AEROBIC BIODEGRADATION OF DOMINANT MICROPOLLUTANTS IN THE ERGENE RIVER BY RIVER MICROCOSMS AND PURE CULTURES OF BACTERIA

by
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ABSTRACT

AEROBIC BIODEGRADATION OF DOMINANT MICROPOLLUTANTS IN THE ERGENE RIVER BY RIVER MICROCOSMS AND PURE CULTURES OF BACTERIA

The Ergene River is the most polluted surface water in Turkey. A great number of micropollutants, which have potential toxic and endocrine-disruptive effects and play a role in antibiotic resistance, are identified in the Ergene River. Once discharged into the environment, micropollutants are exposed to various natural attenuation processes such as dilution, sorption onto solids and sediments, photolysis, hydrolysis, and biodegradation. However, biodegradation is considered the most important process for the removal of most micropollutants. In this study, aerobic biodegradation of forty-three micropollutants belonging to different chemical groups such as industrial chemicals i.e. plasticizers, UV blockers, surfactants etc., antibiotics, pesticides and personal care products i.e. fragrances, pharmaceuticals etc. were investigated in river water samples taken in August 2017, November 2017, February 2018, and May 2018. Analysis of the micropollutants was performed using ultra-high-performance liquid chromatography and tandem mass spectrometry (UHPLC-MS^n). At the end of the study, most compounds such as acetaminophen, celestolide, piperonyl butoxide, oxybenzone, and 3-chloraniline were degraded via river microcosms at different rates whereas diuron, N-Ethyl-p-toluenesulfonamide, benzo[a]pyrene, benzanthrone, terbutryn, prochloraz, and omethoate were not removed in any samples. Aerobic biodegradability of hexa(methoxymethyl)melamine, the most abundant target micropollutant in the river was also investigated using different bacterial strains previously isolated at BIOMIG Lab. A strain named *Pseudomonas* sp. BIOMIG1 transformed almost all hexa(methoxymethyl)melamine into melamine in 45 days. With this study, aerobic biodegradability of various micropollutants in the Ergene River and the biotransformation of hexa(methoxymethyl)melamine have been first revealed.

ÖZET

ERGENE NEHRİ'NDEKİ YAYGIN MİKROKİRLETİCİLERİN NEHİR MİKROKOZMLARI VE SAF BAKTERİ KÜLTÜRLERİ İLE AEROBİK BİYOBOZUNUMU

Ergene Nehri, Türkiye'nin en kirli yüzey suyudur. Ergene Nehri'nde toksik ve endokrin bozucu etkileri olduğu ve antibiyotik direncinde rol oynadığı bilinen çok sayıda mikrokirletici madde tanımlanmıştır. Çevreye salındıktan sonra mikrokirleticiler seyrelme, katılara ve çökeltilere tutunma, fotoliz, hidroliz ve biyolojik bozunma gibi çeşitli doğal azalma süreçlerine uğramaktadır. Bununla birlikte biyolojik bozulmanın çoğu mikrokirleticinin uzaklaştırılmasında en önemli süreç olduğu düşünülmektedir. Bu çalışmada Ağustos 2017, Kasım 2017, Şubat 2018 ve Mayıs 2018 tarihlerinde alınan nehir suyu örneklerinde plastifiyanlar, UV blokerleri, yüzey aktif cisimleri gibi endüstriyel kimyasallar, antibiyotikler ve pestisitler ile kokular ve ilaçlar gibi kişisel bakım ürünleri gibi kırk üç mikrokirleticinin aerobik biyobozunması araştırılmıştır. Mikrokirleticilerin analizi ultra yüksek performanslı sıvı kromatografi ve tandem kütle spektrometrisi (UHPLC-MS^n) ile gerçekleştirilmiştir. Çalışmanın sonunda asetaminofen, selestolid, piperonil bütoksit, oksibenzon ve 3-kloroanilin gibi bileşiklerin farklı oranlarda nehir mikrokozmomları tarafından bozunduğu görülürken diuron, N-Etil-p-toluensülfonamid, benzo[a]piren, benzantron, terbütrin, prokloraz ve ometoatın hiçbir örnekte bozunmaya uğramadığı tespit edilmiştir. Nehirde en bol bulunan hedef mikrokirletici madde olan heksa(metoksimetil)melaminin aerobik biyobozunurluğu, daha önce BIOMIG Laboratuvarı'nda izole edilmiş olan farklı bakteri suşları kullanılarak da incelenmiştir. *Pseudomonas* sp. BIOMIG1'in hemen hemen tüm heksa(metoksimetil)melamini 45 günde melamine dönüştürmüştür. Bu çalışma ile Ergene Nehri'ndeki çeşitli mikrokirleticilerin aerobik biyobozunurluğu ve heksa(metoksimetil)melaminin biyotransformasyonu ilk kez ortaya ortaya çıkarılmıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

Abbreviation	**Explanation**
3-CA	3-Chloroaniline
4-CA	4-Chloroaniline
4-MBTA	4-Methyl-1H-benzotriazole
5,6-DMBTA	5,6-Dimethyl-1H-benzotriazole
5-MBTA	5-Methyl-1H-benzotriazole (5-Tolytriazole)
ACF	Aclonifen
ACMP	Acetamiprid
ACs	Activated Carbos
ADBI	Celestolide
AHDI	Phantolide
AOPs	Advanced Oxidation Processes
APAP	Acetaminophen (Paracetamol)
AUG	August
AZM	Azithromycin
BAC	Benzalkonium Chlorides
BaP	Benzo[a]pyrene
BDMA	N-Benzyldimethylamine
BMA	N-Benzylmethylamine
BOD	Biochemical Oxygen Demand
BP-1	2,4-Dihydroxybenzophenone (Benzophenone-1)
BP-3	Oxybenzone (Benzophenone-3)
BTA	1,2,3-benzotriazole
BTMA	Benzyltrimethylammonium
BZA	7H-Benzo(de)anthracene-7-one (Benzanthrone)
C	Concentration
C_0	Initial Concentration
C_{12}BDMA	Benzyldimethyldodecylammonium
C_{14}BDMA	Benzyldimethyltetradecylammonium
CAM	Clarithromycin
CE	Collision Energy

CF	Ciprofloxacin
CUR	Curtain Gas
CXP	Cell Exit Potential
CYA	Cyanuric Acid
D	Day
DCH	Dicyclohexylamine
DCMU	Diuron
DEA	Atrazine-desethyl
DEET	N,N-Diethyl-m-toluamide
DEHP	Di(2-ethylhexyl)phthalate
DI	Deionized Water
DMMM	Di(methoxymethyl)melamine
DP	Declustering Potential
ESI	Electrospray Ionization
EU	European Union
FEB	February
GAC	Granular Activated Carbon
GS	Ion Source Gas
H	Hour
HHCB	Galaxolide
HMMM	Hexa(methoxymethyl)melamine
HPLC	High-Performance Liquid Chromatography
IS	Ion Spray Voltage
K	Rate Constant
k_H	Henry's Law Constant
kPa	Kilopascal
LB	Luria-Bertani
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MEL	Melamine
MBC	Carbendazim
MBR	Membrane Bioreactor

MF	Microfiltration
mg/L	Milligram/Liter
MITI	Ministry of International Trade and Industry
MMMM	Mono(methoxymethyl)melamine
MP	Methyl Paraben
MQ	Mepiquat
MRM	Multi Reaction Monitoring
MS/MS	Tandem Mass Spectrometry
MS^n	Tandem Mass Spectrometry
N/A	Not Available
NETSA	N-Ethyl-p-toluenesulfonamide
N	Nitrogen
NF	Norfloxacin
NF	Nanofiltration
ng/L	Nanogram/Liter
NOV	November
NP2EO	Nonylphenol Diethoxylate
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NTR	Nitrogen regulatory
OECD	The Organisation for Economic Co-Operation and Development
OF	Ofloxacin
OMA	Omethoate
P	Partition Coeffecient
Pa	Pascal
PAC	Powdered Activated Carbon
PBO	Piperonyl Butoxide
PCZ	Prochloraz
PMMM	Penta(methoxymethyl)melamine
Q_1	Parent Ion
Q_3	Fragment Ion
QNP	Quinalphos
R^2	The Coefficient of Determination
RF	Reverse Osmosis
R_t	Retention Time

sCOD	Soluble Chemical Oxygen Demand
SD	Standard Deviation
SMX	Sulfamethoxazole
SP	Sampling Point
s-Triazines	Symmetric Triazines
T	Time
$T_{1/2}$	Half-Life
TBEP	Tris(2-Butoxyethyl) Phosphate
TBN	Terbutryn
TEM	Temperature
TMMM	Tetra(methoxymethyl)melamine
TriMMM	Tri(methoxymethyl)melamine
UF	Ultrafiltration
UHPLC	Ultra-High-Performance Liquid Chromatography
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
UV	Ultraviolet
VOCs	Volatile Organic Compounds
WHO	World Health Organization
W/M	Weight/Volume
WWAP	World Water Assessment Programme
WWTP	Wastewater Treatment Plant
µg/L	Microgram/Liter
µM	Micromolar
µm	Micrometer

1. INTRODUCTION

Water pollution is one of the most challenging environmental problems around the globe today (Schwarzenbach et al., 2010). Any physical, chemical or biological factors that lead to aesthetic or detrimental effects on aquatic life and on those who consume the water are defined as a water pollutant (Goel, 2006). Over the last few decades, the occurrence of a large group of inorganic and organic chemical substances in the aquatic environment has been taking great attention due to their potential adverse effects on both humans and the environment. Those chemicals generally occur at ng/L and µg/L in the world's water and therefore referred to as 'micropollutants'. Micropollutants include both natural and anthropogenic substances such as pharmaceuticals, personal care products, steroid hormones, and pesticides. Sources of micropollutants encompass agricultural runoff, agricultural reuse of sewage sludge, and irrigation with treated wastewater as well as municipal, industrial and hospital effluents. The release of wastewater treatment plant effluents into water environment has been considered as the primary source of micropollutants since conventional wastewater treatment plants are not designed to target micropollutants and thus generally ineffective on them (Schwarzenbach et al., 2006; Farré et al., 2008; Luo et al., 2014; Kim and Zoh, 2016; Barbosa et al., 2016). Micropollutants have potential toxic (Grenni et al., 2018) and endocrine-disruptive effects (Kim and Farnazo, 2017) on aquatic organisms and play a role in antibiotic resistance (Pruden et al., 2006; Fent et al., 2006).

Micropollutants are exposed to various processes such as sorption onto solids and sediments, photolysis, hydrolysis, and microbial transformation which affect their fate in the environment (Jjemba, 2006; Mompelat et al., 2009). In engineered systems, advanced treatment processes such as activated carbon adsorption, advanced oxidation processes (AOPs), and membrane filtration systems have proven as effective technologies for the removal of micropollutants. However, advanced treatment technologies require high investment and maintenance costs and consist of quite complex procedures (Luo et al., 2014). Compared to physicochemical processes, biological treatment processes are relatively cheaper to build and operate. Moreover, biological transformation is considered as the major removal mechanism of micropollutants in wastewater (Garcia-Becerra and Ortiz, 2018). On the other hand, biodegradation potential of many micropollutants is not known in surface water. Therefore, the risk associated with the micropollutants as well as the mitigation strategies to reduce the risk associated with the chemical pollution are obscure.

The Ergene River, which is located in the north-western part of Turkey and used for irrigation of the most fertile agricultural land, has been suffering from severe organic and metal pollution for a long time. A great number of micropollutants were found at alarming concentrations in the river. In this study, aerobic biodegradation of forty-three micropollutants belonging to different chemical groups such as industrial chemicals i.e. plasticizers, UV blockers, surfactants etc., antibiotics, pesticides and personal care products i.e. fragrances, pharmaceuticals etc. were investigated using river microcosms and pure cultures of bacterial strains that are powerful degraders under aerobic conditions. Quantification of the micropollutants was performed using ultra-high-performance liquid chromatography and tandem mass spectrometry (UHPLC-MS^n). With this study, aerobic biodegradation of a great number of micropollutants in the Ergene River and the biotransformation of hexa(methoxymethyl)melamine by a bacterial strain have been first presented.

2. THEORETICAL BACKGROUND

2.1. Water and Water Pollution

Covering seventy-five percent of the Earth's surface, water is a fundamental need for all living entities. Not only most of the cells are surrounded by water but also cells themselves are made up of seventy to ninety-five percent of water (Reece, 2011). In natural systems, water nourishes vegetation soil and riverbeds, nourishes the full range of animals, removes organic material or transform it through weathering, and moves soil and other inorganic materials. As for humans, water is fundamentally a drinking source. Humans irrigate their cultivated plants to provide food and clothing to themselves. Humans also use water for transportation, construction, recreation, and generating hydroelectric or fossil-fuel energy (Wescoat et al., 2003).

Despite its extensive presence, only about 0.01 percent of the water on Earth is fresh and available as surface water in a liquid form. Of this small percentage, much is contained in lakes and rivers which are far from human populations. As a result, many water reserves are simply not readily accessible for humans (Smol, 2009). Moreover, the problem is more complicated today due to the fact that current patterns of human use of water are unsustainable, from five percent to twenty-five percent of global freshwater use exceeds long-term accessible supplies (Millennium Ecosystem Assessment, 2005).

Vorosmarty and colleagues (2010) have defined global stressors to human water security and river biodiversity. At highly endangered regions, they showed that water resource development and pollution were the main issues threating human society and the nature. It is an undeniable fact that water pollution is a major challenge in almost the whole world today (Schwarzenbach et al., 2010). Any physical, chemical or biological factors that lead to aesthetic or detrimental effects on aquatic life and on those who consume the water are defined as a water pollutant. Suspended and dissolved matters are chemical pollutants which constitute the main part of the water pollutants (Goel, 2006). Chemical pollutants are made up of two different groups; macropollutants and micropollutants. Macropollutants typically occur at the milligram per liter level and include nutrients such as nitrogen and phosphorous species, and natural organic components. Micropollutants involve inorganic and organic trace pollutants occurring at the nanogram to microgram per liter level (Schwarzenbach et al., 2010). Physical factors that can create pollution include heat and radiation. Certain microorganisms like pathogenic species are classified as biological pollutants (Goel, 2006). Due to the consumption of water contaminated with pathogens, each year 4 billion people caught diarrhea and 2.2 million people die, most of which are children under five (WHO and UNICEF, 2000).

Pollutants originate from many sources and are grouped in various ways. With respect to their source, pollutants can be divided into two classes; point source pollutants and non-point source pollutants or diffuse source pollutants. A point source pollutant derives from a clearly defined source, such as a smokestack from a smelter, an outflow pipe from a factory, or a sewage plant draining into a river. Because of having a clearly defined origin, these point source pollutants are generally easier to control. Measurement and monitoring of these pollutants can be done easily. When required, technological fixes can be applied more effectively and economically. However, non-point or diffuse pollutant sources such as pesticide or fertilizer runoff from agricultural fields in a lake catchment are often more difficult for managers and scientists to deal with (Smol, 2009).

One of the main causes of water pollution is urbanization. In larger cities, the problems of pollution are more severe in comparison to the smaller cities and rural areas, indicating there is a direct relationship between population density and pollution. The use of any kind of resources produces a waste, which is the basis of pollution in larger cities. In addition to urbanization; industrialization, civilization and living standards are also related to water pollution since they bring about an increase in resources and energy consumption. This explains why developed countries suffer much more from pollution in comparison to developing countries (Goel, 2006). However, other sources than industrialization, particularly agriculture, require more attention today than before (Abel, 2002). For example, fertilizer application in the croplands is one of the main contributors that made nitrate the most prevalent chemical contaminant in the world's aquifers. (Spalding and Exner, 1993). Agricultural pollution can also be seen in soil, water, and air as pesticide pollution, plastic film pollution, and poultry industry pollution which is caused by untreated manure, sewage and residual feed (Xudong, 2011).

2.1.1. River and River Pollution

A river channel is any linear depression on the Earth's surface regularly conveying surface runoff from a watershed to a natural outlet. The basic morphological features of a river channel include its banks, bed, and its exposed depositional bars and islands. Banks vary in shape and roughness during high and low flow periods while bed varies in material, form, and slope. The water within a channel also display differences in flow paths, velocities, suspended sediments, turbidity, chemical characteristics, and temperature regimes. All these physical factors give shape and alter habitats where diverse species of flora and fauna inhabit (Wescoat et al., 2003).

Rivers have been using by humans more than any other type of ecosystem (Singh, 2007). Humans make use of rivers for drinking, irrigation, transportation, fisheries, recreational activities, obtaining electric power, and other purposes (Abel, 2002). Besides, the riverine ecosystem is a real repository for a wide variety of plants, animals and green germplasms that are important suppliers of animal protein fish (Singh, 2007). Rivers have important self-purification capacities to assimilate and make pollutants less harmless; however, the existing pollution loads are often too much for rivers to cope with. According to the UN, the amount of wastewater produced annually is 1,500 km^3, which is six times more water than that in the world's rivers and two million tons of sewage, industrial and agricultural waste are released into the world's water every day (WWAP, 2003).

The discharge of crude or partially treated sewage into rivers is the commonest form of pollution. Discharge of wastewater from trade premises such as acid coal mining plants, tannery and textile factories is another important cause of river pollution (Klein, 2017). For example, the River Ganga, the most important river of India, has highly toxic pollutants due to domestic sewage and untreated industrial discharges (Goel, 2006).

