and or MK-cysteine-protein adducts in fish liver were used to monitor nitromusks hazards as biomarkers of exposure.

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Table 9. Concentration of nitro musk metabolites in trout liver samples using hydrolyzed extraction. (*SOURCE: Reproduced with permission from reference (42), Copyright© Elsevier*).

Exposure period (Day)	*Exposure level MX or MK (mg/g)*	*Nitro musk metabolites (ng/g)*		
		2-AMX	*4-AMX*	*2-AMK*
	0.01	94.0	2404.4	115.4
	0.03	492.0	12588.5	505.5
	0.10	444.1	10325.9	426.6
1-Day	0.30	259.1	5147.3	396.1
3-Days	0.03	213.6	6097.6	357.8
7-Days	0.03	113.5	2988.3	298.0
Controls	None	Not detected		

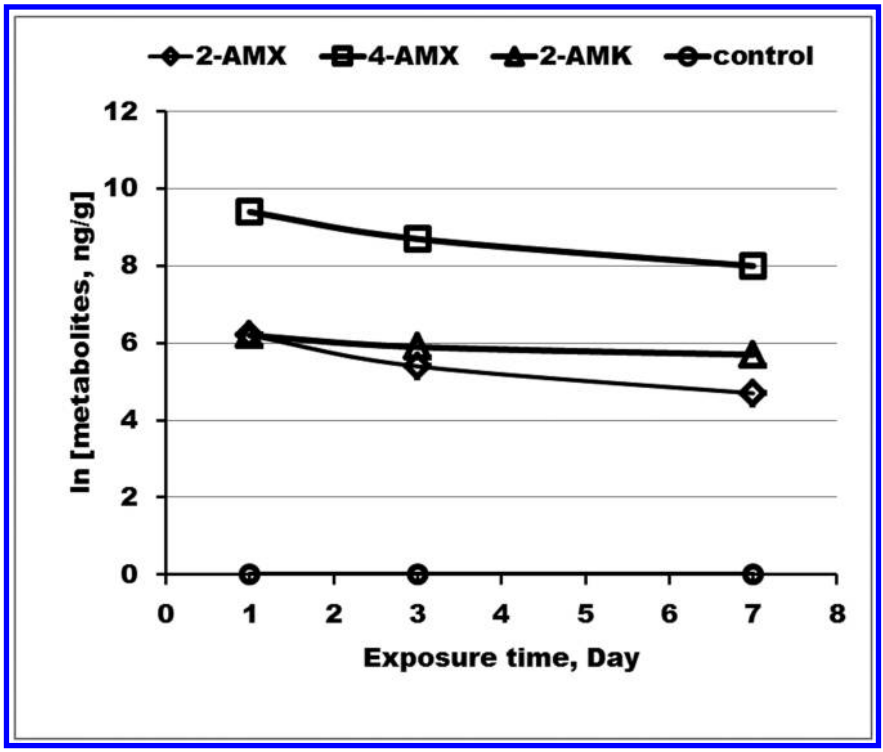

Figure 8. Toxicokinetics plot prepared as natural log (ln) of concentration of the amino metabolites obtained as nitro musk cysteine containing proteins adduct in the fish liver versus nitro musk exposure period for the dosing level of 0.03 mg/g of MX and or MK in fish. The elimination rate constant and half-lives of the metabolites were estimated from this graph. (Reproduced with permission from reference (42), Copyright© Elsevier).

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Table 10. Elimination rate constant (k) and half-life ($t_{1/2}$) of bound metabolites in trout liver on first order kinetics. (*SOURCE: Reproduced with permission from reference (42), Copyright© Elsevier*).

Target metabolites	*ln of concentration of metabolites, (ng/g)*			*Elimination rate constant (k), (Day^{-1})*		*Half-life ($t_{1/2}$), (Day)*	
	Day 1	*Day 3*	*Day 7*	*Day 3*	*Day 7*	*Day 3*	*Day 7*
2-AMX	6.2	5.4	4.7	0.40	0.25	2.0	3.0
4-AMX	9.4	8.7	8.0	0.35	0.23	2.0	3.0
2-AMK	6.2	5.9	5.7	0.15	0.08	5.0	9.0

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Conclusions and Recommendations

Increasingly reported detections of very low level concentrations of PPCPs in various environmental matrices is mainly attributable to technological advances in the sensitivity and accuracy of detection equipment and analytical methods. The GC-SIM-MS or GC-MS/MS and LC-MS/MS are advanced methods that are able to determine the target compounds at low sub-ng/g. The selection of protocols is dependent on the physco-chemical properties of the target compounds. The LC-MS/MS analysis prefers measuring target compounds that are more polar and highly soluble in water, whereas GC-SIM-MS or GC-MS/MS is more suitable for volatile/semi-volatiles compounds that are relatively less polar favoring to dissolve in organic solvents. With such improved analytical capabilities and methods, scientists can correlate the fate and occurrence of PPCPs in the environment, aquatic organisms and human health risks that could be used as integrated human risk assessment methods.

Regulatory approval processes for PPCPs and metabolites require thorough assessments to demonstrate the efficacy and safety of active compounds. These assessments determine the margin of safety associated with human consumption and take into account the risk–benefit equation. The stream of PPCPs and metabolites into the environment is likely to increase in the future because humans and animals are aggressively using more PPCPs for their protection and wellbeing. To save the organisms in ecosystems, relevant regulatory federal agencies should impose and enforce stable rules and regulations about unused, expired and manufacturing residuals of PPCPs disposal. The agencies should recommend improving the capability of wastewater/sewage treatment plants that can effectively remove the PPCPs prior to their disposal into water systems. Consumers need to be aware of the consequences of PPCPs to aquatic organisms and public health and should follow the federal agencies disposal guidelines to make our environment safe for all living organisms. At the same time, scientists and toxicologists should continue to investigate the transport, environmental fate and eco-toxicity of those compounds and their potential for physiological effects on humans and wildlife.

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Chapter 4

Properties of Soil Particle Size Fractions and Their Contribution on Fate and Transport of Hormones in Soil Environment

Yong Qi,[1,*] Tian C. Zhang,[1] and Rui Ma[2]

[1]Civil Engineering Dept., University of Nebraska-Lincoln at Omaha Campus, 1110 S 67th Street, Omaha, Nebraska 68182, United States
[2]Ecological Environment Institute, Chinese Academy for Environmental Planning, Ministry of Environmental Protection, 8 Dayangfang BeiYuan Rd., Chaoyang District, Beijing 100012, China
[*]E-mail: yqi01@unomaha.edu

Hormones detected in environments have aroused wide public concern due to their possible adverse effects to humans and ecosystems. Currently, information is still very limited on the fate and transport of hormones in the soil environment. This chapter describes the methodology of fractionating soil particles into different size fractions, how to evaluate soil particles' sorption-desorption properties for testosterone, and how to link the mass and particle size distribution of the soil with the hormone's transport to surface or ground water. Results show small particles play dominant role in facilitating the transport of hormones due to their high sorption capacity, low desorption potential and easier mobility through runoff. These results provide indirect evidence on the colloids (clay)-facilitated transport of hormones to surface water and to groundwater via preferential flow. Soil particles may be fractionated into a fully- or non-dispersed size distribution. As a future research direction, a new protocol concerning the effects and roles of non-dispersed soil particles on the fate and transport of micro-pollutants should be set up with the corresponding procedures being standardized.

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1. Introduction

During the past decades, natural and synthetic steroid hormones including testosterone, estrone and estradiol have been widely detected in the environment, which have been an increasing concern due to their ability to alter the sexual behavior and endocrine systems of animals and aquatic species (*1*, *2*). For example, trenbolone has been shown to be a potent reproductive toxicant in fathead minnows and has affected the population of wild fathead minnow inihibiting waterbody downstream of a cattle feedlot (*3*, *4*). In the U.S., about 238,000 farms are considered animal feeding operations; many of them are concentrated animal feeding operations (CAFOs). These CAFOs generate more than 500 million tons of animal waste annually (*5*). Persistent pollutants such as hormones and their metabolites have been detected in significant concentrations in cattle waste, and also in runoff from CAFOs (*6*, *7*). Currently, the majority of the manure from CAFOs is applied to agricultural land as a nutrient source, increasing the risk of natural and synthetic hormones in CAFO wastes entering the environment (*8*).

Soil has been identified as one of the main media that control bioavailability, transport and ultimate fate of steroid hormones (*9*, *10*). Being lipophilic (log K_{ow}= 2.6–4.0), most steroid hormones are expected to be sorbed on soils and/or organic matters and are unlikely to be very mobile (*11–13*). However, this conclusion cannot explain the frequent detections of hormones in ground and surface water. For example, It was reported that reproductive hormones were found in approximately 40% of the 139 streams sampled across 30 states in the United States (*8*). Testosterone has been detected in sediments 45 m below a dairy-farm wastewater lagoon (*14*). The traditional tests on sorption-desorption behavior and rainfall field studies of hormones cannot explain the phenomenon very well. This might be, because soils contain a wide range of particles of different diameters that have different mobility during rainfall events, while most of the traditional studies were conducted with the bulk soil that lumped all soil particles in the same samples (*15*, *16*). To date, no field study has been focused on soil particle distributions and their effects on hormones' concentration in either runoff or leachate (*17*, *18*), and therefore, cannot provide reliable information on how particle size distributions contribute to hormone transport in runoff or leachate.

Some studies have reported that a slight change in clay content greatly facilitates transport of soil-sorbed chemicals (*19*). Also, it has been documented that colloidal particles in runoff play an important role in phosphorus transport to waterbodies (*20*). Therefore, in order to fully understand the fate and transport of hormones in soils, two questions should be answered: 1) how hormones will associate and dissociate with soil particles of different size fractions? 2) What are the soil particles size distributions in the runoff and leachate and the contribution of different soil particles to the fate and transport of hormones during rainfall events?

This chapter briefly summarizes the information on hormones and their fate and transport in the soil environment. The chapter presents the studies on fractionation of soil particles into five different size fractions and evaluation of

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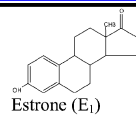
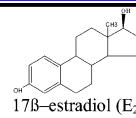
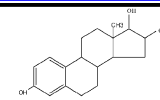
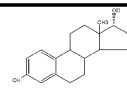
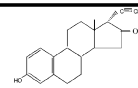
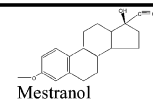
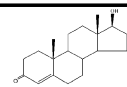
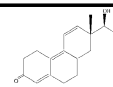
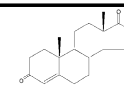
their sorption and desorption behavior for testosterone under the influence of different test conditions. The chapter introduces a soil slab reactor that was used to simulate rainfall tto generate runoff and leachate and also help understand how particle properties (e.g., size distribution and mass) affects transport phenomenon. Finally, the chapter gives conclusions and future perspectives in the discussion section.

2. Hormones and Their Fate and Transport in Soil Environments

For years, beef cattle feedlot operations have been using hormone treatments to increase production (*21*). The growth-promoting steroid hormones approved by the U.S. Food and Drug Administration (FDA) for use in cattle include estrogens (e.g. estradiol and zeranol), androgens (e.g. testosterone and trenbolone acetate), and progestins (e.g. progesterone and melengesterol acetate (Table 1).

Table 1. General properties of selected hormones in environments (*11–13*, *22–24*).[a]

Chemical Structure/Name			
	Estrone (E_1)	17ß–estradiol (E_2)	Estriol (E_3)
Formula	$C_{18}H_{22}O_2$	$C_{18}H_{24}O_2$	$C_{18}H_{24}O_3$
Solubility (mg L^{-1})	0.8–12.4	1.51–12.96	3.2–13.3
$LogK_{ow}$	3.1–3.4	3.8–4.0	2.6–2.8
Chemical Structure/Name			
	17α–estradiol (17α)	17α–ethinylestradiol (EE_2)	Mestranol
Formula	$C_{18}H_{24}O_2$	$C_{20}H_{24}O_2$	$C_{21}H_{26}O_2$
Solubility (mg L^{-1})	3.2–13.3	3.1–10	0.3
$LogK_{ow}$	3.4–4.0	3.7–4.2	4.1–4.7
Chemical Structure/Name			
	Testosterone	Trenbolone	Androstenedione
Formula	$C_{19}H_{28}O_2$	$C_{18}H_{22}O_2$	$C_{19}H_{26}O_2$
Solubility (mg L^{-1})	18–25	20	37–41
$LogK_{ow}$	3.22	NA	2.75

[a] Note: the most common detected natural estrogens include E_2 and its main metabolite E_1, E_3 and 17α (*15*, *25*, *26*)

In general, steroid hormones are classified as natural and synthetic hormones. Synthetic steroid hormones and their metabolites that are persistent in the soil have been detected in significant concentrations in cattle waste, and also detected in runoff from CAFOs (*6*, *7*). Estrogen, androgens, and progestin agonistic and antagonistic activities have been associated with CAFO effluents (*27*, *28*). For example, the concentrations of E2, E1, E3, 17B-estradiol, EE2, testosterone and

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mesthanol (Table 1) were as high as 200, 112, 51, 74, 831, 214, 407 ng L^{-1}, respectively, in streams of the US (*8*). The estradiol and testosterone concentration in runoff is in the range of 4–3500 and 1–1830 ng L^{-1}, respectively. Hormones in environments may cause reproductive abnormalities to aquatic species even at a very low concentration (as low as 1 ng L^{-1}) (*29*).

Few studies have investigated the fate and transport of hormones in agriculture soils. Shore et al. (1995) studied the hormone concentration in a pond and a stream close to a field that received poultry litter (*30*). The authors reported that the testosterone concentration in the pond were 0.5-5 ng L^{-1}, and 1-28 ng L^{-1} in the stream. However, most studies indicate that steroids hormones have a relatively high affinity for soils, which would intuitively retard their movement in runoff and filtration. At the same time it might increase the potential of particle-associated steroids transport in surface waters (*15*). Various laboratory studies have investigated the sorption-desorption of various hormones in bulk soils. These studies have reported that linear sorption isotherm coefficient (K_d, in L kg^{-1}) for estradiol, ethylestradiol, estrone, androstenedione and testosterone are in the ranges of 3.56-84.41, 2.33-23.4, 3.4-48.1, 19.3-142 and 4.56-27.3 L kg^{-1}, respectively (*31–33*).

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3. Effects of Soil Particles of Different Size Fractions on Hormones Fate and Transport in Soil Environments

Generally, soils contain a wide range of particles of different size fractions. Soil erosion is a complex phenomenon involving the detachment and transport of soil particles, storage and runoff of rainwater, and infiltration (*34*). During a storm event, hormones in manure applied to the agricultural land could be carried away through both the dissolved (rainwater) and solid phase (eroded particles with sorbed hormones). Therefore, in order to further understand how the soil particles would facilitate/impede the transport of hormones in soil environments, we fractionated the soil into particles of different size fractions and evaluated their sorption-desorption potential of hormones. The methods used and the major results obtained are described in this section. Combining the particle distributions of different size fractions in runoff and leachate under different rainfall simulation events, the fate and transport of hormones in agricultural soil can be better understood.

3.1. Methods and Materials

3.1.1. Soils Particles Collection

Soils used in these studies were obtained from top 0-5 cm (0-2 inch) at HAL (Haskell Agriculture Laboratory, Concord, NE, USA). The soil is a Nora silty clay loam (fine-silty, mixed, mesic Udic Haplustolls). The soil particle size distribution is 20% clay, 60% silt, 18% sand and 2.2% total organic carbon (TOC). The concentration of Fe^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+} in the soil are 21, 4.7, 2131, 318 mg kg^{-1}, respectively. The cation exchange capacity (CEC) is 13.9 m eq/100 g, and

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total pore volume is 0.0135 mL g^{-1}. The specific surface area of the original soil is 12. 61 m^2 g^{-1}. No residual testosterone was detected in the soil sample. The soil particles of five different size fractions were obtained from the HAL soil by using the mechanical stirring and repeat wet sieve washing methods. The detailed separation procedure is reported by Qi et al (2014) (*35*). The five soil fractions were classified as fine sand (0.425-0.075 mm), medium silt (0.075-0.045 mm), fine silt (0.045-0.002 mm), clay 1 (0.9-2 μm) and clay 2 (0.6-0.9 μm).

3.1.2. Chemicals and Solution

^{14}C-labeled testosterone was purchased from the American Radio Chemicals Society (St. Louis, MO, USA). Low activity stock solution (directly used in the experiment) was made by adding 1 μL of high activity (= 50 μCi) stock solution of ^{14}C-labeled testosterone to 10 mL deionized (DI) water (electrical resistivity = 18 MΩ cm). The DI water was generated through the Millipore Simplicity System (Thermo Fisher Scientific Inc. U.S.). Unlabeled testosterone stock solution (= 10 μg mL^{-1}) was made by dissolving 0.0010 g testosterone into 100 mL methanol. Various other chemical reagents such as $CaCl_2$ (96% purity), urea (99% purity), NaN_3 (99% purity) and HCl were purchased from Fisher Scientific Inc., whereas NaOH (98.9% purity) was purchased from J.T. Baker Inc. (Phillipsburg, N.J. USA). Humic acid (HA) (Catalog # 1S103H) and Fulvic acid (FA) (Catalog # 2S103F) were obtained from International Humic Substances Society (IHSS) (St. Paul, MN) without any additional pretreatment.

3.1.3. Sorption Kinetics

For testing sorption kinetics, soil particles (0.2 g) of each of five fractions were placed into each of two 15 mL glass centrifuge tubes (Corning Screw Thread Finish, Fisher Scientific). Then, 9.8 mL of DI water containing 0.01 M (1.1 g L^{-1}) $CaCl_2$ (for control of ion strength (*16*)) and 200 mg L^{-1} NaN_3 was added to each of the tubes. Previous studies showed the NaN_3 could be used to eliminate the microbial influence on the study of hormone sorption to soils and no influence had been reported (*36*, *37*). The corresponding volume of ^{14}C-labeled low activity stock solution and unlabeled testosterone stock solution were added to each of the tubes to reach the required hormone concentration. And two centrifuge tubes with the same situation of solution and soil without testosterone were treated as blank. The test tubes were sealed with Teflon-lined caps and rotated (top to bottom, 360°/5 s) for 5, 15, 30, 60, 90, 150, 300, 600, or 1440 minutes. At the end of each time, two dedicated tubes were taken randomly for centrifugation (Kendro Inc. Asheville, NC, USA) at 2000 rpm (1350 g) for 15 minutes. A sample containing 200 μL of the supernatant from the centrifuge tubes was pipetted into a 20 mL counting vial (Wheaton Millville, New Jersey, USA) with 5 mL cocktail (PerkinElmer, Waltham, MA, USA) to detect the radioactivity with 2500 TR Liquid Scintillation Counter (Packard, Downers Grove, IL, USA). The sorbed hormone (μg g^{-1}) was calculated by subtracting the hormone in the liquid (ng mL^{-1}) from the orginal

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mass of hormones. The sorption process can be treated as a first-order reaction for the first 2.5 hours, and the sorption coefficient k (h^{-1}) can be calculated as (*16*):

$$\ln(C) = \ln(C_0) - kt \quad (1)$$

where C = aqueous testosterone concentration (ng mL^{-1}); C_0 = original concentration in aqueous phase (ng mL^{-1}); t = time (h).

3.1.4. Sorption Isotherm

Most of the test procedures were the same as sorption kinetics except that the initial hormone concentrations were different, and 24 h reaction time were used to reach equilibrium at the temperature of 4, 23, 35 °C. Seven different initial concentrations were chosen for the five soil particle size fractions. Also two centrifuge tubes with the same situation of solution and soil without testosterone were treated as blank. These concentrations were selected as per reported field concentrations of testosterone (*7*, *14*). The 24-h equilibrium time was selected as per sorption kinetics results and previous literature reports (*16*). The linear, Freundlich and Langmuir models were used to fit sorption data:

$$\text{Linear} \qquad S = K_d C \quad (2)$$

$$\text{Freundlich} \qquad S = K_f C^n \quad (3)$$

$$\text{Langmuir} \qquad S = \frac{q_m C}{1/b + C} \quad (4)$$

where S = concentration of testosterone adsorbed on the solid phase (μg g^{-1}); C = equilibrium aqueous phase concentration (ng mL^{-1}); K_d and K_f are the linear (L kg^{-1}) and Freundlich sorption coefficient ($\mu g^{1-n} L^n kg^{-1}$), respectively; n is a measure of sorption isotherm linearity (unitless); q_m = maximum sorption capacity (μg g^{-1}) and b = sorption equilibrium constant (mL μg^{-1}). Temperature effect on ΔG^0, ΔH^0 and ΔS^0 values are calculated with:

$$\Delta G^0 = -RT \ln K_d \quad (5)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (6)$$

where ΔG^0(kJ·mol^{-1}), ΔH^0 (kJ·mol^{-1}) and ΔS^0 (kJ·mol^{-1}) are Gibbs free energy change, enthalpy change and entropy change, respectively, in the sorption process; T = temperature (°K); and R = ideal gas constant ($J \cdot K^{-1} mol^{-1}$).

3.1.5. Desorption

All of the desorption tests were conducted immediately after the sorption tests. First, the total weight of each dry soil particle sample and centrifuge tube was measured and designated as M_1 (g). After the sorption equilibrium was reached

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i.e. after 24 h, the tubes were centrifuged, and the supernatant of each test tube was decanted. The total weight of the tube and the wet soil sample were measured as M_2 (g). Then, 10 mL of background solution without testosterone (i.e., DI water containing 0.01 M $CaCl_2$ and 200 mg L^{-1} NaN_3) was added to the tube, which was sealed and rotated (top to bottom, 360°/5 s) for 24 h in all of the desorption tests. The tubes were then centrifuged at 2000 rpm (1350 g) for 15 minutes, and hormones in the liquid phase were measured. These steps together are called one desorption cycle. In order to guarantee all the desorbable testosterone to desorb, 4 desorption cycles were conducted. The percentage of testosterone desorbed from the soil particles was calculated for each cycle with Eq. 7:

$$P_{desorb}(\%) = \left\{\frac{\left[C_B \times \left(10\ mL + \frac{M_2 - M_1}{\rho}\right)\right] - \left[C_A \times \left(\frac{M_2 - M_1}{\rho}\right)\right]}{[C_o - C_A] \times 10\ mL}\right\} \times 100\% \qquad (7)$$

where C_B = concentration of testosterone in the aqueous phase after desorption (ng mL^{-1}); C_A = equilibrium aqueous concentration after sorption (ng mL^{-1}); C_0 = original concentration of testosterone in the aqueous phase before sorption. M_1 = total weight of soil particle sample and tube (g), and M_2 = total weight of soil particle sample and tube together with the residual solution left after supernatant being decanted (g); and ρ = density of residual solution (= 1 g mL^{-1}). Similarly, for desorption kinetics, the corresponding centrifuge tubes were taken out and tested at different time intervals.

3.1.6. Control of Test/Environmental Conditions

The rotator (Model # C415110, Thermo Scientific Inc.) with centrifuge tubes was put in low temperature incubator (Model #307, Fisher Scientific Inc.) at 4 °C and Isotemp Oven (Model # 516G, Fisher Scientific Inc.) to maintain the temperature of 23 and 35 °C. Ionic strength was controlled by adding $CaCl_2$ (0.1 M) stock solution to reach a concentration from 0.001 to 0.07 M. The desired pH values were achieved by adding HCl or NaOH stock solution (0.01 M). Corresponding volume of HA stock solution (10 g L^{-1}) were added in the tubes to achieve the desired concentration. While urea was used for studying the hydrogen bonding contribution during the sorption of testosterone to soil. This was controlled by adding different volumes of urea solution to reach 0-2.0 M.

3.1.7. Experiment Setup for Rainfall Simulation Texts

As shown in Figure 1, the soil slab (14” x 14”) is placed into a container. Two glass plates (no sorption to hormones) are inserted into the soil for two test areas (each 8” x 3”); two PVC pipes with a 3-inch wide slot that link the glass plates to collect runoff from each of the two test areas. To collect leachate, two Teflon trays (each 8” x 3” x 1”) connected with flexible pipes are inserted into the soil at the bottom. The system consists of a bucket (11.5” diameter and 11.8” height), a peristaltic pump, pipes, and water distribution devices set up 7-ft above the soil

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slab reactor. Big resistance distribution system is used to distribute artificial rain to the soil surface. Apparently, the reactor can be used to collect runoff and leachate samples simultaneously. The rainfall intensity was controlled under 2, 10, 50 years return frequency corresponding to Area 2 [i=A/(t+B), where A, B are constants that depend on the return period and climatic factors, t=duration] (*38*). The runoff and leachate samples were collected every 30 mins intervals for 2 hours. The properties (e.g., rainwater volume, TS, particle size distribution) of the runoff and leachate were analyzed.

Figure 1. Photo of the soil slab reactor (left) and the rainfall simulation system (right) that was used for conducting the experiments.

3.1.8. Analytical Methods

The total organic carbon (TOC) and soil composition (e.g., the percentage weight of the sand, silt and clay) were analyzed by the Mid-west laboratory (Omaha, NE, USA) (Tru Spec CN628, #622-000-100, LECO Inc.). The Brunauer-Emmett-Teller (BET) surface area of soil particles of the five different size fractions was tested by a commercial laboratory (Micromeritics analytical services, Norcross, GA, USA) with multipoint surface area using Nitrogen gas (ISO 9277). The particle size distribution was analyzed by Zetasizer (Nano ZS90, Malvern, UK) operating with a He-Ne laser at a wavelength of 532 nm. Scanning Electron Microscope (SEM) (Hitachi S-4700, Hitachi, Tokyo, Japan) analysis of clays 1 and 2 were performed at 10 kV. The surface properties of the particles in the five different size fractions before and after sorption tests were analyzed with the FTIR (Fourier Transform Infrared Spectrometry)-AIR method (Thermo Nicolet Avatar 380 FT-IR Spectrometer, UNL, Lincoln, USA). Furthermore, in order to eliminate the influence of testosterone sorbed to Teflon caps and glass tubes, centrifuge tubes with the same amount of testosterone and solution without soil particles were used as control. All the radioactivity detections were based on two blank samples (without hormone) of centrifuge tubes that had the same situation (e.g., $CaCl_2$ and NaN_3 concentration, soil-water ratio) as testing tubes. A microscope (LR33310Mod E, Fisher Scientific, MicroMaster Inc.) was used to

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check the diameter size of fine sand, medium and fine silt. The particle diameter size of clays 1 and 2 were determined and compared by both SEM (Hitachi S-4700) in 10 kV and Zetasizer (Nano ZS90, Malvern, UK) operating with a He-Ne laser at a wavelength of 532 nm.

3.2. Results and Discussion

3.2.1. Characterization of Particle Size Fractions

The diameter size range was determined as 0.07-0.12 mm for sand, 0.04-0.07 mm for medium silt, 0.005-0.04 mm for fine silt, 0.9-2 µm for clay 1 and 0.6-0.9 µm for clay 2 (Figure 2).

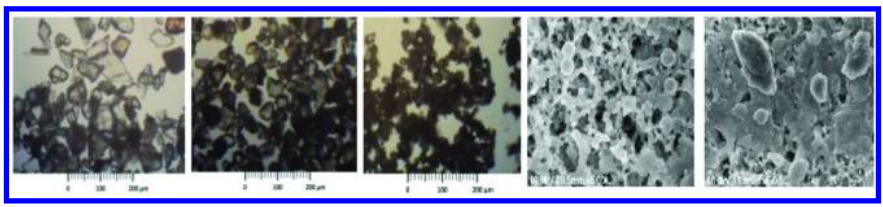

Figure 2. Microscopic and SEM analysis showing particle sizes (in µm) of separated sand (d = 70-120), medium silt (d = 40-70), fine silt (d = 2-40), clays 1 (0.9-2) and 2 (0.6-0.9) (from left to right) (35).

Table 2. Characteristics of soil particles of five different size fractions collected from HAL 0-5 cm top soil

Particles	*Size range (mm)*	*Weight (%)*	*TOC (%)*	*BET surface area (m^2 g^{-1})*
Sand	0.425-0.075	18	0.12	2.23
Medium Silt	0.075-0.045	60[a]	0.09	1.02
Fine Silt	0.045-0.002		1.72	23.30
Clay 1	0.002-0.0009	20[a]	1.97	52.21
Clay 2	0.0009-0.0006		1.97	77.66

[a] The total silt percentage was 60% and the total clay percentage was 20% as per the result analyzed by a commercial laboratory.

Table 2 shows the properties of the soil particles of five fractions. Clay 2 has the largest BET surface area, followed by clay 1 and fine silt (Table 2). Medium silt and sand have the lowest BET surface area, indicating that smaller particles contain more binding sites than bigger particles with the same weight. The TOC of the smaller particles is higher than that of bigger ones (Table 2). While the TOC of fractionated particles are all less than that of the bulk soils (2.2%).

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3.2.2. Sorption and Desorption Kinetics

For sorption kinetics study, the sorbed concentration increased from 0 to 2.5 h for all the particle size fractions. We analyzed the results with Eq. 1, the sorption kinetic coefficients (k in Eq. 1) ranged from 0.27 for sand to 2.04 h^{-1} for clays (Table 3). The concentration of testosterone in the liquid phase appeared to increase slightly after 2.5 hours, indicating desorption of testosterone back to the aqueous phase, which is similar as Lai et al. (2000), who reported that the estrogens were first sorbed rapidly and then started to desorb from the soil particles (*10*). The results are consistent with the previous study reporting that the adsorption of testosterone ranged from 0.08 to 0.64 h^{-1} for five different loam soils (*16*). Also the sorption rates are determined by the particle size, the results show that the time for testosterone to reach the maximum sorption capacity is < 2.5 h for sand, medium silt, fine silt, and < 30 min for clays 1 and 2. All the sorption reactions for these particles of different size fractions are much faster than that of bulk soils that were used in our previous study (*39*), which shows that testosterone sorbed onto HAL soils at 3 depths reached equilibrium between 8-12 h. It was reported that it required 5 h for the testosterone sorbed onto loam soils (*16*). While for soils of Chelsea and Pahokee peat soils used by Kim et al. (*40*), 336-504 h is needed to reach equilibrium. Furthermore, sorption of testosterone appears to be fast in the first few minutes for the particles of the five fractions, which indicates that most sorption will occur once the hormone molecules touch the soil particles. Kim et al. (2007) reported that the sorption of testosterone and androstenedione on soils were very rapid within the first hour of reaction and eventually became slower to approach equilibrium (*40*). The results provide the evidence that the movement of testosterone will be retarded due to the rapid sorption to soil particles particularly when no soil particles are taken away by rainwater.

Table 3. The sorption kinetic coefficient k (h^{-1}) of the first order reaction at different initial testosterone concentrations[a]

Initial concentration (ng mL^{-1})	*100*	*10*
Sand	0.27	0.35
Medium silt	0.29	0.50
Fine silt	0.31	0.54
Clay 1	NA[b]	2.04
Clay 2	NA[b]	1.95

[a] Calculation based on the data of the first 2.5 hours for sand, medium silt, fine silt and 0.5 h for clays 1 and 2 (as per Eq. 1). [b] NA = not available.

Figure 3 shows that it takes ~20 h for clays 1 and 2 and 6 h for sand, medium silt and fine silt to reach desorption equilibrium, indicating that the testosterone

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desorption from smaller particles is much more difficult than bigger ones. Also, sorption of hormones by smaller particles is significantly faster than the desorption processes.

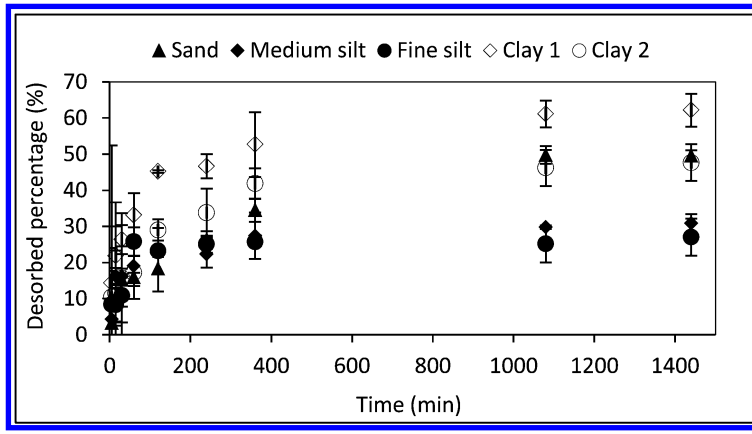

Figure 3. Desorption kinetics for five soil particles in first cycle. Error bars show the standard deviations of duplicate results.

3.2.3. Sorption Isotherm

Sorption isotherms show the sorption affinity and thermodynamic properties of hormones for particles of different size fractions. We fitted the data with three different sorption models (Figure 4). Based on the R^2 values, at 23 °C, the Freundlich model fits the data better for sand, medium silt and fine silt.. The Freundlich sorption coefficient K_f ranges from 80.02 to 87.36 $\mu g^{1-n}L^n\ kg^{-1}$ for sand to silts. At the initial concentration of 0.04–200 ng mL^{-1}, the K_d value is 42.33 L kg^{-1} for sand, 55.12 for medium silt and 80.32 L kg^{-1} for fine silt, which indicate the fine silt has higher sorption affinity than medium silt and sand. For clays 1 and 2, at the initial concentration of 0.04–50 ng mL^{-1}, the K_d values are 329.46 and 803.23 L kg^{-1}, respectively, which are much larger than that of sand and silts. It can be observed from the above findings that the sorption affinity of clay 2 is more than twice the sorption affinity of clay 1.

Although the FTIR results show that clay 1 and 2 have same surface functional groups, higher sorption affinity of clay 2 could be due its relatively higher surface area (Table 2) than that of clay 1. The sorption affinity can be ranked according to particle size distribution as clay 2 > clay 1 > fine silt > medium silt > sand. Other studies show that sandy loam (high sand content) has a relatively lower sorption capacity on hormones than that of silt and clay loam (*41*); fine particle fractions have a higher sorption capacity to hydrophobic chemicals than that of coarse fractions (*42*). The results indicate that smaller particles have higher potential to facilitate the transport of hormones in soil environments.