Discharge of sewage and trade wastes affect physicochemical characteristics of rivers and has an adverse impact on aquatic life. Fermentable organic matters in sewage and trade wastes decompose and cause deoxygenation of rivers, which can lead to fish kill and objectionable smells due to hydrogen sulfide, mercaptans, and organic amines. Corrosive substances such as acids and alkalis and toxic substances such as cyanides and phenols in sewage and trade wastes can kill bacteria, animals, and plants. Pathogenic microorganisms discharged with sewage can induce epidemics. Heated effluents discharged into rivers from power stations can cause harmful rises in the temperature of the rivers. This can lead to undesirable putrefaction in rivers already polluted by organic matter and may also cause the destruction of fish. Undesirable physical effects such as turbidity, discoloration, foam, and radioactivity can also occur because of the discharge of sewage and trade wastes. Certain minerals such as calcium and magnesium can cause excessive hardness in river water, which makes it difficult to use for the certain manufacturing process. Excessive amounts of salt discharged into rivers may be objectionable when the chloride content of the river rises to a value which is harmful to fish and vegetation (Klein, 2017). Furthermore, in many parts of the world, rivers also have become contaminated with heavy metals such as zinc, lead and copper. Metal contamination in aquatic environments is a huge concern due to its toxicity, abundance and persistence in the environment, and subsequent accumulation in aquatic habitats. Heavy metal residues in contaminated habitats may accumulate in microorganisms, aquatic flora and fauna. Heavy metals enter the human body through consumption of these aquatic organisms and can lead to serious health problems (Cooke et al., 1990, Deniseger et al., 1990). Some of the anthropogenic sources of heavy metals pollution are mining and smelting operations, combustion of fossil fuels, processing and manufacturing industries, waste disposal, and agricultural runoff (Sutherland 2000, Chatterjee et al., 2007). In addition, untreated wastewater discharges into water resources is also a cause of heavy metal contamination since domestic and industrial wastewaters often contain heavy metals in high concentrations while non-contaminated fresh and saltwater contains extremely low concentrations of heavy metals, usually at or below $\mu g\ L^{-1}$ levels (He et al., 2005).

2.1.1.1. Pollution in the Ergene River. In Turkey, many rivers have been polluted with organics and heavy metals because of mining activities, agricultural discharges, industrial wastewater discharges, domestic wastewater discharges, and some natural events (Varol 2011, Uckun 2017, Bilgin and Konanc, 2016, Yilmaz and Koc, 2016, Ozseker and Eruz, 2016, Kucuk and Alpbaz, 2008). One of those polluted rivers in Turkey is the Ergene River. The Ergene River is born from Istıranca Mountains in Kavacık area of Vize and joins with the Maritsa River in İpsala passing through Saray, Çerkezköy, Çorlu, Muratlı, Lüleburgaz, Babaeski, Pehlivanköy, Hayrabolu, Uzunköprü and Meriç. After merging with the Maritsa River, it flows from the Saroz Gulf into the Aegean Sea. The Ergene River joins a large number of streams and creeks for 283 km from its source to the point where it meets the Maritsa River (Figure 2.1). Its water potential is 1.71 billion m^3 and 78% of this potential is surface water and while 22% is groundwater. The rapidly increasing distorted industrialization in the Ergene Basin after 1990 has brought about many environmental problems in the region. Most of the industrial facilities in the basin are located in Tekirdağ. Industry in this province has mainly developed around Çerkezköy, Çorlu, Muratlı and Lüleburgaz. The textile sector takes the first place in the sectoral distribution of industry and is followed by food, chemical, leather and mining sectors. In addition to the rapidly developing industry, increasing population, settlements and agriculture in the Ergene Basin have created the problem of water in terms of pollution load and water consumption in the basin. As the Ergene River flows, its flow rate increases six times due to domestic and industrial wastewater discharges. Today, the wastewater discharged without treatment into the Ergene River is 460,000 m^3 per day and corresponds to 65% of the total discharge amount. Industrial discharges of the textile factories concentrated in the region have caused color problems in water. Chemicals such as NaOH used during processing have created pH problems while oil and grease increased biochemical oxygen demand (BOD). In addition, high heavy metal concentrations have been also observed in the river because of the metal industry discharges (Marmara Belediyeler Birliği, 2018).

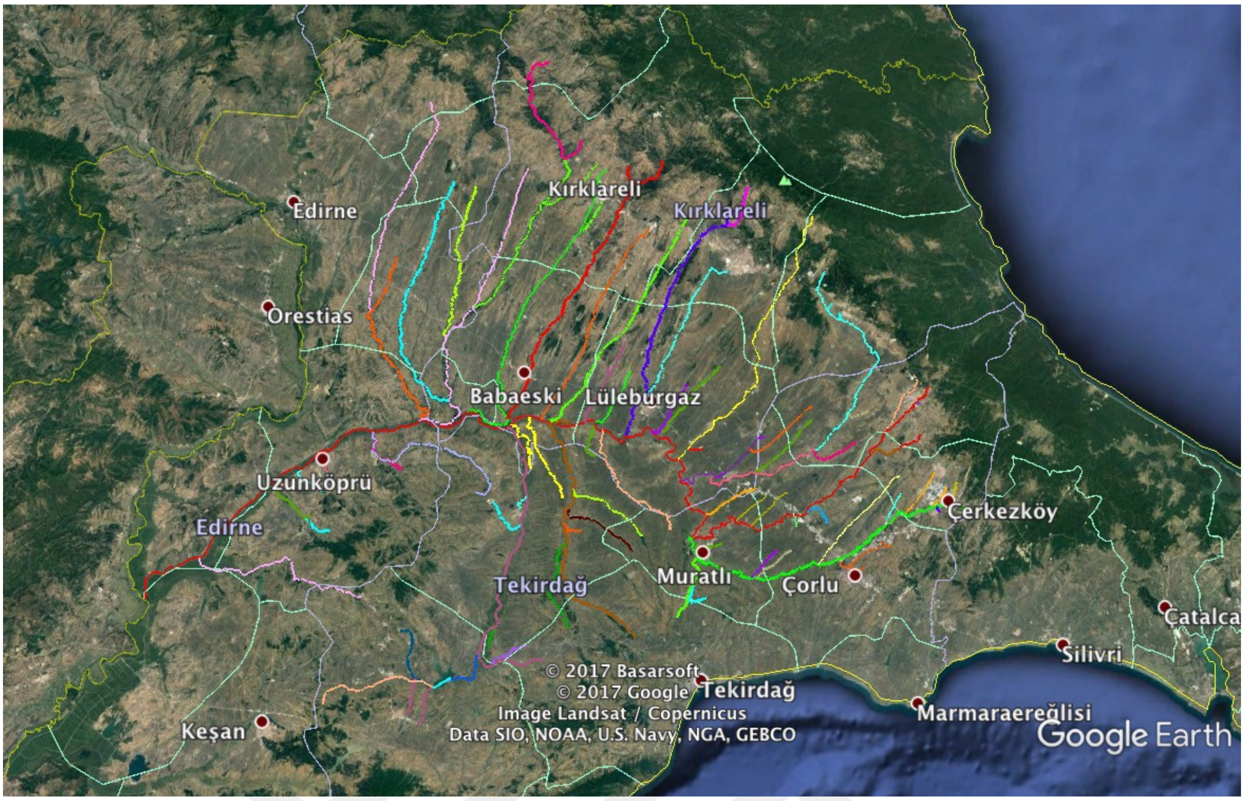


Figure 2.1. Satellite map showing the Ergene River and its tributaries

2.2. Micropollutants and Micropollutant Pollution in Surface Waters

With the advent of highly sensitive analytical techniques, such as liquid chromatography-mass spectrometry (LC-MS), pollutants once thought to have insignificant distribution and concentration in the environment have been largely investigated for the last decades (Farre et al., 2012; Rykowska and Wasiak, 2015). Recently, micropollutants have been drawing great attention due to their hazardous effects on both humans and the environment. These chemicals include both natural and anthropogenic substances such as pharmaceuticals, personal care products, pesticides, steroid hormones, surfactants, and industrial chemicals (Table 2.1). Because the concentration of these chemicals in water is generally between ng/L and µg/L, both their detection and removal are highly challenging. Sources of emerging contaminants are diverse such as municipal, industrial and hospital effluents, agricultural runoff, agricultural reuse of sewage sludge, and irrigation with treated wastewater; however, the release of wastewater treatment plant effluent into water environment has been considered the primary source (Figure 2.2). Conventional wastewater treatment plants are not designed to target micropollutants and consequently not effective on them, many of these compounds end up in surface water and become a threat to aquatic life. (Schwarzenbach et al., 2006; Farré et al., 2008; Luo et al., 2014; Kim and Zoh, 2016; Barbosa et al., 2016). Even at trace concentrations, micropollutants and/or their metabolites and transformation products have been thought to have toxic

(Gu et al., 2017; Grenni et al., 2018) and endocrine-disruptive effects (Vos et al., 2000; Tiwari et al., 2017; Kim and Farnazo, 2017), and play a significant role in the antibiotic resistance (Pruden et al., 2006; Fent et al., 2006; Dantas et al., 2008; Kemper, 2008).

Table 2.1. The major class and subclasses of micropollutants detected in the aquatic environments (Luo et al., 2014)

Class	Subclasses
Industrial chemicals	Fire retardants, plasticizers
Personal care products	Disinfectants, fragrances, insect repellents, UV filters
Pesticides	Fungicides, herbicides, insecticides
Pharmaceuticals	Antibiotics, anticonvulsants, beta blockers, lipid regulator, NSAIDs, stimulants
Steroid hormones	Estrogens
Surfactants	Non-ionic surfactants

Once discharged into the environment, micropollutants are exposed to various natural attenuation processes such as dilution, sorption onto solids and sediments, photolysis, hydrolysis, and biodegradation (Jjemba, 2006; Mompelat et al., 2009). However, micropollutants are affected at different degrees in those processes. For example, personal care products are inclined to sorption rather than biodegradation (Gómez et al., 2012). On the other hand, sorption is just of minor importance for hydrophilic and negatively charged pollutants like most of the pharmaceuticals (Verlicchi et al., 2012).

Having a direct impact on concentration, river water dilution decreases the level of micropollutants in the environment. Similarly, rainfall can lead to dilution of the pollutants so that their concentrations in the environment can become lower than wastewater effluents (Gros et al., 2007). For instance, in the summer, some micropollutant groups such can pharmaceuticals occur at lower levels than in the winter (Wang et al., 2011). This may happen due to the dilution by rainfall in the summer months or increased biodegradation because of warmer temperatures (Luo et al., 2014). However, rainfall can sometimes increase the concentration levels of some micropollutants such as biocides due to the leaching of these chemicals from buildings (Jungnickel et al., 2008).

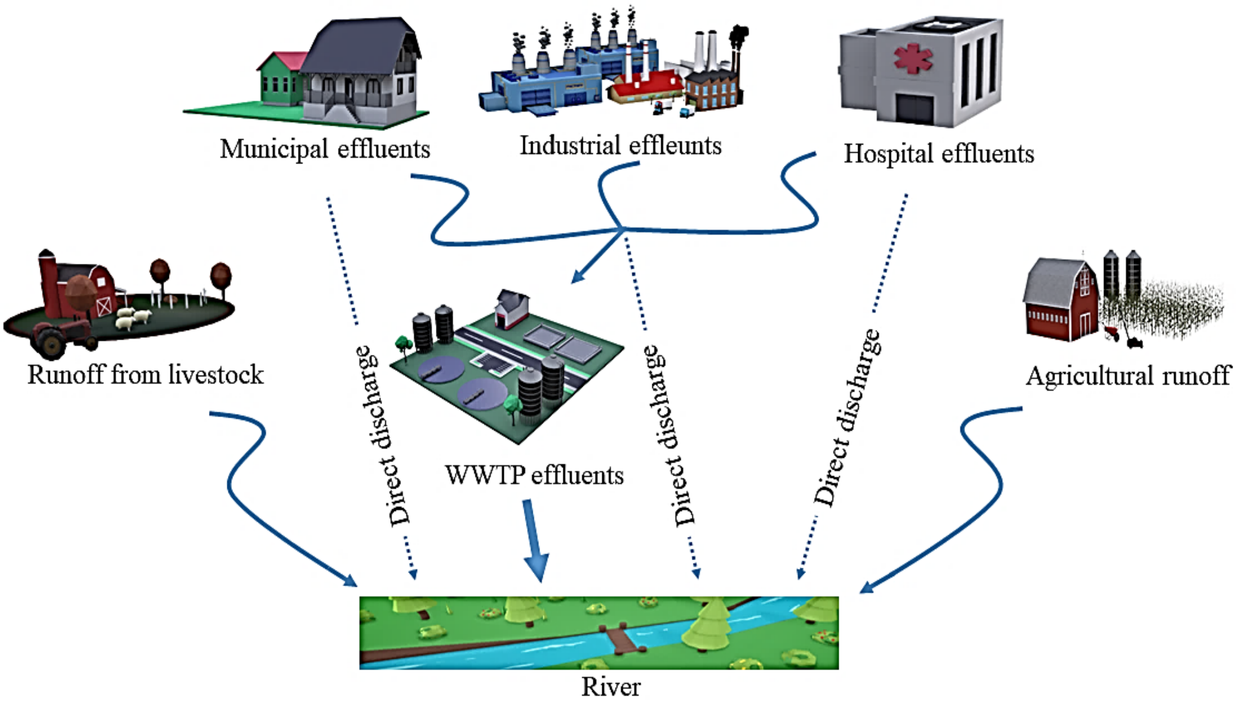


Figure 2.2. The major sources and pathways of micropollutants in rivers

2.2.1. Fate and Removal of Micropollutants in Conventional WWTPs

Municipal wastewater treatment plants are designed to remove soluble organic pollutants, suspended solids and flocculated matter and produce a high-quality effluent before environmental release (Tiwari et al., 2017). Although WWTPs are not designed to target micropollutants and generally don't achieve a proper elimination of them, several compounds can be removed with a moderate or even high efficiency in WWTPs (Luo et al., 2014). Major micropollutant removal mechanisms in WWTPs are comprised of sorption, biological transformation, abiotic degradation, and volatilization (Margot et al., 2015).

Sorption is the movement of a compound from the water compartment to the solid compartment (Margot et al., 2015). For the removal of hydrophobic or positively charged micropollutants, sorption onto sludge or particulate matter is of a great significance and can be achieved either hydrophobic interactions or electrostatic interactions. Hydrophobic interactions occur between hydrophobic micropollutants and suspended solids, extracellular polymeric substances or the lipophilic cell membrane of microorganisms while electrostatic interactions form between positively charged groups of the pollutant and the negatively charged surfaces of microorganisms and effluent organic matter (Ternes, 2006).

Sorption occurs mainly as sorption onto primary sludge in the primary treatment and as sorption onto activated sludge in the secondary treatment. The primary treatment, the main object of which is to remove suspended solids, have been found ineffective for the elimination of most micropollutants. (Luo et al., 2014) For most pharmaceuticals, sorption onto solids does not surpass 5% in most cases (Verlicchi et al., 2012). For example, ibuprofen, naproxen, sulfamethoxazole and estrone don't show a considerable reduction in primary treatment. However, some micropollutants such as galaxolide and tonalide can be eliminated well due to their sorption on primary sludge in primary treatment (Carballa et al., 2004; Carballa et al., 2005).

In the secondary treatment, micropollutants are exposed to different processes such as dispersion, dilution, partition, biodegradation and abiotic transformation (Luo et al., 2014). Biodegradation, biotransformation and sorption are the important removal mechanisms during biological treatment (Verlicchi et al., 2012). Mineralization or incomplete degradation of micropollutants can occur during secondary treatment. Single substrate growth of a specialist oligotrophic organism, cometabolism, and mixed substrate growth are the main micropollutant biodegradation mechanisms in the secondary treatment (Daughton and Ternes, 1999, Vader et al., 2000) However, biodegradation of micropollutants in wastewater treatment plants mostly occur via cometabolism rather than the metabolism pathway since many micropollutants are toxic and resistant to microbes. In addition, the presence of micropollutants in wastewater at trace levels also limits microbial growth and enzyme induction involved in the biodegradation (Tran et al., 2018).

Each micropollutant is considered to have different biodegradation potential. For example, while most antibiotics are not easily biodegradable (Verlicchi et al., 2012), an antibacterial and antifungal agent triclosan was found to show great biodegradation potential in secondary treatment (Samaras et al., 2013). Similarly, nearly % 60 of diuron, a pesticide, was reported to be degraded biologically by activated sludge (Stasinakis et al., 2009). However, in general, most pharmaceuticals, pesticides, and several household chemicals such as corrosion inhibitors, sweeteners, and phosphorus flame retardants are considered resistant to biodegradation (Margot et al., 2015).

The factors affecting micropollutant biodegradation in the secondary treatment are primarily the number of microorganisms available, the type of microorganisms, the biodegradability of the pollutant by these microorganisms, and the hydraulic retention time within the reactor (Joss et al., 2006). In addition, temperature, pH, redox conditions, and the availability of a co-substrate can influence biodegradation rates (Cirja et al., 2008; Ternes, 2006).

Organic micropollutants can also be degraded via abiotic reactions such as photolysis and hydrolysis in WWTPs. Photolysis refers to chemical bond cleavage happened when a photon is absorbed by a compound whereas hydrolysis is breakage of chemical bonds due to a substitution reaction between an atom or group of atoms in an organic compound and a water molecule. Both photolysis and hydrolysis are of negligible importance for the removal of micropollutants in WWTPs (Margot et al., 2015). However, β-lactam, macrolide, and tetracycline antibiotics are known as susceptible to abiotic degradation in WWTPs (Ying et al., 2013; Lee, 2003).