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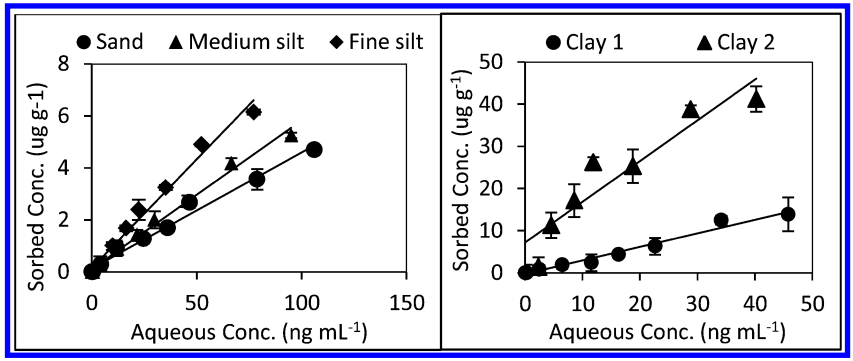

Figure 4. Sorption isotherms of testosterone on sand, medium silt and fine silt with the initial testosterone concentration being 10–200 ng mL^{-1} (left) and on clays 1 and 2 with the initial testosterone concentration being 3–50 ng mL^{-1} (right).

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3.2.4. Desorption

Figure 5 shows that most of the desorption are completed after the third cycle. Sand and medium silt exhibit similar desorption properties, which correleates weel with the FTIR results that revealed that both have similar surface properties.. After 4 cycles of desorption under the initial concentration of 100 ng mL^{-1}, sand desorbs 63.2% while fine silt just desorbs 33.5% of the total sorbed testosterone (Figure 5). The un-desorbed percentage (the irreversible sorption) of testosterone is 36.8% for sand, 38.3% for medium silt, 55.9% for fine silt, 65.4% for clay 1, and 66.5% for clay 2. Thus, the sorbed testosterone will not completely release from the soil particles even after 4 desorption cycles especially for clay particles. Therefore, the results showed that hysteretic phenomena existed for all these particles. Compared to bigger particles, smaller particles have higher desorption hysteresis and hold more irreversible testosterone.

3.2.5. Effect of Organic Matter and pH

HA in the concentration range of 20−500 mg L^{-1} was added to the soil/water system. The sorbed concentration of HA increased from 0.39 to 0.63 ng g^{-1} for sand, and 1.3 to 1.41 ng g^{-1} and 1.09 to 1.32 ng g^{-1} for clay and silt, respectively (Figure 6). We can see the sorption capacity would be increased by the contribution of sorbed HA, which has a relatively higher sorption affinity to testosterone than soil particles. Considering our soil particles were all very clean and thus, did not have funtional organic matter (on the soil surface) to facilitate pollutant sorption onto the soil particles, our tests about HA effects demonstrate that sorption capacity of soil particles would increase if some natural organic matter attaches the particles during their transport.

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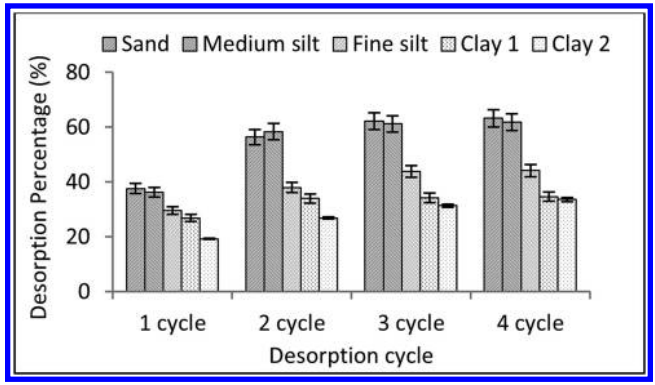

Figure 5. Accumulated desorption percentage of testosterone from particles of five size fractions at the initial testosterone concentration of 100 ng mL^{-1} of four cycles. Error bars show the standard deviations of duplicate experiments.

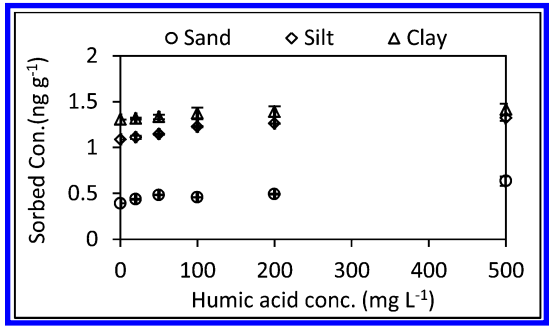

Figure 6. Effects of HA (20−500 mg L^{-1}) on the sorption of testosterone onto sand, silt and clay at the initial concentration of 40 ng L^{-1} at 23 °C. Error bar shows the standard deviation of two results.

The FTIR results indicate that the hydroxyl group (peak 2550) and carboxyl group (peak 2980) were showed up after the HA adsorbed on these soil particles. It was reported that hydroxyl group and carboxyl group were observed and proved to be great contributions to the sorption of E3 (estriol) onto activated charcoal (*43*). Due to the similar structure of E3 and testosterone (*41*), we can conclude that these two functional groups contributed by sorbed HA may enhance the sortion of testosterone on these soil particles.

For pH, the sorption capacity of sand, silt and clays decreased from 0.49 to 0.33 ng g^{-1} and that are 1.15 to 1.05 ng g^{-1} and 1.34 to 1.20 ng g^{-1}, respectively (Figure 7 left). Obviously, the sorption capacity of the particles of the three fractions would decrease with an increase in pH (Figure 7 left). The influence of pH on desorption of testosterone (four cycles) was also determined at the initial concentration of 40 ng L^{-1}. Higher pH will greatly facilitate desorption of testosterone from the soil particles, especially for sand.

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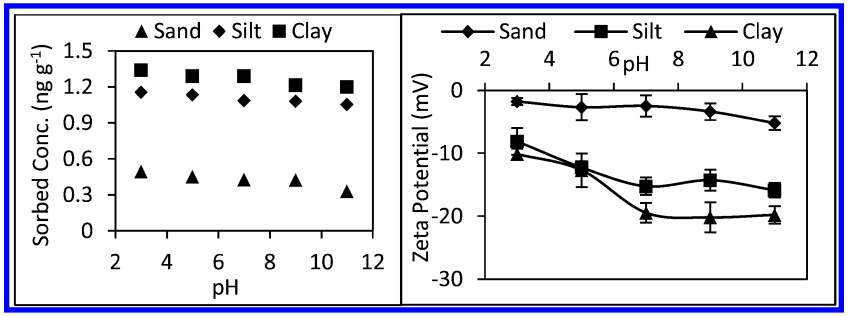

Figure 7. Effects of pH on the sorption of testosterone onto sand, silt and clay (left) and the zeta potential of the systems at 23 °C (right) (Error bar shows the standard deviation of three results). The initial concentration of testosterone = 40 ng L-1, and pH (3 to 11) was adjusted by HCl or NaOH solution.

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The zeta potentials decreased with an increase in pH for these particles, which coincides with the sorption capacity (Figure 7 right). These results indicate that the contribution of electrostatic attraction to hormone's sorption capacity will be affected by surface charge which is changed with solution pH. Therefore, the static attraction force may play an important role in sorption of testosterone to sand particles. The static attraction force will vary with change in pHs affecting the sorption of testosterone. For clay particles, the electrostatic attraction force is not a main factor on the sorption of testosterone because the change in surface charge marginally affects the sorption capacity. Pan et al. (2007) reported that sorption of dissolved HA (DHA) on polyaromatic hydrocarbon (PAH) increased as pH decreased (*42*); at alkaline and neutral conditions (pH 7 and 11), carboxyl and hydroxyl functional groups of organic matter are deprotonated, and the molecules are overall negatively charged.

3.2.6. Effect of Urea

Urea (CH_4N_2O) was used as a hydrogen bonding breaker to study the contribution of hydrogen bonding on the sorption of testosterone onto soil particles at the initial concentration of 10 to 200 ng L^{-1}. Urea was used as a hydrogen bonding breaker to reduce the adsorption of CMC (Carboxymethyl Cellulose) on talc (*43*). Camberato et al. (2001) reported that urea would not sorbed by soil before transformed to ammonium or nitrate (*44*), and therefore, urea would not facilitate the sorption of testosterone onto soil particles by itself. It was reported that hydrogen bonding played an important role on androgen sorption to soils and sediments (*37*). In this study, sorption isotherms of testosterone on three soil particle fractions under four different urea conditions (0−2.0 M) indicated that hydrogen bonding contributed to testosterone sorption onto clay (~10%) and silt (~5%), but not on sand (~0%) (Figure 8).

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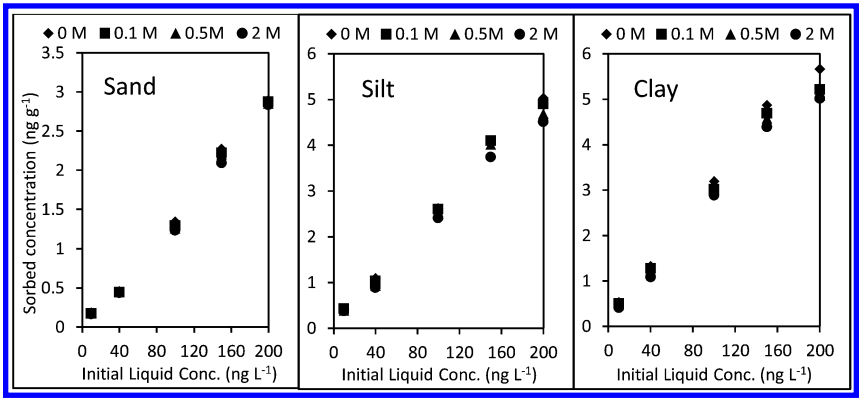

Figure 8. Effects of urea (0 M to 2 M) on sorption of testosterone (at initial concentrations = 10−200 ng L^{-1}) onto sand, silt and clay at 23 °C.

3.2.7. Sorption and Desorption Mechanisms

FTIR were conducted to see the changes in surface properties before and after sorption experiments. BET surface area measurements were also performed for all of three soil particle size fractions. As shown in Table 2, clay has the highest TOC percentage and BET surface area among the particle fractions investigated. In light of the above analyses, the sorption mechanisms of testosterone on the soil particles of three size fractions are:

- ~10% and ~5% of hydrogen bonding contribute the sorption of testosterone to clay and silt, respectively, while hydrogen bonding effects was minimal for sand;
- Electrostatic attraction force play an important role during sorption-desorption of testosterone with sand, while the irreversible function group binding sites are main factors for silt and clay particles; and
- Organic matter in soil particles will greatly facilitate the sorption of testosterone on the soil particles but impede the release (desorption) processes.

3.2.8. TS and Particle Size Distributions in Runoff and Leachate

The results indicate that it takes longer time for leachate generation than surface runoff generation (Table 4). For example, for 2 years return period, it takes 21 minutes to generate the leachate samples, and 7 minutes for runoff. The TS distribution results show (Figure 9) runoff carries much more soil particles than leachate does. Higher rainfall intensity takes more soil particles for both runoff and leachate. During the tests, we found that the mass of particles taken away by rainwater increased at the beginning then decreased when the rills formed.

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Table 4. Time for generation of leachate and runoff under three rainfall intensities

Return Frequency (year)	*Time started (min)*	
	Runoff	*Leachate*
2	7	21
10	4	12
50	2	10

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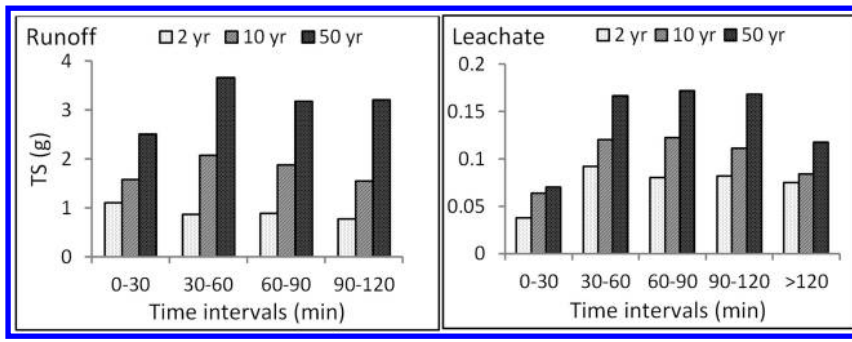

Figure 9. Total solid mass taken away by runoff (left) and leachate (right) of every 30 min intervals under 2, 10 and 50 years return frequency.

The results show that bigger soil particles would be detached by higher rainfall intensity and there exisits an inverse relationship between the diameter and the rainfall time (Figure 10). The runoff can take soil particle less than 2 μm (clay) in diameter much easier which leading to a higher potential for particle facilitated transport of hormone to surface water due to their high sorption affinity and low desorption potential. However, soil particles bigger than 50 nm did not exist in the leachate sample even under the rainfall intensity of 50 years return frequency (data not show), which indicated the transport of hormone through particle-sorbed mechanism to ground water was impossible if no preferential pathways existed. This conclusion is consistent with a column study which showed that the preferential flow plays an important role during the transport of hormones (testosterone and 17β-estradiol) in ground water (*45*).

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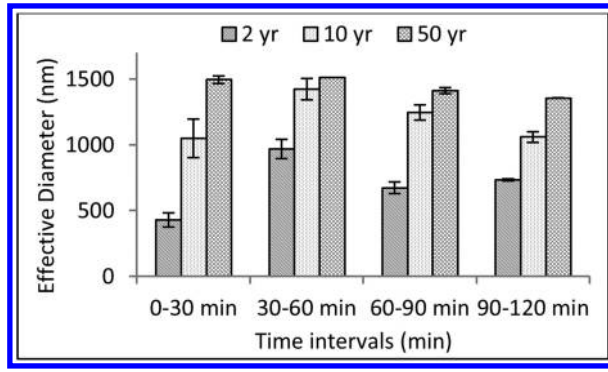

Figure 10. Effective diameter of runoff sample as a function of time intervals under 2, 10 and 50 years return frequency. Error bar shows the standard deviation of three results. Results were obtained by particle size analyzer (NanoBrook 90 Plus/BI-MAS, Brookhaven Instrument Corp).

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3.3. Limitations and Future Directions

It should be pointed out that studies described in Sections 3.1 and 3.2 have some limitations. In general, there are two approaches to conduct the tests to evaluate the size fraction-based contribution of soil particles on sorption-desorption of hormones: a) collecting soil particles into different fractions first and then conducting the tests−first approach; and b) conducting the tests with the 'whole' soil and then separating them into different fractions−second approach. In the first approach, we can obtain the basic properties of the soil particle size fraction directly and easily. However, the problem with first apporach is that: a) it is difficult to avoid changes in the properties of soil particles (e.g., surface charge, TOC, aggregation); and b) it may eliminate the sorption competitions between soil particles of different size fractions which otherwise exist if the "bulk soil" (real world situation) is used. On the other hand, in the second approach we can avoid the problems associated with the first approach, and thus, should be the future direction for research.

Because researchers may use different methods to fractionate soil particles, they may obtain the soil particles with either a fully-dispersed or non-dispersed size distribution (Figure 11). In the past, researchers often used either a) sodium hexametaphosphate $(NaPO_3)_6$, b) high-speed stirring (both belong to the standard method for grain-size analysis, ASTM D 422) (*46*), c) ultrasonic technology (*47*), or d) wet sieve/sedimentation method (*35*, *46*) to fractionate soil particles, and they only obtain soil particles with a fully-dispersed size distribution. Methods a) to d) are important to determine the ultimate texture of the soil with respect to certain chemical or physical properties. However, soil particles dispersed by real world processes, such as soil erosion and sediment transport by overland flow (*19*, *48*, *49*), usually belong to non-dispersed soil particles due to the mild dispersing intensity of the processes.

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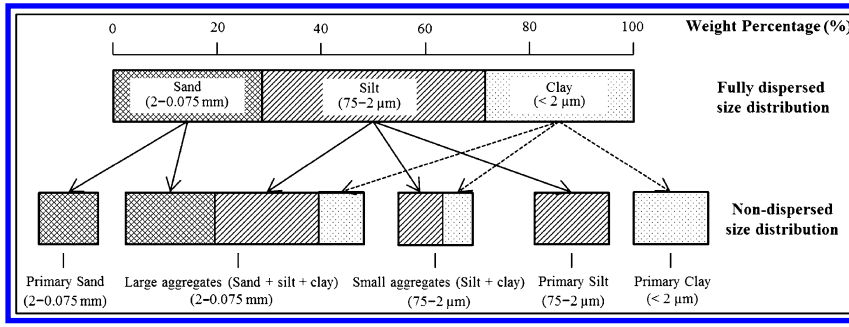

Figure 11. Relationship between the fully dispersed and non-dispersed soil particle size distribution (19, 49).

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Accordingly, to fully understand the basic chemical properties (e.g., TOC, specific surface area, surface charge, and sorption mechanisms) of the original soil, fully-dispersed soil particles should be used as test material (e.g., the use of the soil texture triangle is widely accepted). However, when studying the fate and transport of micro-pollutants in soils, non-dispersed soil particle size distribution may be more important because natural dispersing processes (e.g., rainfall events, irrigation) are not as strong as that of methods a) to d) mentioned above. It has been reported that many soil aggregates are stable under the forces of erosion (*50*). Therefore, a new framework concerning the effects and roles of non-dispersed soil particles on the fate and transport of micro-pollutants should be set up with the corresponding procedures being standardized.

4. Summary

Animal feedlot operations commonly use growth-promoting hormone treatments to enhance animal production. Because the majority of the generated animal waste is applied to agricultural land, there is an increased risk for the natural and synthetic hormones in such waste to enter the environment through surface and ground water. Hormones are known to have toxic effects on certain organisms, and at present, little information is known regarding the fate and transport of hormones in soil–that is, how they move through and are altered in the soil. Laboratory studies indicate that hormones are unlikely to experience significant soil transport, yet field studies frequently detect hormones in ground and surface water. This apparent contradiction may be because different soil particles are involved in these laboratory or field studies.

The results of our studies indicate that small particles have higher sorption affinity and capacity, lower desorption potential and easier mobility through runoff, which provides indirect evidence on the colloids (clay)-facilitated transport of hormones in soil environment. The rainfall simulation tests indicate that particle-sorbed hormone might be the main mechanism for hormones transport to surface water through runoff, while preferential flow should be the major mechanism for hormone transport to groundwater due to the absence of soil particle in leachate.

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Conventional studies on hormone fate and transport use either "bulk" soil (i.e., non-fractionated soil) or fractionated soil particles (with a fully dispersed size distribution) as test material, which may not exist in nature. Natural dispersing processes (e.g., rainfall events, irrigation) may only generate soil particles with a a non-dispersed size distributions. Therefore, a new framework concerning the effects and roles of non-dispersed soil particles on the fate and transport of micro-pollutants should be set up with the corresponding procedures being standardized.

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Chapter 5

Modeling Fate and Transport of Emerging Micro-Pollutants in the Environment

Kurwadkar Sudarshan,* Garrett Struckhoff, Phoolendra Mishra, Nidhi Modha, Emilio Murga, Bruna Amaral, Glenda Machado, and Natalia Gomes

Department of Civil and Environmental Engineering, California State University-Fullerton, 800 N. State Collge Blvd., Fullerton, California 92831, United States
***E-mail: skurwadkar@fullerton.edu**

In recent years, there has been a growing concern regarding the occurrence of micro-pollutants in the environment. Emerging micro-pollutants (EMs) are the ones that have been typically detected at very low concentrations, and are currently not being monitored or regulated. The EMs may or may not be of recent origin, but their environmental detection and ecological implications are only now becoming known. This chapter deals with the mobility of sulfamethazine (SMN) and hydrolysis of three neonicotinoids – dinotefuran (DNT), imidacloprid (IMD) and thiamethoxam (THM) in the environment. Transport of SMN in the sub-surface environment was modeled using a modified one-dimensional advection dispersion equation and showed that the mobility is impacted by the speciation of SMN and as such it may be highly mobile in alkaline soils compared to acidic soils. A classical hydrolysis model suggests that DNT, IMD and THM, all undergo hydrolysis. However, IMD hydrolyzes at a much faster rate suggesting that DNT and THM can persist longer in the environment compared to IMD. The mobility and persistence of these EMs may pose adverse ecological risk.

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1. Introduction

Two of the emerging micro-pollutants (EMs), pharmaceuticals and insecticides, are the focus of this chapter. Particularly, the chapter deals with the transport of sulfamethazine (SMN) – a commonly used antimicrobial in animal agriculture and the fate of neonicotinoid insecticides: Dinotefuran (DNT), imidacloprid (IMD) and thiamethoxam (THM) in the post- application scenario.

The use of antimicrobials in livestock has grown enormously over the past few decades. In 1963, approximately 1 million kg of antimicrobials per year were used in animal feed; within two decades it nearly tripled (*1*). At present, nearly 80% of all antibiotics (estimated 13 million kg annual production) produced in the USA annually is currently used in animal agriculture mostly for sub-therapeutic purposes such as growth promotion (*2*). The use of antimicrobials in animal agriculture is so pervasive that the feed itself comes pre-mixed with antibiotics (*3*). For example, monensin antibiotic is an integral part of feed in the majority of dairy operations. The massive use of antimicrobials in livestock operations is a major concern because the un-metabolized residues can potentially perturb the ecology and breed antimicrobial-resistant micro-organisms (*4*). Now it is demonstrated that, upon administration, the antibiotics are not completely metabolized by the animals and up to 90% of the antibiotic gets excreted back into the environment within 1-2 days (*4*, *5*). The antibiotics could be excreted as parent compound (unmetabolized), or in a partially metabolized form such as an oxidation/hydrolysis byproduct of the parent compound (*6*). In some cases, the excreted conjugates may revert back to the parent compound. For example, N-4-acetylated sulfamethazine may revert back to sulfamethazine (SMN) in liquid manure (*7*, *8*). Understanding the mechanism of transport of antimicrobials in the environment and modeling it using a modified one-dimensional advection dispersion equation (ADE) will be a major focus of this chapter.

Another EM being considered here is the neonicotinoid class of insecticides. For many years, classical nicotinoids have been used for insect management. Neonicotinoids differ from classical nicotinoids in terms of mode of action and structure, and are classified into 1st generation (sub class: chloronicotinyl) and 2nd generation (sub class: thianicotinyl) (*9*). According to Bayer CropScience, IMD (i.e. a 1st generation neonicotinoid) was the first commercially available neonicotinoid introduced in the US market in 1994 and sold under several commercial formulations; such as Gaucho® (seed treatment), Admire® (soil application), Provado® (foliar application), Merit® (turf and ornamental use) and Premise® (termite control). Other neonicotinoids: THM (a 2nd generation neonicotinoid, sold under the trade names Actara®, Cruiser®, Flagship® and Platinum®) and DNT (a third generation neonicotinoid and sold under the trade name Safari® and Venom®) were introduced in the US market in 2001 and 2005, respectively (*10*).

Neonicotinoids are among the most widely-used class of insecticides in a majority of agriculture operations for controlling sucking insects, soil insects, termites, and for protection against Pierces' disease (*11–13*). In 2004, the worldwide annual usage of neonicotinoids accounted for approximately 11% –

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15% of the total insecticide market with sales of nearly one billion dollars (*13*) (Fig. 1).

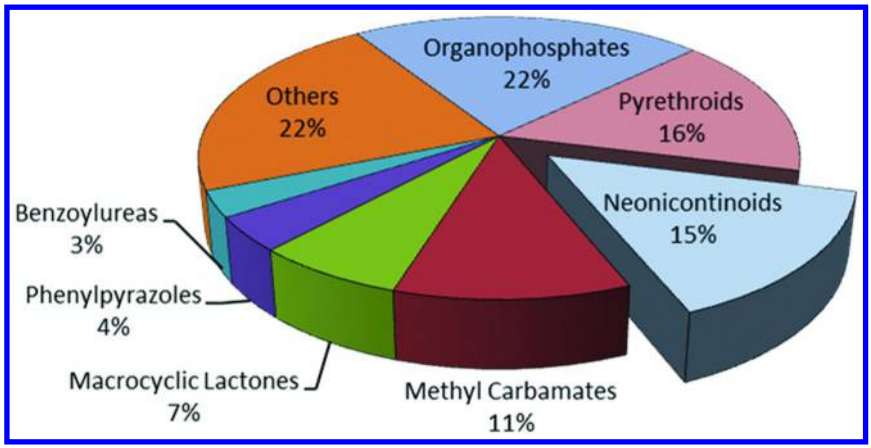

Figure 1. Worldwide annual use of different classes of insecticides

The use of neonicotinoids continued to rise, and in 2006, worldwide annual usage accounted for approximately 17% of the global insecticide market, with sales of nearly 1.6 billion dollars (*14*). This class of insecticide has gained widespread acceptance as a key component of integrated pest management programs because of unique properties, such as broad spectrum activity, low application rate, systemic and translaminar activity, pronounced residual activity and unique mode of action (*15*, *16*).

Although 2nd and 3rd generations of neonicotinoids offered greater protection and have helped reduce the populations of vector species and Pierces' disease outbreaks in vineyards and in other agriculture applications, their persistence in the environment and subsequent ecological consequences have largely been ignored. Neonicotinoids have been shown to exhibit high toxicity to honeybees (*17*). In addition to toxicity, neonicotinoid intoxication has also been implicated in inhibiting the homing instinct of honeybees (*18*). In response to reports such as these, the European Commision banned the use of three neonicotinoids (clothianidin, imidacloprid and thiametoxam) for two years, beginning December 2013 (*19*, *20*). Evidence is also arising that neonicotinoids may affect human fetal brain development (*21*). Recent studies have shown that these insecticides can affect mammalian fetuses during pregnancy via nAChR receptors, the same nueral receptors that are stimulated by nicotine (*22*). This implies that, following parent exposure through ingestion of water contaminated with neonicotinoics, human fetuses may experience similar developmental defects as those exposed to nicotine. Considering potential adverse ecological impact and toxicity concern to non-target species, it is imperative that we understand the fate of neonicotinoids in the post-application scenario. The environmental fate of the 1st generation neonicotinoid IMD was extensively studied. However, not much is known about the environmental fate of the 2nd and 3rd generations of neonioctinoid THM and

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DNT. This chapter explores one of the abiotic fate mechanism – hydrolysis of DNT, IMD and THM in the environment. A classical hydrolysis kinetic model is used for investigating persistence of these insecticides.

2. Transport of Sulfonamides in Soil Environments

2.1. Sulfamethazine Speciation Behavior

Sulfonamides (SMN belongs to this class) are odorless, white or slightly colored N-substituted derivatives of sulfanilamide. Different variants of sulfonamides can be obtained by replacing hydrogen from the sulfanilamide moiety. General speciation equilibria and molecular structures of sulfonamide and SMN are shown in Figure 2. SMN is characterized by two acid dissociation constants (pKa's): $pKa_1 = 2.03$ cationic↔neutral, indicates the protonation of an NH_3^+ group at lower pH (pH 2-3), while $pKa_2 = 7.49$ neutral↔anionic, indicates deprotonation of the $-SO_2NH-$ group at higher pH (pH 5-11) (*5*, *23*, *24*). The pKa of the SMN indicates that it can occur in either neutral form or in anionic form if pH>>pKa (*5*). These pKa values of SMN are in the range of pH observed in most soils, suggesting that SMN could be subjected to protonation and deprotonation in soil solution, hence, speciation largely determines the mobility of SMN in the sub-surface environment (*6*).

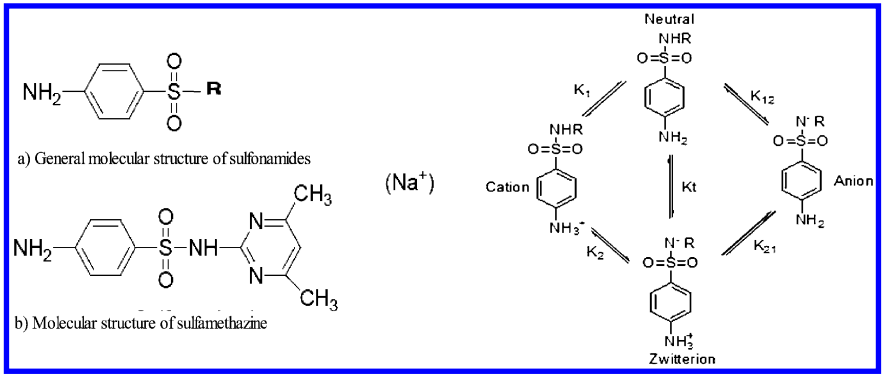

Figure 2. Molecular structure of sulfonamide and SMN (left) and a general speciation equilibria of sulfonamides (right).

2.2. Speciation and Sorption of SMN

The speciating nature of SMN significantly affects its ability to bind to soil surfaces, form complexes with metal ions, or undergo biodegradation or photolysis (*24*). SMN is an ionizing compound (Fig. 2) and as such pH plays a very important role in its sorption. Sorption is a function of both sorbate and sorbent characteristics. Sorption of SMN is dependent on its aqueous solubility. The decrease in sorption coefficient at high pH value was observed to be more pronounced for SMN, mainly because of the rapid increase in solubility of

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SMN at high pH value (*25*). The sorption and transport phenomenon should be understood with reference to the isoelectric point (pKa_1+pKa_2)/2, where SMN has the lowest solubility due to the predominance of the undissociated molecular species (*26*). Higher water solubility (150 mg/100 mL at 29ºC (*25*)) of SMN and lower octanol-water partitioning coefficient (0.89 (*25*)) indicate that SMN has lower potential for sorption in soil and greater affinity for mobility. For simulating the speciation effect on sorption of SMN, sorption coefficients developed for Laboratory Developed Activated Carbon (LDAC) and Powdered Activated Carbon (PAC) were used. These materials represent relatively high and moderate sorption potential with likelihood of representing a wide variety of soils. These sorption coefficient values were developed at pH levels 2, 5 and 8 so that sorption of predominantly cationic, neutral and anionic species can be studied. These values were then used for calculating the net sorption coefficient for simulating the mobility of SMN.

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2.3. Speciation Based Transport Model

To assess the mobility of SMN in saturated soil environments, one-dimensional ADE-reaction is adopted. The classical one-dimensional ADE for conservative solute in homogeneous porous media under steady state of water flow (*27*) can be written as:

$$\frac{\partial C}{\partial t} = D_x \frac{\partial^2 C}{\partial x^2} - V_x \frac{\partial C}{\partial x} \qquad \text{(equation 1)}$$

where C is the concentration of solute in liquid phase (mg/L), t is time (s), D_x is the hydrodynamic dispersion coefficient (m^2/s) and V_x is the average linear pore water velocity (m/s). Due to ion exchange, SMN will also adhere to soil particles and become immobile. This would result into the overall retardation of the transport process. Since this ionic exchange reaction equlibrates on a relatively smaller time-scale than advection-dispersion process, instantaneous kinectics can be adopted. Considering a linear adsorption isotherm (adsorbed phase concentration $S = k_{deff}C$), the modified one-dimensional ADE (*28*) with retardation factor can be expressed as:

$$R_f \frac{\partial C}{\partial t} = D_x \frac{\partial^2 C}{\partial x^2} - V_x \frac{\partial C}{\partial x} \qquad \text{(equation 2)}$$

Where,

$$R_f = 1 + \frac{\rho_b k_{deff}}{n_e} \qquad \text{(equation 3)}$$

Where,

R_f – Retardation factor indicating relative mobility of SMN with respect to average linear groundwater velocity

k_{deff} – pH dependent effective sorption coefficient (L/kg)

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ρ_b – Bulk density of the aquifer (g/cm^3)

n_e – Effective porosity of soil media (%)

Since SMN speciates, the retardation factor can be different for each of the speciated forms. The retardation factor directly depends on the sorption coefficient at neutral, cationic and anionic pH levels. The overall sorption of SMN was modeled using the weighted sorption coefficient approach. The rationale for choosing this approach is to account for the contribution of all speciated forms of SMN existing at any given pH value. The effective or weighted sorption coefficient k_{deff} is represented by (*29*):

$$k_{deff} = k_{d0}\alpha_0 + k_{d1}\alpha_1 + k_{d2}\alpha_2 \qquad \text{(equation 4)}$$

Where,

k_{d0}, k_{d1}, k_{d2}, and α_0, α_1, α_2 represent the individual sorption coefficients (k_d) and fractions (α) of SMN in cationic, neutral and anionic forms, respectively.

The fraction of speciated forms is dependant on the pKa of the compound and pH of the solution. The fractions of neutral, cationic and anionic forms can be represented as shown in (Fig. 3.

$$\alpha_0 = \frac{1}{1+10^{(pH-pK_1)}+10^{(2pH-pK_1-pK_2)}}$$

$$\alpha_1 = \frac{1}{1+10^{(pK_1-pH)}+10^{(pH-pK_2)}}$$

$$\alpha_2 = \frac{1}{1+10^{(pK_2-pH)}+10^{(pK_1+pK_2-2pH)}}$$

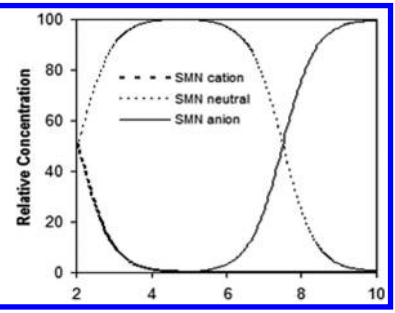

Figure 3. Fractions of speciated forms of SMN and speciation diagram of SMN.

Present research considers only an instantaneous pulse injection. The solution to equation (2) is obtained by assuming the pulse to be introduced at x =0, with zero background concentration. As the pulse moves downstream with velocity V_x in the +x direction, it spreads out due to hydrodynamic dispersion. The analytical solution for equation (2) for a pulse mode scenario can be written as (*28*):

$$C(x,t) = \frac{M}{n_e\sqrt{4\pi R_f D_x t}} e^{-\left(\frac{(R_f x - V_x t)^2}{4R_f D_x t}\right)} \qquad \text{(equation 5)}$$

Where,

M – Injected mass per unit cross-sectional area (mg/m^2)

x – Travel distance in (m)

C– Conc. of solute in liquid phase (mg/L)

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D_x – Dispersion coefficient (m^2/s)
V_x– Average linear groundwater velocity (m/s)
t – Time (s)

3. Hydrolysis Kinetics of Neonicotinoids

3.1. Hydrolysis of Neonicotinoids

With relatively high solubility and mobility, neonicotinoids are expected to reach the aqueous environment (*30*). As such, understanding hydrolysis behavior is important to better asses persistence of neonicotinoid and toxicity to non-target species (*31*). Hydrolysis kinetics help in better understanding the stability of neonicotinoids in aqueous environment. Hydrolysis is one of the major abiotic degradation mechanism which results in breaking of one or more bonds of organic compound by water with reaction products resulting in substitution of OH^-, H^+ or both groups from water. As such, the net hydrolysis rate would then be the contribution coming from each of these elements. The acid catalyzed rate constant (k_A) indicates the contribution from hydronium ion, the base catalyzed rate constant (k_B) contribution from the hydroxide ion and the neutral rate constant (k_N) contribution resulting from reaction of water with the organic compound. Hydrolysis rate constants for all three neonicotinoids at acidic, neutral and basic pHs were obtained after an extensive literature review.