Volatilization of micropollutants can occur as surface volatilization but it is achieved mainly by stripping during aeration in WWTP (Margot et al., 2015). Agitation, temperature, and atmospheric pressure are also known to affect volatilization during the treatment operations (Pomies et al., 2013).

Vapor pressure and Henry's law constant (k_H) are commonly used to characterize the volatility of a compound. Organic compounds whose vapor pressure are higher than or equal to 0.01 kPa at 20 °C are defined as volatile organic compounds (VOCs) by The European Parliament and The Council of The European Union (Directive, 2010). The k_H ranging from 10^{-2} to 10^{-3} ($m^3 \cdot Pa/mol$) mostly indicates a high tendency of volatilization (Stenstrom et al., 1989). For micropollutants, volatility is generally considered insignificant and negligible. For example, volatility is totally negligible for pharmaceuticals and estrogens and is of minor importance for fragrances tonalide and galaxolide. (Suárez et al., 2008). On the other hand, volatilization plays a major role in the loss of another fragrance compound celestolide (Suarez et al., 2010).

Based on their removal degree, Luo and colleagues (2014) classified the micropollutants that are widely detected in WWTPs. More than half of the compounds e.g. acetaminophen, benzophenone-3, galaxolide, N,N-Diethyl-meta-toluamide were found to show high removal in WWTPs. On the other hand, several compounds e.g. atrazine, metoprolol, erythromycin, and carbamazepine were found to show poor removal. while diuron, sulfamethoxazole, tebuconazole, and atenolol displayed a moderate removal. Classification of widely detected micropollutants based on their removal degree in WWTPs is presented in Table 2.2.

Table 2.2. Classification of widely detected micropollutants based on their removal degree in WWTPs (Luo et al., 2014)

Degree of Removal	**Compounds**
Poorly removed (<40%)	Atrazine, carbamazepine, diazinon, diclofenac, erythromycin, metoprolol, mefenamic acid, tris(2-carboxyethyl) phosphine, tris(1,3-dichloro-2-propyl) phosphate
Moderately removed (40-70%)	Atenolol, bezafibrate, clofibric acid, diuron, ketoprofen, nonylphenol, sulfamethoxazole, tebuconazole, trimethoprim
Highly removed (>70%)	Acetaminophen, benzophenone-3, bisphenol A, caffeine, clotrimazole, dibutyl phthalate, N,N-Diethyl-meta-toluamide, bis(2-ethylhexyl) phthalate, estradiol, estriol, estrone, ethinylestradiol, galaxolide, gemfibrozil, ibuprofen, naproxen, nonylphenol, octylphenol, salicylic acid, tonalide, triclosan

2.2.2. Removal of Micropollutants by Advanced Treatment Processes

Some micropollutants showing insufficient or moderate removal during conventional wastewater treatment can be well eliminated with modern biological treatments that provide high removal of BOD and ammonium. On the other hand, refractory and hydrophilic micropollutants such as several pharmaceuticals, pesticides, phosphorus flame retardants, and corrosion inhibitors require advanced treatment processes (Margot et al., 2015) such as advanced oxidation processes (AOPs), microfiltration (MF), reverse osmosis (RO) (James et al., 2014), membrane bioreactor (MBR) (Kovalova et al., 2012), or granular activated carbon (GAC) (Choi et al., 2008).

Ozonation and advanced oxidation processes (AOPs) are non-selective redox technologies achieving high degradation rates (Luo et al., 2014). A low dose of O_3 like 5 mg/L has been found enough to remove more than %90 of carbamazepine, diclofenac, metoprolol and trimethoprim (Sui et al., 2010). Similarly, an application of 5 mg/L ozone with 3.5 mg H_2O_2 has been achieved a considerable removal (>%90) of sulfamethoxazole, triclosan, carbamazepine, and estrone. While atrazine has been removed moderately by this method (Gerrity et al., 2011), complete elimination of the chemical has been accomplished by a UV/H_2O_2 treatment (De la Cruz et al., 2012). However, oxidation processes generally bring about the formation of oxidation by-products of micropollutants instead of achieving their complete mineralization (Luo et al., 2014).

Adsorption by activated carbons (ACs) such as powdered activated carbon (PAC) and granular activated carbon (GAC) is typically used for controlling taste and odor in drinking water. In order to achieve a better removal of micropollutants, this technique can be employed on secondary effluent (Luo et al., 2014). A highly efficient removal is obtained for especially non- polar micropollutants when adsorbents having heterogeneity in pore size and shape are applied (Rossner et al., 2009; Verlicchi et al., 2012).

Membrane processes such as microfiltration (MF) and ultrafiltration (UF) are efficient processes to remove turbidity. However, these processes generally achieve only a small elimination of most micropollutants because membrane pores are much larger than micropollutants (Luo et al., 2014). Therefore, MF and UF were combined with other processes such as nanofiltration (NF) or reverse osmosis (RO) to obtain a higher removal efficiency. For example, MF/RO process was found to achieve very effective removal (>95) of many personal care products (Sui et al., 2010). Similarly, the same process obtained a better removal of pharmaceuticals than the MF process alone (Garcia et al., 2013). On the other hand, membrane processes demand high energy, and disposal of the material retained by them is needed. Membrane fouling and desorption of sorbed chemicals from the membrane are other important problems limiting the use of membrane processes (Luo et al., 2014).

Membrane bioreactor (MBR) process couples activated sludge biological treatment and membrane filtration such as MF or UF (Luo et al., 2014). This process has an important place in water recycling practice and wastewater reclamation (Santos et al., 2011; Melin et al., 2006). Besides, MBR is also considered a promising technology for the removal of micropollutants. For example, hydrophobic micropollutants such as 4-tert butylphenol, estrone, bisphenol A, triclosan, and 4-n-nonylphenol were eliminated almost completely by MBR in one study. In addition, non-hydrophobic compounds such as salicylic acid, ibuprophen, and carbamazepine were also removed with very high efficiency (> 90) in the same study (Hai et al., 2011). Since MBRs have high SRT, slow-growing bacteria such as nitrifying bacteria can flourish in the reactors, which leads to higher biodegradation efficiency for certain micropollutants (Roh et al., 2009). For example, 99% of 17α-ethinylestradiol was removed owing to the application of a nitrifier enrichment in an MBR system (De Gusseme et al., 2009). However, membrane bioreactor systems, like other membrane-containing systems, require high energy. MBR systems also suffer from other drawbacks such as membrane fouling and inconsistent removal of polar and resistant compounds (Luo et al., 2014).

Physical or chemical treatment systems can be combined with biological treatment processes to remove micropollutants. Such combined systems, called hybrid systems, can achieve higher removal of micropollutants compared to single processes. For example, ozonation-biological activated carbon system, one of the most widely used combined system, can achieve almost complete elimination of some beta-blockers such as atenolol, metoprolol and propranolol while these pollutants are poorly removed during activated sludge treatment. The same system also removes analgesics pharmaceuticals such as carbamazepine, codeine, diclofenac, ibuprofen, naproxen, paracetamol and tramadol with very high efficiency (> %94). Similarly, MBR-reverse osmosis system is also considered effective on the removal of pharmaceuticals. More than 99% of azithromycin, clarithromycin, erythromycin, ofloxacin, sulfamethoxazole, diazepam, lorazepam, famotidine, ranitidine and clopidogrel can be eliminated with MBR-reverse osmosis system (Ahmed et al., 2017).

2.2.3. Micropollutant Biodegradation/Biotransformation

2.2.3.1. Biotransformation and biodegradation in general. Biotransformation is literally the same as metabolism but mostly used for biological transformation of xenobiotics such as drugs and carcinogens in mammals and environmental pollutants in prokaryotes. Biodegradation refers to an enzymatic process in which a microorganism takes a compound away or ideally converts a toxic compound into a non-toxic one (Wackett and Hershberger, 2001). It can also be defined as the breakdown of organic contaminants by microbial activity (Maier and Gentry, 2015).

Biodegradation can occur as primary biodegradation (partial biodegradation) or ultimate biodegradation (mineralization). In primary biodegradation, the chemical structure of a substance is altered, by which the substance loses its specific property (Mandaric et al., 2016; Maier and Gentry, 2015). In this type of biodegradation, the substance does not undergo complete oxidation and breakdown. This can result from the absence of the appropriate degrading enzyme since each step in the biodegradation pathway is catalyzed by a specific enzyme. In addition, cometabolism can be another reason for incomplete biodegradation. In cometabolism, partial degradation of the substance occurs but the energy derived from the oxidation is not used for microbial growth (Maier and Gentry, 2015). In ultimate biodegradation, on the other hand, cells meet their carbon and energy demand for growth and reproduction by breaking down the substance to fully oxidized or reduced simple molecules such as carbon dioxide/methane, nitrate/ammonium, and water (Mandaric et al., 2016; Maier and Gentry, 2015).

Biodegradation is governed by several factors that can be divided into three groups; biological factors, contaminant-related factors, and environmental factors. First, the presence and expression of appropriate degrading genes by the indigenous microbial community is essential for biodegradation. In order that a biodegradation pathway is maintained within an adapted community, previous exposure of microorganisms to contaminants is known as a common cause (Maier and Gentry, 2015). Adaptation of microbial populations generally occurs by induction of enzymes needed for biodegradation followed by an increase in the number of degrading microorganisms (Leahy and Colwell, 1990). In addition to previous exposure to contaminants, genetic changes such as mutations or gene transfers play a role in the microbial adaptation by creating new metabolic capabilities (Maier and Gentry, 2015) For example, transposable elements and insertion sequences are known to assist the dissemination of catabolic genes (Janssen et al., 2005). IS*1071,* which flanks the atrazine degradative genes *atzA*, *atzB* and *atzC* on plasmid pADP-1 in *Pseudomonas* sp. ADP is an example of the role of insertion elements in the catabolic gene transference (Wackett, 2004).

Contaminant-related factors include toxicity, bioavailability, and structure of the contaminant. Toxicity of a contaminant to microbial population inhibits or slow biodegradation. For example, non-ionic pollutants such as petroleum and organic solvents can disrupt membrane integrity by partitioning into the lipophilic layer of the cell membrane. Bioavailability of a compound determines its uptake by the cell which is the first step in a biodegradation reaction. Since microorganisms often obtain substrates from the surrounding aqueous phase, compounds with limited water solubility or high sorption potential to soil or sediments have low bioavailability and therefore limited biodegradation (Maier and Gentry, 2015). For example, low bioavailability of atrazine limits their biodegradation and increases its persistence in the environment (Mudhoo and Garg, 2011). Effect of contaminant structure on biodegradation can be examined under two headings. Steric effect, which occurs when the reaction site is blocked due to the presence of branching or functional groups in the substance, can slow biodegradation. Branching or functional groups can also affect biodegradation by influencing the transport of the substrate across the cell membrane, especially if transportation occurs with the help of an enzyme. Functional groups may also contribute to electronic effects that impede biodegradation by affecting the interaction between the contaminant and the enzyme. The electron density of the reaction site can be changed by functional groups that can be electron-donating (e.g., CH_3) or electron-withdrawing (e.g., Cl). In general, biodegradation rates increase in the presence of functional groups which add to the electron density of the reaction site while functional groups that decrease the electron density of the reaction site decrease biodegradation rates (Maier and Gentry, 2015). The number of electron-withdrawing groups in a pollutant also affect its biodegradation. For example, heavily chlorinated compounds often cannot be degraded by aerobic microorganisms while monochlorinated ones are easily degraded (Bhatt et al., 2007).

Environmental factors affecting biodegradation are the amount and content of organic matter, oxygen availability, nutrient availability, temperature, pH, salinity, and water activity. A high and variable organic matter content promotes high microbial numbers and diverse metabolic activities, which support biodegradation. Oxygen is another important factor affecting biodegradation rates and extent. Aerobic biodegradation is known much faster than anaerobic biodegradation. For example, petroleum-based hydrocarbons entering the aerobic zones of freshwater lakes and rivers are generally inclined to microbial degradation while oils accumulated in anaerobic sediments can be highly persistent (Maier and Gentry, 2015). Warmer temperatures can promote biodegradation and increase biodegradation rates. (Luo et al., 2014). Seasonal temperature fluctuations in the natural environment have been reported to influence the rate of biodegradation (Palmisano et al., 1991; Nie et al., 2012, Qiang et al., 2013). Biodegradation of hydrocarbons in the soil is generally faster at neutral pH (Maier and Gentry, 2015) while moderate-to-high levels of salinity tends to limit hydrocarbon degradation (Ulrich et al., 2009). Water activity, which is related to the amount of water available for the metabolic reactions within the cell, affects microbial growth and activity (Húngaro et al., 2014). For example, aerobic microorganisms display the highest activity when 38-81% of the soil pore space is filled with water since water and oxygen availability are maximized in this range (Maier and Gentry, 2015).

2.2.3.2. Biodegradation of micropollutants. Micropollutants can be degraded by microbial communities such as activated sludge, river and sediment microcosms or a specific microbe/group of microbes. Biodegradation of micropollutants is influenced by physicochemical conditions such as pH, temperature, and redox conditions (Luo et al., 2014). Moreover, it was reported that addition of yeast extract and/or carbon sources such as succinate, glucose, and acetate could enhance biodegradation (Zhang et al., 2010; Shafiee et al., 2006).

There are numerous studies investigated the biodegradation of micropollutants in different environments and conditions. For example, WWTPs from different regions were evaluated for their micropollutant removal efficiency (Luo et al., 2014; Tran et al., 2018). Researches investigating biodegradation of micropollutants in the environment have been mostly conducted by incubating collected environmental samples in the laboratory rather than measuring the biotransformation *in situ*. In the laboratory, micropollutant membrane bioreactors and batch reactors are featured systems to study micropollutant biodegradation (Kagle et al., 2009).

So far, no studies have investigated biodegradability of micropollutants in the Ergene River. However, the target micropollutants of this thesis, but not all of them, were investigated in many studies for their biodegradability. For example, acetaminophen, norfloxacin, ciprofloxacin, ofloxacin, azithromycin, sulfamethoxazole and clarithromycin were investigated for their removal in a WWTP containing a membrane bioreactor. In that study, acetaminophen, azithromycin, and sulfamethoxazole were degraded by %100, %49, and % 65, respectively. However, norfloxacin, ciprofloxacin, ofloxacin, and clarithromycin showed no degradation in the same study (Kim et al., 2014). On the other hand, another study reported the aerobic biodegradation of ofloxacin, norfloxacin, and ciprofloxacin by a strain named *Labrys portucalensis* F11in a medium containing acetate as a carbon source (Amorim et al., 2014).

Another target group of micropollutants of this thesis, benzotriazoles were reported to be biodegradable in different studies. For example, aerobic biodegradation of 1,2,3-Benzotriazole and 5-Methyl-1-H-benzotrizole by activated sludge microorganisms in a batch-type reactor was investigated in a study and 100% of 5-Methyl-1-H-benzotrizole and 49% of 1,2,3-Benzotriazole were reported to be degraded. Another study also reported the degradation of benzotriazoles by activated sludge microorganisms. In that study, more than 99.5% of 1,2,3-Benzotriazole and 5-Methyl-1-H-benzotrizole were degraded in 168 h. However, another benzotriazole, 4-Methyl-1-H-benzotrizole showed only partial removal in the same study (Huntscha et al., 2014).

Benzalkonium chlorides (BACs) are an important class of QACs. QACs are cationic biocides, which are classified as emerging contaminants because of their continuous release into the environment (Buffet-Bataillon et al., 2012; Tezel and G. Pavlostathis, 2011). QACs have been known to susceptible to biodegradation in the environment for a few decades (Dean-Raymond and Alexander, 1977). *Pseudomonas* genus has been especially related to BACs degradation in the environment. (Takenaka et al., 2007; Oh et al., 2014; Ertekin et al., 2016). *Pseudomonas sp.* BIOMIG1 is one of the isolates used in this thesis and is an important BAC degrader shown to mineralize and use BACs as a carbon and nitrogen source. The target BACs in this thesis, benzyldimethyldodecylammonium and benzyldimethyltetradecylammonium can be degraded by *Pseudomonas sp.* BIOMIG1. Moreover, N-benzyldimethylamine, one of the metabolites formed during BAC biodegradation, also found degraded by this microorganism (Ertekin et al., 2016).

Biodegradation of pesticides is of great importance since pesticides are among the most widely detected chemicals in the environment (Ortiz-Hernández et al., 2013). A great number of microorganisms capable of degrading pesticides have been isolated and identified. For example, *Ochrobactrum* sp. strain HZM, a bacterium capable of degrading organophosphate pesticides, was isolated from the pesticide-contaminated soil samples. This bacterium was reported to utilize different organophosphate pesticides such as quinalphos, profenofos, parathion-methyl and chlorpyrifos as growth substrates. Sasikala and colleagues (2012) also isolated several microorganisms degrading chlorpyrifos from agriculture soil. Among these microorganisms, *Pseudomonas putida* (NII 1117), *Klebsiella sp.,* (NII 1118)*, Pseudomonas stutzeri* (NII 1119) and *Pseudomonas aeruginosa* (NII 1120) were shown to degrade approximately 250 mg/L in 7 days. In another study, a bacterium identified as *Pseudomonas putida* DTB was found to utilize insect repellent N,N-diethyl-m-toluamide (DEET) as a sole carbon and energy source (Rivera-Cancel et al., 2007). Similarly, insecticide acetamiprid is also susceptible to biodegradation. A bacterium isolated from soil and named *Pigmentiphaga* sp. strain AAP-1 was reported to degrade acetamiprid completely in 2.5 hours (Rivera-Cancel et al., 2007). Carbendazim, which is a systemic fungicide used broadly to control various fungal diseases in agriculture and forestry, was found to be degraded by a few bacteria (Holtman and Kobayashi, 1997; Zhang et al., 2005). For example, *Rhodococcus jialingiae* djl-6-2 was found capable of using carbendazim as the sole carbon and nitrogen source. Diuron, a herbicide used to control the development of weeds, was reported to be degraded by different microbial strains such as *Stenotrophomonas acidaminiphila* TD4.7*, Bacillus cereus* TD4.31*, Delftia* sp. TD3.31*, Alcaligenes faecalis* TG4.48*,* and *Pseudomonas aeruginosa* TD2.3 (Egea et al., 2017).

Symmetric triazines (s-triazines) are N-heterocycles containing 1,3,5-triazine ring. Various members of commercial products such as bleaches and disinfectants, dyestuffs, explosives, resins and polymers, and pesticides are in the structure of s-triazine; however, herbicides are the most widely investigated group of s-triazine compounds (Cook, 1987). Cyanazin, simazine, propazine, and atrazine are the main triazine herbicides used in agriculture. Triazine herbicides are considered resistant to biological and chemical degradation. (Klementova and Keltnerova, 2015). However, their hydrolysis and dealkylation can occur in the environment via abiotic and biotic processes (Erickson et al., 1989). Udikovic-Kolic and colleagues (2012) repeatedly detected triazine herbicides and their degradation products such as deethylatrazine and deisopropylatrazine in surface waters at significant concentrations.

Owing to its low cost and high effectiveness, atrazine is the most widely used s-triazine herbicide in weed control (Udikovic-Kolic et al., 2012). Even though most xenobiotics are utilized as carbon sources, atrazine and other s-triazines are mostly used as nitrogen sources by microorganisms. Moreover, atrazine consumption by these microorganisms is known to be repressed in the presence of preferential nitrogen sources such as ammonium, urea, and nitrate (Govantes et al., 2009). Several microorganisms capable to perform partial (e.g. (Mulbry, 1994)) and complete degradation (e.g. (Mandelbaum et al., 1995); Struthers et al., 1998)) of atrazine have been isolated and identified. The initial step in atrazine degradation is to the conversion of atrazine to cyanuric acid, which is achieved via hydrolytic or oxidative-hydrolytic pathways (Govantes et al., 2009). Three enzymes product of the genes *AtzA*, *AtzB*, and *AtzC* are found responsible for the hydrolysis of atrazine to cyanuric acid (Martinez et al., 2001) while cytochrome P450 (Nagy et al., 1995) and AtzA (Shao and Behki, 1995) catalyse oxidative reactions.

2.2.3.2.1. Modelling biodegradation of micropollutants. When substrate concentrations are not high enough to support microbial growth, a concurrent utilization of various compounds present in a mixture is a common response (Harder and Dijkhuizen, 1982). The amounts of micropollutants in the environment are generally too low to support microbial growth and the cometabolism is therefore the dominating biodegradation process for micropollutants (Fischer and Majewsky, 2014; OECD, 2004). In this situation, biodegradation of compounds is anticipated to fit the first-order kinetic model. The first-order kinetics indicates that the rate of degradation (mg/L/day) is proportional to the concentration of substrate decreasing over time and the degradation rate constant, k, is independent of time and concentration (OECD, 2004). Concentration of a compound can be found using the first-order kinetics equation below (Anderson, 1986).

$$C = C_0e^{-kt} \tag{1}$$

Where C is the concentration of the compound at time t, C_0 is the initial concentration of the compound, k is rate constant, and t is time.

3. MATERIALS AND METHODS

3.1. River Water Samples

River water samples were taken from 29 different points on the Ergene River in November 2017, August 2017, February 2018, and May 2018. The points were selected due to their proximity to various resources of wastewater discharges such as a textile factory and a wastewater treatment plant (WWTP). Temperature and pH of the samples were measured *in situ.* The samples were collected in 1L-glass bottles with Teflon cap and preserved at 4°C in dark through the transportation to the lab. In the lab, all the sample bottles were stored at 4°C in dark until the characterization of the samples and biodegradability experiments for 3 days at most.

A map showing the locations of the sampling points is presented in Figure 3.1. Locations of the sampling points as latitude and longitude and their pH and temperatures are given in Appendix A.

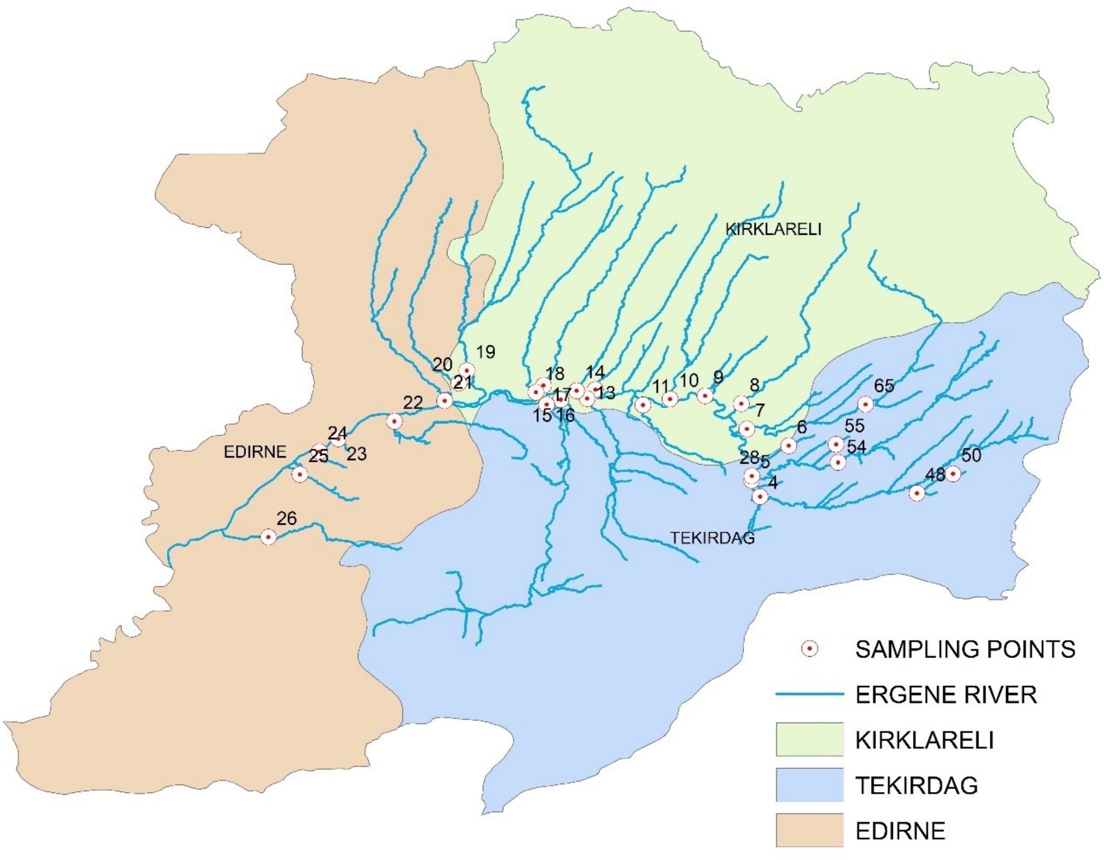


Figure 3.1. The locations of the sampling points on the Ergene River

3.1.1. Biodegradability Experiments with River Microcosms

Biodegradability of the target micropollutants, which were selected due to their high detection frequencies in the river observed within the scope of a project entitled "Development Of A Geographical Information Systems Based Decision-Making Tool For Water Quality Management of Ergene Watershed Using Pollutant Fingerprints'' funded by TUBİTAK, were evaluated in those sampling points for all months. Biodegradability experiments with river microcosms were performed in 500-mL Erlenmeyer flasks with screw caps. About 100 mL of samples from each point were transferred into the flasks, aseptically. The caps were left loose to allow oxygen entrance into the bottles. A flask containing sterile 100 mL DI water served as a control. In order to ensure that the samples contain an active microbial population, acetaminophen (Paracetamol) was added from a sterile 10 g/L stock (1g in 100 mL water) after serial dilutions into the flasks at 10 µg/L initial concentration as reference chemical. All flasks were agitated at 100 rpm on an orbital shaker (Stuart®) at room temperature. In every other day, 1 mL samples from the flasks were mixed with MS grade methanol in 1:1 ratio in 2-mL microcentrifuge tubes. The tubes were centrifuged at 10000 rpm in 4^0C for 10 minutes. Then, the supernatants were filtered through UNIFLO 13/0.2 RC filters. Filtrated samples were stored in 2 ml amber glass vials at -20^0C for analysis. Concentration of the target micropollutants were measured by LC-Ms^n/MRM as described in section 3.2.1.1.

3.2. Chemical Substances, Solutions, and Media

3.2.1. Chemical Substances

In this thesis, all the chemicals used in media preparations were purchased from Sigma Aldrich Chemicals Company (Germany). Analytical standards of 43 target chemicals were also purchased from Sigma Aldrich Chemicals Company (Germany) except hexa(methoxymethyl)melamine which is purchased from Tokyo Chemical Industry Co., Japan. Triphenyl phosphate was used as internal standard in all LC-MS/MS analysis. CAS numbers, the chemical structures, and the purpose of usage of these target compounds are presented in Table 3.1.

Stock solutions of the target chemicals were individually prepared in methanol (Merck KGaA, Germany) and formic acid (Sigma Aldrich) or water (Merck KGaA, Germany) and formic acid according to their solubilities (Appendix B). These stock solutions were stored at -20°C until used for two months at most. Stock solutions of hexa(methoxymethyl)melamine, cyanuric acid purchased from Merck KGaA (Germany), and melamine purchased from Alfa Aesar (United States) were prepared in DI water and used immediately.

3.2.1.1. Analysis of the chemical substances. The analysis of the target micropollutants was performed by using liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). An AB SCIEX QTrap 4500 linear ion trap tandem mass analyzer system coupled with Eksigent Ekspert UltraLC 110 ultra-high-performance liquid chromatography (UHPLC) unit was used in quantification of the target chemicals. UHPLC is equipped with Phenomenex Kinetex C18 2.6μ column (50 x 3 mm). A binary solvent system composed of MS grade methanol (A) and MS grade water (B) both buffered with 0.1% formic acid were used as mobile phases, respectively. The gradient elution starting at 100% solvent B for 0-1.5 min, followed by 50% A (2-4 min.) and increased to 100% A (4-8 min.) and maintained at 100% A for the last 6 minutes was applied. The flow rate was constant at 0.5 mL/min, the column was kept at 40 ºC and the injection volume was 10 µL. For the detection and the quantification of the chemicals scheduled multiple reaction monitoring (MRM) was performed. Optimized MRM parameters were scanned at the determined retention times of the chemicals during the gradient elution (Appendix C). Instrumental control, data acquisition, and processing were performed using Analyst Software (AB Sciex) while Multiquant 3.0.1(AB Sciex) was used for the peak control and quantitation.

The method LOD and LOQ for the target compounds were determined using the signal-to-noise ratio method (Shrivastava and Gupta, 2011) by spiking each compound within the range of 0.05-5 µg/L at 5 concentrations. The method LOD and LOQ for melamine and cyanuric acid were determined using the linear regression method (Magnusson and Ornemark, 2014) by spiking each compound within the range of 0.01-5 µg/L at 3 concentrations in triplicate. LOD of the method for the target chemicals ranged between 0.0032 and 2.6823 µg/L.

The measurement of acetic acid, glucose, and succinic acid were performed using an Agilent 1260 Series HPLC (Agilent Technologies, Palo Alto, CA, USA) unit equipped with a Hi-Plex H ion exclusion column (300 × 7.8 mm) (Agilent Inc., USA) and UV/visible diode array and refractive index detectors. A 0.01N H_2SO_4 solution was used as the mobile phase with a flow rate of 0.6 mL/min and the column was maintained at 65ºC. The samples were centrifuged, and the supernatant was acidified with 0.01N H_2SO_4 in 1:1 ratio. The acids were detected by the UV detector at 210 nm wavelength, whereas the glucose and alcohols were detected by the refractive index detector (RID).

Calibration curves of glucose, succinic acid, and acetic acid are presented in Figure 3.2.

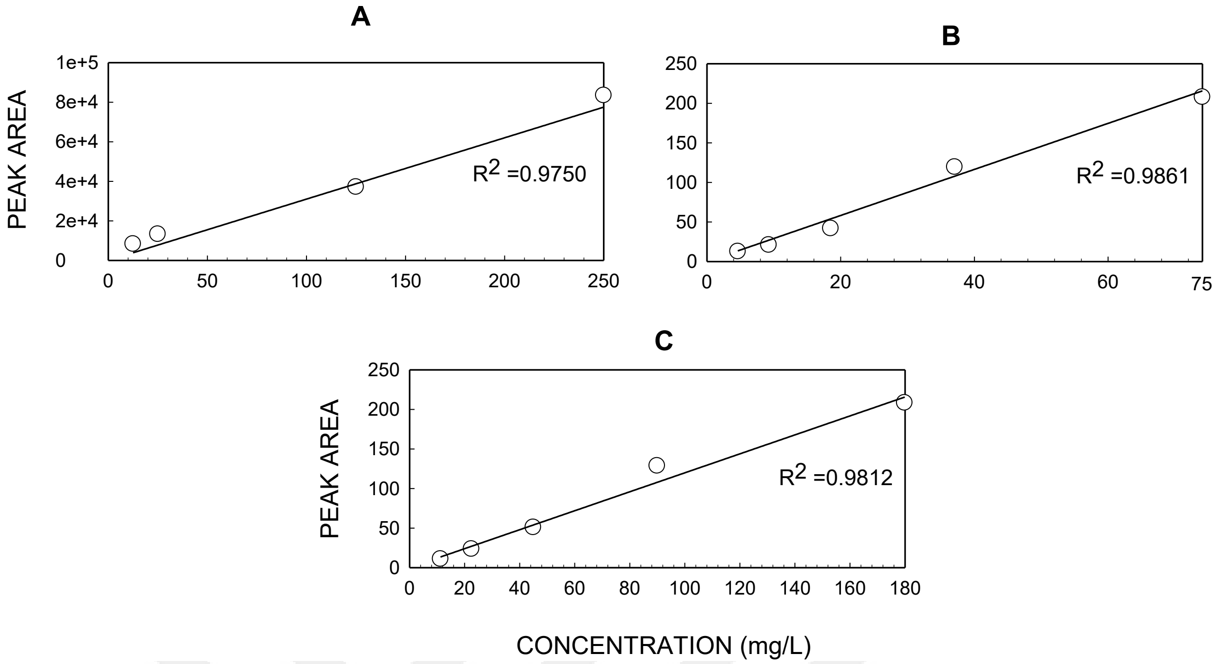


Figure 3.2. Calibration curves of (A) glucose, (B) succinic acid, and (C) acetic acid

Table 3.1. CAS numbers, molecular formulas, structures, and purpose of use of the target chemicals

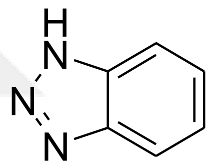
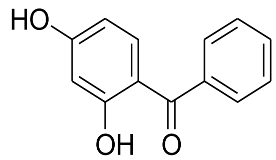
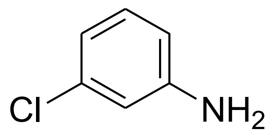
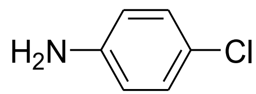
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
1,2,3-Benzotriazole	95-14-7	$C_6H_5N_3$		Corrosion inhibitor, ultraviolet light stabilizer for plastics, and antifoggant in photography (Davis et al., 1977)
2,4-Dihydroxybenzophenone (Benzophenone-1)	131-56-6	$C_{13}H_{10}O_3$		Ultraviolet light absorbent (He et al., 2011)
3-Chloroaniline	108-42-9	C_6H_6ClN		An ingredient for production of pesticides, dyes, and several pharmaceuticals (Boon et al., 2001; Zhang et al., 2010; Yao et al., 2011)
4-Chloroaniline	106-47-8	C_6H_6ClN		An ingredient for production of pesticides, dyes, and several pharmaceuticals (Boon et al., 2001; Zhang et al., 2010; Yao et al., 2011)

Table 3.1. Continue

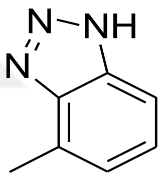
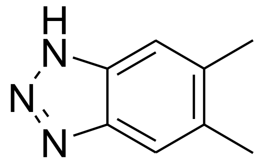
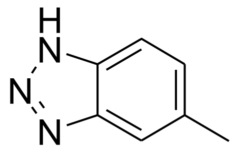
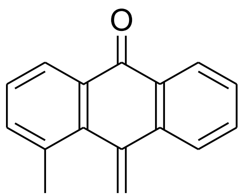
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
4-Methyl-1H-benzotriazole	29878-31-7	$C_7H_7N_3$		Corrosion inhibitor, ultraviolet light stabilizer for plastics, and antifoggant in photography (Davis et al., 1977)
5,6-Dimethyl-1H benzotriazole	4184-79-6	$C_8H_{11}N_3O$		Corrosion inhibitor, ultraviolet light stabilizer for plastics, and antifoggant in photography (Davis et al., 1977)
5-Methyl-1H-benzotriazole (5-Tolytriazole)	136-85-6	$C_7H_7N_3$		Corrosion inhibitor, ultraviolet light stabilizer for plastics, and antifoggant in photography (Davis et al., 1977)
7H-Benzo(de)anthracen-7-one (Benzanthrone)	82-05-3	$C_{17}H_{10}O$		Dye intermediate (Tewari et al., 2015)