3.2. Hydrolysis Kinetic Model Development

As discussed earlier, hydrolysis is a pH-dependent process. For example, at neutral pH it is facilitated by nucleophilic attack by H_2O while at the same time, it is also dependent on relative predominance of H^+ and OH^- ions for acid and base catalysis to occurr. As such, hydrolysis kinetics must take into account the potential for H_2O to dissociate. Even at neutral pH, acid or base catalysis can significantly accelerate hydrolysis. The hydrolysis kinetics can be expressed as a function of cationic, neutral and basic pH. Mathematically, the hydrolysis kinetics can be modeled by modifying the traditional first order reaction rate kinetics model:

$$\frac{dC}{dt} = -k_A[C][H^+] - k_N[C] - k_B[C][OH^-] \qquad \text{(equation 6)}$$

Where,

C – Concentration of neonicotinoids (mol/L)

k_A, k_N and k_B – first order hydrolysis rate constants at acidic, neutral and basic pH respectively

t – time (days)

The proposed model can be simplified by rearranging it as:

$$\frac{dC}{dt} = -[C]\left\{k_A[H^+] + k_N + k_B[OH^-]\right\} \qquad \text{(equation 7)}$$

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At any given pH, the hydrolysis rate of neonicotinoids would remain constant, so the net hydrolysis rate can be modeled using pseudo-first order reaction rate kinetics:

$$\frac{dC}{dt} = -k_{hyd}[C] \qquad \text{(equation 8)}$$

Where,

$$k_{hyd} = \left\{k_A[H^+] + k_N + k_B[OH^-]\right\} \qquad \text{(equation 9)}$$

$$k_{hyd} = \left\{k_A 10^{-pH} + k_N + k_B \frac{k_w}{10^{-pH}}\right\} \qquad \text{(equation 10)}$$

Where,

k_{hyd} – Pseudo-first order hydrolysis rate constant

The analytical solution to the hydrolysis kinetic equation can be represented as:

$$\ln\left(\frac{C(t)}{C_0}\right) = -k_{hyd}t \qquad \text{(equation 11)}$$

$$\ln\left(\frac{C(t)}{C_0}\right) = -\left\{k_A 10^{-pH} + k_N + k_B \frac{k_w}{10^{-pH}}\right\}t \qquad \text{(equation 12)}$$

$$C(t) = C_0 e^{-\left\{k_A 10^{-pH} + k_N + k_B \frac{k_w}{10^{-pH}}\right\}t} \qquad \text{(equation 13)}$$

Where,

C_0 – Concentration of neonicotinoids at t = 0

C(t) – concentration of neonicotinoids at any time t

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4. Results and Discussion

4.1. Simulated Transport of Sulfamethazine

4.1.1. Effect of Speciation on Net Effective Sorption

Prior to assessing the mobility of SMN, the pH dependent sorption behavior of SMN was investigated in LDAC and PAC. The k_{deff} values were calculated using the experimentally determined sorption coefficients at pH 2, 5, and 8 and the acid dissociation constants (pK_1 and pK_2) of SMN. For both LDAC and PAC, the k_{deff} values decreased with increase in pH levels. For PAC, between pH 2-4, k_{deff} rapidly decreased, whereas such initial rapid drop in k_{deff} is not evident in LDAC. For LDAC, between pH 6-10, there was significant drop in k_{deff} whereas it is gradual in the case of PAC (Fig.4). These modeling results clearly demonstrate

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which material is more likely to undergo rapid decrease in k_{deff} in acidic and basic pH values. While the PAC shows gradual decrease with near-steady state at high pH levels, the LDAC practically ceased to have any sorption potential at high pH. In general, at lower pH, sorption of SMN is enhanced primarily due to the predominance of cationic species. Conversely, the observed reduction in the k_{deff} at high pH could be attributed to the cumulative effect of increased solubility and the prevalence of the anionic form (*25*).

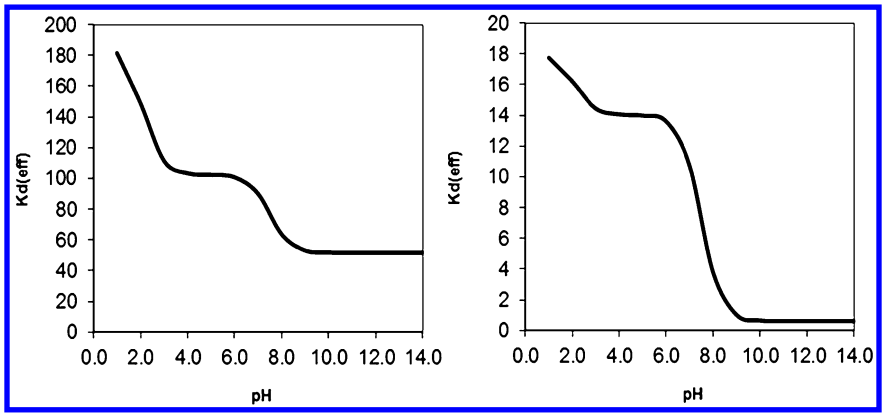

Figure 4. Effect of speciation on net sorption coefficient for PAC (left) and LDAC (right).

4.1.2. Effect of Speciation on Mobility of SMN

A one-dimensional ADE with pH-based retardation factor was used to simulate mobility of SMN. The sorption coefficients developed for PAC and LDAC were used as a surrogate to simulate sorption behavior of a variety of soils from high to low sorption potential. As shown in Fig. 4, the sorption potential of LDAC and PAC is dependent on pH and as such the mobility also depends on pH. These results show that speciation significantly impacts the sorption and mobility of SMN. For both LDAC and PAC, at high pH, fewer bed volumes are required for SMN to breakthrough compared to the bed volumes required at low pH (Fig. 5). For example, in highly acidic soils, SMN can be expected to partially occur in the cationic form, and become attached to the clay surface by cation exchange and become immobile. At soil pH closer to the isoelectric point, SMN exists in transient equilibrium between neutral to zwitterionic form. Previous research has shown that the zwitterionic form constitutes less than 2% of the net neutral species and hence, negligible (*23*). Thus at neutral or slightly acidic pH levels, the SMN may tend to be in the neutral form typically partitioning into the soil organic carbon and as such be less mobile (*32*). On the other hand, in alkaline soils with pH levels well above the isoelectric point; SMN will speciate to the anionic form and be expected to be much more mobile. pH of soil is only one of the important factors that could increase mobility, and its role must be judged in conjunction with other soil properties that may affect sorption and mobility. Besides pH, other

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parameters such as, % organic carbon, clay fraction, ionic strength and surface area affect the mobility of ionizable compounds in the environment (*32*).

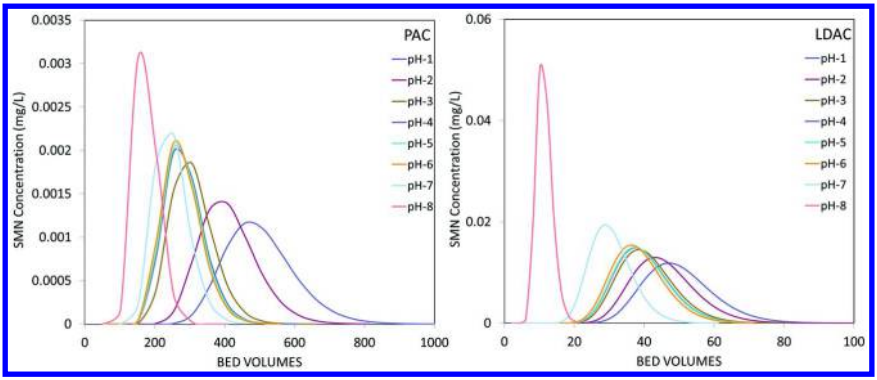

Figure 5. Effect of pH on the mobility of SMN showing direct effect of pH on mobility of SMN with significantly higher mobility observed at higher pH levels

4.2. Modeling Hydrolysis Kinetics of Neonicotinoids

4.2.1. pH Dependent Hydrolysis of Neonicotinoids

Hydrolysis rate constant values were collected from the literature at approximately pH 4, 7 and 9 for all three neonicotinoids (*33–37*). These rate constants were developed at room temperature. From the rate constant values, it was evident that all three neonicotinoids undergo hydrolysis with rate constant $k_{DNT}<k_{THM}<k_{IMD}$. The hydrolysis rate constant for DNT, IMD and THM varies with pH and is higher in alkaline conditions than acidic and neutral conditions. From the hydrolysis kinetic model discussed above, it can be clearly seen that pseudo-first order hydrolysis rate constant is fixed at given pH and is independent of the concentration of neonicotinoids. Our extensive literature review revealed that DNT is practically stable towards hydrolysis; however, in extremely alkaline pH conditions, it slowly undergo hydrolysis.

All three neonicotinoids undergo hydrolysis with net pseudo first-order hydrolysis rate constants for k_{DNT}, k_{THM} and k_{IMD} were found to be 0.0048 d^{-1}, 0.010 d^{-1} and 0.031 d^{-1} respectively. These results demonstrates that DNT and THM may persist longer in the environment compared to IMD (Fig 6). Note that there are other scientific databases such as Pesticide Properties Database (PPDB) that reported that these neonicotinoids are stable towards hydrolysis (*38*). A similar observation was also reported that DNT is stable at ambient temperature but has a half-life of 165 days at pH 9 and 40 °C (*39*).

4.2.2. Temperature Effect on Hydrolysis of Neonicotinoids

When the effect of pH and temperature on net hydrolysis of IMD and THM were compared at room temperature; both THM and IMD hydrolysed slowly

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whereas at elevated temperature the net hydrolysis rate increases (Fig. 7). Please note that these hydrolysis rate constatnt data is used from the published studies (*33*). The hydrolysis rates presented in this study are remarkably low, in fact the hydrolysis rate constant at elevated temperature is much less than some of the earlier investigation. The rationale for presenting the these results here is to highlight the effect of temperature and pH on net hydrolysis of THM. Temperature had a pronounced effect on net hydrolysis rate of both IMD and THM. In both cases, at elevated temperature and pH, IMD appears to favor rapid hydrolysis compared to THM.

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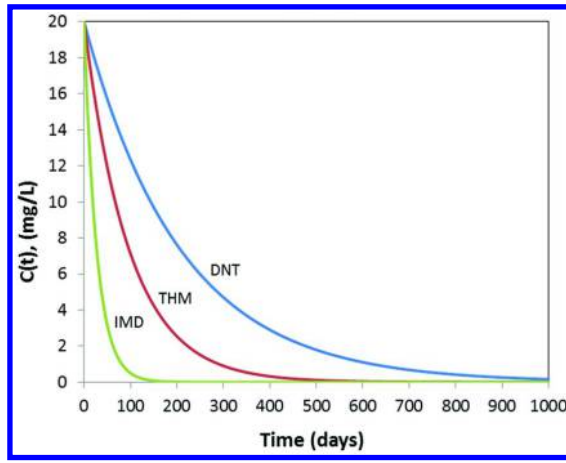

Figure 6. Modeled hydrolysis rate kinetics of DNT, THM and IMD using pseudo-first order reaction rate constants at pH 4, 7 and 9 at 25 °C

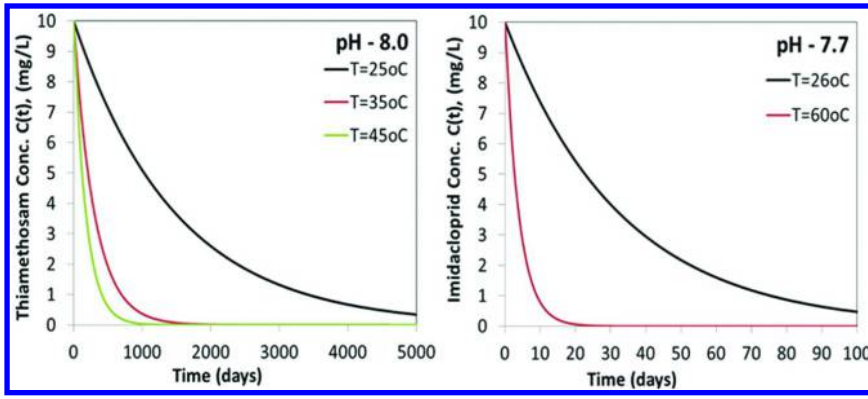

Figure 7. Overall hydrolysis of IMD and THM , shows that at nearly same pH level and temperature, IMD hydrolyses much more rapidly compared to THM

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When the net hydrolysis rate of THM was investigated at a variety of temperatures and at two pH levels, it was observed that elevated temperature combined with the high pH had a significant impact on the net hydrolysis of THM; however, these conditions were not environmentally relevant (Fig. 8).

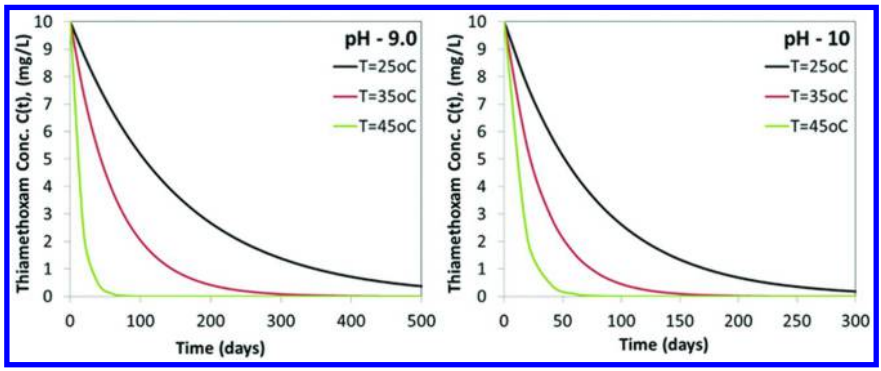

Figure 8. Effect of pH on hydrolysis kinetic under varying temperature conditions.

4.2.3. pH Dependent Net Hydrolysis Rate Constant of Neonicotinoids

The modeled overall hydrolysis rate constant (k_{hyd}) depends on the pH and the magnitude of the respective rate constants at acidic (k_A), neutral (k_N) and basic (k_B) pH levels. To understand the relative contribution of acid or base catalyzed hydrolysis constant, we modeled the variation of these rate constants with regard to pH. In all three neonicotinoids, the acid-catalyzed hydrolysis rate term contributes to the overall hydrolysis term, at acidic level, the rate of IMD hydrolysis is much higher compared to THM and DNT. From fig. 9, it can be clearly seen that, below pH 2, the acid rate (k_A) contributes to the overall hydrolysis rate. Whereas, at environmental relevant pH range (4 to 8), the neutral hydrolysis rate (k_N) will dominate.

Modeled hydrolysis rate kinetics of neonicotinoids demonstrate that at 11<pH<2 hydrolysis rates were stable and governed by rate constant at neutral pH whereas, beyond this pH range significant increase in hydrolysis rate was observed. The base hydrolysis rate (k_B) for IMD, DNT and THM will not compete with neutral hydrolysis until pH 11, which is well above the range of environmentally relevant pH in natural aquatic ecosystems. From fig. 9 it is clear that all three neonicotinoids are stable towards hydrolysis under environmentally relevant temperature and pH. This bring credence to various other studies that reported that neonicotinoids are stable towards hydrolysis.

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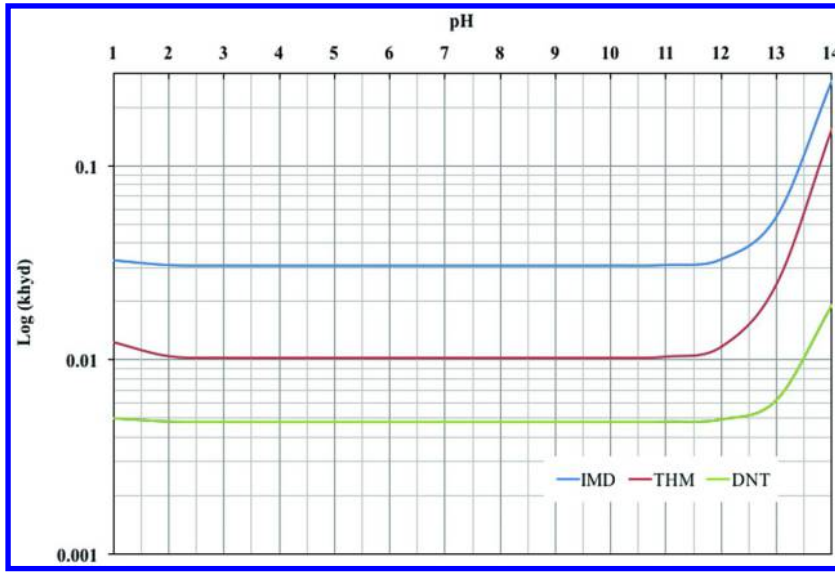

Figure 9. pH-rate profiles for the hydrolysis of DNT, IMD and THM

5. Summary

The occurrence, distribution and fate of EMs is of particular concern due to their toxicity and potential risks to non-target species. The chapter emphasized the mobility of a commonly used sulfonamide - SMN and the widely debated neonicotinoid class of inseticides. Mobility of SMN in the sub-surface environment could potentially impact groundwater resources thereby causing an unknown risk to human health and soil biota. We investigated the mobility of SMN in sub-surface environment using sorption coefficient of LDAC and PAC as a surrogate representing a wide range of soil types. Modeling mobility using the unique speciation chemistry of SMN showed that mobility of SMN is pH dependent with higher mobility being observed at high pH and vice versa.

Neonicotinoids are one of the most widely used classes of insecticides. Neonicotinoids have offered greater crop protection; however, their persistence in the environment may result in an adverse ecological impact. The results of hydrolytic kinetic behavior modeled using pseudo-first order reaction kinetics clearly demonstrate that among the neonicotinoids investigated, IMD exhibits rapid hydrolysis compared to both THM and DNT. In fact, various studies have even reported that DNT is practically stable to hydrolysis. The effect of pH on hydrolysis revealed that under basic conditions, hydrolysis was much faster compared to the neutral and acidic conditions. These results demonstrate that both IMD and THM undergo hydrolysis and pseudo-first order reaction rate kinetics can be used to model the hydrolysis behavior. Based on the hydrolysis rate kinetic modeling, we conclude that the net rate of hydrolysis is dependant on both pH and temperature with increased hydrolysis rate observed at higher temperature and pH above 11. Rapid hydrolysis was also observed below pH 2 and above pH

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11, which are not environmentally relevant, hence neutral hydrolysis rate controls the environmental persistence of neonicotinoids. Of the three neonicotinoids the hydrolysis pattern from low to high is DNT<THM<IMD. DNT is stable under hydrolytic conditions and as such might pose greater ecological risk compared to IMD and THM. Hydrolysis of THM at two different pH levels under varying temperature conditions demonstrated that elevated temperature and basic conditions enhanced net hydrolysis rate of THM.

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Chapter 6

A Comparative Study of the Aqueous-Phase Adsorption of Sulfamethazine onto Commercially Available and Laboratory Developed Activated Carbon

Kailas R. Malwade,[1] David Ramirez,[*,1] and Sudarshan Kurwadkar[2]

[1]Department of Environmental Engineering, Texas A&M University-Kingsville MSC 213, 700 University Blvd., Texas 78363
[2]Department of Civil and Environmental Engineering, 800 N. State College Blvd., California State University-Fullerton, California 92831
[*]E-mail: David.Ramirez@tamuk.edu

Sulfamethazine (SMN) is a sulfonamide antimicrobial agent extensively used in cattle and swine operations for therapeutic and sub-therapeutic treatments. Upon administration, SMN is partially metabolized by cattle and swine and is then released into the environment through excretion. Because of its high solubility and potential threat to surface water, soil and groundwater, it is important that SMN sorption mechanism is well understood so that SMN removal technology can be developed. Commercially available powdered activated carbon (C-PAC) and laboratory developed powdered activated carbon (LD-PAC) were used as adsorbents for the removal of SMN from aqueous solution. Physical characterization of C-PAC and LD-PAC showed N_2-BET surface areas of 617 m^2/g and 329 m^2/g, respectively. Sorption of SMN was investigated at a fixed sorbent dose of 5 mg and with varying adsorption time at pH 7. Experimental results demonstrated that the maximum SMN adsorption capacity for C-PAC and LD-PAC were ~ 99 % and ~ 95 %, respectively at the concentration of 3 mg/L of SMN. C-PAC showed more rapid and higher SMN adsorption in comparison to LD-PAC at SMN concentrations ranging from 3 mg/L to 20 mg/L. Second order kinetic model can be approximated to describe the SMN adsorption kinetics

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onto C-PAC and LD-PAC. The Freundlich isotherm model predicted the SMN adsorption on C-PAC and LD-PAC with good agreement with the experimental results. From the results it can be seen that both C-PAC and LD-PAC were effective adsorbents for the removal of SMN from aqueous solution.

Keywords: sulfamethazine; powdered activated carbon; adsorption; adsorption model; kinetic model

Introduction

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Veterinary antibiotics such as sulfamethazine (SMN) are routinely used as an antimicrobial agent for the treatment, control and prevention of diseases and as husbandry growth promoters in poultry, swine, cattle and other food producing animals (*1–3*). The unintentional release of antibiotics into the environment poses a risk even though such antibiotics are often released at low concentrations. The administered antibiotics molecules are poorly absorbed and distributed in the gut of the animals and nearly 75% to 90% of the antibiotics remain un-metabolized in body of the animal (*4*). Antibiotics are then involuntarily discharged through excretion in parent form, as conjugates or partially metabolized form in the fecal matter or dissolved in urine (*2*, *5*). The excreted antibiotics do not degrade readily and can retain in the manure up to one year (*6*). High solubility of these antibiotics tends to facilitate their movement through sub-surface environment through leaching from soil and surface and ground waters (*6*, *7*). Antibiotics such as SMN enter water resources through urine and feces typically stored in an on-site lagoon which are not typically designed to remove low levels of antibiotics. Furthermore, the wastewater treatment facilities are also poorly equipped to remove antibiotics during the wastewater treatment process (*5*, *8*). Antibiotics can also interfere with the treatment system by inhibiting bacterial growth by obstructing their metabolic process (*3*, *4*, *8*). There is also a concern that, non-target species such as bacteria and other aquatic and soil organisms may develop resistance due to continued exposure to low levels of antibiotics.

Antibiotics are used for therapeutic, sub-therapeutic and prophylactic purposes. Although, they are used worldwide for disease prevention and growth promotion, there is a limited information about the consumption of antibiotics in different countries (*9*). The global annual usage of antibiotics has been estimated to be between 100,000 to 200,000 tons (*10*, *11*). In the United States, over 22,000 tons of antibiotics are produced and about 40% are used for veterinary purposes (*5*). For example, the total consumption of antibiotics in the fish industry has been estimated to be 92 tons to 196 tons in the mid-1990s and about 2% of the nonmedical use in cattle, swine and poultry (*2*, *12*). The annual production of environmental matrices having residual antibiotics such as in feces and urine is over 100 billion tons in the U.S. This significant production of residual antibiotics account for their entry into surface water sources or other aquatic environment (*13*).

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The removal of antibiotics such as SMN is important. Few studies have reported the removal of SMN using activated carbon or biochar (*14*). Rajpaksha et al. (2014) used biochar produced at different temperatures from waste tea with and without nitrogen and steam activation for SMN removal (*14*). A study done by Choi et al. (2008) and Lertpaitoonpan et al. (2009) investigated the effect of organic matter in the soil on the adsorption of SMN (*15*, *16*). This study focused on the determination of the adsorptive capacity of SMN on commercially available powdered activated carbon (C-PAC) and laboratory developed powdered activated carbon (LD-PAC) and to find out the most effective powdered activated carbon between C-PAC and LD-PAC for the adsorption of SMN from water. SMN adsorption and kinetic studies were conducted and modeled using Langmuir and Freundlich isotherm models and first order, second order and intra-particle diffusion kinetic models.

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Materials and Methods

Chemicals

Sulfamethazine (4-amino-N-[4, 6-dimethyl-2-pyrimidinyl] benzenesulfonamide, $C_{12}H_{14}N_4O_2S$, ≥98% purity, CAS # 1981-58-4) was obtained from Sigma-Aldrich. Hydrochloric acid (ACS grade) was obtained from EMD Chemicals and sodium hydroxide (99.8% purity) was purchased from Fischer Scientific. Orthophosphoric acid (ACS grade, BDH Acids) was purchased from VWR International. A standard solution of 1,000 mg/L was prepared by weighing 0.51 g SMN and mixing in 500 mL of nano pure water.

Activated Carbon Manufacturing and Characterization

Two types of powdered activated carbons were used for the SMN adsorption studies because it was necessary to check the applicability of manufactured activated carbon for SMN removal from aqueous solution and compare the results of LD-PAC with commercially available activated carbon to verify the effectiveness in SMN removal. Figure 1 shows the manufacturing protocol for the LD-PAC. The mesquite waste sawdust collected from a sawmill factory in Kingsville, Texas was used to manufacture the LD-PAC because the mesquite sawdust precursor a waste product with an important content of carbon that is needed for an effective SMN removal. The sawdust was sieved to obtain desirable size fraction (200 to 400 μm) and washed 3 times with de-ionized water to remove any impurities such as dust and ash. The washed material was dried in an oven (Model 1300U, Sheldon manufacturing, Inc.) at a temperature of 105 °C for 24 h. The dried material was mixed with ortho-phosphoric acid at the weight/volume ratio of 1:1 (1 g of raw material/1mL of ortho-phosphoric acid) and impregnated for 24 hours (*17*).

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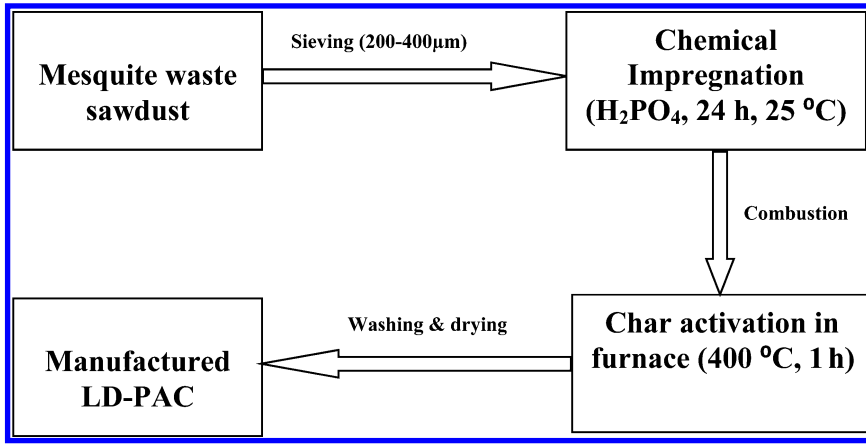

Figure 1. Flow chart for the manufacture of LD-PAC

The impregnated material was activated in the furnace at a temperature of 400 °C for 1 hour. The formed activated carbon was again washed with de-ionized water. The washed and dried powdered activated carbon was used to conduct the SMN adsorption study.

The C-PAC (CAS#7440-44-0) was purchased from Norit Americas Inc. The specific surface area of the C-PAC was measured using a high speed surface area and pore size analyzer (NOVA 2200, Quantachrome) with N_2 and the Brunauer, Emmet and Teller (BET) method (*18*). The proximate analysis of the LD-PAC and C-PAC was carried out using ASTM Standards 3173-11, 3174-12, and 3175-11.

Adsorption Experiments

Adsorption experiments of SMN on C-PAC and LD-PAC were conducted using adsorption test OECD guidelines (*19*). Eleven standard solutions ranging from 0.0125 mg/L to 20 mg/L were prepared by spiking selected volumes of 1,000 mg/L of SMN solution in nano pure water to achieve a volume of 40 mL and initial concentrations of 3, 5, 10, 15 and 20 mg/L of test solution (*20*, *21*). The 5 mg of C-PAC and LD-PAC were separately mixed with 40 mL of each prepared SMN solution and agitated in a gyro-shaker (G10 Gyrotory Shaker, New Brunswick Scientific) at 100 rpm. The adsorption experiments were conducted at 25 °C and pH 7. Control samples (only substance test solution) were also examined in order to check the stability of the test solution, meaning possible adsorption to the glassware surfaces during experiments and any loss due to degradation. The samples were prepared in duplicates and filtered with 0.45 μm syringe filters (Polyethersulfone, VWR International). The filtrate was transferred into a vial for HPLC analysis.

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HPLC Protocol

SMN concentrations were measured using Waters Alliance High Performance Liquid Chromatography system equipped with e2695 separation module with integrated solvent and sample management functions. This HPLC system was also equipped with 2998 photodiode array detection with analytical flow cell that was used for optical detection and quantitation along with the spectral analysis. Chromatographic separations were achieved using a Phenomenex Luna *3 μm* phenyl-hexyl 100A (150x3.0 mm) column with a binary gradient with solvents A and B. Solvent A comprised of 90% by volume of 20 mM ammonium acetate solution and 10% acetonitrile with glacial acetic acid. Solvent B was prepared with 20% of solvent A and 80% acetonitrile. The analytical separation was achieved through isocratic separation with mobile phase configuration of 70% A and 30% B with an injection volume 50 μL with 10 minute retention time.

Calculations

The percent SMN removal and equilibrium adsorption capacity, q_e (mg/g) was determined by using the following equations (*22*):

$$\% \text{ Removal} = \frac{(C_0 - C_e)}{C_0} \times 100$$

$$q_e = \frac{(C_0 - C_e) \times V}{m}$$

Where, C_0 is the initial SMN concentration (mg/L); C_e is the SMN concentration at equilibrium (mg/L); V is the volume of SMN solution (mL) and m is the mass of activated carbon (g).

Adsorption Kinetic Modeling

The reaction-based (first and second order) and diffusion-based (intra-particle diffusion) sorption kinetic models were chosen to analyze the SMN adsorption data because these models were commonly used for sulfonamide group antibiotics using several adsorbents and serve as a useful tool in the interpretation of SMN uptake rate and residence time of the adsorbate at the solid-liquid interface (*23–30*). Moreover, Weber-Morris equation was used to investigate the intra-particle diffusion, a rate controlling step in SMN adsorption, which could not be described by first or second order kinetic models (*31–33*). The chosen kinetic models are represented as follows.

First-order-kinetic model

$$Log(q_e - q) = Log(q_e) - \frac{k_f}{2.303} t$$

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Second-order-kinetic model

$$\frac{t}{q} = \frac{1}{k_s q_e^2} + \frac{1}{q_e} t$$

Weber-Morris intra-Particle diffusion model

$$q = k_{id} t^{1/2} + C$$

Where, q is the adsorption capacity at any time t (mg/g); k_f is the first order kinetic coefficient (1/h); t is the adsorption time (h); k_s is the second order kinetic coefficient (g/mg.h); k_{id} is an intra-particle diffusion rate constant and C is the thickness of boundary layer.

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Adsorption Isotherm Modeling

The adsorption isotherms provide the information about distribution of adsorbed molecules between adsorbate and adsorbent surface when adsorption process reaches at equilibrium step. Consequently, the interaction of adsorbate molecules with adsorbent surface can be described using adsorption isotherms (*28*). The Langmuir and Freundlich adsorption isotherm models were used to understand the equilibrium characteristics of adsorption and optimize the design of adsorption system (*34*).

Langmuir Isotherm

This equation is based on the assumptions that only monolayer adsorption is possible, adsorbent surface is uniform in terms of energy of adsorption, no interaction of adsorbed molecules with each other, and that there is no migration of adsorbed molecules on the adsorbent surface (*35*).

The Langmuir model is expressed as,

$$q_e = \frac{q_m K C_e}{1 + K C_e}$$

The linear form of the Langmuir model is the following

$$\frac{1}{q_e} = \left(\frac{1}{K q_m}\right)\left(\frac{1}{C_e}\right) + \left(\frac{1}{q_m}\right)$$

Where q_m is the maximum adsorption capacity of SMN (mg/g) and K is a constant related to the energy of adsorption (L/mg).

Freundlich Isotherm

The Freundlich isotherm assumes a heterogeneous surface with a non-uniform distribution of heat of adsorption over the surface. The heat of adsorption in

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many instances decreases in magnitude with increasing extent of adsorption. This decline in heat of adsorption is logarithmic, implying that adsorption sites are distributed exponentially with respect to adsorption energy. This isotherm does not indicate an adsorption limit when coverage is sufficient to fill a monolayer (*35*). The equation of Freundlich Isotherm is given as

$$q_e = K_f C_e^{\frac{1}{n}}$$

The linear form of the Freundlich equation is

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e$$

Where K_f and n are constants.

Average Relative Error (ARE)

ARE was estimated to compare adsorption isotherm model results with experimental equilibrium results. ARE is defined as:

$$ARE = \frac{100}{n} \sum_{i=1}^{n} \left| \frac{(q_{e,exp} - q_{e,Calc})}{q_{e,exp}} \right|_i$$

Where, n is the number of data points; $q_{e,exp}$ and $q_{e,calc}$ are experimental and calculated equilibrium adsorption capacities (mg/g).

Results and Discussion

Table 1 shows the physico-chemical properties of C-PAC and LD-PAC. The adsorbent pores can occupy moisture, volatile matter in the pores and affect the adsorption process. Hence, it was necessary to remove moisture and volatile matter before adsorption process. The specific surface area of C-PAC was approximately two times higher than the specific surface area of LD-PAC. Total pore volume for C-PAC was 0.54 cm^3/g, which is twice the pore volume of LD-PAC. The moisture content of the C-PAC was 4% lower than the moisture content of LD-PAC. However, the ash content and volatile content values were comparatively higher for C-PAC (19%) than LD-PAC (11%). The fixed carbon content for C-PAC was 26% lower than the fixed carbon content of LD-PAC.