Table 3.1. Continue

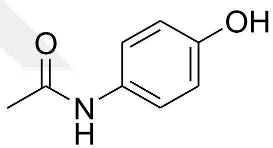
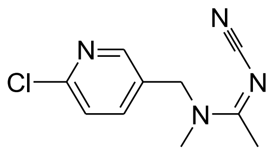
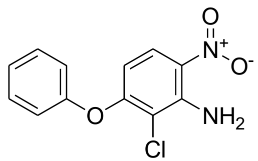
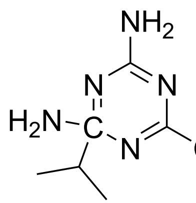
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Acetaminophen (Paracetamol)	103-90-2	$C_8H_9NO_2$		Analgesic and antipyretic drug (Wu et al., 2012)
Acetamiprid	135410-20-7	$C_{10}H_{11}ClN_4$		Insecticide (Jeschke et al., 2011)
Aclonifen	74070-46-5	$C_{12}H_9ClN_2O_3$		Herbicide (Kilinc et al., 2011)
Atrazine-desethyl	6190-65-4	$C_6H_{10}ClN_5$		A metabolite of herbicide atrazine (Peng et al., 2007)

Table 3.1. Continue

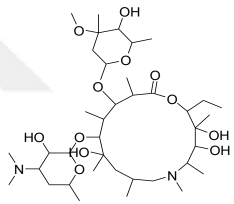
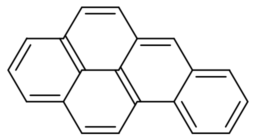
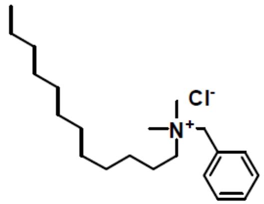
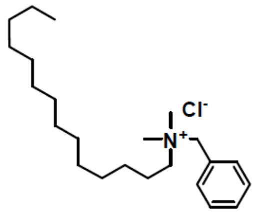
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Azithromycin	83905-01-5	$C_{38}H_{72}N_2O_{12}$		Antibiotic (Kümmerer and Henninger, 2003)
Benzo[a]pyrene	50-32-8	$C_{20}H_{12}$		Combustion byproduct (Boubel and Ripperton, 1963)
Benzyldimethyldodecylammonium chloride	139-07-1	$C_{21}H_{38}ClN$		Disinfectant (Tezel and Pavlostathis, 2015)
Benzyldimethyltetradecylammonium chloride	139-08-2	$C_{23}H_{42}ClN$		Disinfectant (Tezel and Pavlostathis, 2015)

Table 3.1. Continue

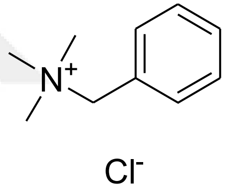
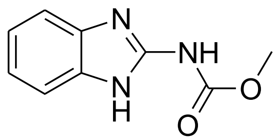
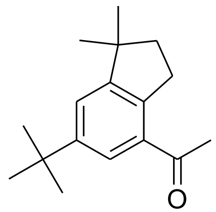
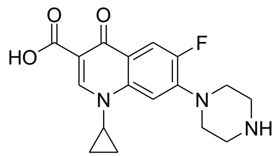
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Benzyltrimethylammonium chloride	56-93-9	$C_{10}H_{16}ClN$		Phase-transfer catalyst (Starks and Halper, 2012)
Carbendazim	10605-21-7	$C_9H_9N_3O_2$		Fungicide (Wang et al., 2010)
Celestolide	13171-00-1	$C_{17}H_{24}O$		Fragrance (WHO and UNICEF, 2000)
Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$		Antibiotic (Kümmerer and Henninger, 2003)

Table 3.1. Continue

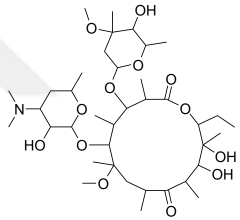
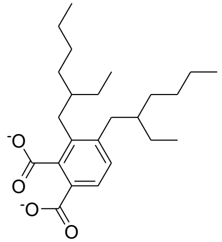
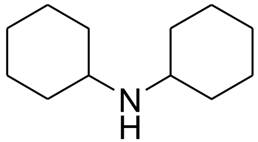
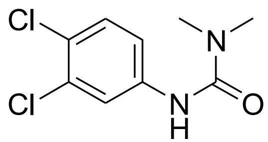
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$		Antibiotic (Kümmerer and Henninger, 2003)
Di(2-ethylhexyl)phthalate	117-81-7	$C_{24}H_{36}O_4$		Plasticizer (Erythropel et al., 2014)
Dicyclohexylamine	101-83-7	$C_{12}H_{23}N$		Corrosion inhibitor (Anderson et al., 2009)
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$		Herbicide (Coelho-Moreira et al., 2013)

Table 3.1. Continue

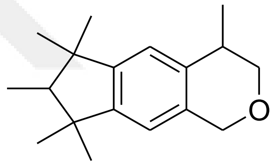
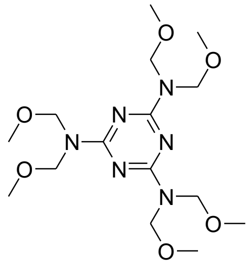
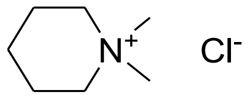
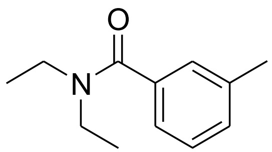
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Galaxolide	1222-05-5	$C_{18}H_{26}O$		Fragrance (WHO and UNICEF, 2000)
Hexa(methoxymethyl)melamine	3089-11-0	$C_{15}H_{30}N_6O_6$		Ingredient in the production of coatings and plastics (Dsikowitzky and Schwarzbauer, 2015)
Mepiquat chloride	24307-26-4	$C_7H_{16}ClN$		Plant growth regulator (Hodges et al., 1991)
N,N-Diethyl-m-toluamide	134-62-3	$C_{12}H_{17}NO$		Insect repellent (Aronson et al., 2012)

Table 3.1. Continue

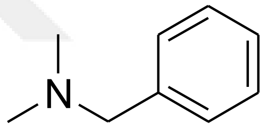
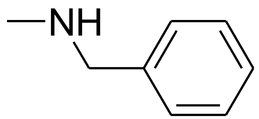
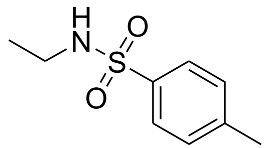
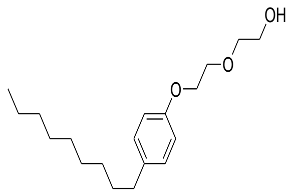
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
N-Benzyldimethylamine	103-83-3	$C_8H_{11}N$		An intermediate compound formed during the biodegradation of benzalkonium chlorides (Patrauchan and Oriel, 2003; Ahn et al., 2016; Ertekin et al., 2016)
N-Benzylmethylamine	103-67-3	$C_8H_{11}N$		An intermediate compound formed during the biodegradation of benzalkonium chlorides (Ahn et al., 2016, Patrauchan and Oriel, 2003)
N-Ethyl-p-toluenesulfonamide	80-39-7	$C_9H_{13}NO_2S$		Herbicide (Alink et al., 2007)
Nonylphenol diethoxylate	20427-84-3	$C_{19}H_{32}O_3$		Surfactant (Liu et al., 2016)

Table 3.1. Continue

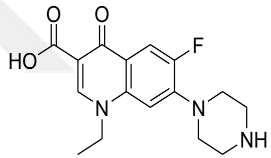
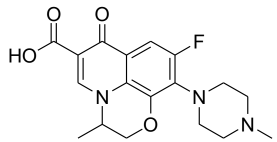
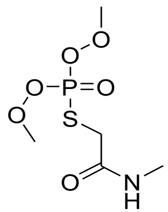
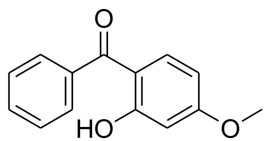
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$		Antibiotic (Kümmerer and Henninger, 2003)
Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$		Antibiotic (Kümmerer and Henninger, 2003)
Omethoate	1113-02-6	$C_5H_{12}NO_4PS$		Insecticide (Zhao et al., 2009)
Oxybenzone	131-57-7	$C_{14}H_{12}O_3$		Ultraviolet filter (Balmer et al., 2005)

Table 3.1. Continue

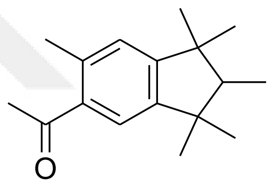
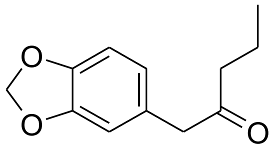
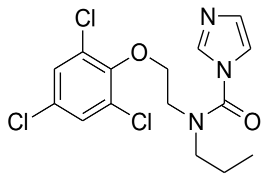
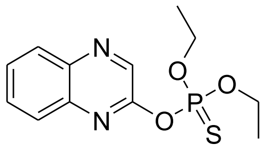
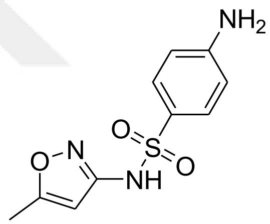
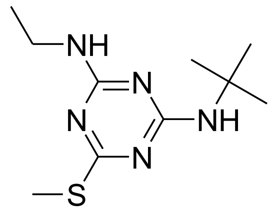
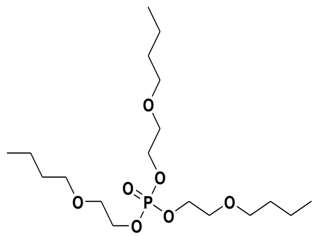
COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Phantolide	15323-35-0	$C_{17}H_{24}O$		Fragrance (WHO and UNICEF, 2000)
Piperonyl butoxide	51-03-6	$C_{19}H_{30}O_5$		Pesticide synergist (Franklin, 1972)
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$		Fungicide (Vinggaard et al., 2006)
Quinalphos	13593-03-8	$C_{12}H_{15}N_2O_3PS$		Pesticide (Hayes and Laws, 1991)

Table 3.1. Continue

COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	STRUCTURE	PURPOSE OF USAGE
Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$		Antibiotic (Kümmerer and Henninger, 2003)
Terbutryn	886-50-0	$C_{10}H_{19}N_5S$		Herbicide (Quednow and Püttmann, 2007)
Tris(2-butoxyethyl) phosphate	78-51-3	$C_{18}H_{39}O_7P$		Plasticizer (Sager and Little, 1989)

3.2.2. Solutions and Media

3.2.2.1. Mineral salt medium. Mineral salt medium (modified M9) was composed of 7.4 g/L K_2HPO_4, 3.0 g/L KH_2PO_4, 0.5 g/L NaCl, 1.00 g/L NH_4Cl, 0.1 M $MgSO_4.7H_2O$, 0.01 M $CaCl_2$ and 0.1% (v/v) trace metal solution (0.5g/L $ZnCl_2$, 0.30g/L $MnCl_2.4H_2O$, 3.0g/L H_3BO_3, 2.0g/L $CoCl_2.6H_2O$, 0.10g/L $CuCl_2.2H_2O$, 0.20g/L $NiSO_4.6H_2O$ and 0.30g/L $Na_2MoO_4.2H_2O$). After dissolving these ingredients in 979 mL DI water, the content was autoclaved at 121°C for 15 min. Sterile 0.005 M $FeCl_2.4H_2O$ (0.25 g in 250 mL DI water), 0.1 M $MgSO_4.7H_2O$ (6.16 g in 250 mL DI water) and 0.01 M $CaCl_2.2H_2O$ (0.37 g in 250 mL DI water) solutions were added as 1 mL, 10 mL, and 10 mL into the content under aseptic conditions respectively. All the chemicals used in M9 medium preparation were purchased from Sigma Aldrich Chemicals Company (Germany).

Nitrogen-free mineral salt medium contains the same ingredients in mineral salt medium except NH_4Cl and was prepared as same as mineral salt medium.

3.2.2.2. Saline solution. A 0.85% (w/v) saline solution was prepared dissolving 8.5 g NaCl in 100 mL DI water. The solution was autoclaved at 121°C for 15 min for sterilization.

3.2.2.3. Glucose, sodium acetate, and sodium succinate. Stock solutions of glucose (2%, m/v), sodium acetate (2%, m/v), and sodium succinate (2%, m/v) were prepared adding 20 g glucose, 20 g sodium acetate, 20 g and sodium succinate into 1 L DI water individually. The stocks were autoclaved at 121°C for 15 min for sterilization.

3.2.2.4. LB-BAC broth, LB-MP broth, LB-APAP broth. Luria-Bertani (LB) medium was composed of 10 g/L tryptophan, 5g/L yeast extract, and 5g/L NaCl and was sterilized by autoclaving at 121°C for 15 minutes. After the medium cooled down to 50ºC, a BAC mixture, methyl paraben (MP), and paracetamol (APAP) were added into the separate bottles containing LB medium at the final concentration of 50 ppm for each, aseptically.

The BAC mixture (Lonza Inc., Switzerland) was composed of 40% (w/w) benzyldimethyldodecylammonium chloride (C_{12}BDMA-Cl, $C_{21}H_{38}NCl$, 340 g/mole), 50% (w/w) benzyldimethyltetradecylammonium chloride (C_{14}BDMA-Cl, $C_{23}H_{42}NCl$, 368 g/mole), and 10% (w/w) benzyldimethylhexadecylammonium chloride (C_{16}BDMA-Cl, $C_{25}H_{46}NCl$, 396.1 g/mole).

Stocks for MP and APAP at a concentration of 10 g/L were prepared in DI water and autoclaved at 121°C for 15 min for sterilization.

3.3. Bacterial Cultures

3.3.1. Culture Preparation

A pure culture of bacteria belonging to species *Pseudomonas*, *Nocardia, Nubsella, Microbacterium, Rhodococcus, Sphingobium* and *Flavobacterium* were previously isolated in our lab. Cultures of these isolates used in all experiments were prepared as follows: 1 mL stocks of each strain previously stored at -80°C in a 1.5 mL microcentrifuge tube were taken out and thawed. The stocks were added into LB broth containing 50 mg/L BAC, 50 mg/L methyl paraben (MP) and 50 mg/L paracetamol (APAP) for the BAC degrader, methyl paraben degraders, and paracetamol degraders respectively to a final volume of 5 mL in a 50 mL falcon tube aseptically. The cultures were grown overnight on an orbital shaker at 130 rpm at 25ºC. Serial dilutions of 10^{-6}, 10^{-7} and 10^{-8} with a sterile saline solution (0.85% NaCl, w/v) were prepared in and spread onto appropriate agar plates containing 50 mg/L BAC, 50 mg/L MP or 50 mg/L APAP the next day. After an overnight growth on the plates at 25°C, the single colonies were patched onto appropriate LB agar plates via sterile wooden applicator. After overnight growth on the plates at 25°C, a single colony for each isolate was transferred into 50 mL falcon tubes containing 5 mL LB-BAC broth, LB-MP broth, or LB-APAP broth. The next day, 1 mL was taken from each overnight grown culture and transferred into separate 1.5 mL sterile microcentrifuge tubes aseptically. The rest overnight grown cultures were mixed with glycerol in 5:1 ratio in 1.5 mL sterile microcentrifuge tubes and stored at -80°C individually. These pure bacterial cultures were used in used in hexa(methoxymethyl)melamine biodegradability assays and presented in Table 3.3.

Using the software Geneious version 7.1.8, a phylogenetic tree of relationships based on 16S rDNA sequences of the isolates, determined by maximum likelihood followed by neighbor-joining tree building method and Hasegawa-Kishino-Yano genetic distance model, is presented in Figure 3.3. In the figure, branches in red denote the isolates whereas branches in black are species with the closest sequences to the isolates. *Methanosarcina bakeri* was used as the outgroup. Branch labels show consensus support as percentage.

Table 3.3. Bacterial strains used in hexa(methoxymethyl)melamine biodegradability assays

Strains	Origin	Reference
Pseudomonas sp. BIOMIG1	Sewage, BAC degrader	Ertekin et al., 2016
Pseudomonas vancouverensis BIOMIG-MP03	Soil, methyl paraben degrader	N/A
Nocardia globerula BIOMIG-MP04	Soil, methyl paraben degrader	N/A
Nubsella zeaxanthinifaciens BIOMIG-MP22	Soil, methyl paraben degrader	N/A
Microbacterium suwonense BIOMIG-MP46	Soil, methyl paraben degrader	N/A
Rhodococcus erythropolis BIOMIG-P19	Soil, paracetamol degrader	N/A
Pseudomonas nitroreducens BIOMIG-P2	Soil, paracetamol degrader	Akay, 2017
Sphingobium sp. BIOMIG-P36	Soil, paracetamol degrader	Akay, 2017
Flavobacterium sp. BIOMIG-P32	Soil, paracetamol degrader	Akay, 2017

N/A: Not available

3.3.2. Hexa(methoxymethyl)melamine (HMMM) Biodegradability Experiments with the Cultures of Bacterial Strains

Utilization of HMMM as a sole carbon and as a sole nitrogen source by each strain was tested individually in mineral salt medium and nitrogen-free mineral salt medium, respectively. For these experiments, each culture in 1.5 mL microcentrifuge tubes was centrifuged at 10000 rpm for 10 minutes and wash 3 times with saline solution. Cells were resuspended in 1 mL saline solution at final. For testing HMMM utilization as the sole carbon source, each resuspended bacterial cell was transferred into individual flasks containing mineral salt medium. For testing HMMM utilization as the sole nitrogen source, each resuspended bacterial cell was transferred into individual flasks containing nitrogen-free mineral salt medium after sterile 2% glucose (5 g in 250 mL), 2% sodium acetate (5 g in 250 mL) and 2% sodium succinate (5 g in 250 mL) were added into the flasks as final concentration of each was 2 g/L. For the both experiments, a flask containing sterile DI water was used as a control. Initial HMMM concentration in each flask was 10 mg/L (25 µM). All flasks were agitated at 100 rpm on an orbital shaker (Stuart®) at room temperature. Sampling and measurements of HMMM concentration in flasks were performed as same as in the river microcosms experiment.

3.3.3. Utilization of Hexa(methoxymethyl)melamine (HMMM) by *Pseudomonas* sp. BIOMIG1 in Mineral Salt Medium and Nitrogen-free Mineral Salt Medium

3.3.3.1. Utilization of hexa(methoxymethyl)melamine (HMMM) by Pseudomonas sp. BIOMIG1 in nitrogen-free mineral salt medium. After determining that Pseudomonas sp. BIOMIG1 removed HMMM in the biodegradability experiment with the cultures, the experiment with Pseudomonas sp. BIOMIG1 was repeated to determine biotransformation by-products. For this purpose, Pseudomonas sp. BIOMIG1 was grown overnight in LB-BAC broth and transferred into the flasks containing 10 mg/L HMMM as described in section 4.3. and 4.3.1. A flask containing sterile DI water and 10 mg/L HMMM was used as a control. The experiment was done in triplicate.

3.4. Biodegradability Predictions

Biodegradability of the target compounds was predicted running the program BIOWIN™ v 4.10 present in The EPI (Estimation Programs Interface) Suite™ v 4.11. BIOWIN™, which requires only a chemical structure to make predictions, estimates the probability of rapid aerobic and anaerobic biodegradation of an organic compound in the presence of mixed populations of environmental microorganisms by using seven separate models. Biodegradability estimates are based upon fragment constants developed using multiple linear or non-linear regression analyses, depending on the model. BIOWIN1 and BIOWIN2 models are used to obtain a general idea of biodegradability under aerobic conditions. BIOWIN3 and BIOWIN4 produce estimates for the time needed to achieve an ultimate or primary biodegradation in aquatic environments. BIOWIN5 and BIOWIN6, which use an approach similar to that used to develop BIOWIN1 and BIOWIN2, are predictive models for evaluating a compound’s biodegradability in the Japanese MITI ready biodegradation test such as OECD 301C. The most recent model, the anaerobic biodegradation model BIOWIN7 predicts probability of rapid degradation in the serum bottle anaerobic biodegradation screening test. Biodegradability scores of the target compounds estimated using six BIOWIN models are given in Appendix I.

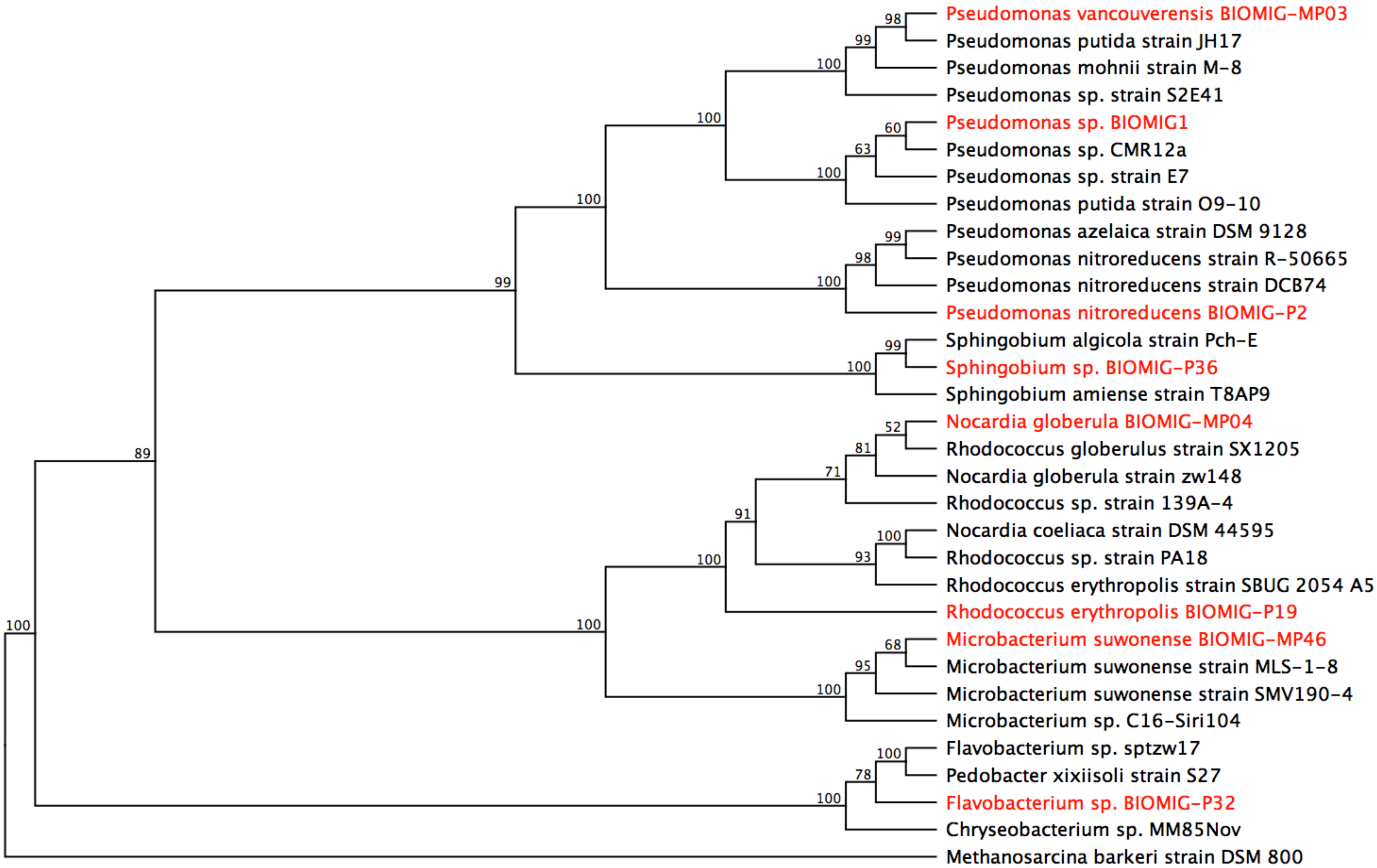


Figure 3.3. Phylogenetic tree of relationships of the bacterial isolates based on their 16S rDNA sequences

4. RESULTS AND DISCUSSION

4.1. Optimization of LC-MS/MS Analysis

MRM transitions and MS/MS conditions for each compound were obtained by infusing each compound manually as 10 µL/min with a syringe pump at 10 µg/L. Optimization of the parameters for ESI source was performed in 'Manual Tuning' mode. When ESI probe was operated in positive ionization mode, the mass analysis' parameters were adjusted as follows: Curtain gas (CUR)=30, Ion spray voltage (IS)=5500 V, Temperature (TEM)= 550ºC, Ion source gas 1 (GS1) = 50; Ion source gas 2 (GS2)= 60. In negative ionization mode, the mass analysis' parameters were adjusted as follows: Curtain gas (CUR)=30, Ion spray voltage (IS)=-4500 V, Temperature (TEM)= 550ºC, Ion source gas 1 (GS1) = 50; Ion source gas 2 (GS2) = 60.

After adjusting the appropriate ESI source parameters, the parent ion (Q1) of a chemical was determined. Two ions with the highest intensities were assigned as daughter ions (Q3s), which were generated after the fragmentation of the parent ion in the collision cell. Optimum parameters for de-clustering potential (DP), collusion energy (CE), and collision cell exit potential (CXP) to generate daughter ions were obtained in "Compound Optimization" mode. Optimized parameters for each compound and its daughter ions with method LOD and LOQ values are presented in Appendix C.

The retention time of each compound was identified by LC/MS-MS system running mixed standards in unscheduled mode. All the compounds were detected within 9 minutes at optimized conditions. Figure 4.1 shows the chromatogram of the target compounds given to the system as a mixture of 15 µg/L and Figure 4.2 shows the chromatogram of hexa(methoxymethyl)melamine, melamine, cyanuric acid, and the internal standard run at the concentration of 1 µg/L, 1 µg/L, 10 µg/L, and 1 µg/L respectively.

4.2. Biodegradability Experiments with River Microcosms

The reference chemical acetaminophen was completely removed in all river water samples in four days. In control flasks, no significant reduction was observed in acetaminophen concentration except for November flask where a 25% reduction occurred. These observations were demonstrated that microbes were active in river water samples and acetaminophen was mostly removed via microbial activity. Figure 4.3 shows the concentration of acetaminophen in the control flasks through 30 days.

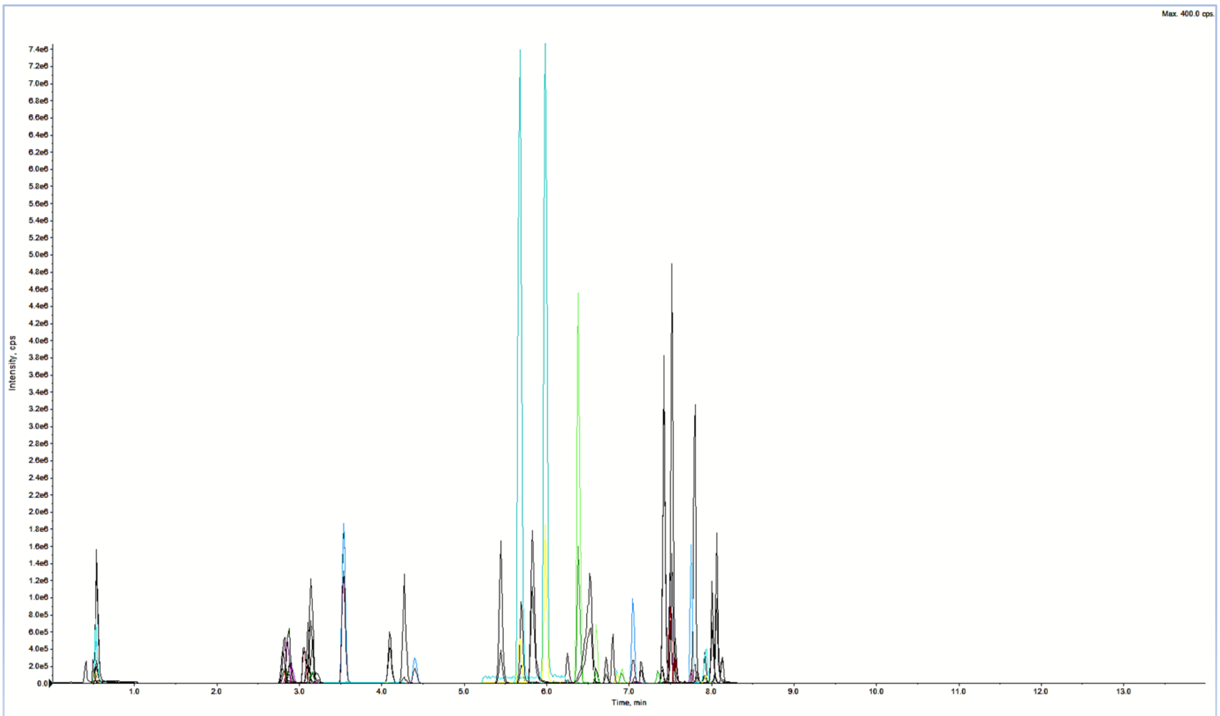


Figure 4.1. LC-MS/MS chromatogram of the target compounds

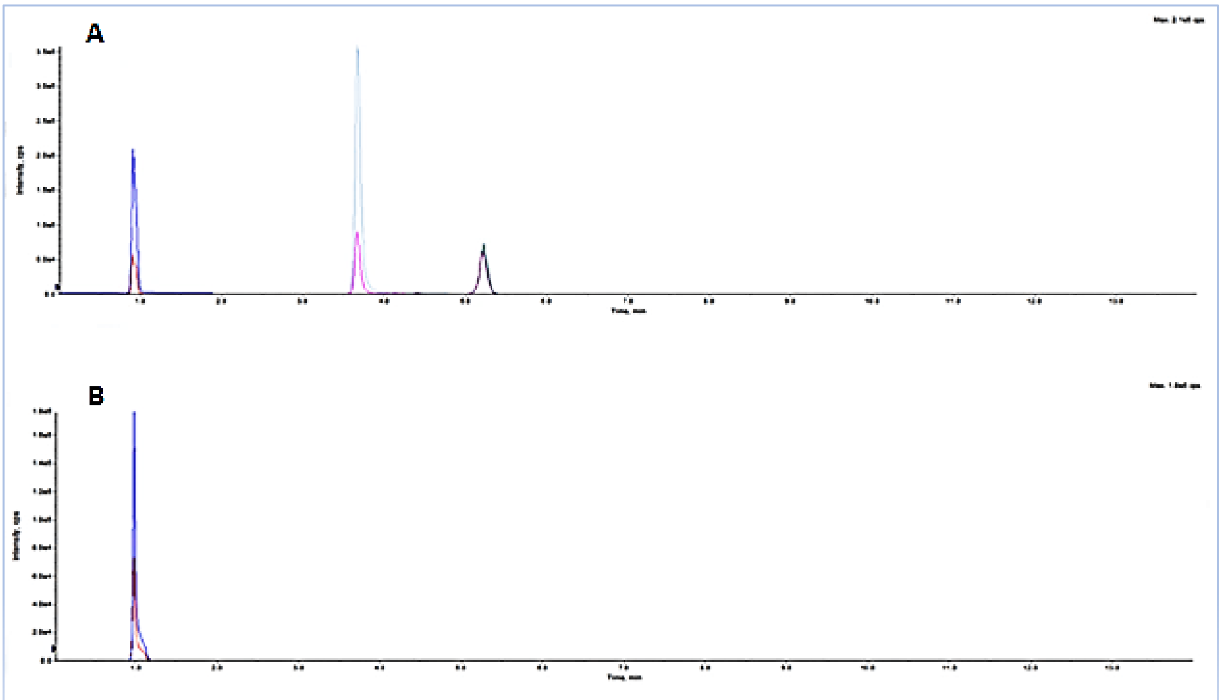


Figure 4.2. LC-MS/MS chromatogram of (A) melamine, internal standard, hexa(methoxymethyl)-melamine, and (B) cyanuric acid

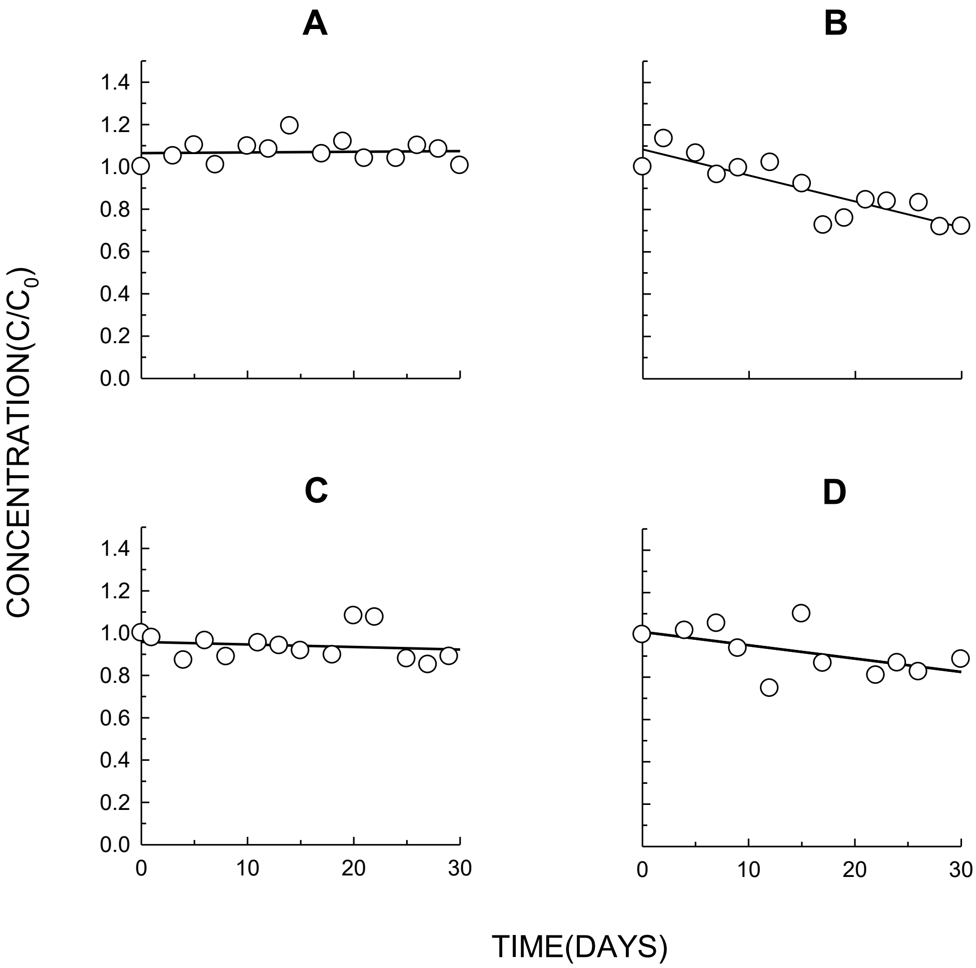


Figure 4.3. Acetaminophen concentration in (A) May, (B) November, (C) August, and (D) February control flasks through 30 days

4.2.1. Biodegradation Rate Constants and Half-lives of the Target Compounds

4.2.1.1. Biodegradation rate constants. Biodegradation rate constants and the corresponding half-life (t1/2) of the chemicals were calculated by fitting the first-order decay kinetics equation to the experimental data obtained for 30 days using Sigma Plot v10.2 Software.