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Table 1. Physico-chemical properties of PAC samples

Properties	*C-PAC*	*LD-PAC*
N_2-BET surface area (m^2/g)	617	329
Total pore volume (cm^3/g)	0.54	0.20
Moisture content (%)	8	12
Ash content (%)	39	20
Volatile matter (%)	25	14
Fixed carbon (%)	28	54

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Adsorption Kinetics of SMN on C-PAC and LD-PAC

Figures 2A and 2B show the percent removal of SMN by C-PAC and LD-PAC, respectively at initial SMN concentration range from 3 mg/L to 20 mg/L, pH 7 and 25 °C. Figure 2A shows the rapid removal of SMN in the first hour; thereafter, the percent SMN removal was slow and gradual. The percent SMN removal increased with a decrease of initial SMN concentration from 20 mg/L to 3 mg/L. After 1 h of adsorption time, the SMN percent uptake by C-PAC increased from 79.1 % for 20 mg SMN/L to 99.6 % for 3 mg SMN/L. After 12 h, the percent SMN removal increased from 89.9 % to 99.9 % as the SMN concentration decreased from 20 mg/L to 3 mg/L. After 24h, removal efficiency averaged from 91 % to 99 % for SMN concentration variation of 20 mg/L to 3 mg/L. The difference in SMN removal between 1 h and 12 h of adsorption time ranged from 0.36% to 12.87% for initial concentrations of 3-20 mg/L. However, the difference in SMN removal between 12 h and 24 h of adsorption time only ranged from 0.05% to 1.78%. Therefore, the steady state conditions were assumed at 12 h of adsorption time with C-PAC. The lower initial SMN concentration showed rapid and higher SMN removal efficiency by C-PAC. This kind of behavior was reported in the previous literature for adsorption of amoxicillin, cephalexin, tetracycline, penicillin-G and ciprofloxacin antibiotics on activated carbons. This can be explained by considering that adsorbents have a limited number of active adsorption sites and at certain concentration the active sites become saturated (*36*, *37*). Secondly, the hydrophobicity could be responsible for SMN adsorption onto C-PAC because SMN contains two methyl groups which are related to the hydrophobic tendency (*15*). The SMN adsorption for initial concentration of 3 mg/L showed the lowest SMN residual concentration (0.09%) in aqueous solution at 12 h of adsorption time. For SMN concentration of 3-20 mg/L, the percent SMN residual concentration was in the range of 0.09% - 10.13% for 12 h. The necessary equilibrium time for SMN adsorption on C-PAC was about 12 h.

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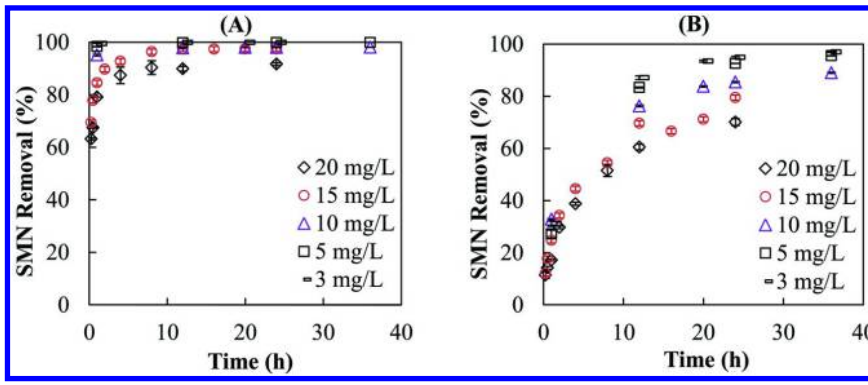

Figure 2. Adsorptive removal of SMN on A) C-PAC and B) LD-PAC at 25 °C and pH 7

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Figure 2B shows the percent SMN removal as a function of time for 3-20 mg/L of initial concentration for LD-PAC. The percent adsorptive uptake of SMN by LD-PAC was slow and gradually increased with time during the adsorption process. The percent SMN removal lowered from 31.1% - 17.2% at 1 h, 87.1% - 60.5% at 12 h and 94.9% - 70.1% at 24 h with SMN concentration between 3 to 20 mg/L. The percent residual SMN concentration at 12 h varied from 12.9 % for 3 mg/L to 39.47 % for 20 mg/L, which was observed lower at 24 h of adsorption time and varied from 5.0 % to 29.88 %. Hence, the adsorption time of about 24 h was considered as equilibrium time for LD-PAC.

In comparison, C-PAC demonstrated rapid and higher percent SMN adsorptive uptake than that of LD-PAC because C-PAC has more specific surface area and consequently more adsorption sites availability than LD-PAC. The SMN adsorption on C-PAC required about 12 h of equilibrium time, which was less than the equilibrium time required for SMN adsorption on LD-PAC. The SMN adsorption on C-PAC reported very less residual concentration compared with the use of LD-PAC.

First and Second Order Kinetic Models

First and second order kinetic models (*23*, *24*) were used to obtain the best fit model with the experimental adsorption kinetic data of SMN on C-PAC and LD-PAC.

Figures 3A and 3B shows the 1st and 2nd order kinetic modelling of SMN, respectively at pH 7 and 25 °C for C-PAC. The kinetic model results for LD-PAC are shown in Figure 4.

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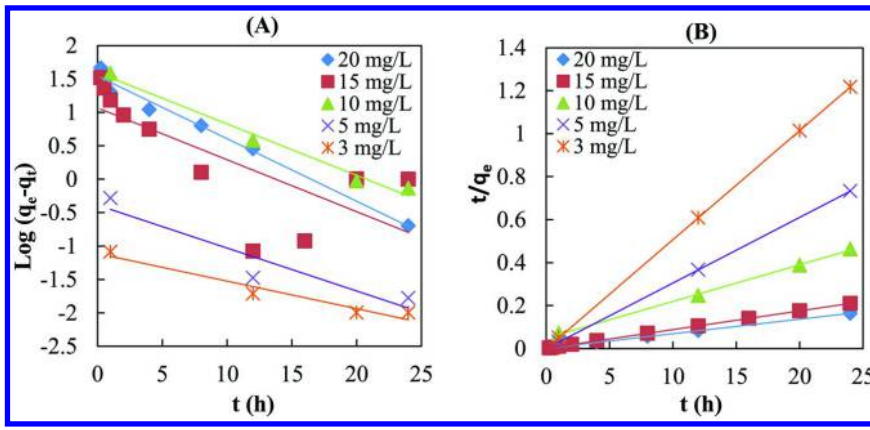

Figure 3. Adsorption kinetic modeling for SMN on C-PAC using A) 1st order and B) 2nd order models at pH 7 and 25 °C

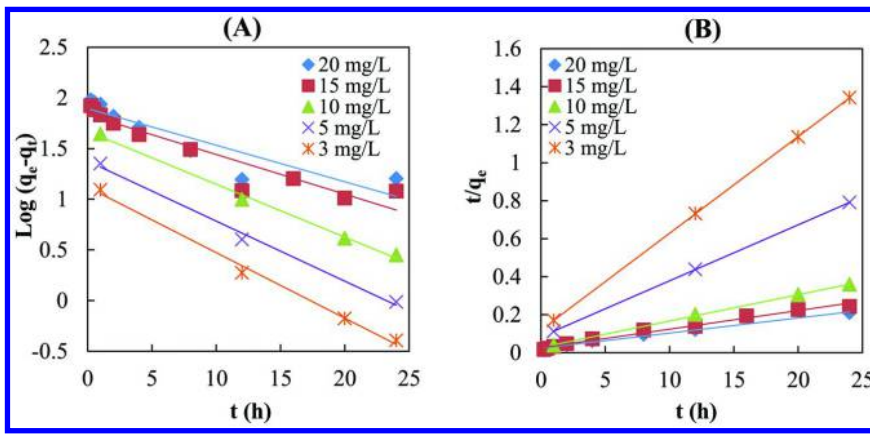

Figure 4. Adsorption kinetic modeling for SMN on LD-PAC using A) 1st order and B) 2nd order models at pH 7 and 25 °C

1st and 2nd order kinetics parameters k_f, k_s, $q_{e,cal}$ and correlation coefficients were obtained from the slope and intercept of the plot, Log(q_e-q_t) versus time and t/q_e versus time for C-PAC and LD-PAC. The values of the adsorption kinetic parameters are shown in Table 2. The correlation coefficients for second order kinetic model are consistent and close to one for C-PAC and LD-PAC as compared to correlation coefficients of first order kinetic model. Moreover, the predicted adsorption capacities for first and second order kinetic models were compared to choose the suitable adsorption kinetic model for SMN. For second order kinetic model, the predicted SMN adsorption capacities for C-PAC and LD-PAC are close with experimental SMN adsorption capacities as shown in Table 2. The sum of square errors for the predicted and experimental adsorption capacities for second order kinetic model varied from 0 - 119, which are lower in comparison with the sum of square errors (46 - 12477) for SMN adsorption capacities for C-PAC and

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LD-PAC with first order kinetic model. The k_s values in Table 2 shows that the adsorption rate decreased with increase in SMN concentrations for C-PAC and LD-PAC. However, the lower adsorption rate was observed for LD-PAC compared with C-PAC. The k_s values showed in Table 2 for C-PAC and LD-PAC are higher in comparison to k_s values found in the literature. For sulphonamide, the literature reported k_s values are in the range of 0.007-0.004 g/mg.h for coal based powdered activated carbon (*29*).

Table 2. Adsorption kinetics parameters and R^2 values for 1st and 2nd order models

Adsorbent	C_o *(mg/L)*	$q_{e,exp}$ *(mg.g^{-1})*	*1st Order*			*2nd Order*		
			k_f *(h^{-1})*	$q_{e,cal}$ *(mg/g)*	R^2	k_s *(g/mg.h)*	$q_{e,cal}$ *(mg/g)*	R^2
C-PAC	3	19.7	0.29	12.9	0.95	8.57	19.7	1.00
	5	32.8	0.15	2.4	0.88	1.86	32.8	1.00
	10	51.8	0.18	39.5	0.98	0.01	58.8	0.99
	15	114.3	0.18	11.7	0.55	0.06	115	1.00
	20	146.6	0.21	34.9	0.98	0.03	147	0.99
LD-PAC	3	17.9	0.15	13.2	0.99	0.02	19.6	1.00
	5	30.4	0.14	23.8	0.99	0.01	33.9	1.00
	10	66.9	0.12	46.7	0.99	0.01	77.9	1.00
	15	98.4	0.09	67.9	0.89	0.004	102	0.99
	20	114.1	0.08	78	0.81	0.003	125	0.99

Intra-Particle Diffusion Model

The Weber and Morris is an intra-particle diffusion model used in this study to understand the diffusion mechanism of SMN on C-PAC and LD-PAC (*38*). The mechanism of adsorption generally includes three steps, the first step is transport of adsorbate molecules across the external boundary layer film of liquid surrounding the adsorbent, the second step is diffusion of the adsorbate molecules onto the interior surface of adsorbent by a pore diffusion process through the liquid filled pores or by a solid surface diffusion mechanism, and final step is adsorption at a site within the pores of adsorbent (*39*, *40*).

In this diffusion model, the fractional uptake of adsorbate varies almost proportionately with the square root of time (*41*). The plot of q_t versus $t^{1/2}$ should be a straight line when intra-particle diffusion is a rate controlling step (*42*). Previous studies also showed that Weber-Morris plot may contain a multi-linearity or more than two stages (*23*). The first stage is the external surface adsorption or rapid adsorption stage. The second stage is the gradual adsorption stage, where the intra-particle diffusion is controlled. The third stage is an equilibrium stage

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where the intra-particle diffusion starts to slow down due to solute concentrations in the water (*23*, *43*). Figures 5A and 5B show the Weber-Morris plots of q_t versus $t^{1/2}$ for C-PAC and LD-PAC, respectively at initial SMN concentrations from 3 mg/L to 20 mg/L. Figure 5A shows two stages, one with a rapid adsorption stage, and a second stage when then gradual adsorption stage occurred and dominated by intra-particle diffusion for SMN concentrations from 10 mg/L to 20 mg/L. Figure 5B shows an external surface adsorption dominance by LD-PAC for SMN concentration of 3 mg/L to 20 mg/L. However, no line passed through origin and therefore intra-particle diffusion was not a rate controlling step for C-PAC and LD-PAC (*42*). The values of k_{di} and C obtained from the slope and intercept of the straight lines of Figure 5A and 5B are shown in Tables 3A and 3B. The increase in C_1 and C_2 values indicates the increase in boundary layer thickness, consequently decreasing the change of external mass diffusion (*27*).

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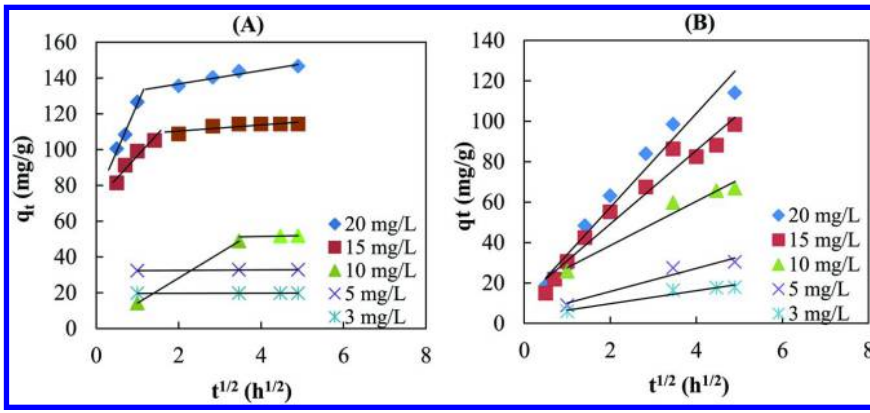

Figure 5. Weber-Morris kinetic models for the adsorption of SMN on (A) C-PAC and (B) LD-PAC at pH 7 and 25 °C

Based on the correlation coefficient close to one and consistent q_e and q_{exp} values for C-PAC and LD-PAC between first and second order kinetic models, the second order kinetic model can be fitted more suitably with experimentally obtained kinetic adsorption data for C-PAC and LD-PAC.

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Table 3A. Weber-Morris Kinetic Parameters

Adsorbent	C_0 *(mg/L)*	K_{id1} *(mg/g.h)*	C_1	R^2
C-PAC	3	0.02	19.6	0.94
	5	0.12	32.2	0.87
	10	14.0	0.13	1.00
	15	25.1	71.5	0.93
	20	52.9	72.9	0.98
LD-PAC	3	3.20	3.4	0.94
	5	5.7	4.4	0.94
	10	10.9	16.7	0.96
	15	18.2	12.7	0.96
	20	23.2	10.8	0.96

Table 3B. Weber-Morris Kinetic Parameters

Adsorbent	C_0 *(mg/L)*	K_{id2} *(mg/g.h)*	C_2	R^2
C-PAC	10	0.47	49.5	1.00
	15	1.72	107	0.70
	20	3.73	129	0.93

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SMN Removal Efficiency and Capacity

Figure 6A shows the SMN removal efficiency of C-PAC and LD-PAC at pH 7, 25° C. The given amount of adsorbent mass adsorbed a fixed amount of adsorbate. As expected, the percent SMN removal decreased with increase in SMN concentrations. The maximum SMN removal of ~ 99% for C-PAC and ~ 95% for LD-PAC at 3 mg/L of initial SMN concentration were observed.

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Figure 6B shows the equilibrium uptake by C-PAC and LD-PAC at changed initial SMN concentrations. It is evident that the equilibrium adsorption capacity increased with increase of initial SMN concentration. The increasing trend of equilibrium adsorption capacity with initial SMN concentrations is due to the increasing concentration gradient, which acts as a driving force to decrease the resistances to mass transfer of SMN ions between the aqueous solution and the adsorbent surface (*36*). The higher SMN adsorption uptake of 143.8 mg/g by C-PAC and 114.1 mg/g by LD-PAC was observed at 20 mg/L of initial SMN concentration.

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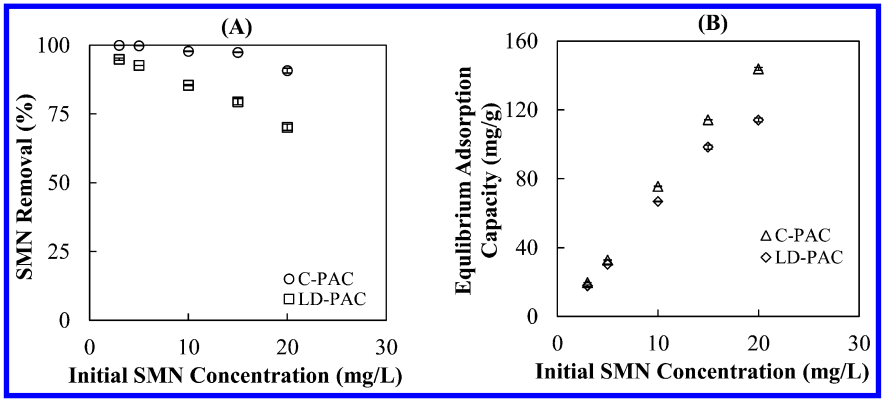

Figure 6. (A) Adsorption efficiency and (B) adsorption capacity of C-PAC and LD-PAC for SMN at pH 7 and 25 °C

Equilibrium Adsorption Isotherm Models

Equilibrium adsorption isotherms describe the interaction of adsorbates with the adsorbent. Therefore, the practical design and operation of adsorption systems need an appropriate correlation of data obtained by empirical equations and experimental data (*44*). The linearized Langmuir and Freundlich isotherm models results are plotted in Figure 7. The Langmuir and Freundlich isotherm parameters and R^2 values were determined from the plots shown in Figure 7 and listed in Table 4.

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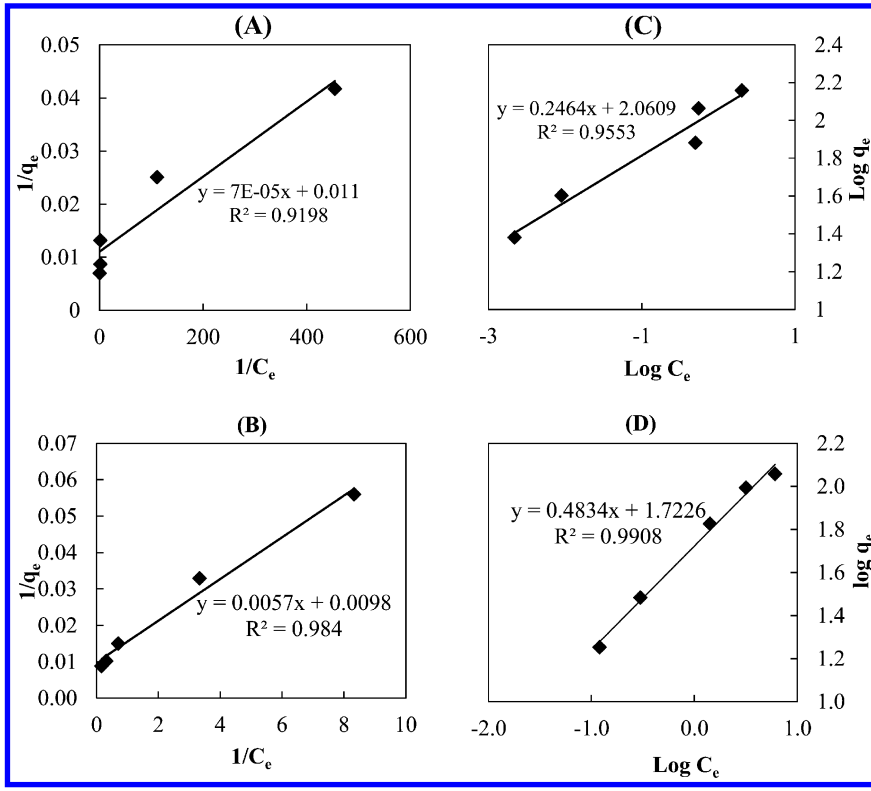

Figure 7. Linearized Langmuir model on A) C-PAC and B) LD-PAC and linearized Freundlich model on C) C-PAC and D) LD-PAC atpH = 7 and 25 °C

Table 4. Langmuir and Freundlich model parameters for C-PAC and LD-PAC

Adsorbent	*Langmuir Model*			*Freundlich Model*		
	b (L/mg)	*q_m (mg/g)*	*R^2*	*K_f (L/mg)*	*1/n*	*R^2*
C-PAC	157	90.9	0.92	115	0.25	0.96
LD-PAC	0.61	102	0.98	33.8	0.48	0.99

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The linear regression correlation coefficient value for Freundlich isotherm model for C-PAC was higher than Langmuir isotherm model. On the other hand, the correlation coefficient of Langmuir model for LD-PAC was higher than Freundlich model.

By comparing the results of average relative errors listed in Table 5, the Freundlich model fitted SMN equilibrium adsorption data better than the Langmuir model. Figure 8 shows a comparison of experimental adsorption capacity with Langmuir and Freundlich isotherm model results for the adsorption of SMN on C-PAC and LD-PAC. The Freundlich model best described the SMN equilibrium adsorption data for C-PAC and LD-PAC.

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Table 5. Average relative errors for Langmuir and Freundlich model for C-PAC and LD-PAC

Adsorbent	*Isotherm Model*	*ARE*
C-PAC	**Langmuir**	22.70
	Freundlich	12.50
LD-PAC	**Langmuir**	39.96
	Freundlich	6.47

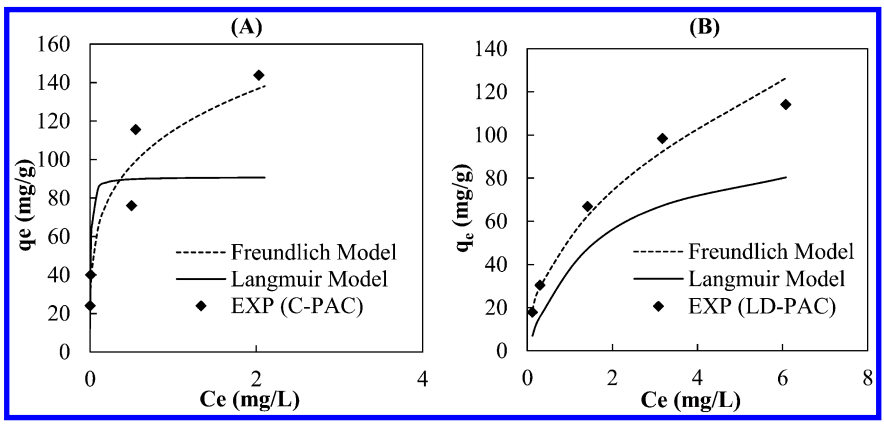

Figure 8. Comparison of Langmuir and Freundlich models with experimental equilibrium adsorption capacities for A) C-PAC and B) LD-PAC

Conclusion

Comparative study of SMN adsorption on C-PAC and LD-PAC showed that C-PAC has higher adsorption capacity than LD-PAC probably because of having higher specific surface area and total pore volume of C-PAC. The adsorption of SMN on C-PAC showed 12 h of equilibrium time which was lower in comparison with 24 h for LD-PAC. This study demonstrated that second order kinetic model

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can be suitably approximated with experimental SMN adsorption data obtained using C-PAC and LD-PAC. It also revealed that second order SMN adsorption rate was decreasing with increase of initial SMN concentration. However, C-PAC showed higher adsorption rate in comparison to LD-PAC. The intra-particle diffusion model exhibited that SMN adsorption on C-PAC and LD-PAC was not a rate controlling step. The intra-particle diffusion for C-PAC was dominated by SMN concentration from 10 to 20 mg/L. The Freundlich isotherm model predicted the SMN equilibrium adsorption data better than the Langmuir model for C-PAC and LD-PAC. Comparative study of adsorption of SMN using C-PAC and LD-PAC confirmed that C-PAC showed more efficient adsorbent in comparison to LD-PAC.

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Chapter 7

Identifying Knowledge Gaps in Assessing Implication of Engineered Nanomaterials on Wastewater Reuse

Divya Singh and Arun Kumar*

Department of Civil Engineering, Indian Institute of Technology, New Delhi, India
***E-mail: arunku@civil.iitd.ac.in; Phone: +91-11-2659-1166; Fax: +91-11-2658-1117**

In recent years there has been increased usage of wastewater residuals (wastewater effluent and biosolids) in agriculture for irrigating crops and improving nutrient concentrations in soil. Engineered nanomaterials (ENMs) have been reported to be toxic to plants, aquatic species and human beings, hence, it becomes imperative to understand the effects of their presence in wastewater residuals on growth of edible plants and subsequently on human health. The objective of this study was to understand effect of use of engineered nanomaterials-contaminated wastewater effluent as irrigation water on growth of seeds and plants. To illustrate this, a hypothetical study was carried out for assessing risk due to consumption of tomato grown using ENM-contaminated wastewater. The analysis of published reports and literature data indicates the need of following future efforts: (1) Determination of occurrence of ENMs in wastewater effluents before it is used as irrigation water, (2) Determination of levels of ENMs in plants and edibles grown using wastewater effluents, and (3) Development of a health risk-based criteria for deciding treatment goals of ENMs in wastewater effluent before it can be used for irrigation activities. These efforts are needed to successfully reuse wastewater effluent as well as to protect human health.

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Introduction

Agriculture sector demands almost 70% of the total water consumption, leading to burden on water source. According to the published reports, between years 2000-2030 developing countries are expected to expand irrigated area from 202 million hectares to 242 million hectares (*1*), which might result in an additional requirement of water for irrigation purposes. Currently around 7% of world's agricultural land is irrigated using raw or partially treated wastewater directly or indirectly (*2*).

In recent years, occurrence of engineered nanomaterials in wastewater have been reported (*3*). This information indicates the possibility of their exposure during use of wastewater for irrigation purposes. As these ENMs pose risks to health and toxicity to plants and aquatic species, it is important to know if use of wastewater effluent as irrigation water is appropriate for plant growth and for irrigating edible plants.

Safe and well managed wastewater reuse is need of the hour, especially for countries with increasing demand of irrigation water. Table 1 shows parameters considered by various national and international agencies in determining the use of wastewater for irrigation purposes. From Table 1, it is clear that currently ENMs are not considered as one of the parameter for deciding the suitability of wastewater for irrigation purposes.

Table 1. Parameters considered in guidelines for selecting water for irrigation purposes

*Agencies**	*Parameters considered in Guidelines and their values*[a]
FAO (*1*)	Total dissolved solids=450 mg/L, Na=3 me/L, Cl^- =0.7-4 me/L, B=10 mg/L, N=<5 mg/L; HCO_3^- =< 1.5 me/L, pH=6.5-8.4, SAR= Maximum=3
CPCB (*4*)	pH=6-8.5, EC (at 25 °C)=25250 μΩ/cm, SAR=26, Boron=2 mg/L
BIS (*5*)	Total salt (EC)=1500-6000 μΩ/cm, Residual sodium carbonate=1.5-6 me/L; Boron content=1-4 mg/L
WHO (*6*)	Maximum allowable concentration in soil (mg/Kg soil) Sb=36, As=8, Ba=302, Be=0.2, B=1.7, Cd=4, F=635, Pb=84, Hg=7, Mo=0.6, Ni=107, Se=6, Ag=3, Tl=0.3, V=47 Organic: Alderin=0.48, Benzene= 0.14, Chlorobenzene=211, Chloroform=0.47, 2,4-D=0.25, DDT=1.54, Dichlorobenzene=15, Dieldrin=0.17, Dioxins=0.00012, Heptachlor=0.18, hexachlorobenzene=1.4, Lindane=12, Methoxychlor=4.27, PAHs= 16, PCBs=0.89, Trichloromethane=0.68, Toluene=12, Toxaphene=0.0013, Tetrachloroethylene= 0.54, Tetrachloroethane=1.25, 2,4,5-T=3.82, Styrene=0.68, Pyrene=41, Phthalate=13733, Pentachlorophenol=14

[a] me/L= millimole/Liter; SAR= Sodium absorption ratio

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The objective of this study is to understand effect of ENMs in wastewater on reuse of wastewater as irrigation water. To illustrate this, a hypothetical study was carried out for assessing risk due to consumption of tomato grown using ENM-contaminated wastewater. This study aimed to provide a systematic approach for selecting wastewater effluent as irrigation water with regards to ENMs in water using human health risk- based approach.

Proposed Framework for Assessing Implications

To investigate implications of using wastewater effluents as irrigation water with regards to ENMs in water, this study proposed a structure (Figure 1) which consists of following two aspects: (1) Assessment of toxic effects of ENMs on plant growth and (2) Estimating health risks due to exposures of ENMs from consuming edible parts of plants, irrigated with wastewater. Findings of published reports were analyzed to understand present status of knowledge and to identify data gaps. For the first part, effects of ENMs on plant growth (in terms of root and shoot lengths and biomass yield) were studied. For the second part, risk characterization consisting of four steps: Hazard identification, Exposure assessment, Dose response assessment and risk estimation was used.

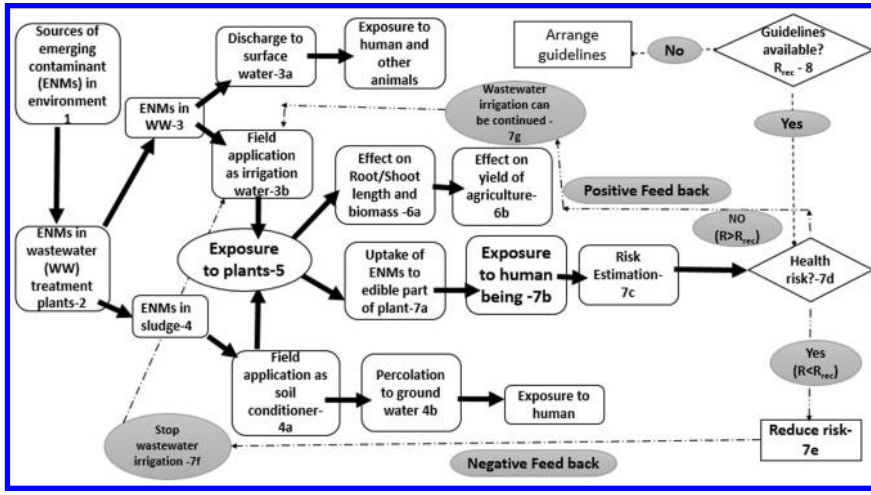

Figure 1. Proposed structuring for assessing implications of Engineered Nanomaterials on wastewater reuse (R_{rec} = Recommended value)

Part 1. Assessment of Toxic Effects of ENMs on Growth of Plants

This part addresses assessment of toxic effects of ENMs of growth of plants. It consists of review of findings of toxicity of ENMs to plants in full growth studies (parameters evaluated: biomass yield and root and shoot lengths). The sequence for understanding toxic effects of ENMs to plants is shown in Figure 1 *(sequence of nodes: 1-2-3-3b-5-6a-6b).*

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Studies focusing on toxicity of various NPs to plants were reviewed (Table 2). From this review, it became apparent that knowledge gaps exist in following three areas. The first gap is lack of use of exposure conditions similar to the exposure conditions studied for environmental exposure to toxic compounds. In most of the studies, effect of lower concentration of ENMs (concentration <10mg/L) on plant growth has not been discussed. Studies have used higher concentration to observe effects, however, these findings do not provide information about toxic effects of exposure of low concentration of ENMs from environmental water to plants. As reported environmental concentrations of ENMs in wastewater effluent and stream water are below 10 mg/L, it is highly probable that plants might be exposed to ENMs at low concentrations. Thus, use of low concentration of ENMs in toxicity studies could aid in knowing toxic effects to plants exposed to environmentally relevant concentrations. Secondly, effect on ENMs exposure on change in plant biomass (an important parameter of agricultural yield) has not been studied in most of the studies. Information on change in biomass due to exposure to ENMs could aid in understanding overall impact of using ENMs-contaminated wastewater as irrigation water on plant growth and productivity. Thirdly, some of the toxic studies have used soil media while other studies have conducted toxicity studies in hydroponic/water media. As wastewater irrigation is generally done with plants grown in soil, findings of toxicity studies using soil medium is more representative of field conditions and useful as compared to that obtained using hydroponic/water media.

Overall, there is a need for conducting detailed toxic studies for following additional conditions: (1) Use of environmentally-relevant ENM concentration in water (<10mg/L), (2) Use of toxic studies using soil as medium, (3) Using biomass yield as one of the parameters to assess toxic effects. This approach could provide information on toxic effects of ENMs on plant growth and help us in deciding the suitability of using wastewater contaminated with ENMs for irrigation purposes.

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Table 2. Summary of studies reporting toxicity of ENMs on plants

NPs/Plant	*Conditions*	*Findings*
TiO_2 and ZnO (< 100 nm)/ *Triticum aestivum* (*10*)	8–9 weeks exposure; 0, 50, or 500 mg/kg (dry weight) of ZnO; Medium: Garden soil (pH=7.36)	In case of control, shoot biomass was 320 g higher than that grown with TiO_2 NPs (277.1 g) and ZnO NPs (295.7 g).
CeO_2 NPs (8nm)/ *Coriandrum sativum* (*7*)	30 days exposure; 0, 62.5, 125, 250, and 500 mg/kg in Millipore water; Medium: Garden soil (pH=7.4)	At 125 mg/kg: significantly longer shoots (5.2 cm) than control plants (~4.9cm); significantly larger roots (~12.9 cm) compared with the other treatments (~7.0 cm); Biomass production at 125 mg/kg was statistically higher only compared to the 250 mg/kg treatment.

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Table 2. (Continued). Summary of studies reporting toxicity of ENMs on plants

NPs/Plant	*Conditions*	*Findings*
AgNPs (8 ± 1 nm)/ *Arabidopsis thaliana* (*8*)	2 weeks exposure; 50 mg/L, 100 mg/L, and 200 mg/L in DI water. Medium: Murashige and Skoog agar plates	Root length of seedlings exposed to 0.5 and 3.0 mg/L AgNPs were 75.4% and 34.5% for 1st week and 91.67% and 58.6% for 2nd weeks. After exposure to 3 mg/L AgNPs for one and two weeks, the fresh weight of the seedlings was reduced to 57.3% and 46.1% of the control, respectively, and was not affected by exposure to 0.2 and 0.5 mg/L.
ZnO/*Glycine max* (*9*)	8–9 weeks exposure; 0, 50, or 500 mg/kg (dry weight) of ZnO OECD standard soil (pH=4.8 to 5.2)	Root length: decreased by 10% and 89% percent for 50 and 500 mg/kg ZnO NPs treatments respectively. The EC50 value of root and shoot length was 160 and 227 mg/kg.
MWCNT, Ag, Cu, ZnO, Si/ Zucchini (*11*)	14 days exposure; 1000 mg/L Medium: 25% Hoagland solution	Cu NPs reduced root length by 77% and 64% as compared to control; biomass was reduced to 60%, 78-90% and 75% for MWCNT, ZnO NPs and AgNPs respectively. Exposure of AgNPs at 500 and 100 mg/L decreased plant biomass by 57% and 41% respectively.
MWCNT/ red spinach, lettuce, rice, cucumber, chili, lady's finger, and soybean (*12*)	15 days exposure; 0, 20, 200, 1000, and 2000 mg/L in DI water Medium: Hydroponic culture	Reduction in (a) shoot fresh weight of red spinach, lettuce, rice, and cucumber plants at 2000 mg/L: 88%, 63%, 46%, and 36%, respectively (b) root fresh weight for red spinach and lettuce at 2000 mg/L declined 81% and 79% respectively. (c) Shoot length for Red spinach, lettuce, cucumber and rice shoot heights at 2000 mg/L exposure decreased by 80%, 50%, and 66%, 48% (200 mg/L) respectively (d) root lengths of red spinach and lettuce at 2000 mg/L exposure were 67% and 45%, respectively.

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Table 2. (Continued). Summary of studies reporting toxicity of ENMs on plants

NPs/Plant	*Conditions*	*Findings*
CeO_2 (~8nm) *Phaseolus vulgaris* (*13*)	15 days exposure; 62.5, 125, 250 and 500 mg/L were prepared in modified Hoagland nutrient solution (NS) (pH 5.8)	In this study, no apparent signs of toxicity such as stunted growth, mortality, chlorosis, or wilting were observed.
CuO (~50 nm) and ZnO (~100 nm), deionized water (pH=7.5) *Triticum aestivum* (*14*)	14 days exposure; 500 mg metal/kg sand Commercial white silica sand	Shoot length was reduced by 13 % and 8% by CuO and ZnO NPs respectively. Root length was reduced by 59 % for the CuO NPs, and 53 % for the ZnO NPs.

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Part 2. Estimation of Health Risks

This part addresses estimation of health risks of exposure to ENMs during consumption of edible portion of plants irrigated with wastewater. The sequence for understanding effects of ENMs exposures on health is shown in Figure 1 *(sequence of nodes: 1-2-3b-5-7a-7b….7g)*. This part consists of (1) compilation of information on uptake of ENMs by plants and (2) use of human health risk assessment (HHRA) structure for assessing health risks. Information on uptake of ENMs by plant is compiled which is used in estimating amounts of ENMs ingested during consumption of edible portion of plants irrigated with ENM-contaminated wastewater. The HHRA structure includes hazard identification, exposure assessment, dose-response effects assessment, risk estimation and characterization and risk management.

Uptake of ENMs by Plants

Studies focusing on uptake of various NPs in plants during full growth were compiled and analyzed (Table 3). Review of studies indicated following three data gaps. Firstly, none of the studies have reported uptake in plants during full growth of plant. Secondly, very few studies have used NPs concentrations less than 10 mg/L in uptake studies. As ENMs have been reported up to 10 mg/L in environmental water, findings of these studies do not provide information on uptake of ENMs in plants in field conditions. Thirdly, studies have reported uptake in terms of ion contents and not in terms of NPs concentrations (*10*). Uptake of ENMs by plants is a very recent field of study and most of the data corresponds to the germination stage only. Studies have used various characterization methods to qualitatively indicate presence of NPs in plants but they have not quantified concentrations of NPs in terms of mass concentrations of NPs or number concentrations of NPs. As protocol for quantification of NPs inside the plant (especially edible part of plant) is not yet well-defined, the discussion is mainly oriented towards uptake

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in the form of metal ions. As NPs and ions have different toxicities to human (*15*), information about uptake in terms of NPs and ion concentrations in plant are required so that these concentrations values could be used in estimating NPs dose during consumption of edible portion of plant.

Table 3. Summary of studies reporting uptake of ENMs in plants

References/ NPs/ Plant	*Conditions*	*Findings*
CeO_2 *Zea mays* (*16*)	30 days exposure; 100, 200, 400, and 800 mg NPs/kg soil in deionized water (pH=7); Garden Soil	Uptake reporting as metal content; at highest exposed concentration (800 ppm), concentration of Ce in root for organic soil was around 150 mg/kg dry weight and for shoot 6 mg/kg dry weight of corn biomass.) Minimum Concentration showing uptake is 100 mg/ kg soil.
CeO_2 (7 nm and 25 nm) (pH=7) *Cucumis sativus* (*17*)	14 days 2, 20, and 200 mg/L Modified half-strength Hoagland's solution	For 20 mg/L of ceria NPs, leaves portion accumulated about 33.7 ng/gram fresh weight of plant tissues 25 nm size and 140 ng/gram fresh weight of plant tissues 7 nm size. Minimum Concentration showing uptake in shoot is 2 mg/L.
CuO (43 ± 9 nm) *Oryza sativa* (*18*)	2 weeks 100 mg/L Hydroponic (half-strength nutrient solution) (pH=7)	The Cu contents in the mature leaves, stems, and young leaves exposed to 100 mg/L CuO NPs were nearly 4.3, 2.3, and 1.9 times greater than those of the control plants, respectively.
CuO (20–40 nm) *Zea mays* (*19*)	2 weeks 2, 5, 10, 20, 30, 40, 50, 100 mg/L Hydroponic (25% strength nutrient solution) (pH=6.8)	The Cu content of plants exposed to 100 mg/L CuO NPs was 3.6 times for root and was 7 times higher than the control. Minimum concentration showing uptake is 100mg/L.
ZnO *Zea mays* (*21*)	30 days 100, 200, 400, and 800 mg NPs/kg soil (pH=7-8) Garden soil	The uptake (mg/kg) of Zn by one-month old corn plants varied from 69 to 409 in roots and from 100 to 350 in shoots, respectively, in soils contaminated with different concentrations of ZnO NPs (from 100 to 800 mg NPs/kg soil). Minimum concentration showing uptake is 100mg/L

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Table 3. (Continued). Summary of studies reporting uptake of ENMs in plants

References/ NPs/ Plant	*Conditions*	*Findings*
Fe@ZnO (22nm) *Pisum sativum* (*22*)	25 days 10% Fe@ZnO NPs at 0–500 mg/kg Soil culture (pH of 8.39 ± 0.3)	In stem, Zn uptake increased by 31%, 36%, and 48% at 125, 250, and 500 mg/kg treatments respectively, compared to control. Leaves, only 500 mg/kg treatment showed significantly higher Zn (72%) than that of control. The rate of translocation of zinc (i.e., zinc concentrations in stem to those in roots) for control, 125, and 500 mg/kg treatments were: 0.73, 0.25, and 0.20.
ZnO *Vigna unguiculata* (*20*)	4 weeks Hydroponic pH 6.1 (25 mg/L) Garden soil culture pH 6.7 (500 mg/L)	Zn concentrations of roots (1003 µg/g DM), stems (108 µg/g DM), leaves (155 µg/g DM), or seeds (43.3 µg/g DM) for ZnO-NP treatments. As a consequence, the Zn transfer coefficient (i.e., the ratio of Zn in the leaf relative to the root) was 4.7-times lower in the ZnO-NP treatment. Transfer coefficients in soil culture (0.155 and 0.154) were substantially higher than those in solution culture.
TiO_2 (7 nm) and Ag (10-15 nm) *Lycopersicon Esculentum* (*23*)	Five weeks 0, 50, 100, 1000, 2500 and 5000 mg/L Soil culture	Tomato fruits TiO_2 at 1000 mg/L and 5000 mg/L shown Ti uptake as 2.6±0.2 and 3.1±0.4 mg/g dry weight. For AgNP at 1000 mg/L and 5000 mg/L shown Ag uptake as 0.4±0.2 and 3.7±0.0 mg/g dry weight.
Fe_3O_4 20 nm *Cucurbita maxima* (*24*)	20 days 500 mg/L Hydroponic culture	The strongest magnetization (27.64×10^{12} Fe_3O_4 particles/g) was detected right above the roots, which might be due to NP agglomeration and no magnetization signal was detected from the control plant.
CNTs 239.70nm *Oryza sativa* (*25*)	6 months 20 to 800 mg/L Soil culture, pH 5.7	Uptake of C_{70} harvested stem, leaves and seeds was found to be around 60%, 30% and 20% at 20 mg/L of exposure concentration Robust transport from roots to leaves as no C_{70} found in roots.

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Human Health Risk Assessment

Hazard Identification

This step involves compilation of health effects due to ENMs exposure. Toxicity of ENMs to different organs depends on parameters, such as toxicity, size, shape, surface area and chemistry, porosity, reactivity in different matrix, chemical composition, availability, usage, toxicokinetics, and toxicodynamics physicochemical properties (*26*). Information about health effects due to ENMs are obtained from different governmental agencies, such as OECD, USEPA and US. Federal Environmental, Health, and Safety (EHS) and toxicity studies.

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Exposure Assessment

This step involves estimation of exposed dose of NPs. For this, first scenario of exposure is defined and characterized. Figure 1 shows different parts leading to exposure of ENMs during consumption of edible portion of plants. Exposure dose is estimated in terms of average daily dose (ADD) (mg/kg of body weight/day). It is defined as ENMs ingested every day through oral exposure route and calculated using Eq. (1) as per the USEPA guidelines (*27*):

$$ADD = \frac{IR \times ED \times C_{edible}}{BW \times AT} \quad (1)$$

Where IR is daily ingestion rate of edible portion of plants (mg/day), ED is exposure duration for risk assessment (years), C_{edible} is concentration of ENMs in edible potion of plants (mg/kg dry weight of plant), BW is the body weight (kg) and AT is the averaging time for toxic effects (days). Here C_{edible}, i.e., value of concentration of ENMs in edible portion of plant is taken from findings of uptake studies (for example, uptake data mentioned in Table 2).

Dose-Response Assessment

This step requires information about relationship of dose-response of ENMs for different target organs which is used in deciding about extent of toxic effects for a given exposure dose of ENMs. For ENMs with non-carcinogenic health effects, reference dose (RfD) (the lowest dose causing effect) values are required. For ENMs with carcinogenic effects, values of potency factors (PF) are required. This information is generally obtained from dose-response data of toxicity studies, conducted either using rats as animal models or animal or human cell lines. PF value is slope of the dose response curve at low dose values. RfD is calculated using Eq. 2, where point-of-departure (POD) value is obtained from dose-response curve. It is generally equal to either no-observable-adverse effect level (NOAEL) or low-observable-adverse effect level (LOAEL) divided by the

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uncertainty factors. NOAEL is generally used as POD and LOAEL is used as POD only if NOAEL value is unavailable. Uncertainty factors describe following five aspects: interspecies extrapolation, intraspecies extrapolation, short-term to long-term extrapolation, data quality, LOAEL to NOAEL extrapolation. This step consists of following knowledge gaps (*26*): (1) lack of availability of toxicity data using rats for different OECD-listed ENMs, (2) lack of availability of toxicity data using human cell lines, (3) lack of explicit guidelines in obtaining NOAEL value from dose-response data, (4) difficulty in assigning values to different uncertainty factors. If RfD is not available for a given NP-organ pair, TTD value (i.e., target organ toxic dose) is used (*28*).

$$RfD = \frac{POD}{UF_1 \times UF_2 .... \times UF_5} \quad (2)$$

Risk Estimation and Characterization

This step estimates risks of exposures of ENMs using dose-response information and exposure dose of ENMs. For ENMs giving non-carcinogenic health effect, hazard quotient (HQ) is calculated. For ENMs giving carcinogenic health effects, life time excess risk of cancer (LCR) is calculated. HQ is calculated by dividing average daily dose (ADD) to RfD value. HQ value less than 1, does not indicate the possibility of any health effect while the HQ value greater than 1 indicates a chance of having adverse health effects. LCR is calculated using chronic daily intake (CDI) and PF values.

$$HQ = \frac{ADD}{RfD} \quad (3a)$$

$$LCR = PF \times CDI \quad (3b)$$

Risk Management

This step is required to manage risks after the exposure event. Risk estimate of ENMs exposure is compared with recommended guideline values (Box 8, Figure 1) of different agencies, such as U.S. Environmental Protection Agency, WHO, Indian Central pollution Control Board, etc. to decide if risk is significant and needing attention. Currently, regulatory guidelines for ENMs for different exposure media are being prepared (*26*) and as this represent an important knowledge gap.

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Illustrative Example: Calculation of Health Risks of Hypothetical Exposures of TiO_2 and AgNPs from Consumption of Contaminated Tomato

A hypothetical case of exposures of TiO_2 and AgNPs due to consumption of contaminated tomato was considered and health risks was estimated. NP contents in tomato were determined and dose-response information about NPs were obtained from literature. It is assumed that reported metal uptake information in tomato represents NPs concentration (as uptake) in tomato. 'C_{edible}' is concentration of NPs in plants (mg/kg dry weight of plant) = Ti (2600 mg/Kg) and Ag (400 mg/kg) (*23*). Using these values, ADD was calculated to be 2.971 g/kg body weight/day for TiO_2 and 0.457 g/kg body weight/day for AgNPs (Table 4). Dose-response effect information for titanium dioxide and silver NPs were obtained from the study (*28*) (Table 4). This study calculated target organ toxic dose (TTD) of two types of NPs. TTD for titanium oxide NPs was found to be 0.00833 mg/L and TTD for AgNPs was found to be 0.00033 mg/L (*28*). Using these values, HQ values were calculated and found to be greater than 1 (Table 4), indicating the chance of adverse health risks.

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Table 4. Average daily dose and risk estimates of hypothetical exposures of TiO_2 and AgNPs during consumption of contaminated tomato (IR=80000mg/d; ED=1 year; BW=70kg; AT=1 year) (*29*)

Hazard (NPs)	*Concentration in NPs in tomato g/Kg (23)*	*ADD g/Kg/day (Eq.1)*	*TTD in mg/L (27)*	*HQ (Eq. 3a)*	*Risk (Yes If HQ>1)*
Ag	0.4	0.457	0.4	89.04	Yes
TiO_2	2.6	2.971	5.00E-03	10958	Yes

Identified Data Gaps in the Proposed Framework

Table 5 summarizes the data gaps identified in 3 steps of the proposed framework for conducting risk assessment. In step 1 of HHRA, i.e., primary issue regarding information on toxicity and uptake was lack of data on fate of ENMs in wastewater effluent and stream water, lack of use of soil as medium, and lack of use of biomass yield as one of the parameters to assess toxic effects. For step 2 (i.e., exposure assessment) identified issues were lack of uptake data in edible part of plant in the form of NPs and not in the form of ions, exposure duration and exposure frequency of contaminated vegetable/fruit. Finally, identified data gap for step 3, (i.e., dose- response assessment step) was the knowledge of effect of NPs exposure to human

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Table 5. Identified data gaps for each step of proposed schematic

Step of risk assessment	*Identified data gap*	*Suggestions for identified gap*	*References for implementing suggestion*
Toxicity and uptake data	Lack of (a) Use of environmentally-relevant ENM concentration in water (<10mg/L) (b) Fate of ENMs in wastewater effluent and stream water (c) Lack of use of soil as medium (d) Lack of use of biomass yield as one of the parameters to assess toxic effects	(a) Study on lower concentration range of NPs (b) Monitor and occurrence of NPs in wastewater effluent and in irrigation water coming from wastewater source (c) More studies on soil as media not hydroponic (d) Studies on effect of NPs on yield of plants	(*5*, *25*, *26*)
Exposure assessment	Lack of (a) uptake data in edible part of plant (b) Uptake data in terms of ions not NPs (c) Exposure duration and exposure frequency of contaminated vegetable/fruit	Study on (a) uptake data of NPs in edible part of plant (b) Uptake data in edible parts of plants in form of NPs needed as NPs and ions have different toxicities (c) Amount and frequency of exposure of contaminated part of plant to human	(*28–32*)
Dose response	Dose response of NPs exposure to human	Develop guidelines for regulating ENMs in environment; calculated maximum allowable concentrations of NPs	(*33–37*)

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Summary and Conclusion

The objective of this study was to understand effect of engineered nanomaterials in wastewater on reuse of wastewater as irrigation water. To investigate implications of using wastewater effluents as irrigation water with regards to ENMs in water, this study proposed a structure (Figure 1) which consists of following two aspects: (1) Assessment of toxic effects of ENMs on plant growth and (2) Estimating health risks due to exposures of ENMs from consuming edible parts of plants, irrigated with wastewater. Major identified data gaps (Table 5) were as follows: (1) Environmental concentration of NPs in river water is unknown, (2) Uptake data of NPs in edible part of plant is unknown as it is present in form of ions not in terms of NPs (This information is important as

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ions and NPs exhibit different toxicities), and (3) Dose- response relationship of NPs exposure to human is also not known.

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Chapter 8

Toxicity of a Mixture of Metal Oxide Nanoparticles on Activated Sludge

Baranidharan Sundaram and Arun Kumar*

Department of Civil Engineering, Indian Institute of Technology, New Delhi, India
***Phone: +91-11-2659-1166; Fax: (+91)-11-2658-1117;**
E-mail: arunku@civil.iitd.ac.in

The objective of this study was to understand the toxic effect of a mixture of metal oxide nanoparticles (NPs) on biological functioning of activated sludge. Two NPs (Ag_2O and TiO_2) were selected to illustrate the toxic effect of a mixture of NPs on biological functioning by performing 5-day biochemical oxygen demand (BOD_5) (concentration tested: 0.1, 1 and 10 mg/L), specific oxygen uptake rate (SOUR) inhibition and time-dependent chemical oxygen demand (COD) study (concentration tested: 1 mg/L; 5-h exposure duration). The mixture of NPs at 10 mg/L in solution pH inhibited SOUR by 34.17±2.4%. It was found to be more toxic to activated sludge (maximum reduction in BOD_5 =22% in comparison to control) than single NPs. The rank of toxicities of NPs, based on minimum concentrations, that yielded significant effects compared to the control was found to be the following: (least toxic) Ag_2O < TiO_2 < Mixture of TiO_2 and Ag_2O (most toxic). COD reduction in 5-h was found to be most affected by TiO_2 NPs (24% reduction) than the other two types of NPs (22%) when compared to the control. These findings indicate the effect of a mixture of NPs on biological functioning of activated sludge in a quantitative manner, for the first time as per author's knowledge. This information is useful to wastewater treatment plant operators in deciding about modification required to address issues of toxic effects of a mixture of NPs on biological treatment processes.

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Introduction

Nanotechnology promises great societal rewards, with potentially widespread application of nanoparticles (NPs) in various fields of industry (semiconductor, medicine) and daily life (consumer goods) (*1*). Due to their unique properties, NPs are incorporated into numerous household and personal care products. According to the nanotechnology inventory by the Project on Emerging Nanotechnologies (PEN), over 1,701 manufacturer-identified, nanotechnology-enabled products have entered the global commercial marketplace since 2005 (*2*). According to PEN, the most commonly found NPs in consumer products are silver (Ag), titanium dioxide (TiO_2), carbon (C_{60}) zinc oxide (ZnO), copper oxide (CuO) and gold (Au^0). These NPs are mostly found in sunscreens, paints, paper, plastics, food fungicides and cosmetics (*3–5*).

Due to large scale production and usage, a significant fraction of NPs reach wastewater treatment plants (WWTPs) (*6*). Currently, WWTPs play an important role by controlling the release of NPs into the environments (e.g. into surface waters via effluent discharges and to land via sewage sludge disposal) (*7*). Still, quantification of NP concentrations in the environment is completely missing. One study modeled and predicted the environmental concentrations of 10 ng/L in natural waters (*8*). However, real-time information on concentrations of NPs in natural waters is still unavailable. Recently it was found that while washing nanosilver coated socks, a considerable fraction of Ag NPs gets easily released into wastewater (*9*, *10*). Another study found the occurrence of TiO_2 NPs in treated effluent from different WWTPs in the USA at concentrations ranging from <5 to 15 µg/L (*11*). In addition, recent studies of WWTPs in Germany found that Ag NP concentrations in influent wastewater varied between 0.06 and 1.5 µg/L (*12*). Further, field studies discovered that Ag NPs got transformed to silver sulfide particles with the individual particle size ranging between 5–20 nm (*13*). Although WWTPs are the last barriers prior to the release of NPs into the environment (i.e. lakes, rivers, streams, oceans), little information is currently available about the fate of NPs in WWTPs (*14*). From these studies, it can be inferred that there are no regulatory guidelines on NP application and release due to the risk-based policy formulation approach of "no data, no regulation" (*15*).

NPs differ in chemical composition, shape, size, density, aggregation, and surface charge, and can differ substantially from those of larger counterparts. Further, it is not clear that these different properties require a new rigorous environmental risk assessment compared to their bulk materials (*16*). Currently, an abundance of literature is available on NP synthesis and characterization at nanoscale, yet the associated environmental risks have largely been ignored.

The effect of NPs on microorganisms in wastewater is not yet fully understood. Table 1 presents a summary of various studies attempting to understand the effect of NPs on microorganisms in both aerobic and anaerobic processes. Studies in the past have been conducted to examine the potential impacts of NPs on wastewater treatment in a conventional activated sludge (CAS) processes (*17–20*). Studies conducted in a lab scale aerobic treatment process using synthetic wastewater reported removal of around 94 % CeO_2 NPs (*21*).

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When exposed to anaerobic sludge, C_{60} fullerenes had no significant effect on anaerobic microbial structures at the concentration of 50 g/kg of biomass (*14*). In a sequencing batch reactor, the removal efficiency of TiO_2 NPs was reported to be 88% (*11*). Recently, researchers have found that Ag NPs form new products such as Ag_2S when adsorbed to aerobic sludge (*22*). Whereas other studies found that 30 mg/L ZnO NP was sufficient to inhibit CH_4 production by 18.3% (*23*). Further studies have confirmed that concentrations of Ag NPs up to 40 mg/L did not cause any effect on CH_4 production and methanogens while no Ag^+ ions were released from Ag NPs under anaerobic conditions (*24*). While investigating the effect of three different NPs on mesophilic and thermophilic anaerobic bacteria, it was found that CeO_2 NPs were more toxic than Au and TiO_2. EC_{50} values (concentration giving 50% toxic effects) of CeO_2 NPs for mesophilic and thermophilic biomass equaled 0.26 mg/mL and < 0.32 mg/mL, respectively (*25*).

Overall, these investigations in Table 1 provide insights about the behavior of NPs during wastewater treatment. Researchers are currently working towards understanding the effects of NPs on pure bacterial cultures and microbial communities and their impacts on biological functioning of activated sludge. However, knowledge about the effects of mixtures of NPs on microbial communities and their possible impacts on biological functioning of activated sludge is not available. Several studies in the past have investigated the effect of individual NPs on both aerobic and anaerobic microorganisms, but still the effect of mixture of NPs towards microorganisms have not been studied extensively.

The effect of mixtures of NPs on microorganisms needs to be addressed, as most of the studies have only focused on studying individual effects of NPs on microorganisms. Exposure of bacterial communities to mixtures of NPs is a realistic exposure scenario compared to the exposure of individual NPs. In the past, researchers have studied the toxic effect of mixtures of ions on aquatic species and reported that the mixture toxicity of metals depends on dissolved metal concentrations and ratios, as well as the background solution composition (i.e., temperature, pH, and concentrations of major ions and dissolved organic carbon) (*39*). Further it was also reported that mixtures of chemicals can possibly have greater adverse impacts than an individual constituent of a mixture (*40*) . Considering these aspects, it is important to understand the effect of mixtures of NPs on the bacterial communities.

The overall objective of this study was to understand the effect of a mixture of metal oxide nanoparticles on biological functioning of activated sludge. Two NPs (Ag_2O and TiO_2) were selected to illustrate the toxic effect of a mixture of NPs. Specific oxygen uptake rate (SOUR), 5-d biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) were measured to understand the effect. The findings of this study could provide the quantitative estimate of toxicity of a mixture of NPs to activated sludge.

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Table 1. Summary of studies focusing on toxic effects of NPs on biological activities

Ref	*NP/Size (nm) /Dosage*	*Process/Operating Conditions*	*Findings*
(*26*)	CeO_2/55 mg Ce/L	Lab scale activated sludge (A/S) system/70 days	At 950 mg/L: 50% microbial inhibition.
(*27*)	Ag	Membrane Bioreactor/60 days	No effect on membrane fouling rate; extracellular polymeric substances (EPS) concentration increased significantly after NP dosing. No effect of Ag NPs (< 0.10 mg/L) on activated sludge.
(*28*)	Al_2O_3	Nutrient removal/70 days	At 50 mg/L: Total nitrogen (TN) removal decreased from 80.4% to 62.5%, and NPs decreased denitrifying bacteria in activated sludge. Al_2O_3 NPs had a marginal effect on wastewater phosphorus removal.
(*29*)	Nano ZnO, bulk ZnO and Zn ion	Activated sludge	> 40 mg-Zn/L: soluble Zn exhibited greater toxicity than nano-ZnO; nano-ZnO had more toxicity than bulk ZnO. The maximum inhibition rates for nano-ZnO and soluble Zn were about 80%, while that for bulk ZnO was only about 40%. Concentration for 50% inhibition (IC_{50}): 164 (ZnO) and 59 (soluble Zn) mg-Zn/L. No IC_{50} for bulk ZnO up to 803 mg-Zn/L.
(*30*)	ZnO/1 mg/L	Lab scale SBR/120 days	At 1 mg/L: NPs did not impact phosphorus, nitrogen and COD removal. ZnO NPs affect the settling ability of activated sludge.
(*31*)	Ag NPs (14 nm), Ag^+ Ions, AgCl colloids (0.1 to 2 μm)/1 mg/L of Ag	Nitrifying Bacteria and *E.coli*/Batch Mode	On nitrifying bacteria: Ag NPs were more toxic (86 %) than ionic Ag (42 %). On *E.coli*: Ag^+ ions were more toxic (100 %) than Ag NPs (54%).

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Ref	*NP/Size (nm) /Dosage*	*Process/Operating Conditions*	*Findings*
(*32*)	Ag NPs (29 nm)/1 mg/L	Autotrophic and Heterotrophic bacteria/30 days	At 1 mg/L nitrification inhibitions: 41.4 ± 13% for Ag NPs and 13.5 ± 6.7% for Ag^+ ions. No variations in COD values before and after shock load. *Nitrospira*, experienced population decrease while *Nitrobacter* was washed out completely. Nitrification inhibition lasted for more than one month after a 12-h shock loading of Ag NPs to reach a final peak Ag^+ concentration of 0.75 mg/L in activated sludge treatment system
(*33*)	Ag NPs/1,50, 200 mg/L	Activated sludge system/1 day	Ag^+ ions were more toxic than Ag NPs. Inhibition towards biofilm: At 200 mg/L but not at 1 and 50 mg/L. After 24-h treatment at 200 mg Ag/L, no significant change was detected in the viability of heterotrophic bacteria.
(*17*)	TiO_2 NPs/1 and 50 mg/L	Activated sludge (SBR)/70 days	Short term exposure (1 day): 50 mg/L had no influence on nitrogen and phosphorus removal. Long-term exposure (70 days): 50 mg/L significantly decreased the TN removal from 80.3% to 24.4%, whereas phosphorus removal was unaffected. Ammonia-oxidizing bacteria highly decreased at 50 mg/L.
(*18*)	Ag, TiO_2, Fullerenes/0.5-2.5 mg/L	Activated sludge/150 days	No effect of nanomaterials on COD removal; After 4 months of continuous exposure of C_{60}: removal of nanomaterials was dependent on biomass concentration. After 15 days of exposure: 98% TiO_2 NP removal was obtained in a sequential batch reactor (SBR).
(*34*)	ZnO/34.5 mg Zn/L	Long Term effect on lab scale UASB reactor and Short term effect on methanogens/75 days	Decline in the CH_4 production and the removal of acetate and propionate. At 34.5 mg of Zn/L: Complete inhibition of CH_4 production was observed after 7 days of operation. Batch toxicity assays were not suitable to predict the long-term inhibitory effect of sub-ppm ZnO NPs concentrations.

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Table 1. (Continued). Summary of studies focusing on toxic effects of NPs on biological activities

Ref	*NP/Size (nm) /Dosage*	*Process/Operating Conditions*	*Findings*
(*35*)	CuO/ 37 /1.4 mg Cu/L	Long Term effect on lab scale UASB reactor and Short term effect on methanogens /107 days	At 1.4 mg Cu/L: CH_4 production decreased by more than 50%; inhibition to acetoclastic and hydrogenotrophic methanogens was reported to be at 6% and 16% and at 19 mg Cu/L the inhibition to both methanogens was reported to be nearly 50%.
(*36*)	Ag^0, Al_2O_3, CeO_2, Cu^0, CuO, Fe^0, Fe_2O_3, Mn_2O_3, SiO_2, TiO_2, and ZnO at 1500 mg/L	Inhibitory effect of acetoclastic and hydrogenotrophic methanogens /Batch Mode	Cu^0 and ZnO NPs caused high levels of inhibition to both the acetoclastic and hydrogenotrophic methanogenic activity. CuO NP caused high toxicity to acetoclastic methanogens. IC_{50}: For acetoclastic methanogens were 62, 87, and 223 mg/L for Cu^0, ZnO, and CuO NPs, respectively. For hydrogenotrophic methanogens were 68, 250, and >1500 mg/L, respectively. $Cu2^+$ and $Zn2^+$ salts caused similar levels of inhibition as Cu^0 and ZnO NPs thus suggesting that the toxicity was due to the release of metal ions by NP-corrosion.
(*37*)	Nano-TiO_2, nano-Al_2O_3, nano-SiO_2 and nano-ZnO	Waste activated sludge Anaerobic digestion/18 days	Up to 150 mg/g-TSS, Nano-TiO_2, nano-Al_2O_3 and nano-SiO_2 showed no inhibitory effect, whereas nano-ZnO showed inhibitory effect with its dosages increased. CH_4 generation was the same as that in the control when in the presence of 6 mg/g-TSS of nano-ZnO, however, which decreased respectively to 77.2% and 18.9% of the control at 30 and 150 mg/g-TSS.
(*38*)	ZnO NPs/10-50 (mg/g TSS)	Anaerobic granular sludge (AGS) /Batch Mode	CH_4 production decreased to 74.9% of the control, further decreased to 56.5% of the control as the dosage of ZnO NPs was increased to 200 mg/g-TSS. AGS was exposed to the released $Zn2^+$. It was observed that CH_4 production was 80.8% and 62.4% of the control at 30.5 and 40.9 mg/L of $Zn2^+$, respectively. ATP content decreased with the ZnO dosage, in parallel to the decrease of the methanogenic activity.

Materials and Methods

Nanoparticles

Silver (I) oxide (Ag_2O) and Titanium dioxide (TiO_2) were purchased from Sigma-Aldrich, St. Louis, Missouri, USA (Ag_2O Purity: 99%, CAS Number: 20667-12-3, color: dark grey; TiO_2 Purity: 99.7 %, CAS Number 1317-70-0, color: white). NP stock suspensions (100 mg/L) were prepared in deionized water and sonicated for 15 minutes. Three different NP concentrations (0.1, 1, and 10 mg/L) were chosen based on the previous observation in a WWTP (*10*, *41*). Higher concentration of 10 mg/L was chosen considering the potential increase in release of NPs in future. Also, scientists recommend that a much higher concentration should be investigated to understand the toxicity of NPs (*14*).

Z-average size of Ag_2O and TiO_2 NPs in ultrapure water at 10 mg/L were measured by collecting 4.5 mL of NP sample in polystyrene cuvettes which were then placed inside the dynamic light scattering-based particle size analyzer (Nicomp 380 ZLS-Particle sizing system). The z-average diameter values of Ag_2O and TiO_2 NPs in ultrapure water were found to be 301.03 ± 33.2 nm and 614.4 ± 158.47 nm, respectively. X-ray diffraction measurements of Ag_2O and TiO_2 NPs show Ag_2O to be in cubic form and TiO_2 NPs in tetragonal form. The specific surface area of Ag_2O and TiO_2 NPs were analyzed by Micrometrics ASAP 2010 instrument at 77 K using the Brunauer–Emmett–Teller (BET) method. The specific surface areas of Ag_2O and TiO_2 NPs were found to be 57.6 and 74.23 m^2/g, respectively.

Activated Sludge

Activated sludge was obtained from an aeration tank of a local sewage treatment plant in South Delhi, India (capacity: 14 million liters per day). The sewage treatment plant was based on the extended aeration process and sampling was carried out for a period of 3 months from March 2013 to May 2013 (three sampling events). All analyses were carried out as per the Standard Methods (*42*). The following characteristics of activated sludge were determined (average and one standard deviation of three replicates are presented) pH: 7.82 ± 0.11; turbidity: 617 ± 17.55 NTU; COD: 650.33 ± 19.85 mg/L; BOD_5: 261.33 ± 15.87 mg/L; dissolved oxygen (DO): 2.63 ± 0.32 mg/L; mixed- liquor suspended solids of aeration tank (MLSS): 3863 ± 310 mg/L. These characteristics of sludge were found to be consistent with that reported in a previous study (*43*). All chemicals used were of analytical grade. pH was adjusted using HCl and NaOH solutions. Ionic strength (IS) of solution was maintained using NaCl.

Methods

Specific Oxygen Uptake Rate (SOUR)

The impact of NPs on sludge was assessed in terms of oxygen uptake rate (OUR). The activated sludge sample collected from the treatment plant was aerated with pure oxygen for a period of 2 hours, and 300 mL of sample was placed inside

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a BOD bottle along with the respective NP concentration (0.1, 1 and 10 mg/L). In order to ensure that the experiment was not substrate limited, 100 mg/L of soluble COD was added inside the BOD bottle prior to the analysis. DO readings were continuously monitored using a dissolved oxygen probe (Hach HQ 40d, USA) equipped with a magnetic stirrer. DO readings were measured every 1 minute up to 20 minutes or until the DO concentration reached less than 1 mg/L for all NP concentrations tested. Control tests were also performed where activated sludge samples were not spiked with NPs. Similar tests were carried out at NP solution pH of 5 and 10. In addition, IS of the NP solution was also varied for two levels: 0 M and 0.1 M. All the experiments were conducted in triplicates and at room temperature. The OUR was determined from the absolute value of the slope of the linear portion of DO reading versus time plot. SOUR was then calculated by dividing the OUR with mixed liquor volatile suspended solids (MLVSS) concentration. SOUR inhibition was calculated using the following equation (Eqn.1.):

$$SOUR_{inhibition}\% = [1-(SOUR_{NP}/SOUR_{control})] \times 100 \quad (1)$$

where $SOUR_{NP}$ is the SOUR of the mixed liquor in the presence of a given NP concentration, and $SOUR_{control}$ is the average SOUR without any NP addition.