Biodegradation rate constant of the compounds ranged between 0 and 3.565 $days^{-1}$ (d^{-1}). All SigmaPlot graphics of the target micropollutants identified in August, November, February and May samples are given in Appendix D, Appendix E, Appendix F, and Appendix G respectively. The initial concentrations, biodegradation rate constants and half-lives of the all compounds are given in Appendix G.

Based on the occurrence and biodegradation rate constants of the compounds, two heatmaps for the target compounds detected at twenty-nine sampling points in November (Figure 4.4) and for common compounds at selected five points at four months (Figure 4.5) were constructed. The scale bar in the graphics represented biodegradation rate constants. A compound that was not detected in a sample was in dark blue color having a degradation rate equal to -1.

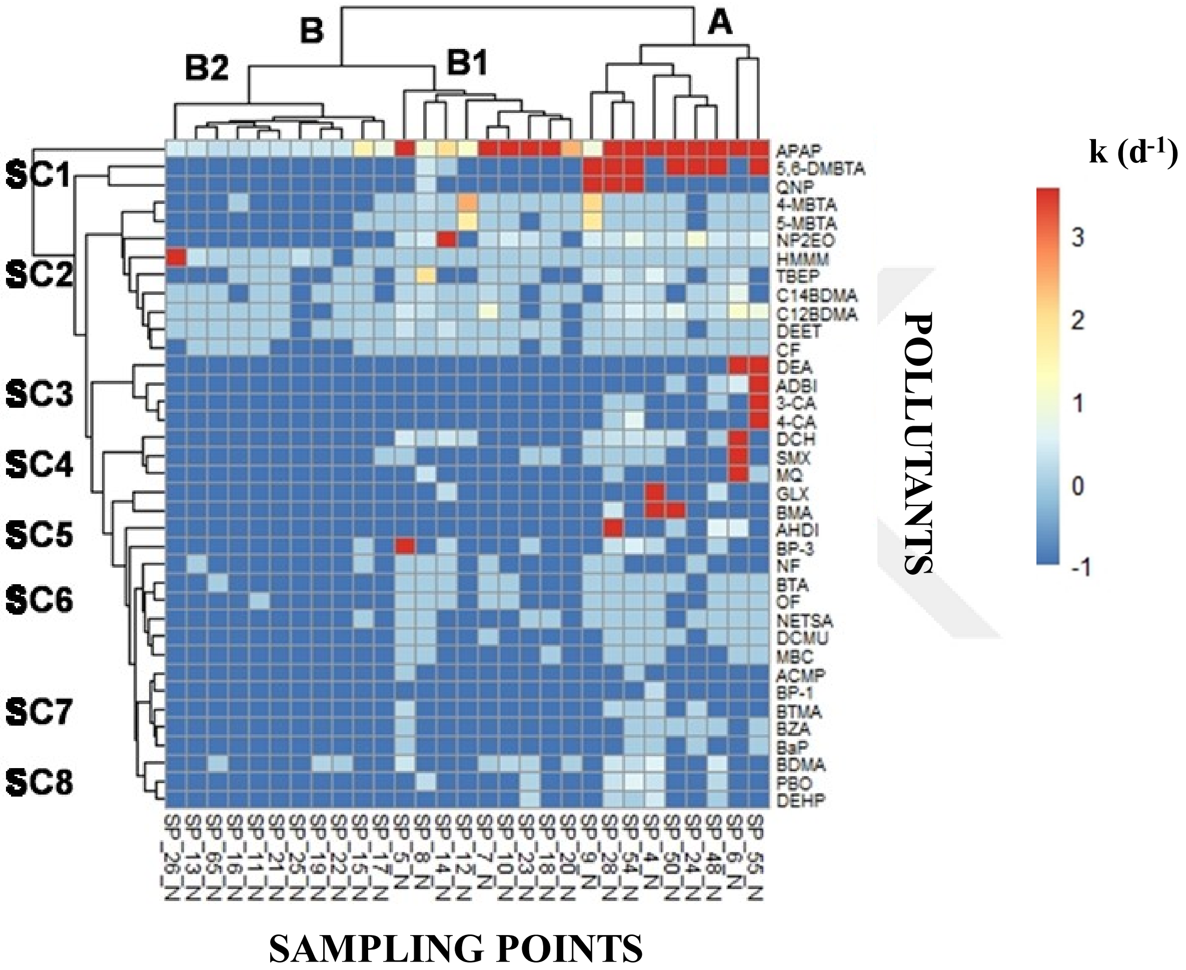


Figure 4.4. Clustering of sampling points and chemical substances based on occurrence and biodegradation rate constants in November

As seen in Figure 4.4, there were two main clusters of the sample points (Cluster A and Cluster B). Cluster A was made up of most contaminated sample points by the target micropollutants. Cluster B was composed of less contaminated sample points and was divided into two sub-clusters (Cluster B1 and Cluster B2). Sample points in Cluster B1 were more contaminated and had generally greater biodegradation rate constants of the compounds compared to Cluster B2. For example, reference chemical acetaminophen (APAP) showed a greater biodegradation rate constant at the points in Cluster A such as Sample Point 55, Sample Point 6, Sample Point 48, and Sample Point 24. On the other hand, this compound showed the slowest biodegradation at the points in Cluster B2 such as Sample Point 26, Sample Point 13, and Sample Point 65.

Targets compounds were grouped into two main clusters based on their occurrence and biodegradation rate constants. The first main cluster contained solely reference chemical APAP which was the only compound present in every sample. The second main cluster was made up of eight sub-clusters (SC1-SC8). SC1 was composed of 5,6-Dimethyl-1H-benzotriazole (5,6-DMBTA) and quinalphos (QNP), which were identified at nine and four points in Cluster A, respectively. These two compounds generally had the highest biodegradation rates among the compounds in all the sub-clusters. SC2 contained compounds with a broader occurrence in the river such as ciprofloxacin (CF), N,N-Diethyl-m-toluamide (DEET), C_{12}BDMA, hexa(methoxymethyl)melamine (HMMM), tris(2-butoxyethyl) phosphate (TBEP), benzyldimethyltetradecylammonium (C_{14}BDMA), 4-Methylbenzotriazole (4-MBTA) and 5-Methylbenzotriazole (5-MBTA), which the last two were grouped further into another cluster due to their higher biodegradation rate constants compared to the others in the same sub-clusters. On the other hand, those compounds generally showed slower biodegradation than APAP, 5,6-DMBTA, and QNP.

Atrazine-desethyl (DEA), celestolide (ADBI), 3-Chloroaniline (3-CA), 4-Chloroaniline (4-CA), mepiquat (MQ), sulfamethoxazole (SMX), dicyclohexylamine (DCH), galaxolide (HHCB), and N-Benzylmethylamine (BMA) showed a rare occurrence in the river and were concentrated at the points in Cluster A. Biodegradation rate constants of these compounds ranged between 0 and 3.565 d^{-1}. Chemicals that showed the fastest degradation at the same point were clustered together. For example, DEA, ADBI, 3-CA, and 4-CA were degraded fastest at Sample Point 55 and were clustered in SC3. MQ, SMX, and DCH were degraded fastest at Sample Point 6 and were clustered in SC4 whereas HHCB and BMA showed the highest biodegradation rate at Sample Point 4 and were clustered in SC5. Those points might host the appropriate degraders and/or have favorable physicochemical conditions that supported microbial growth and biodegradation.

SC6 was composed of phantolide (AHDI), oxybenzone (BP-3), norfloxacin (NF), 1,2,3-Benzotriazole (BTA), ofloxacin (OF), N-Ethyl-p-toluenesulfonamide (NETSA), diuron (DCMU), and carbendazim (MBC). These compounds generally showed a broader occurrence in the river compared to the compounds in SC5. Biodegradation rate constants of the compounds in this sub-cluster ranged between 0 and 3.565 d^{-1}. Biodegradation rate constant of AHDI and BP-3 were 3.565 d^{-1} at Sample Point 28 and Sample Point 5, respectively. On the other hand, most compounds of this cluster either showed slow biodegradation or did not get degraded at all. For example, NETSA and DCMU were identified at thirteen and ten points respectively and stayed unchanged in all of them.

Acetamiprid (ACMP), benzyltrimethylammonium (BTMA), 2,4-Dihydroxybenzophenone (BP-1), benzo[a]pyrene (BaP), and benzanthrone (BZA), which were clustered in SC7, showed a rare occurrence in the river, mostly identified at the points in Cluster A, and resisted to biodegradation. While ACMP, BaP, and BZA showed no removal at any points, BP-1 and BTMA were degraded at one point with a biodegradation rate constant of 0.205 d^{-1} and at four points with biodegradation rate constant that ranges between 0.025 and 0.154 d^{-1}, respectively.

The cluster SC8 was composed of N-Benzyldimethylamine (BDMA), piperonyl butoxide (PBO), and di(2-ethylhexyl)phthalate (DEHP), which were identified together in Sample Point 4, 54, 28, and 23. BDMA was identified at twelve points and its biodegradation rate constant was between 0 and 0.511 d^{-1}. PBO was identified in six samples and degraded in all of them with a biodegradation rate constant that ranged between 0.062 and 0.557 d^{-1}. Similarly, DEHP was identified at five points and its biodegradation rate constant was between 0 and 0.397 d^{-1}.

Compounds with high water solubility usually show faster biodegradation than water-insoluble compounds. Chemical groups that are susceptible to enzymatic hydrolysis such as esters, amides, hydroxyls, aldehydes, carboxylic acids, and ketones are also considered to increase aerobic biodegradability (Boethling et al., 2007). Acetaminophen (APAP), the reference chemical of this study, was completely removed in all river water samples in 4 days. APAP has both amide and hydroxyl groups that increase biodegradability and considered as moderately soluble in water. Several microorganisms were reported as acetaminophen degraders (De Gusseme et al., 2011; Zhang et al., 2013). Hydrolysis of acetaminophen by those microorganisms was reported to yield 4-aminophenol which is a toxic and teratogenic chemical (Wu et al., 2012).

2,4-Dihydroxybenzophenone (BP-1) and oxybenzone (BP-3), which are benzophenones that have ketone and hydroxyl groups that are known to increase biodegradability and used as UV filters, were found highly susceptible to biodegradation. Half-life of BP-1 ranged between 1.9 and 3.7 days while half-life of BP-3 was between 0.2 and 40.3 days. Median half-lives of the both compounds were found 3.3 days in the river. A previous study reported transformation of BP-3 to BP-1 via activated sludge under aerobic conditions with a half-life of 10.7 (0.54) days (Liu et al., 2012).

Celestolide (ADBI), galaxolide (HHCB), and phantolide (AHDI), which are polycyclic musk compounds used in personal care and cosmetic products (Tumová et al., 2019), were susceptible to biodegradation in the river. ADBI and AHDI have a ketone group that increases biodegradability. ADBI was degraded in six of seven samples and its half-life ranged between 0.2 and 6.3 days whereas AHDI was degraded in ten of eleven samples and its half-life was between 0.2 and 5.1 days. HHCB has an ether group which generally considered as stable. A previous study reported transformation of HHCB to HHCB-lactone by a few fungi strains (Martin et al., 2007).

Benzyldimethyldodecylammonium (C_{12}BDMA) and benzyldimethyltetradecylammonium (C_{14}BDMA) were mostly degraded in the river. Benzalkonium chlorides (BACs) are an important class of cationic biocides and are classified as emerging contaminants because of their continuous release into the environment (Tezel and G. Pavlostathis, 2011, Buffet-Bataillon et al., 2012). BACs have been known to susceptible to biodegradation. Pseudomonas genus has been especially related to BACs degradation in the environment (Takenaka et al., 2007, Oh et al., 2014, Ertekin et al., 2016). Oxygenases are known responsible to initiate the biodegradation of BACs. Initial reaction can occur as ω-hydroxylation or α-hydroxylation of the carbon atom adjacent to the central nitrogen, or hydroxylation of the methyl carbon attached to central nitrogen (Tezel and Pavlostathis, 2015).

N,N-Diethyl-m-toluamide (DEET) was one of the most frequently detected micropollutants in the Ergene River. Half-life of DEET was between 2.2 and 34.1 days. DEET is used as an active ingredient in insect repellents and known to be highly susceptible to biodegradation with a half-life of several days up to a few weeks (Weeks et al., 2012). A study showed that *Pseudomonas* putida DTB can use DEET as a sole source of carbon and energy. This bacterium can metabolize DEET by hydrolyzing the amide bond to yield diethylamine and 3-methylbenzoate which was further metabolized to 3-methylcatechol via meta ring cleavage (Rivera-Cancel et al., 2007). In a previous study, DEET was also shown to be affected by both biotic and abiotic processes and several transformation by-products were identified in river water (Calza et al., 2011). However, DEET was not degraded in twenty-four of thirty-six river water samples suggesting that DEET could show persistence in water.

Norfloxacin (NF), ciprofloxacin (CF), and ofloxacin (OF) did not get degraded in most samples. Half-lives of NF and OF ranged between 28.1 and 52.5 days and 9.7 and 35.4 days, respectively. OF was removed only in one sample with a half-life of 19.7 days. NF, CF, and OF contain a fluorine-substituted central ring and are among the most widely prescribed fluoroquinolones, which is a subgroup of quinolones. (Sukul and Spiteller, 2007). In consistent with their broad usage, these antibiotics generally have the highest detection frequency and the highest concentration in sewage compared with other quinolones (Zhang and Li, 2011). Many studies found that while biodegradation was of minor importance, sorption via sludge was the dominant mechanism for the removal of fluoroquinolones in WWTPs (Golet et al., 2003, Jia et al., 2012, Lindberg et al., 2006). However, biotransformation of norfloxacin (Adjei et al., 2006, Kim et al., 2011), ciprofloxacin (Wetzstein et al., 1999, Liu et al., 2013, Liao et al., 2016), and ofloxacin (Maia et al., 2016) by single and mixed cultures were also reported. In addition to their single utilization by microorganisms, Amorim and colleagues (2014) has also reported biodegradation of these antibiotics as both single and mixed substrates in the presence of an easily degradable carbon source. On the other hand, quinolone antibiotics are generally considered as persistent in water. Moreover, they were reported to have toxic effects on aquatic microorganisms and reduced microbial diversity (Naslund et al., 2008, Johansson et al., 2014).

Sulfamethoxazole (SMX), carbendazim (MBC), and hexa(methoxymethyl)melamine (HMMM), which are used as an antibiotic, a fungicide, and a coating agent respectively, were degraded in the river but in few samples. SMX was degraded only in Sample Point 6 in November with a biodegradation rate constant of 3.565 d^{-1}. HMMM was degraded in Sample Point 13, 23, 26, and 65 in November and Sample Point 4 in February. Biodegradation rate constant of HMMM was found between 0.017 and 3.565 d^{-1}. MBC was degraded in Sample Point 4 and Sample Point 28 in February with biodegradation rate constants of 0.127 and 0.121 d^{-1}. Several bacterial isolates were reported to degrade SMX (Gauthier et al., 2010, Reis et al., 2014) and MBC (Holtman and Kobayashi, 1997, Zhang et al., 2005, Wang et al., 2010). Those reported degraders may be present at those points but missing at others.

Biodegradation rate constants of some compounds such as TBEP, PBO, and AHDI varied among seasons while some compounds including DEET, BTA, MBC, HMMM, DCH, BP-1 and BP-3 did not show any differences. The fastest biodegradation of target micropollutants generally occurred in May samples (Figure 4.5), which may be a result of favorable environmental conditions that promote microbial diversity and growth in this season. On the other hand, acetaminophen biodegradation was slower in this season compared to the other months. The amount and content of organic matter, temperature, oxygen and nutrient availability, temperature, pH, and salinity are known to affect the biodegradation of pollutants (Maier and Gentry, 2015).

TBEP was the only chemical identified at each point and in each season. The fastest biodegradation of the compound was observed at Sample Point 8 in November and Sample Point 4 in May. QNP was also identified at sample Point 8 at each season and showed the fastest biodegradation in May. At other points and in seasons, on the other hand, TBEP showed a similar biodegradability while quinalphos was not identified (Figure 4.5).

PBO was identified at Sample Point 8 in each season and showed the fastest biodegradation in May. Similarly, it showed faster biodegradation at Sample Point 48 in May than in other three seasons. On the other hand, PBO showed faster biodegradation in February than in August at Sample Point 50 (Figure 4.5).