BOD_5

The effect of NPs on oxygen consumption capacity of activated sludge was studied using BOD_5, which is a commonly used test to examine the biodegradability of wastewaters. The effect was studied by varying the NP concentration (0.1, 1 and 10 mg/L), NP solution pH (5 and 10), and IS of the NP solution (0 and 0.1 M). The test was carried out by preparing high quality organic free dilution water (adding 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions to a liter of distilled water) which was aerated for at least 12 hours and allowed it to get stabilized at 20˚C for 4 hours until it got saturated with oxygen. Then the activated sludge was added at the rate of 3 mL per 300 mL of dilution water into a 300 mL BOD bottle. Pre-sonicated NP stock solution was then dispersed into the BOD bottle to achieve the desired target NP concentrations. Further, NP solution already adjusted for pH and IS was added inside the BOD bottle. For all of the combinations, initial DO was measured on the same day (0^{th} day) while final day samples were incubated at 20˚ C for the period of 5 days. All experiments were conducted in triplicates. BOD_5 was calculated using 0^{th} and 5^{th} day DO measurements. For every experiment, percent decrease in BOD_5 values with regard to control (i.e., samples without exposure to NPs) was calculated and used as an indicator of NP toxicity to activated sludge. Obtained BOD_5 values for different samples were expressed as average ± standard deviation and compared using a student's t-test for $p \leq 0.05$ (95% confidence level). The minimum concentration (MC) giving statistically significant BOD_5 reduction with regards to control was calculated.

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Time-Dependent COD Removal Study in Biological Reactors

NPs effect on activated sludge was also studied in biological reactors for a period of 5 hours by measuring COD values at different time periods. Reduction in COD values with time was used as a metric to understand the effect of toxicity of NPs on COD. Activated sludge was exposed to the desired NP concentration in 250 mL volumes conical flasks. Two out of four reactors (R1 and R2) were used as control reactors (no exposure of NPs). Except for control reactors (R1 and R2), all other reactors (R3 to R5) were spiked with Ag_2O NPs, TiO_2 NPs, and a mixture of both NPs at 1 mg/L concentration. Every reactor was filled with 100 mL of fresh activated sludge taken from a previously well-established aerobic reactor in the laboratory. The fresh activated sludge had an initial COD of 102 ± 18 mg/L. All reactors were aerated continuously for 5 hours to ensure proper DO concentration at a rate of approximately 0.25 L/min. During the study, pH of biological reactors remained constant at pH=7.7. Up to 5 hours, samples were collected at the every 1 hours and analyzed for COD, pH and DO. For every sampling period, % reduction in COD values with respect to initial COD values was calculated. These values were then plotted with sampling time to study trends of reduction in COD due to the effect of NPs with time. Data follows a linear model, and slope, intercept, and coefficient of determination (R^2) values were calculated. Slope values indicate the rate of change of % COD reduction with time.

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Results and Discussion

Effect on Specific Oxygen Uptake Rate

Figure 1 shows specific oxygen uptake data for different NPs. SOUR was found to vary with NP type and concentration. SOUR was found to decrease with NP concentration. DO data was found to be linearly dependent on time (R^2>0.98). The maximum inhibition was found to be 34.17±2.4% during exposure of the mixture of NPs (at 10 mg/L concentration at solution pH) to sludge. Among the individual NPs, TiO_2 NPs were found to be more toxic than Ag_2O NPs (at 10 mg/L). During exposure of 0.1 mg/L NPs, both Ag_2O NPs and the mixture of NPs resulted in 5±1 % inhibition, whereas TiO_2 NPs showed greater inhibition (11.11±1.4 %).

Oxygen uptake inhibition was found to vary with solution pH. It was found to be maximum in alkaline compared to acidic conditions. The mixture of NPs at 10 mg/L imparted higher inhibition at both pH conditions (pH=5 and pH=10) but inhibition was found to be maximum in alkaline pH (inhibition at pH=5: 18.5 ± 1.6%; inhibition at pH=10: 35.2 ± 2.1%). Among NPs, TiO_2 NPs at 0.1 mg/L were found to inhibit more in acidic solutions. At 1 mg/L NPs concentration, the mixture of NPs was found to inhibit more in alkaline pH. At 10 mg/L, the mixture of NPs gave 1.9 times more inhibition in alkaline pH than in acidic pH. After adjusting the NP solutions to 0.1 M, SOUR values were found to be different for the combinations tested. Among the pH conditions tested, 10 mg/L TiO_2 NPs in alkaline pH was found to inhibit oxygen uptake more than other NPs.

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Different NPs were found to have different levels of inhibition on OUR of activated sludge ($p < 0.05$). Irrespective of pH and IS values, OUR was found to decrease with increasing NP concentration. Similar observations have also been confirmed by other researchers (*44*). In the past, researchers have observed that the OUR of the flocculent sludge was inhibited by Ag NP concentrations as low as 1 mg/L, while that of the granular sludge was only affected at much higher Ag NP concentrations (50 and 100 mg/L) (*45*). Further studies found that silica NPs at 1 mg/L affect the activated sludge process through inhibition of oxygen uptake and smaller size NPs are stronger inhibitors than larger size NPs (*46*). With these experiments, the mechanisms behind the inhibition by respective NPs were clearly unexplainable, but it can be concluded that the decrease in total oxygen uptake was significantly associated with respective NPs coming into contact with microbial cells. Further, this study reported higher inhibition due to a mixture of NPs than individual NPs (TiO_2 and Ag_2O NPs), indicating higher toxicity. This aspect needs to be further investigated to improve understanding.

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Effect on BOD_5

Figure 2 shows % reduction in BOD_5 values of solutions containing NPs. At various solution pH, Ag_2O NPs at 10 mg/L induced higher toxicity (18.18% reduction) than that at other NP concentrations (0.1 and 1 mg/L) ($p < 0.05$). For other Ag_2O concentrations (0.1 and 1 mg/L), BOD_5 values were found to be comparable to that of control ($p > 0.05$), indicating no effect of concentration on BOD_5. Exposure of 10 mg/L Ag_2O NP concentration resulted in higher toxicity to activated sludge than 1 mg/L Ag_2O NP concentration ($p < 0.05$).

TiO_2 NPs gave statistically significant lower BOD_5 values than control ($p < 0.05$). Compared to control, 10 mg/L TiO_2 NP resulted in 19.19% reduction in BOD_5 values ($p < 0.05$). At 1 mg/L, TiO_2 NPs were found to be more toxic than Ag_2O NPs ($p < 0.05$).

The mixture of NPs at 10 mg/L resulted in higher reduction in BOD_5 values (22%) compared to that of individual NPs (Ag_2O NPs: 18.18 % and TiO_2 NPs: 19.91%). At 1 mg/L, the mixture of NPs resulted in statistically different BOD_5 reduction than that of control (14.29% reduction). These results in general were found to be consistent with the findings of SOUR experiments.

A comparison of MC values of NPs provided insights about relative toxicities of solutions containing two individual NPs and their mixture. At solution pH, the MC value was found to lie between 1 and 10 mg/L for Ag_2O NPs and between 0 and 0.1 mg/L for TiO_2 NPs. The difference in observed values of MC for the two NPs indicated that Ag_2O NPs are less toxic to activated sludge than TiO_2 NPs. For the mixture of NPs, the MC value was found to lie between 0.1 and 1 mg/L, indicating that the mixture of NPs is less toxic than TiO_2 NPs but more toxic than Ag_2O NPs. Using MC values, the two NPs and their mixture were ranked in following manner: Ag_2O (lowest toxicity) < mixture of TiO_2 and Ag_2O < TiO_2 (highest toxicity). Overall, it indicates that TiO_2 NPs impart the highest reduction in BOD_5 values of activated sludge and need to be considered first.

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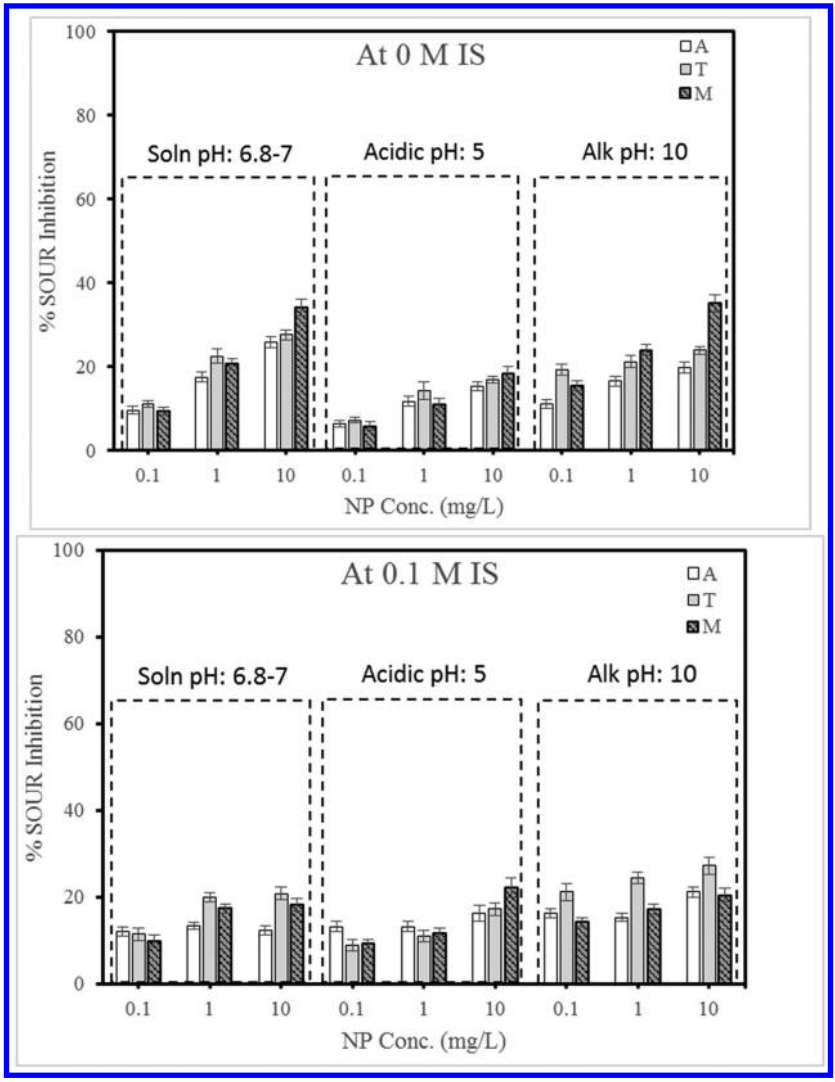

Figure 1. Inhibition of specific oxygen uptake rate (SOUR) of activated sludge exposed to different nanoparticle (NP) concentrations at different pH and ionic strength (IS) conditions (A denotes Ag_2O NPs, T denotes TiO_2 NPs and M denotes Mixture of NPs; Soln: solution; IS: ionic strength).

BOD_5 values were also analyzed to understand effects of NPs by varying the pH and IS of the NP solution. At alkaline pH, a higher reduction in BOD_5 values was found than at acidic pH. In the alkaline range, the mixture of NPs at 10 mg/L gave the maximum reduction in BOD_5 values (27.8±1.71%) than individual NPs (TiO_2 NPs: 26.34±1.4%; Ag_2O NPs: 24.5±1.5%). In the alkaline range, the

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mixture of NPs appeared to be more toxic than individual NPs. In acidic pH, TiO_2 NPs were observed to be toxic at 0.1 and 1 mg/L, while the mixture of NPs was found to be most toxic at 10 mg/L. The pattern of inhibition was found to be consistent with findings of the SOUR experiments. Among all of the pH conditions tested, higher percent reduction in BOD_5 values were found to be at alkaline pH followed by NP solution pH and then acidic pH.

To study the effect of IS, the NP solution was adjusted with NaCl to maintain the NP solution at 0.1 M IS. When adjusted in solution pH, among the NPs, TiO_2 NPs were found to be more toxic than other NPs at every concentration tested. The highest reduction was found to be at 10 mg/L (20.8±1.5%) followed by 1 mg/L (19.9±0.98%) of TiO_2 NPs. At acidic pH, TiO_2 NPs at 0.1 and 1 mg/L were found to be more toxic than others whereas at 10 mg/L the mixture of NPs was found to be toxic. At alkaline pH, as like the solution pH, TiO_2 NPs were found to be more toxic than other NPs (31±1%). Among three conditions tested, TiO_2 NPs imparted highest percent reduction in BOD_5 when compared to other NPs (31±1%). When tested for their statistical significance, all combinations were found to be different from the control data ($p < 0.05$). At solution pH, the percent reduction in BOD_5 at 0 M IS was found to be low when compared to 0.1 M IS. For all the concentrations tested among the NPs in both solution pH and at acidic pH, TiO_2 NPs was found to be more toxic than other NPs at 0 M IS. At alkaline pH at 0.1 M IS, TiO_2 NPs were found to be more toxic at 1 and 10 mg/L, whereas the mixture of NPs were found to be toxic at 0.1 mg/L. Among the NPs at 10 mg/L, TiO_2 NPs has a one-fold increase in reduction of BOD_5 values followed by the mixture of NPs and Ag_2O NPs when the IS of the solution was increased from 0 to 0.1 M. From these results it can be concluded that both pH and IS variation in the NP solutions had significant effect on the oxygen uptake capacity of activated sludge.

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Effect on COD Removal

Figure 3 shows variation of percent reduction in COD values with time for biological reactors exposed to 1 mg/L NP concentrations. As expected, COD values were found to decrease with time. Remaining COD values were found to be significantly higher in exposed reactors than in the control reactor, irrespective of NP types studied ($p < 0.05$). Among NPs, % removal in COD values with time was found to be highest during exposure of 1 mg/L TiO_2. This indicates that NPs cause toxicity to bacteria and thus affect its COD exertion.

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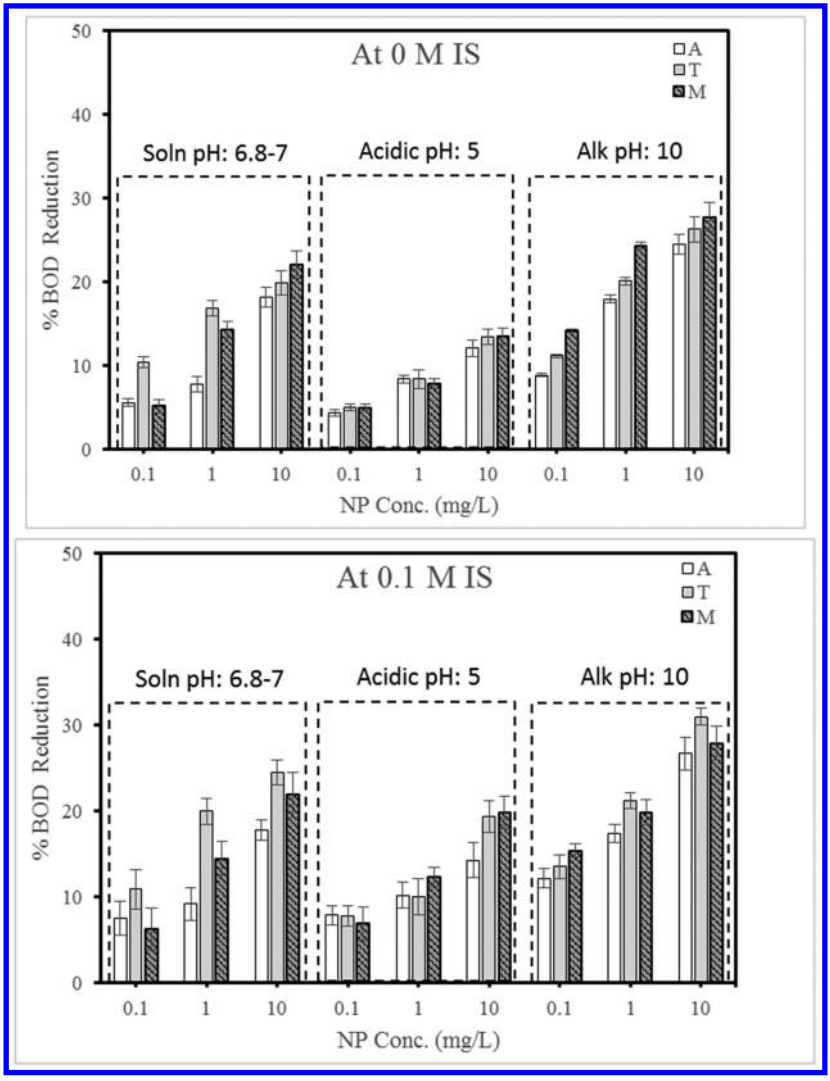

Figure 2. Percent Reduction in BOD_5 values at different NP concentrations (A=Ag_2O NPs; T=TiO_2 NPs; M= mixture of NPs; Soln: solution; IS: ionic strength).

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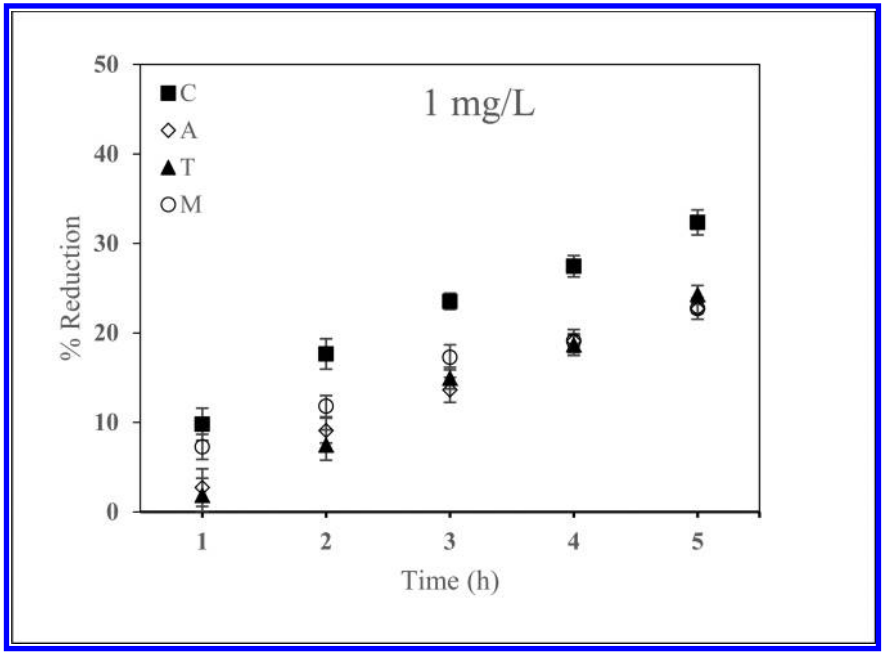

Figure 3. Variation of percent reduction in COD values with time (error bars show one standard deviation around average value) (C: control reactor, A: reactor exposed to Ag_2O NPs, T: reactor exposed to TiO_2, M: reactor exposed to mixture of two NPs)

Overall, this study showed that the mixture of Ag_2O and TiO_2 NPs imparts higher toxicity to activated sludge than individual NPs tested. On the contrary, some studies showed no significant effect on bacterial strains when Ag and TiO_2 NPs were studied alone or in combination (*47*). The observed difference in findings of our study (i.e., reduction in BOD values and COD values for activated sludge) and that in reported studies (i.e., no effect on COD for activated sludge; growth inhibition for *E.coli*) could be attributed to the difference in the type of NPs studied (Ag_2O NPs versus Ag NPs versus TiO_2 NPs), organisms studied (activated sludge versus *E.coli*), exposure duration (5 days in present study versus 15-150 days in published studies), and exposed NP concentrations (0.1-10 mg/L in present study versus 0.1-50 mg/L in published studies). In addition, findings of the time-dependent COD removal study were also compared with that reported in literature. Previously, studies did not find any effect of Ag and TiO_2 NPs on COD values in the activated sludge process (*18*, *19*) (Table 1). For example, studies have found that COD values were not affected by the addition of Ag NPs (*32*). In a sequential batch reactor, it was reported that no change in COD removal was found after a period of 150 days during exposure of Ag, TiO_2, and C_{60} NPs (concentrations: 0.5-2.5 mg/L) to activated sludge (*18*) . Observed differences in trends of COD variation of our study and that reported in published reports could be attributed to the combined effect of NP type, NP concentration, and exposure period. It is important to note that COD removal was affected in

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all studies, which differed with time during long-period exposure. It highlights the need for conducting long-term studies to understand the effect of mixtures of NPs on the organic matter utilization function of activated sludge. Further research is required to understand the effect of exposures of mixtures of other NPs, commonly found in wastewater, on biological functioning of microbial communities in a long-term basis.

Summary and Conclusions

The overall objective of this study was to understand the toxic effect of mixture of metal oxide nanoparticles on biological functioning of activated sludge.

Maximum inhibition in SOUR was found to be 34.17±2.4% due to exposure of microorganisms to 10 mg/L of mixture of NPs at solution pH. Higher inhibition to SOUR was found in alkaline pH conditions than in acidic pH conditions. Exposure of activated sludge to NPs resulted in reduction of BOD_5 values in the range between 18-22% (at maximum exposure concentration of 10 mg/L).

The minimum concentration of NPs giving statistically significant BOD_5 reduction with regard to control was found to be between 1 and 10 mg/L for Ag_2O NPs, between 0 and 0.1 mg/L for TiO_2 NPs, and between 0.1 and 1 mg/L for the mixture of NPs. The rank of toxicity of NPs based on MC values was found to be: (least toxic) Ag_2O < TiO_2 < mixture of TiO_2 and Ag_2O (most toxic). Maximum percent inhibition of BOD_5 was found in the solution containing a mixture of NPs (acidic solution pH condition: 13.52±1%; alkaline solution pH condition: 27.8±1.78 %).

Exposure of activated sludge to 1 mg/L of NPs in 5-h COD reduction study showed that TiO_2 NPs had a higher influence on organic matter utilization function of activated sludge than other NPs.

This is one of the first studies that provides a quantitative estimate of toxic effect of a mixture of two NPs on the organic matter utilization function of activated sludge. Findings of this study are expected to be useful to wastewater utilities and regulators in arriving at the modification of the operations required in biological processes.

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Chapter 9

Toxicity of Long Single-Walled Carbon Nanotubes to the Activated Sludge Process: Examination of the Effects of Extracellular Polymeric Substances

Harshrajsinh Thakor,[1] Alex Parise,[2] and Xiaoqi (Jackie) Zhang[3,*]

[1]MassDEP 20, Riverside Drive, Lakeville, Massachusetts 02347
[2]Loureiro Engineering Associates, Inc., 779 South Main Street, Manchester, New Hampshire 03102
[3]Department of Civil and Environmental Engineering, University of Massachusetts Lowell, Lowell, Massachusetts 01854
[*]Phone: (978) 934-2194; Fax: (978) 934-3052;
E-mail: Jackie_zhang@uml.edu

In this paper, we examined the effect of extracellular polymeric substances (EPS) in protecting the microbial communities when the activated sludge was dosed with long single-walled carbon nanotubes (LSWCNTs). Mix-cultured activated sludge from a local wastewater treatment plant was used in the study and a respiratory inhibition test was performed on the activated sludge without shearing and after shearing to determine the respiratory activity of the activated sludge when the LSWCNTs were introduced. The results showed that the LSWCNTs exhibited toxicity towards the activated sludge microbial communities and more respiratory inhibition was observed when the EPS of the activated sludge was sheared off, illustrating the protective ability of EPS.

Keywords: long single-walled carbon nanotubes (LSWCNTs); activated sludge; extracellular polymeric substances (EPS); respiratory inhibition

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Introduction

Nanotechnology is an applicable aspect of a broader area of nanoscience. Carbon nanotubes (CNTs) can be released into the environment via wastewater discharge and point source emissions from manufacturing industries (*1*). Animal inhalation and aspiration studies have demonstrated that exposure to single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) results in acute pulmonary inflammation and long term chronic effects such as thickening of the bronchial wall, fibrosis and granulomas (*2*). Antimicrobial activity of SWCNTs has been reported after *E.coli* had been exposed to SWCNTs (*3*).

An activated sludge process is a biological process that is used in the wastewater treatment facility to treat wastewater (*4*). Parise (*5*) reported the toxicity effect of different types of SWCNTs on the activated sludge microbial community. Their results showed that long SWCNTs (LSWCNTs) and functionalized SWCNTs were more toxic than the short SWCNTs and non-functionalized SWCNTs.

Extracellular polymeric substances (EPS) form a protective layer outside of the microorganisms (*6*). EPS plays a significant role in the formation and the function of microbial aggregates, including adhesion phenomena, matrix structure formation, microbial physiological processes such as protecting cells against environmental changes in pH, water quality, salt content and hydraulic pressure (*7*). When the activated sludge is sheared, the EPS layer breaks off and dissolves, and the EPS content can be measured as soluble protein, carbohydrates, deoxyribonucleic acid (DNA), etc. (*8*). The amount of dissolved protein and carbohydrates detected after shearing indicates the amount of EPS separated from the microorganisms. The ability and degree of EPS protection against a toxin is dependent on the nature of toxin (*6*).

Presently, only limited information is available on the effect of EPS in protecting the microbial communities when CNTs are present. Luongo and Zhang (*9*) demonstrated that EPS played a significant role in protecting the microbial communities in the activated sludge against the toxicity posed by the MWCNTs. However, the effects of EPS on the activated sludge when the LSWCNTs are present are not yet understood. The objective of this paper is to determine the effect of EPS in protecting the microbial communities when the activated sludge was dosed with the LSWCNTs.

Methods

Sludge Preparation

Fresh activated sludge was collected from the Lowell Regional Wastewater Utility (Lowell, MA). The sludge was concentrated to 4,000 mg TSS/L. To achieve that, the initial mixed liquor suspended solids (MLSS) and Chemical Oxygen Demand (COD) (*10*) were measured as soon as the sludge was brought to the environmental laboratory at the University of Massachusetts Lowell (UML). After

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that the sludge was allowed to settle and washed with tap water three times before decanting the supernatant and measuring the MLSS of the sludge again. After washing, tap water was added so that the sludge had 4000 mg/L of MLSS.

Synthetic Feed and Sludge Storage

Sludge was stored overnight before the experiment. Synthetic feed was added to the sludge during storage to maintain the same Food/Microorganism ratio as in the original sludge. During storage, the sludge was aerated continuously with an aeration stone. The aeration was set at a rate so that only small bubbles were formed. For every 100 mL of synthetic feed, 1.6 g peptone, 1.1 g meat extract, 0.3 g urea, 0.07 g NaCl, 0.04 g $CaCl_2 \cdot 2H_2O$, 0.02 g $MgSO_4 \cdot 7H_2O$, and 0.28 g K_2HPO_4 were added (*11*). To avoid bacterial growth in the feed, fresh synthetic feed was prepared prior to every experiment.

Nanotube Preparation

LSWCNTs were purchased from Cheap Tubes Inc. (Brattleboro, VT). The characteristics of the nanotubes are shown in Table 1. The CNTs were sonicated according to Parise, *et al*. (*5*).

Table 1. Nanotube Compositions

	Long Single-Walled CNTs (LSWCNTs)
Outer Diameter (nm)	1-2
Length (μm)	5.0-30
Purity	> 90wt%
Functional Content	0
Ash	<1.5wt%

The LSWCNTs were prepared by combining 75 mL of deionized water (DI), 5 mL of concentrated synthetic feed and 125 mg of LSWCNTs. The mixture was then allowed to mix on a magnetic stir plate for 10 minutes prior to sonication. A Misonex Sonicator 3000 with cup horn was used to disperse the samples. The mixture was sonicated at a dial setting of 7.5 for 2 hours, pulsing ON and OFF for 30 second intervals. Water cooled with ice cubes was pumped through the sonication assembly to ensure no rise in temperature during sonication. Following sonication, the mixture was placed in a refrigerator on a magnetic stir plate overnight to promote further dispersion of CNTs.

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Activated Sludge Shearing to Release EPS

The effect of EPS was evaluated by comparing all the analyses from two experimental groups: the activated sludge with the EPS attached and the activated sludge with the EPS sheared-off. Similar experimental procedure as described in Luongo and Zhang (*9*) was followed. Modification was made on the shearing method. Instead of shearing the sludge for 5 min at 22,000 rpm, a 5-times pulsated shearing method (1 sec/pulse, pulsing ON and OFF for 1 second interval) was conducted by using a commercial Waring blender (Model 5011). The EPS was quantified by measuring the soluble concentrations of carbohydrates, protein and DNA. The Dubois method was used for carbohydrate analysis (*12*). A total protein kit (Thermo Scientific) was used to measure the protein content (*13*). The DNA concentration was measured by using a Nanodrop 2000 (Thermo Scientific).

Respiratory Inhibition

A respiration inhibition test for sparingly soluble chemicals published by EPA (*11*) was used. The detailed experimental procedure was also presented in Luongo and Zhang (*9*) and Parise *et al*. (*5*) Conditions for each beaker were kept identical by supplying air at 1 L/min and providing mixing at 90 rpm. At the end of each beaker's three-hour run, the dissolved oxygen (DO) readings were recorded using a YSI Model 52CE meter and probe.

This procedure requires a reference chemical (3, 5-dichlorophenol) with known concentrations (5, 10, and 25 mg/L) and two controls. The effective concentration (EC_{50}) of the reference chemical must fall within 5-30 mg/L and the respiration rates from the two controls must fall within 15% of each other.

Two different experimental samples were also part of the setup. They were the unsheared activated sludge dosed with 250 mg/L of LSWCNTs and the sheared activated sludge dosed with 250 mg/L of LSWCNTs.

The percentage inhibition for the samples was calculated by the equation given below.

$$\%\ inhibition\ of\ samples = \left[1 - \frac{2R_S}{R_{C1} + R_{C2}}\right] * 100\%$$

where:

R_{C1} = Respiration rate of control 1, mg/L/hr
R_{C2} = Respiration rate of control 2, mg/L/hr
R_S = Respiration rate of the sample, mg/L/hr

Results and Discussion

Effect of LSWCNTs on the Respiratory Activity of the Activated Sludge

At 250 mg/L, the LSWCNTs caused respiratory inhibition to both the unsheared activated sludge and the sheared activated sludge (8.04±0.40% and 24.27±6.02%, respectively) (Figure 1). The respiratory inhibition data

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demonstrate that the LSWCNTs can be toxic to the microbial communities and the EPS layer can protect the microbial communities from toxicity. When this protective layer is sheared off, the toxin will cause higher respiratory inhibition (24.27±6.02%) towards the microbial communities.

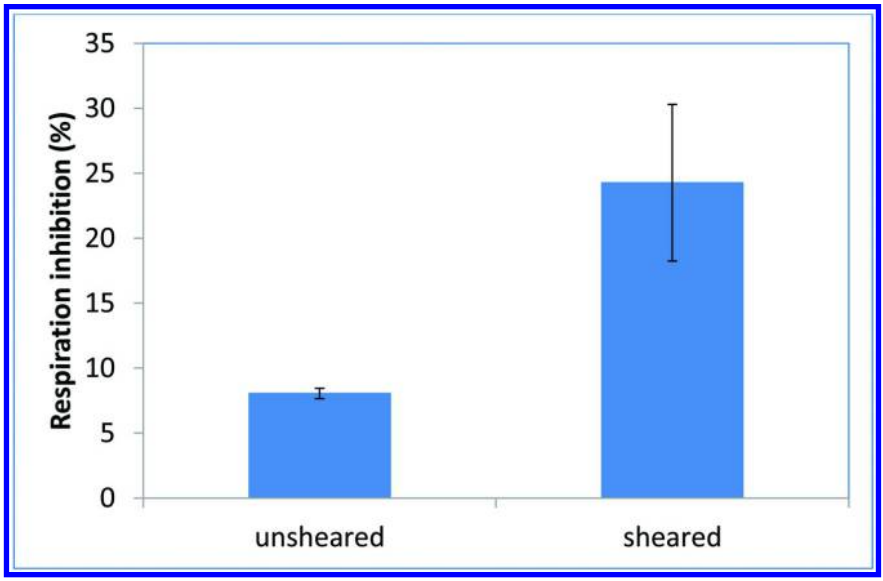

Figure 1. Respiratory inhibition from 250 mg/L of LSWCNTs towards the unsheared activated sludge and the sheared activated sludge

Luongo and Zhang (*9*) studied the respiratory inhibition of MWCNTs at higher concentrations (640 mg/L-3240 mg/L, purchased from Sigma-Aldrich) to the activated sludge microbial communities. By extrapolation, we estimated that 250 mg/L of MWCNTs would cause 5% and 8% of respiratory inhibition to the unsheared and sheared activated sludge, respectively. Therefore it appears that the LSWCNTs used in this study caused more respiratory inhibition towards the activated sludge microbial communities compared to the MWCNTs. This finding supports the general understanding that the physiochemical properties of the CNTs affect their behavior and toxicity (*14*) and the single-walled CNTs are more toxic than the multiwalled CNTs (*15*).

However, care should be taken when comparing results from different studies. Details such as characteristics of the CNTs used, manufacturers, shearing method, experimental setups, analytical methods, etc. should all be considered.

Effect of LSWCNTs on Soluble Organic Matter Release

The soluble protein, carbohydrates and DNA concentrations were all increased after the activated sludge (unsheared and sheared) reacted with the LSWCNTs for three hours. Much more release of the soluble protein, carbohydrates and DNA was detected when the EPS was sheared off from the

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activated sludge comparing to that when the EPS was still attached. After the unsheared activated sludge had reacted with the LSWCNTs for three hours, the soluble protein, carbohydrates and DNA concentrations were increased by 54.73%, 47.19%, and 496.8% respectively compared to the soluble protein, carbohydrates and DNA concentrations in the control sample (Figure 2). After the activated sludge with the EPS sheared off reacted with the LSWCNTs for three hours, the soluble protein, carbohydrates and DNA concentrations were increased by 141.02%, 68.37%, and 600.52% respectively compared to those soluble concentrations in the respective control (Figure 2). DNA release is a good indicator of cell lysis (*16*). A significant increase in the DNA concentrations (496.8%-660.52%) after the activated sludge reacted with LSWCNTs was found regardless of whether the activated sludge was sheared or unsheared. The effect of mixing alone during the three-hour reaction period on the release of soluble organic matter is none to minimal (results not shown). The higher concentrations of the soluble organic matter at the end of the three-hour reaction seems to indicate cell lysis and more release of the EPS material (the tightly bound portion) due to the toxicity of the LSWCNTs. These effects are more significant in the sheared activated sludge samples. The respiratory inhibition observed in the activated sludge could be the result of the cellular destruction caused by the LSWCNTs.

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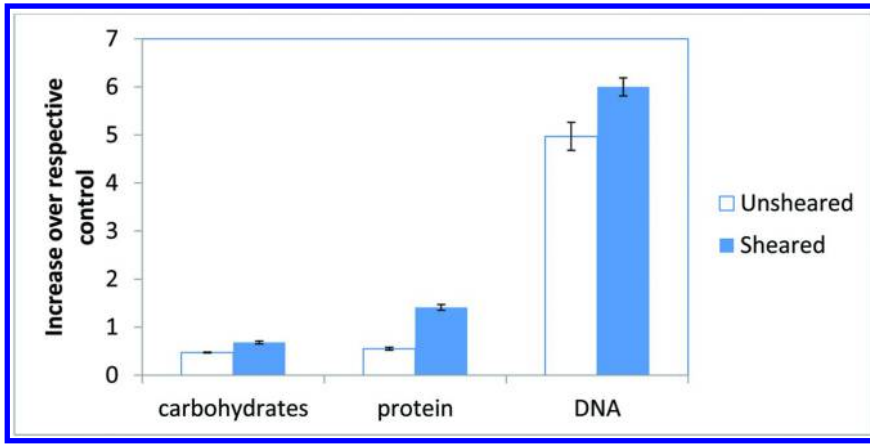

Figure 2. Increase in the concentrations of carbohydrates, protein and DNA in the activated sludge solution after reacting with LSWCNTs.

Conclusions

The LSWCNTs caused respiratory inhibition to the activated sludge microbial communities and caused more respiratory inhibition when the EPS was absent. The LSWCNTs caused significant release of the soluble carbohydrates, protein and DNA from the activated sludge communities and much more release of these soluble organic matters in the sheared activated sludge study. The results illustrated the protective ability of the EPS.

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Chapter 10

Assessment of Anthropogenic Contribution to Perchlorate in the Environment Using an Ice Core Record

K. M. Peterson,* J. Cole-Dai, D. L. Brandis, and E. Manandhar

Department of Chemistry and Biochemistry, South Dakota State University, Box 2202, Brookings, South Dakota 57007
***E-mail: Kari.Peterson@sdstate.edu**

Perchlorate in the environment derives from both natural and anthropogenic sources, and constitutes a significant threat to human health. The relative contribution to perchlorate in the current environment from the natural and anthropogenic sources can be assessed with chronological records of perchlorate in polar ice cores. This information is critical to the efforts to reduce the environmental threat of perchlorate. We have begun to measure perchlorate concentrations in a well-dated Greenland ice core to reconstruct a 500-year perchlorate deposition history. Increased concentrations in post-1980 samples over that in the time period prior to the beginning of the Industrial Revolution suggest that human activities likely to have contributed a significant portion to the perchlorate in the current environment. In addition, the ice core perchlorate record appears to provide evidence of atmospheric perchlorate production influenced by volcanic eruptions and tropospheric ozone levels.

Introduction

Human exposure to perchlorate (ClO_4^-) can lead to adverse health effects. Perchlorate inhibits the uptake of iodine by the thyroid gland, which, with sustained inhibition, can lead to hypothyroidism (*1*, *2*). This can result in serious health problems, including complications in the development of the central nervous system in infants and problems with metabolism in adults (*3*, *4*).

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Perchlorate rarely undergoes degradation or biotransformation and therefore is exceptionally stable in the environment. Because it is highly soluble in water, the fate of perchlorate is likely aquatic systems, such as groundwater and surface water systems, which are often sources of drinking water. Thus, humans are widely exposed to low levels of perchlorate (*5*, *6*) through the consumption of food and drinking water (*7–11*).

The U.S. Environmental Protection Agency (EPA) is developing a National Primary Drinking Water Regulation for perchlorate (*12*). Environmental perchlorate likely derives from both natural and anthropogenic sources. Therefore, effective mitigation of the perchlorate risk through regulation is dependent on the knowledge of the contribution to perchlorate in the current environment from anthropogenic sources, relative to that from natural sources. Our knowledge of the relative contributions, however, is rather limited. Perchlorate has been found in pre-anthropogenic groundwater, suggesting that natural sources exist (*13–15*). Anthropogenic sources, on the other hand, mainly derive from the use of perchlorate as a component in rocket propellants, munitions, and fireworks/pyrotechnics (*16*). In addition, the legacy use of perchlorate-rich Chilean nitrate fertilizers may be viewed as another source of anthropogenic contribution.

The anthropogenic contribution to perchlorate in the current environment can be evaluated, if the magnitude of the natural source is known. Assuming that the magnitude of the natural source has not varied significantly over time, it may be determined by a careful examination of environmental perchlorate records covering time periods when the anthropogenic contribution was negligible. Since significant perturbation of the environment by human activities began with the onset of the Industrial Revolution in the nineteenth century, chronological records covering at least last 200 years are needed. Polar ice cores are often used to reconstruct records of chemical substances in the environment (*17–19*). Thus, perchlorate levels found in polar snow, fallen prior to the Industrial Revolution would represent the magnitude of the natural perchlorate source, while perchlorate levels found in recent snow are likely the combined contribution of both natural and anthropogenic sources.

The objective of this study is to develop a long and detailed (high temporal resolution) record of perchlorate in the environment from a well-dated Greenland ice core. A systematic examination of such a record will enable an assessment of the anthropogenic contribution to perchlorate in the current environment, as well as the natural environmental perchlorate level and its variability. In addition, the high temporal resolution will allow for the exploration of factors influencing the natural production of perchlorate, including climatic conditions and volcanic activities.

Experimental Methods

Ice Core Sample Preparation

In June and July 2007, four ice cores were drilled near Summit Station, Greenland (72°34′44.10″N, 38°27′34.56″W). Each meter section of the cores was

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placed inside a plastic bag, then in a reflective cardboard tube; the tubes were packed in insulated boxes for transport. The temperature of the cores during transport and storage was maintained at or below -20 °C. Core 2 (SM07C2) (150 m) of the four cores was used for perchlorate analysis.

Core 1 (SM07C1) from Summit Station was sampled approximately every 3 cm and analyzed for concentrations of major ions, including Ca^{2+}, Mg^{2+}, SO_4^{2-}, and NO_3^-, with ion chromatography instruments interfaced with a continuous flow ice core melter. The concentrations of Ca^{2+}, Mg^{2+}, and NO_3^- in snow oscillate on an annual basis and can therefore be used to discern and count the annual layers in the ice core as a function of depth. This method of annual layer counting was used to develop the ice core time scale (*20*). Because SM07C2 was drilled in close proximity to SM07C1 and distinctive features, such as the prominent signal of the 1815 Tambora volcanic eruption, occur at essentially the same depth in both cores, the time scale for SM07C1 was adopted for SM07C2. The age of the ice at 150 m was determined to be 1488 C.E (*20*).

Samples at a depth interval of 4-5 cm were taken from continuous sections of SM07C2 and were decontaminated with the following cleaning procedures: the outer surfaces of each sample below 57 m were removed by thorough washing with ultrapure deionized water (18 MΩ); for samples above 57 m, the outer surfaces were removed by cutting with a band-saw. The decontaminated samples were allowed to melt in clean sample containers at room temperature, followed by perchlorate concentration determination. To date, a total of 806 samples have been analyzed for perchlorate covering the depth ranges of 0.00-25.55 m (1950-2007) and 84.14-97.80 m (1701-1753). Analysis of ice between 25.55-84.14 m (1754-1949) and below 97.80 m is ongoing.

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Analytical Measurement

Perchlorate concentrations in the melted ice core samples were determined using the technique of ion chromatography-tandem mass spectrometry with electrospray ionization (IC-ESI-MS/MS). Separation of perchlorate from ice core matrix species (e.g., NO_3^-, SO_4^{2-}) was accomplished using a ThermoFisher (formerly Dionex, Sunnyville, CA) DX500 ion chromatography system consisting of an IP25 isocratic pump, a CD25 conductivity detector, and a Dionex IonPac AS16 (2×250 mm) analytical column. Perchlorate was eluted with 60 mM NaOH at 0.3 mL min^{-1}, which was suppressed using an AERS-500 (2 mm) suppressor after analytical separation. Prior to delivery to the mass spectrometer, the effluent exiting the suppressor was mixed with a 90% (v/v) acetonitrile and 10% water solution at 0.3 mL min^{-1}, to aid in the ionization of the sample. This mixture was allowed to proceed to the nebulizer inlet of an AB SCIEX (Framingham, MA) QTRAP 5500 triple quadrupole mass spectrometer, operating in multiple reaction monitoring (MRM) mode.

A set of perchlorate standard solutions (0.30, 0.75, 2.00, 5.00, 10.00 ng L^{-1}) in deionized water was used for external calibration. Under optimized conditions, the limit of detection (LOD) and lower limit of quantification (LLOQ) of the analytical method are 0.1 ng L^{-1} and 0.3 ng L^{-1}, respectively. The linear dynamic

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range is 0.3 to at least 10.0 ng L^{-1}. Ice blanks, from frozen deionized water, were prepared using the same sampling procedures as the ice core samples and analyzed each day of analysis as part of the quality control protocol. The perchlorate concentration was below the LOD in 54% of the ice blanks. On average, perchlorate concentration (0.1 ng L^{-1}) in the ice blanks was approximately 7% of the typical perchlorate concentration in the samples.

Results and Discussion

The perchlorate concentrations in the Summit, Greenland core (SM07C2) varied from below the LOD (0.1 ng L^{-1}) to 11.9 ng L^{-1}. The sample concentrations were converted to mass deposition (ng m^{-2}) by multiplying the concentration of each sample with the water equivalent sample depth interval. The mass deposition is positively correlated with perchlorate concentrations ($R = 0.892$, $p < 0.001$). To compare perchlorate deposition at different time periods, we calculated perchlorate annual flux (ng m^{-2} yr^{-1}) by summing up mass deposition of all samples in a year.

Perchlorate from Natural Sources

The average annual perchlorate flux and the average perchlorate concentration in pre-industrial (1705-1752) samples are 186.4 ± 155.6 (where $\sigma = 155.6$ is the standard deviation) ng m^{-2} yr^{-1} and 0.8 ± 0.8 ng L^{-1}, respectively. In the time period of 1950-1979, the perchlorate flux, 165.3 ± 86.7 ng m^{-2} yr^{-1} (0.8 ± 0.6 ng L^{-1}), is similar to that of the pre-industrial samples. In contrast, the average annual perchlorate flux in the most recent (1980-2006) ice core samples is 626.9 ± 327.5 ng m^{-2} yr^{-1} (2.7 ± 2.1 ng L^{-1}), approximately three times that of the pre-industrial samples (Figure 1). The similarity of annual perchlorate flux in the periods of 1705-1752 and 1950-1979 in the Summit core suggests that perchlorate, most likely from natural sources, had not varied significantly since the pre-industrial times, though this needs to be confirmed with additional perchlorate data for the period of the mid-eighteenth century to the mid-twentieth century. The increase in recent (post-1980) snow is similar to that found by Rao et al. (*21*) in an ice core from the Eclipse Icefield in the Yukon Territory, Canada, where the average perchlorate concentration in samples dated between 1970 and 1973 was 0.6 ± 0.3 ng L^{-1}, whereas perchlorate concentrations were 2.3 ± 1.7 and 2.2 ± 2.0 ng L^{-1} in the time periods of 1982-1986 and 1999-2002, respectively (*21*).

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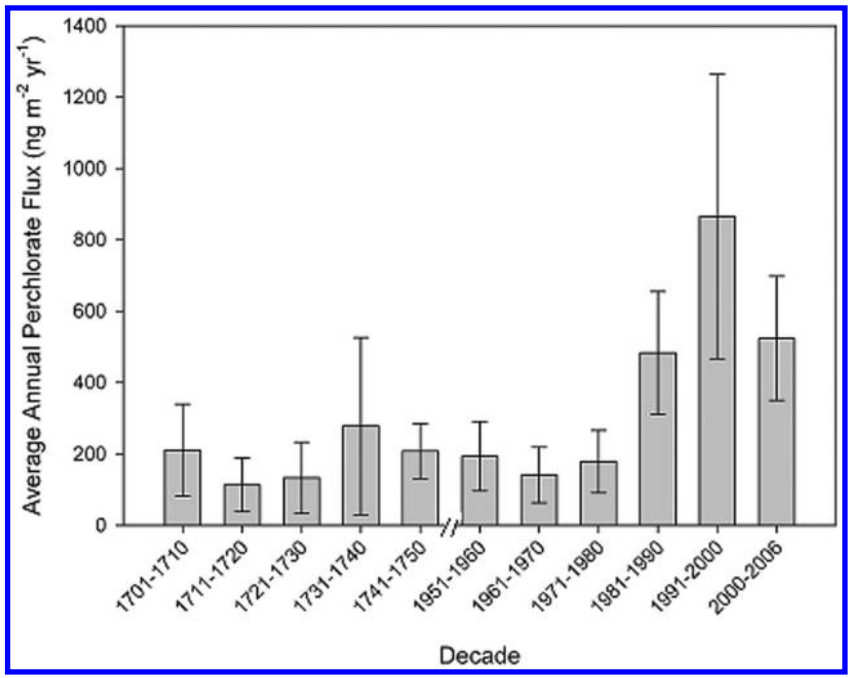

Figure 1. Average annual perchlorate flux for decades in two time periods (1701-1750 and 1950-2007) in the Summit ice core. The error bars represent the standard deviation of the annual perchlorate fluxes.

Anthropogenic Contributions

The data (Figure 2) clearly show that the perchlorate flux in recent (since 1980) Arctic snow is higher, by a factor of 3, than those in the previous time periods including the pre-industrial times. Because the annual perchlorate flux from natural sources does not appear to vary significantly (Figures 1 and 2), it is likely that the higher annual perchlorate fluxes since 1980 are due to anthropogenic sources. The average annual flux of 178.3 ± 133.0 ng m^{-2} yr^{-1} in the time periods of 1705-1752 and 1950-1979 is presumed to be only from natural sources. The difference between the average annual flux (626.9 ± 327.5 ng m^{-2} yr^{-1}) in the period of 1980-2006 and the average flux for the prior time periods (1705-1752, 1950-1979) is 448.6 ng m^{-2} yr^{-1}, suggesting that approximately 70% of the perchlorate in the current environment is probably from anthropogenic sources. Even after excluding fluxes for 1982-1983 and 1991-1993, which may be impacted by volcanic eruptions (discussed below), the average annual perchlorate flux since 1980 is 541.9 ± 243.6 ng m^{-2} yr^{-1}. This is approximately three times the presumed annual flux from natural sources.

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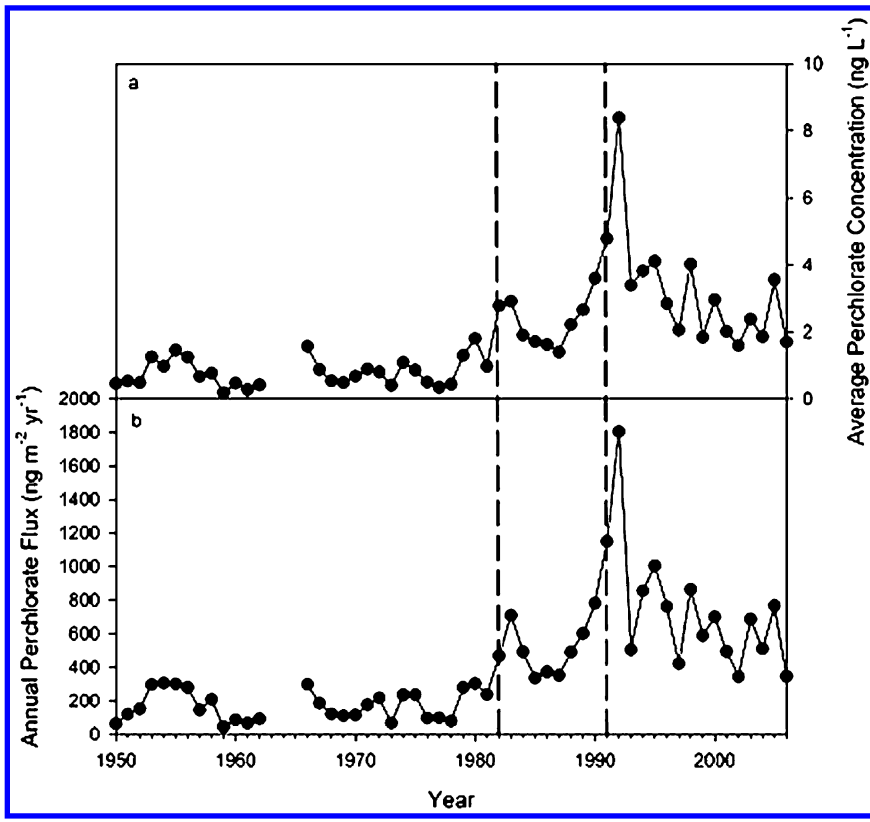

Figure 2. The average perchlorate concentration (a) and the average annual perchlorate flux (b) over the time period of 1950-2007. The dashed lines mark the eruptions of El Chichón (March-April 1982) and Pinatubo (June 1991).

One possible contributor to increased perchlorate since 1980 is the disbursement of perchlorate used as a component of propellants in rocket launches during space operations. For example, the United States National Aeronautics and Space Administration (NASA) began launching space shuttles in 1981. Each space shuttle launch used approximately 500 tons of propellant, of which 70% was ammonium perchlorate (*22*). However, the analysis of a space shuttle plume revealed elevated levels of chloride, but no clear evidence of elevated perchlorate levels (*16*). Thus, it is unlikely that space shuttle launches are the only significant contributor to environmental perchlorate since 1980. Identifying specific anthropogenic contributors to perchlorate is complicated by the lack of easily accessible perchlorate production and usage data due to its consideration as a strategic chemical in the U.S. Nonetheless, Dasgupta et al. (*16*) reported perchlorate production figures based on the EPA archives for three perchlorate production plants and estimates for one additional plant. According to these figures and estimates, perchlorate production generally increased after 1980 (*16*). Presumably, the increased production led to more perchlorate used in applications

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resulting in increased release into the environment. This may be responsible for the elevated perchlorate levels seen in post-1980 Greenland snow samples.

Perchlorate and Volcanic Eruptions

Explosive volcanic eruptions emit large amounts of sulfur dioxide (SO_2) into the atmosphere, where the SO_2 is oxidized to sulfuric acid and subsequently deposited on Earth's surface areas including ice sheets (*23*). Therefore, elevated sulfate concentration in polar ice cores serves as a marker for volcanic eruptions (*24*). The 1991 eruption of the Mt. Pinatubo volcano in the Philippines is one of the largest eruptions during the twentieth century. As seen in Figure 3(b), the higher-than-usual sulfate concentrations in the 8-9 m depth interval (1991-1993) are likely caused by the fallout from the Pinatubo eruption. A marked increase in the annual perchlorate concentration and flux is observed (Figure 2) during these years. Also, although not unambiguously identifiable in the sulfate data (Figure 3(b)), the slightly elevated perchlorate levels between 12.3–13.1 m (1982-1983) (Figures 2 and 3) could correspond to the 1982 eruption of El Chichón in Mexico. Although it is unlikely that volcanoes are a direct source of perchlorate, the significantly increased perchlorate levels following volcanic eruptions point to a potential link between perchlorate and volcanic eruptions. A possible mechanism for the link may be through UV and ozone-mediated oxidation of ClO_2 (*25*, *26*), a perchlorate precursor which was observed to increase over Antarctica due to the effects of the Mt. Pinatubo eruption (*27*). Another possible scenario is enhanced perchlorate production due to elevated tropospheric ozone levels, caused by the penetration of solar UV radiation into the troposphere as a result of the weakening of the stratospheric ozone shield by volcanic aerosols. In fact, Furdui and Tomassini (*28*) noted a strong correlation ($R = 0.78$, $N = 45$, $p = 2\times10^{-10}$) between perchlorate concentrations in snow from Devon Island (Canada) and column ozone measurements at nearby Resolute Bay (Canada).

Furdui and Tomassini (*28*) also postulated a link between volcanic eruptions and perchlorate. They observed that perchlorate flux decreased between 1996 and 1999 following the observed decrease in sulfate from the 1991 Mt. Pinatubo eruption. However, the atmospheric residence time of volcanic sulfate is usually less than 3 years; therefore, reduction in sulfate fallout 5-8 years after the Pinatubo eruption was unlikely to be associated with Pinatubo. In addition, because air pollution from the Northern mid-latitudes contributes significantly to sulfate in recent Arctic snow, it would be difficult to attribute the decreasing trend of sulfate during this time period entirely to the diminishing stratospheric presence of Pinatubo aerosols.

The suggestion of volcanic impact on perchlorate is based on limited data – only the possible effect of the Mt. Pinatubo and El Chichón eruptions have been studied – and is, therefore, tentative. Further, the discussion of potential mechanisms for increased perchlorate concentration through either increased precursor (e.g., ClO_2) levels or enhanced oxidant (e.g., ozone) concentration should be considered speculative. Additional and direct evidence is needed to demonstrate the connection between perchlorate and the levels of precursors and/or oxidants.

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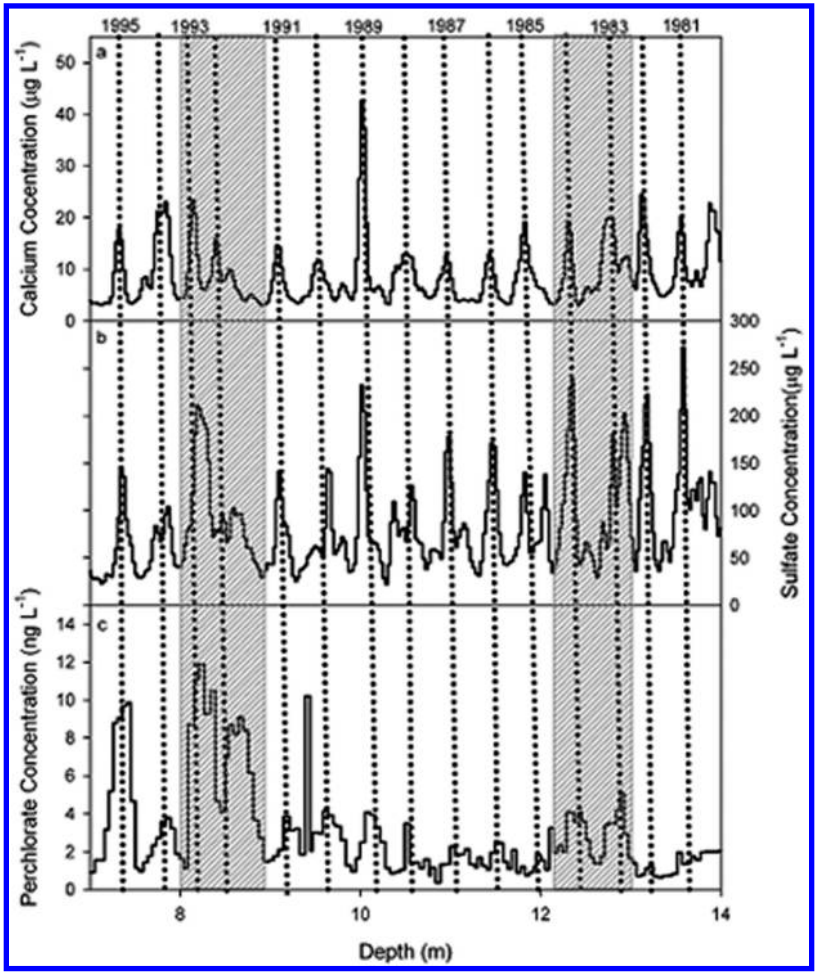

Figure 3. Calcium (a), sulfate (b), and perchlorate (c) concentration profiles between 7-14 m in SM07C2. Gray cross-hatched areas indicate the El Chichón (1982-1983) and Pinatubo volcanic signal (1991-1993) when a significant correlation is apparent between perchlorate and sulfate. The vertical dotted lines designate the spring (March-May) of each annual snow layer, as indicated by the spring calcium concentration maximum.

Seasonal Variation of Perchlorate

Perchlorate levels in the Summit core appear to exhibit a pattern of seasonal variation, as seen in Figure 3(c) where the concentration reaches a maximum in 10 of the 15 years between 1980 and 1995. The apparent annual maximum is reached at approximately the same time of a year as the maximum concentration of calcium (Figure 3(a)), which is known to peak in the spring (March-May). Furdui and Tomassini (*28*) and Rao et al. (*21*) both observed a similar seasonal pattern in the variation of perchlorate concentrations in snow sampled at other Arctic

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locations (Devon Island and Eclipse Icefield). Due to strong correlation between perchlorate and ozone in the Arctic troposphere, which varies in a strongly seasonal pattern, Furdui and Tomassini (*28*) suggest that the seasonal signal of perchlorate is evidence that perchlorate is likely formed in the atmosphere in a photochemical process involving tropospheric ozone. The apparent seasonal pattern in the detailed perchlorate record from the Greenland ice core is consistent with the suggested connection between perchlorate and tropospheric ozone. However, as shown in Figure 3(c), the perchlorate seasonal cycle is less regular than that of calcium concentration and is difficult to discern in some years.

Conclusions

A high resolution perchlorate record is being developed from the analysis of a central Greenland ice core. Examination of the perchlorate data from samples analyzed to date shows that perchlorate levels in recent snow are approximately 3-3.5 times that in the ice dated between 1705 and 1752, suggesting that anthropogenic sources may be responsible for ~70% of the perchlorate in the current environment. A significant increase in annual perchlorate flux, however, was not observed until about 1980. The ice core data alone are insufficient to permit identification of specific anthropogenic sources of perchlorate; but, by comparison with perchlorate production estimates, the results so far allow us to conclude with confidence that human activities are responsible for the significantly higher perchlorate levels since 1980. Additionally, an apparent link between perchlorate concentrations and recent volcanic eruptions (El Chichón (1982) and Mt. Pinatubo (1991)) has been identified. However, the validity of the link between perchlorate and volcanism needs to be tested with the examination of other major volcanic eruptions in ice cores. Finally, it appears that perchlorate concentration in Arctic snow follows a somewhat seasonal cycle, with the highest concentrations in the spring. This seasonal pattern of variation supports the hypothesized atmospheric photochemical formation of perchlorate involving ozone.

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Chapter 11

Applying Advanced Mass Spectrometry Techniques to Emerging Pollutant Detection: Differential Mobility Spectrometry

J. Larry Campbell,* J. C. Yves Le Blanc, Matthew R. Noestheden, and André Schreiber

SCIEX, 71 Four Valley Drive, Concord, Ontario L4K 4V8, Canada
***E-mail: larry.campbell@sciex.com**

The analysis of environmental contaminants has become ever more challenging over the years. More sensitive, selective, and robust workflows have become necessary to determine analyte concentrations in very complex chemical matrices. However, with each sample handling step or derivatization, analyte losses, as well as time and financial costs, are incurred. With that in mind, mass spectrometry-based technologies have advanced to face these challenges, including the adoption of a rapid, orthogonal separation technology – differential mobility spectrometry (DMS). DMS acts to filter analytes in the gas phase from closely related interfering background species based upon subtle differences in their chemical structures. Here, we highlight two case studies of DMS applied to challenging environmental problems: (1) separation of fungicidal triazoles from complex plant matrices with minimal sample preparation and (2) rapid identification of naphthenic acids from oil sands process affected waters.

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Introduction to Differential Mobility Spectrometry (DMS)

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Differential mobility spectrometry (or DMS) is an orthogonal separation technique often coupled to mass spectrometers, though DMS's original implimentations involved no MS whatsoever (*1*). Several reviews of the fundamentals of DMS (a form of high-Field Asymmetric waveform Ion Mobility Spectrometry or FAIMS) have been published elsewhere (*2–4*). Briefly, in our modern implementation, the DMS cell is physically positioned between the sample introduction probe (typically an electrospray ionization – ESI – electrode) and the sampling orifice of the mass spectrometer (Figure 1, top) (*5*). Ionized analytes emitted from the ESI probe are entrained by a flow of carrier gas (pure nitrogen) and dragged through the DMS cell toward the mass spectrometer (under high vacuum). The primary components of the DMS cell are two planar electrodes (30 x 10 mm) separated by a 1-mm gap. Across this gap, a high-voltage rf asymmetric waveform (*6*) is applied (Separation Voltage – SV, Figure 1, bottom) that is perpendicular to the flow of gas and ions. Each waveform cycle consists of a longer low-field component and a shorter high-field component. Under the influence of the SV, ions begin to oscillate rapidly toward one electrode or the other, depending upon the mobility behavior of the ion. During the low-field period, ion/molecule clustering processes occur and the mobility of the ions decreases, while under the high-field conditions, the ion mobility increases as de-clustering takes place. The difference between the analyte's mobility during the high- and low-field portions of the waveform determines how DMS separates these ions. To insure that an ion is detected by the MS, a dc voltage (Compensation Voltage – CV, Figure 1B, bottom) serves to deflect ions from collisions with the electrodes and steer them for successful passage through the DMS cell.

Besides the SV and CV parameters, the gas-phase environment of the DMS cell plays a critical role in these experiments. This environment can be altered by doping the carrier gas with a small amount (typically $\geq$ 1.5% v/v) of volatile chemicals, such as isopropanol or water (*7*, *8*). In the DMS cell, the transient binding (clustering) interactions of the solvent molecules with the analyte ions (*9*) serve to shift their CV values (sometimes dramatically) away from the CVs of isomeric or isobaric impurities. In many case, the addition of chemical modifiers spreads the analytical signals across a wider range of CV values while maintaining the peak widths of the analytes, thereby increasing the overall peak capacity of the DMS experiments (*10*)

Ultimately, using careful SV/CV optimization and chemical modifier selection, DMS has been employed to separate isobaric ions (*5*), structural isomers (*9*, *11–13*), stereoisomers (*5*, *14*), and ions that differ only in their sites of charging (*15*, *16*). In fact, with its speed and lower resource cost compared to chromatographic alternatives, DMS can represent a "greener" analytical technique to conventional workflows (*17*). This can be important for environmental applications since excessive solvent or energy usage in executing these assessments come with their own environmental burdens.

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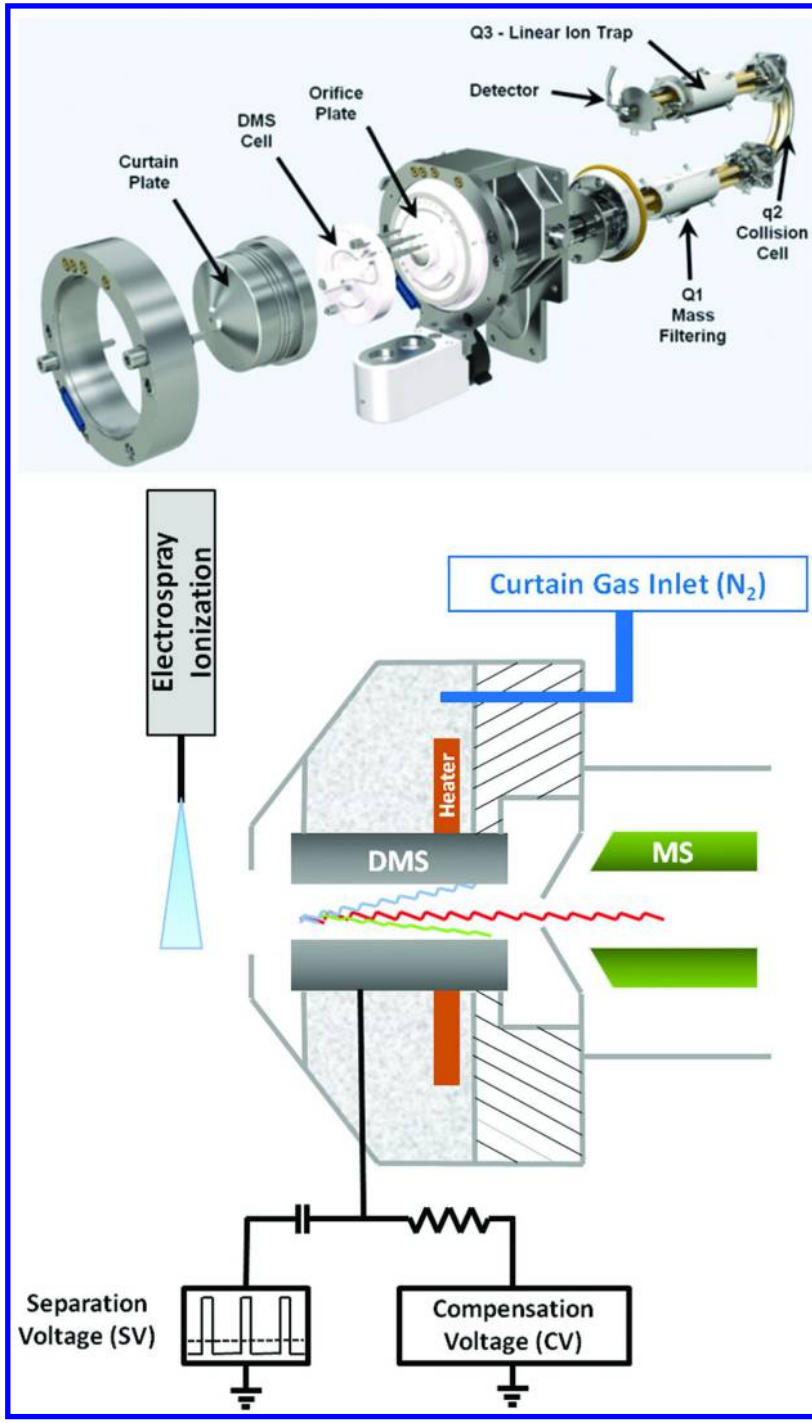

Figure 1. Schematic exploded diagram of a differential mobility spectrometer (DMS) as coupled to a quadrupole-based mass spectrometer. (see color insert)

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Case Study #1: The Challenge of Triazole Fungicide Metabolites in Plant Materials (Jasak et al., 2012)

Generally, DMS finds its greatest utility in the most challenging analytical workflows, where chromatographic retention is sub-optimal, isobaric chemical noise is debilitating to analyte detection, and the potential for isomeric forms of the analyte(s) is present. All of these are commonly encountered in environmental analyses (*18*, *19*). While several DMS-based environmental workflows have appeared in the recent literature (*20–24*), the application of DMS for detecting triazole-derivative metabolites in plant materials highlights all of these attributes of DMS (*25*).

At the structural core of many fungicides, such as triadimefon, cyproconazole, propioconazole, epoxiconazole, and tebuconazole, is 1,2,4-triazole (TRZ), a low molecular weight, highly polar compound, which is the main degradation product of these commercial products. With its suspected deleterious health effects (*26*), TRZ and its derivatives have been highlighted by the US EPA as an environmental contaminant. Upon its uptake into plant tissues, TRZ is rapidly metabolized initially to triazole alanine (TAL), then triazole lactic acid (TLA), and triazole acetic acid (TAA) (Figure 2) (*27*) – all of which are generally targeted as analytes to examine the metabolism of TRZ-based fungicides.

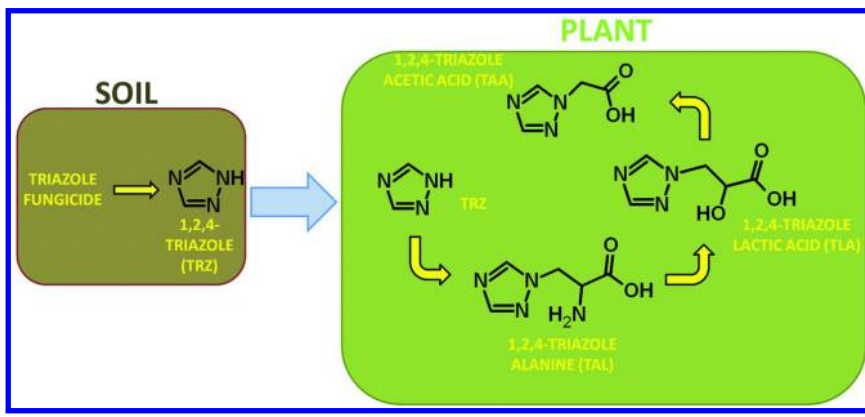

Figure 2. Simplified diagram outlining the flow of triazole-based fungicides in soil through their metabolism in plants. (Adapted from Jasak et al., 2012, Reference (25)) (see color insert)

From an analytical standpoint, these TRZ-based fungicides and their metabolites required quantitation limits of at least 10 µg/kg material, even though the analyses exhibit all three of the aforementioned challenges (sub-optimal chromatography, high chemical noise levels, potential for isomers). For example, while the QuEChERs method (*28*) works well for the fungicides, TRZ and its metabolites – with their low molecular weights and higher polarities – are not extracted as efficiently.

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Alternative workflows employing solid-phase extraction (*29*) or analyte derivitzation (*30*) have been explored but, even using these alternative workflows, analyses continued to struggle against high background signal levels. Ultimately, the desired limits of quantitation (LOQs) were obtained, at the cumulative costs of time-consuming sample handling and derivatization reagents. Lastly, the possibility of the presence of the 1,2,3-triazole isomer of 1,2,4-triazole could not be discounted and, if present and not LC-separable, could confound results.

The new simplified extraction method consisted homogenization of the plant material with methanol/water, filtration, evaporation to an aqueous residue, addition of stable-isotope labeled internal standards, and a final filtration through syringe filters. Then, samples were injected onto the LC column for elution into the DMS-MS/MS system. A diverse selection of plant materials were chosen to validate this new, more economical method: high water content (lettuce head, tomato fruit, and broccoli curd), high protein content (dried French bean, dried soy bean seed), high starch content (turnip root, carrot root, and barley grain), high acid content (orange fruit and grapes), and high fat content (flax seed).

In addition, just as all of the plant materials were subjected to the same extraction method, each subsequent extract was analyzed using identical LC and MS conditions (save for the DMS and its requisite settings). For example, the same solvent gradients, columns, temperatures, etc. were employed to separate the TRZ, TAA, TLA, and TAL from the background matrices. In addition, identical ESI (positive ion mode, $[M+H]^+$ for each analyte) and MS/MS parameters (collision energies, dwell times, etc.) were employed for each analyte – for both DMS and non-DMS experiments. Thus, an equivalent set of experimental parameters – with the exception of the activated DMS cell – were established to observe any benefits of this technology.

The optimal DMS parameters for separation of analytes from chemical noise were easily obtained. This process began by T-infusion of a solution containing four TRZ-based analytes into a stream of LC eluent flowing at the final LC method's flow rate and the gradient compositions at which each analyte elutes from the LC column. Then, DMS-specific parameters, such as SV, CV, cell temperature, and choice of chemical modifier (if any) were evaluated to maintain optimal signal conditions. Ultimately, for the TRZ-based analytes, the highest signals were obtained at SV = 3000 V where analytes and co-extracted matrix compounds were separated well in terms of CV. The DMS system was optimized without use of a chemical modifier (i.e., nitrogen only in curtain gas), which is not uncommon if there are great enough structural differences between the analytes and the underlying chemical noise components, resulting in different mobilities of each species.

Results

The benefits of employing DMS for the analysis of the extracts spiked with TRZ-based analytes were immediately evident. Without the DMS, analyses done at the 0.01 mg/kg analyte levels yielded LC chromatograms rife with high levels of ambient background noise and spurious peaks near and at the predicted retention times of the analytes (Figure 3, top two rows). Obviously, quantitation was

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impossible at these levels using LC alone as the separation mechanism. However, implementation of the DMS (with optimal SV and CV for each analyte engaged) yielded clearly discernable peaks in all of the LC-DMS-MS/MS chromatograms (Figure 3, bottom two rows).

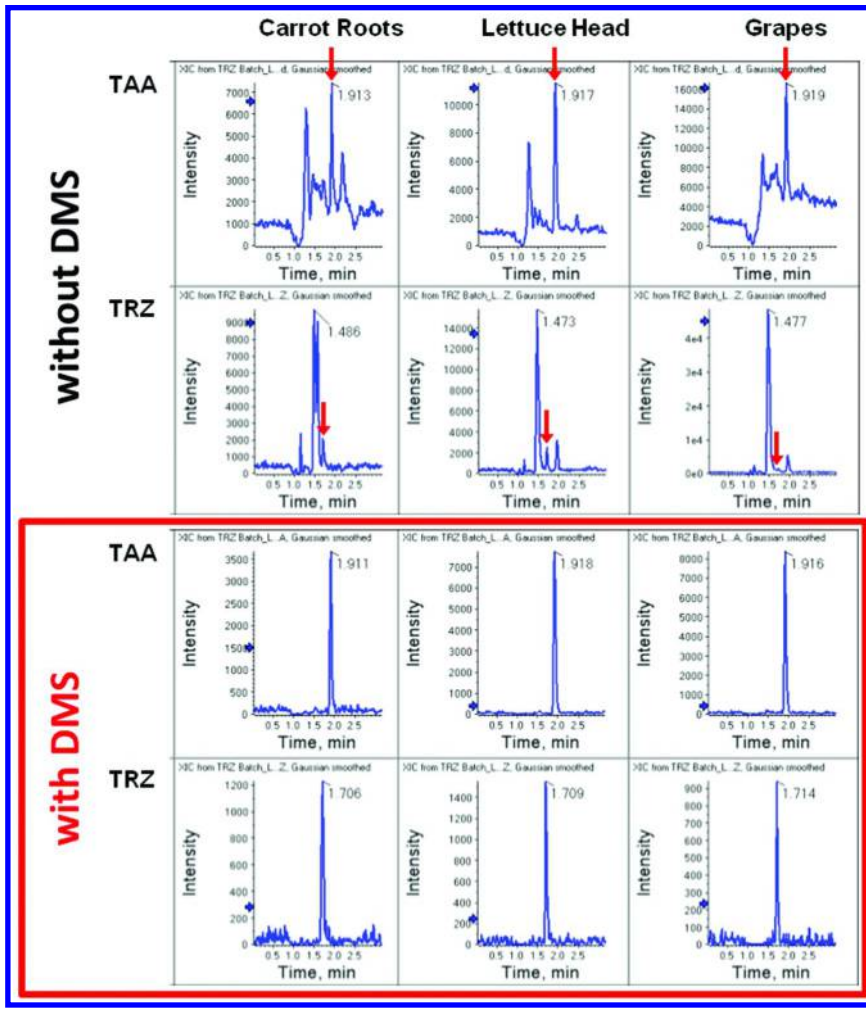

Figure 3. Signals derived from LC-MS/MS analyses of two metabolites of triazole-based fungicides from three different plant extracts. Note the vast improvement (bottom two rows) of the signal-to-noise ratio and spectral clarity when DMS is employed versus without DMS (top two rows). (Adapted from Jasak et al., 2012, Reference (25)) (see color insert)

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While the success of the LC-DMS implementation boded well, the overall success of the new workflow depended equally on the recoveries obtained from the simplified extraction method, the linearity of the analyte response, and obtaining meaningful LOQs for the TRZ-based metabolites. To meet established guidelines (*31*), the analyses at 0.01 – 0.10 mg/kg must have mean recoveries between 70 and 120% and relative standard deviations (RSDs) below 20%. In all but three individual cases (TAA, French bean, 0.01 mg/kg, 129%; TRZ, soy bean, 0.01 mg/kg, 124%; and TAL, flax seed, 0.10 mg/kg, 65%), the new simplified method for TRZ-based analyte met guideline acceptance criteria. However, some of the materials used for the recovery experiments contained appreciable levels of endogenous analytes. This occurred most often for TAL (e.g., high ambient levels in broccoli curd) such that recoveries were only performed at 0.10 mg/kg for that matrix. Lastly, the linearity of signal response was tested for each compound in a range of 0.5 to 50 ng/L. All correlation coefficients were ≥0.9998 for the analytes, revealing that the LC-DMS-MS/MS system provides linear responses for all four TRZ-based metabolites within the analytical range.

Isomer Separation by DMS

The presence of other isomeric forms of 1,2,4-triazole cannot be discounted as being endogenous in plant materials and their resultant extracts. But, can the LC-DMS methodology distinguish 1,2,3-triazole from its 1,2,4-triazole isomer? As displayed in Figure 4, both the LC and the DMS parameters employed here can separate these isomers. In fact, when the DMS' CV was kept at -14.6V (i.e., the optimal setting for only the 1,2,4-triazole isomer) during the LC analysis, the only signal detected for a triazole occurred at the retention time for 1,2,4-triazole (Figure 4).

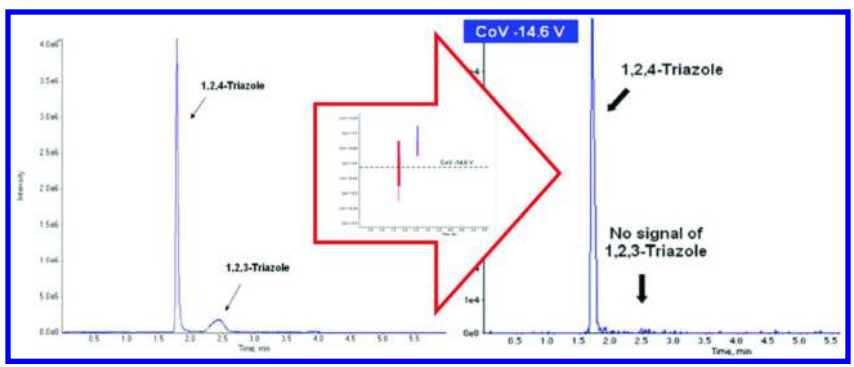

Figure 4. Isolation of two triazole isomers using both LC and DMS technologies. (Adapted from Jasak et al., 2012, Reference (25)) (see color insert)

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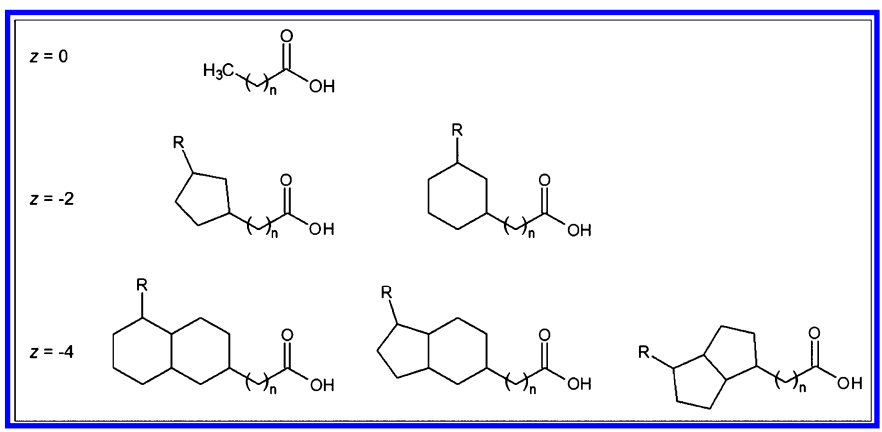
Summary

In summary, this LC-DMS-MS/MS workflow was validated for the analyses of the four TRZ-based metabolites typically found in plant materials treated with fungicides containing a 1,2,4-triazole functionality. Despite the challenges presented by the 11 representative materials from the five relevant crop groups, an average recovery of 96.0±4.6% (RSD) was achieved at the desired LOQ of 0.01 mg/kg. In addition, the simple, quick, and inexpensive sample preparation protocol required to meet these criteria was made feasible by the added separation power provided by the DMS. Without added clean-up or derivatization processes, much higher sample throughput was obtained, with laboratory costs and working time lowered by a factor of 3-5x.

Case Study #2: The Separation and Identification of Naphthenic Acids from Complex Oil Sands Process-Affected Waters Using DMS (Noestheden et al., 2014)

Naphthenic acids (NA) from oil sands process-affected water (OSPW) have been the subject of numerous mass spectrometry-based environmental studies (*32*, *33*). While most NAs have the generic molecular formula of $C_nH2_{n+z}O_2$ (where z is an even negative integer representing hydrogen deficiency) (Scheme 1), a more recent term, naphthenic acid fraction component (NAFC), has been created to include unsaturated and aromatic NAs, as well as heteroatom containing molecules (*32–34*). In the oil sands of northern Alberta, NAFCs have been the subject of much concern as these bitumen extraction byproducts produces large volumes of OSPW (*35*).



Scheme 1. Some representative structures of simple naphthenic acids, indicating the effect of z-number on the structural forms of these species.

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From an analytical standpoint, NAFC analyses often involve direct infusion of samples into an ultrahigh resolution mass spectrometer. Such systems with resolving powers >100,000 can provide reliable elemental compositions. However, deeper structural information from such systems is not straightforward and generally relies on chromatographic separations. Unfortunately, OSPW extracts are quite complex and often require long run times, multiple sample handling steps, or multi-dimensional chromatography. In addition, liquid and gas chromatography are both inherently serial processes, with limited time available to perform deeper structural interrogation of individual analytes.

An alternative to these workflows involves the combination of quadrupole time-of-flight mass spectrometer (qToF-MS) with differential mobility spectrometry (DMS) (*36*). As described earlier in this Chapter, DMS provides gas-phase separation capabilities that can provide "on-demand" access to selected analytes. By combining DMS with sample introduction via direct-infusion of OSPW extracts, the DMS-qToF MS workflow can resolve NAFCs rapidly (< 2 min). Such rapid, information rich methods could be important as regulatory guidelines evolve and as laboratories require faster and more economical workflows (like the aforementioned triazole example).

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Results

Given the complexity of the OSPW samples, the peak capacity of the DMS separations was optimized by adding a series of chemical modifiers to the transport gas. Since each ion was transmitted with a full-width half maximum of ~2.5 V, the greater the spread in total CV space covered by all of the NAFC ions, the greater the peak capacity of the DMS separation. Among the modifiers examined, methanol yielded the greatest total spread in CV (Figure 5 and 6).

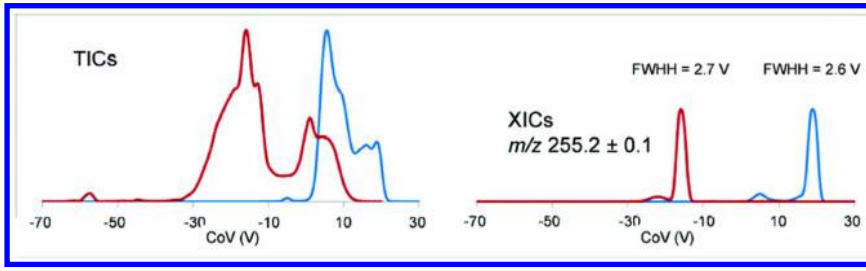

Figure 5. Total ion chromatograms (TICs) showing Merichem separation using DMS operating without chemical modifier (blue trace) and with methanol as chemical modifier (red). Overall, the peak capacity is increased since the red trace is broader (left) but individual ions display peaks having similar full-width at half-maximum (FWHM) (right). (Adapted from Noestheden et al., 2014, Reference (36)) (see color insert)

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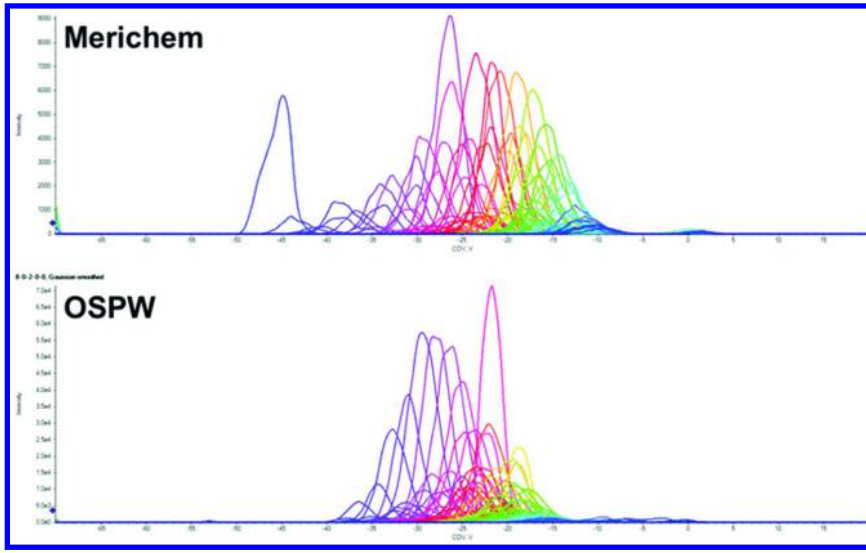

Figure 6. Extracted ion chromatograms showing the same ions present in both Merichem (top) and in OSPW (bottom) as separated by DMS. (Adapted from Noestheden et al., 2014, Reference (36)) (see color insert)

The DMS separation of individual NAFCs revealed a correlation between ion mobility and structure (Figure 7). For example, m/z 181.1233 ($C_{11}H_{17}O_2^-$) was separated from the more saturated analogue at m/z 183.1390 ($C_{11}H_{19}O_2^-$), only two hydrogens added) by +2 V. However, this $C_{11}H_{17}O_2^-$ ion was only separated by +3 V from $C_{12}H_{17}O_2^-$ (an acid one carbon atom heavier) and by +4 V separated from $C_{12}H_{19}O_2^-$ (one CH_2 unit heavier). The more unsaturated analogues exhibited more negative CVs, and the ring/double bond analogues displayed different mobility behavior than the linear chain extended analogues. Such subtle structural differences yield separation of closely related ions in the DMS, demonstrating the potential of this technology. In addition, correlations between CVs and homologous carbon series and z-series were also observed (Figure 7).

Another feature of the DMS separation of NAFCs was its ability to separate isobaric and isomeric ions. In the OSPW extract, m/z 143.1080 ($C_8H_{15}O_2^-$, 1.7 ppm) was transmitted at two CVs (Figure 8). The separation of these two potential isomers was rapid (~3 sec) and due to the infusion-based sample analysis, MS/MS analysis of each m/z 143.1080 ion was easily performed by fixing the appropriate CV values. Each MS/MS spectrum was consistent with the presence of distinct isomeric species, which was verified by analysis of three authentic $C_8H_{16}O_2$ isomers: valproic acid, 2-ethylhexanoic acid and n-octanoic acid (Figure 8). Separation of such isomers by GC or LC generally requires minutes of elution time, but using the DMS, separated required mere seconds and could be analyzed on-demand.

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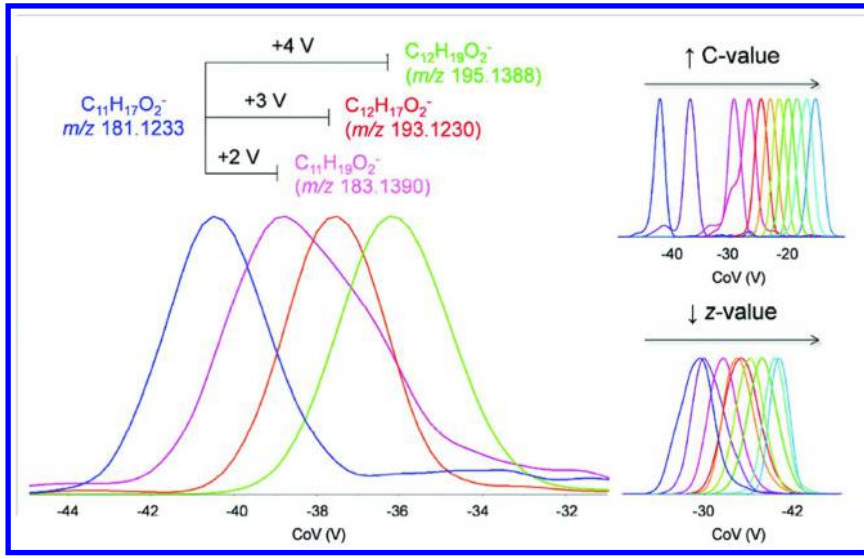

Figure 7. Total ion chromatograms (TICs) showing Merichem separation using DMS operating without chemical modifier (blue trace) and with methanol as chemical modifier (red). Overall, the peak capacity is increased since the red trace is broader (left) but individual ions display peaks having similar full-width at half-maximum (FWHM) (right). (Adapted from Noestheden et al., 2014, Reference (36)) (see color insert)

There were many examples where DMS separated isobaric species, including palmitic acid ($C_{16}H_{31}O_2^-$), a known contaminant in laboratory environments. This lipid was observed at m/z 255.2329 (Figure 9) and was found in the presence of an isobaric ion at m/z 255.1405. While TOF-MS can resolve these ions, further interrogation by MS/MS would be complicated without DMS. Both closely spaced ions would be sampled by the quadrupole mass filter simultaneously and would yield heavily convolved MS/MS spectra. Like the separation of the $C_8H_{16}O_2$ isomers, the CV was optimized to isolate each ion in real-time. Fragmentation of the anion at m/z 255.2329 (CV = -21 V) proceeded via loss of water (m/z 237.2234, 4.3 ppm), consistent with palmitic acid, while the MS/MS of m/z 255.1405 (CV = -26 V) showed carbon dioxide loss (m/z 211.1520, 2.3 ppm), suggesting a different carboxylic acid - $C_{17}H_{19}O_2$ (5.7 ppm) (Figure 9).

In LC-MS analyses of NAFCs, interferences like the aforementioned palmitic acid, stearic acid, or dodecyl sulfate, are generally present as contaminants that are ionized continuously throughout a chromatographic run. Unfettered, these background ions may ultimately deteriorate mass spectrometer performance. However, DMS efficiently removes background ions by sequestering them in discrete CV ranges, outside of which they will not appear in the analytical data. This capability allowed an accurate assessment of the interfering species present in the analytical OSPW samples, ensuring that only authentic NAFCs were reported from the database searches.

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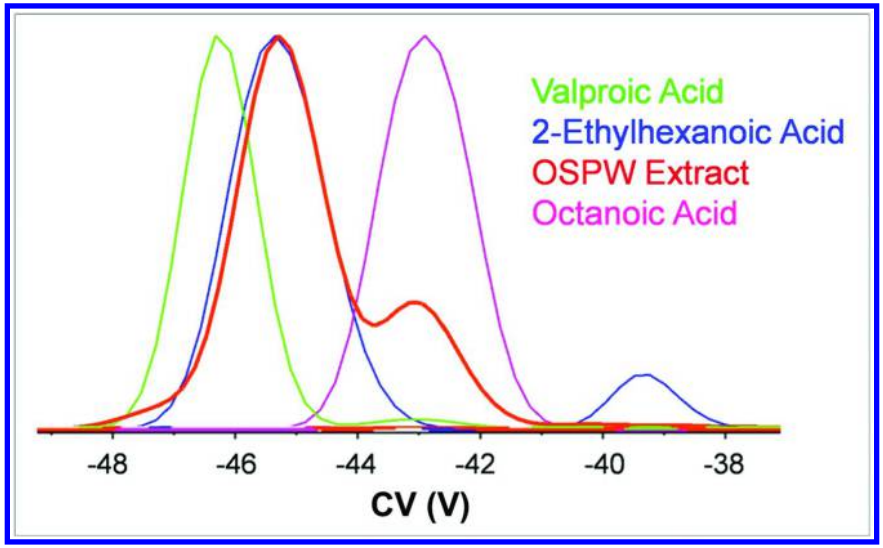

Figure 8. Structural isomers of $C_8H_{16}O_2$ identified in the OSPW extract (red). Subsequent analysis of authentic samples of valproic acid (green), 2-ethylhexanoic acid (blue) and octanoic acid (pink) revealed their presence in the OSPW extract. (Adapted from Noestheden et al., 2014, Reference (36)) (see color insert)

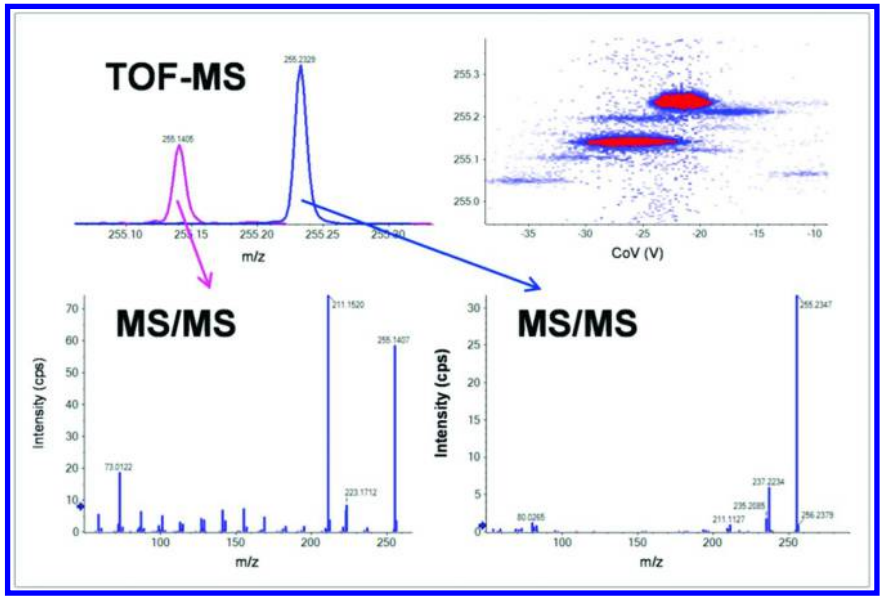

Figure 9. DMS-resolved isobaric ions from an OSPW extract via mass resolution (top left) and ion mobility (top right). MS/MS analysis of m/z 255.2329 is consistent with palmitic acid (bottom right), while the m/z 255.1405 is consistent with another carboxylic acid (bottom left). (Adapted from Noestheden et al., 2014, Reference (36)) (see color insert)

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A popular tool in petroleum analyses, Kendrick mass plots, which display Kendrick mass defect as function of Kendrick mass (*37*), provide a high level visualization of the composition of complex mixtures, like OSPW extracts. In the Kendrick mass plot (Figure 10) all relevant mass peaks in a spectrum are normalized against methylene, such that a horizontal line represents a homologous carbon series for a particular compound class. Moreover, as the degree of unsaturation increases, so does the Kendrick mass defect. This translates to easy to assess trends. For instance, in Figure 4 the red lines indicate a series of simple acids (i.e., only O_2 compounds) with increasing z-values, while the green lines represent a homologous series of O_3 compounds at differing degrees of unsaturation. Finally, the blue lines demonstrate the observable trends in unsaturation for a given carbon number.

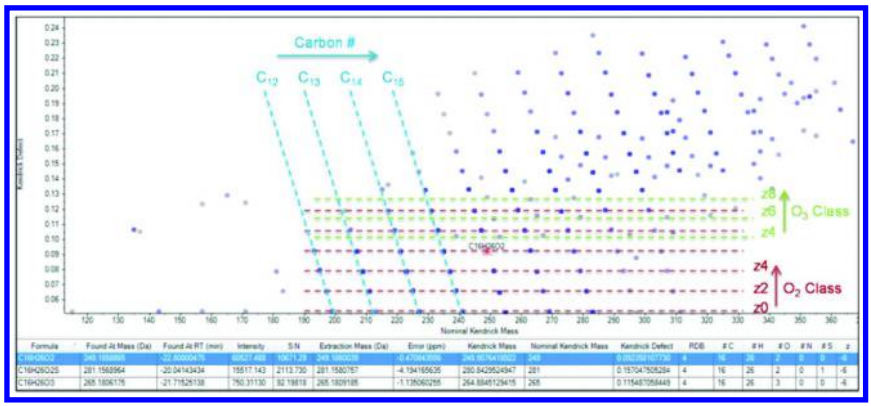

Figure 10. Kendrick mass plot of the OSPW extract. (see color insert)

After DMS separation, the ions detected in the Merichem standard and OSPW extract were searched against a curated database, and matches were compiled as a function of compound class and z-value. Results were broken down further based on total response and the number of homologues identified (Figure 11). Exact agreement between the distribution of ion classes observed in the current study and literature values was not expected given the heterogeneity of OSPWs. By area response (99%) and the number of identified homologues (97%), the Merichem standard consisted primarily of O_2 species. Analysis of the Merichem results by z-value showed a significant number (42%) of homologues $z \leq -6$ (i.e., - 8, -10, etc.). This result seemed contradictory to reported compositions, which state that the Merichem standard consists predominantly of compounds $z \geq -4$.(32, 34) However, these literature composition assessments were all response comparisons, not an identification of the number of homologues present. The response data in the current study showed that those compounds $z \geq -4$ accounted for 82% of the total area response, consistent with literature reports. The results by compound class (log10 scale) supported this comparison, with the Merichem standard containing almost exclusively O_2 species and a small amount of higher oxygen content and heteroatom-containing species.

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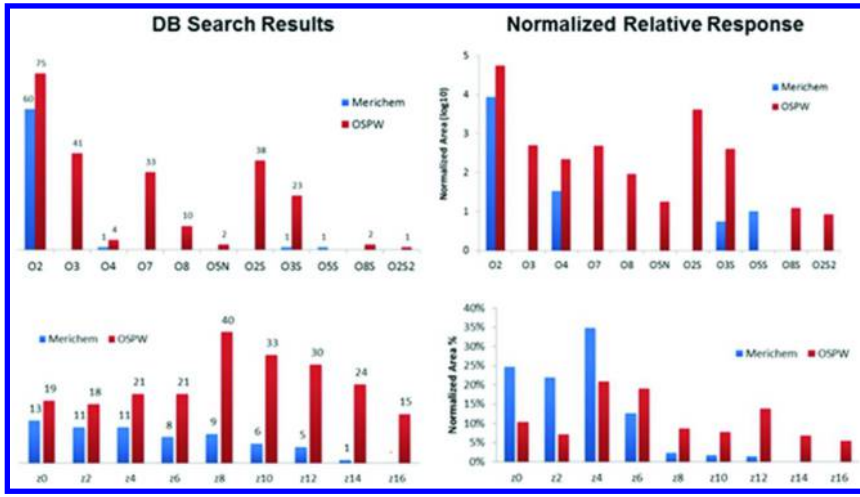

Figure 11. Evaluation of Merichem and OSPW samples as a function of the number of database-identified compounds grouped by class (left) and area response (right). Results are further broken down by compounds class (top) and z-value (bottom). Values shown above the homologue plots are the number of homologues identified during database (DB) searching. Due to the large differences in absolute response between compound classes this data was converted to a log10 scale. (Adapted from Noestheden et al., 2014, Reference (36)) (see color insert)

The OSPW extract showed a more widely distributed number of database matches across the compound classes and z-values evaluated (Figure 11). Interestingly, despite comprising 49% of the positive DB matches, O_3 to O_8 compounds only accounted for 2.8% of the total area response. As with the Merichem results, such compounds either have low response factors or are present at very low levels. Conversely, the z-value results for the OSPW extract showed a relatively even distribution across the number of database matches and the relative area responses of the different z-classes. These results are consistent with the demonstrated composition of OSPW extracts, which are known to contain higher oxygen content and increased unsaturation/polycyclic compounds due to weathering and metabolic processes (*32*, *34*). A detailed breakdown of the compound classes (log10 scale) by area response showed that the OSPW extract was composed of mostly O_2-species (86%), with significant amounts of O_2S (6.6%), O_3 (0.8%) and O_3S (0.6%) compounds (Figure 11). In addition, several potentially interesting compound classes (e.g., O_2NS, O_5NS, and $O_4N_2S_2$) were observed at very low abundances (<0.08% of total area).

Summary

Overall, the DMS-qToF-MS workflow generates complex and insightful datasets very quickly (< 2 min), including the resolution of isomers and isobaric

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ions. The ability to scan the DMS cell to target specific analytes provides on-demand access to deeper structural interrogation. With its speed and lower resource cost compared to chromatographic alternatives, DMS represents a greener analytical technique that requires much less organic solvent or gas consumption. Analysis of a technical Merichem standard and an OSPW extract demonstrated results that are consistent with literature values for similar samples. Owing to the unique separation mechanism of DMS, structural isomers can be resolved and rapidly interrogated in real-time. Finally, compared to standard infusion-based NAFC analyses, DMS-qToF-MS should provide more accurate qualitative and quantitative results owing to the mitigation of background ions and deleterious space charge effects possible when directly infusing complex mixtures.

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Future Outlook

As stated previously, DMS can play a key role in environmental mass spectrometry labs when chromatographic separation is problematic, when isobaric chemical noise cannot be resolved, or when isomeric forms of the analyte(s) are present. As noted recently (*19*), there are several challenges that DMS could address in the analysis of isomeric species, be they enantiomers (*38*) or structural isomers. These are challenges that have been addressed by using DMS in other application spaces, such as lipid analyses (*12*, *13*).

In addition, the expansion of the orthogonal separations provided by combining LC, DMS, and mass spectrometry have been explored in only a handful of instances (*25*, *39*, *40*). The possibilities for these combinations are vast and will undoubtedly flourish in the coming years, and could serve environmental mass spectrometry very well.

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