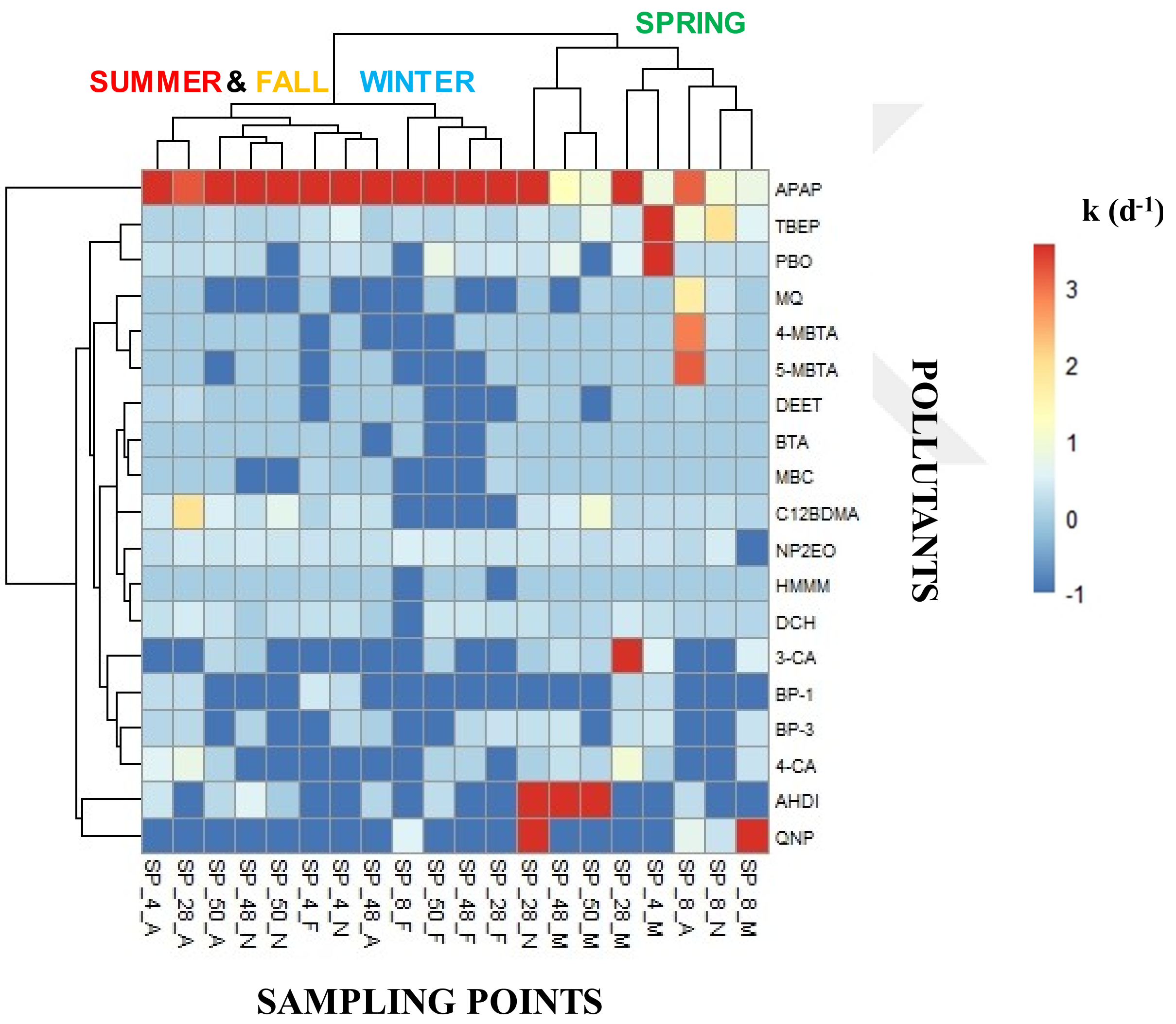


Figure 4.5. Clustering of five sampling points and common chemical substances detected at those points based on occurrence and biodegradation rate constants in four seasons

MQ was identified at Sample Point 8 in May, November, and August and the compound was degraded fastest in August at this point. Similarly, 4-MBTA and 5-MBTA was identified at Sample

Point 8 in those three months and showed fastest biodegradation in August. However, MQ showed similar biodegradability at Sample Point 28 in those three months (Figure 4.5).

AHDI was identified at Sample Point 50 in each season and showed the fastest biodegradation in May. Similarly, it was also identified at Sample Point 48 in May, August, and November and had the highest biodegradation rate constant in May at this point. On the other hand, it was only identified in November at Sample Point 28 and showed similar biodegradability to Sample Point 50 and Sample Point 48 in May (Figure 4.5).

C_{12}BDMA was identified at Sample Point 28 in August, November, and May and showed fastest biodegradation in August. On the other hand, the compound showed faster biodegradation at Sample Point 50 in May among those three months (Figure 4.5).

3-CA was identified at Sample Point 28 in May and November and showed its fastest biodegradation at this point in May. On the other hand, the compound was detected at Sample Point 50 in May and August and Sample point 48 in May and November with no significant differences with respect to biodegradation rate constants (Figure 4.5).

In order to get insight into the effect of the molecular structure of a chemical on its biodegradability, the target compounds sharing the same moieties were grouped together in box plots graphics showing the biodegradation rate constants of the compounds in the four months. The compounds were classified according to the information on their reactive group/groups on NCBI database and the literature. The graphs were plotted using SigmaPlot v.10.0. These graphics revealed that even though chemicals shared similar structures, their biodegradability could be different. For example, 1,2,3-benzotriazole (BTA) did not get degraded in most samples whereas 5,6-Dimethyl-1H-benzotriazole (5,6-DMBTA) mostly showed rapid biodegradation. On the other hand, biodegradation rate constants of two other benzotriazoles, 4-Methyl-1H-benzotriazole (4-MBTA) and 5-Methyl-1H-benzotriazole (5-MBTA) were very closed in river water samples (Figure 4.6). While 4-MBTA and 5-MBTA got degraded slower than 5,6-DMBTA, their biodegradations were faster than BTA, indicating that methyl groups increase biodegradation rates of benzotriazoles. These results are consistent with a previous finding that 1,2,3-benzotriazole was more resistant to biodegradation than 5-Methyl-1H-benzotriazole under aerobic conditions (Liu et al., 2011). Prochloraz (PCZ), which is a diazole, was only identified in one sample point in February and was not degraded. Boxplot graphic of azoles showing their biodegradation rate constants at the four months is presented in Figure 4.6. In all boxplot graphics, each circle represents the first-order biodegradation rate constant of the compounds in sampling points while circle colors indicate different months. The mean was represented as a short dash line in purple.

Biodegradation rate constants of the chemicals belonging to the group anilines ranged between 0 and 3.565 d^{-1}. The median and mean biodegradation rate constants of 3-Chloroaniline (3-CA) were 0.164 d^{-1} and 0.794 $d^{-}1$, respectively. 4-Chloroaniline (4-CA) showed similar biodegradability to 3-Chloroaniline. The median and mean biodegradation rate constants of 4-Chloroaniline were 0.294 d^{-1} and 0.615 d^{-1}, respectively. These results are consistent with a previous finding that 3-Chloroaniline and 4-Chloroaniline were degraded at very close rates by a strain named *Delftia tsuruhatensis* H1. Average utilization rates of 3-Chloroaniline and 4-Chloroaniline as a sole carbon and nitrogen source by the strain were reported 13.0±0.008 (mg/h) and 12.6±0.008 (mg/h) respectively in that study (Zhang et al., 2010) (Figure 4.7).

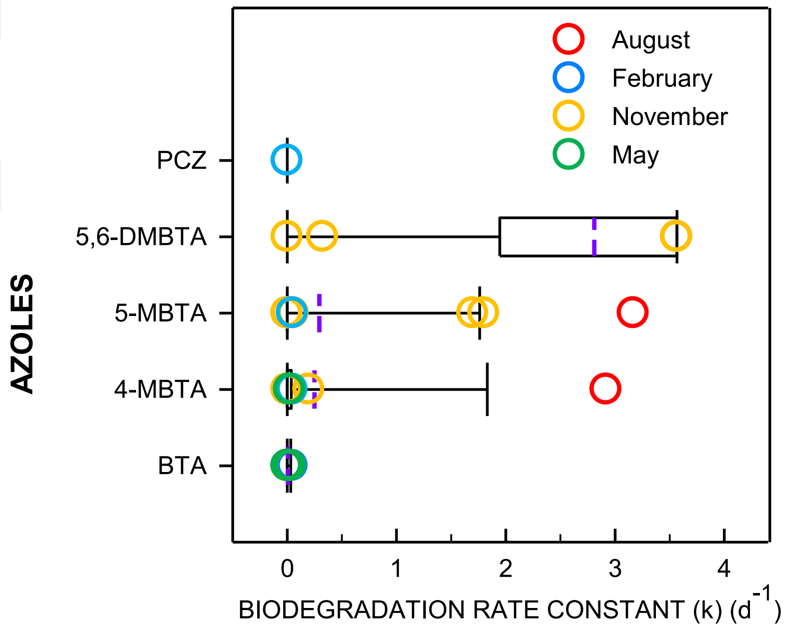


Figure 4.6. The seasonal biodegradation rate constants of azoles

Acetaminophen (APAP), which has an aniline and an acetanilide moiety used as an analgesic and antipyretic drug, was degraded in all samples. The median and mean biodegradation rate constants of acetaminophen were 3.127 d^{-1} and 2.200 d^{-1}, respectively. Even though there is a lack of information about the biodegradation of acetaminophen in the environment compared to other pharmaceuticals (Wu et al., 2012), there are several species and strains reported as acetaminophen degraders. For example, (Zhang et al., 2013) reported that *Stenotrophomonas* sp. f1, which was isolated from paracetamol-degrading aerobic aggregate, consumed 400 mg/L acetaminophen in 116 hours. In the same study, *Pseudomonas* sp. f2 and *Pseudomonas* sp. fg-2 also completely degraded

2500 mg/L and 2000 mg/L paracetamol in 70 and 45 hours respectively. In another study, *Cupriavidus necator* F1, isolated from activated sludge samples, was reported to degrade 400 mg/L acetaminophen in 48 hours (Wei et al., 2011). The result of this thesis supports these previous findings that acetaminophen was highly susceptible to biodegradation. Boxplot graphic of anilines showing their biodegradation rate constants in the four months is presented in Figure 4.7.

Biodegradation rates of benzyldimethyldodecylammonium (C_{12}BDMA) and benzyldimethyltetradecylammonium (C_{14}BDMA) were significantly different. C_{14}BDMA was degraded approximately three times slower than C_{12}BDMA, which may be a result of the increased size of C_{14}BDMA. The median and mean biodegradation rate constants of C_{12}BDMA were 0.195 d^{-1} and 0.306 d^{-1} respectively. The median and mean biodegradation rate constants of C_{14}BDMA were 0.060 d^{-1} and 0.111 d^{-1} respectively. Benzyltrimethylammonium (BTMA) was degraded in all eight samples where it was detected. The median and mean biodegradation rate constants of BTMA were 0.131 d^{-1} and 0.617 d^{-1} respectively. Mepiquat (MQ) was degraded in four samples while found unremoved in nine samples. The median and mean biodegradation rate constants of MQ were 0 and 0.566 d^{-1} respectively. Boxplot graphic of quaternary ammonium compounds (QACs) showing their biodegradation rate constants in the four months is presented in Figure 4.8.

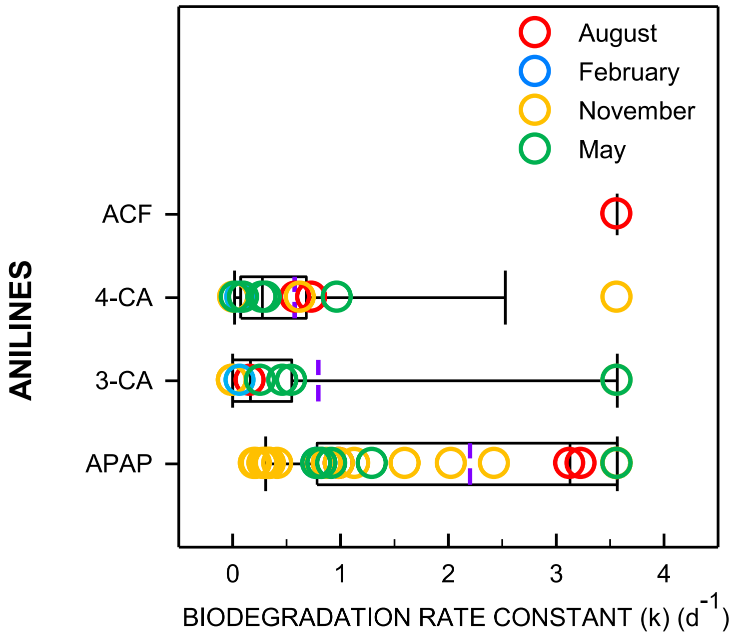


Figure 4.7. The seasonal biodegradation rate constants of anilines

N-benzyldimethylamine (BDMA) and N-benzylmethyhlamine (BMA), which are benzylamines, were mostly degraded in the samples. BDMA was degraded in twelve samples while it did not show any removal in three samples. The median and mean biodegradation rate constants of BDMA were 0.147 d^{-1} and 0.191 d^{-1} respectively. BMA was degraded in all eight samples where it was detected. The median and mean biodegradation rate constants of BMA were 0.431 d^{-1} and 1.287 d^{-1} respectively. Boxplot graphic of benzylamines showing their biodegradation rate constants in August and November is presented in Figure 4.9.

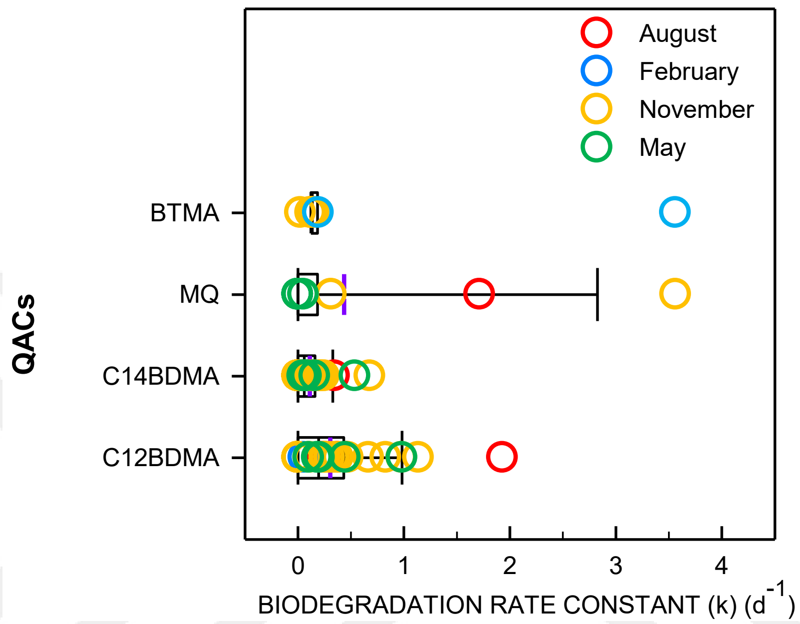


Figure 4.8. The seasonal biodegradation rate constants of quaternary ammonium compounds (QACs)

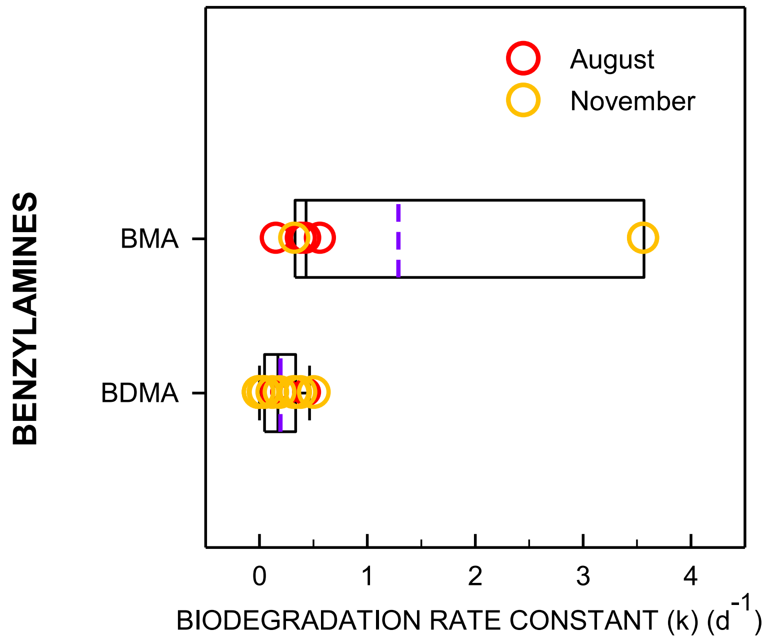


Figure 4.9. The seasonal biodegradation rate constants of benzylamines

Target micropollutants belonging to the chemical group amides showed resistant to biodegradation. Herbicide diuron (DCMU) did not get degraded in twenty-one samples where it was identified. Similarly, antibiotic sulfamethoxazole (SMX) was only degraded in one sample while it was not degraded in fifteen samples. N-Ethyl-p-toluenesulfonamide (NETSA), which is an industrial chemical used in dye, pharmaceutical, and pesticide formulations, was not degraded in all nineteen samples where it was detected. It is noteworthy that these three amides are used as biocides which are synthesized as biodegradation resistant. Another amide N,N-Diethyl-m-toluamide (DEET), which is an insect repellent, was degraded in twelve samples while it was not removed in twenty-four samples. The median and mean biodegradation rate constants of DEET were 0 and 0.040 d^{-1} respectively. Boxplot graphic of amides showing their biodegradation rate constants in the four months is presented in Figure 4.10.

Quinolone antibiotics showed resistance to biodegradation. Ciprofloxacin (CF) was degraded in five samples whereas it was not removed in twenty samples. The median and mean biodegradation rate constants of CF were 0 and 0.006 d^{-1} respectively. Ofloxacin (OF) was not degraded in seventeen samples. OF was degraded in just one sample and its biodegradation rate constant was 0.036 d^{-1} in this sample. Another quinolone norfloxacin (NF) was not removed in eight samples while it was degraded in three samples. The median and mean biodegradation rate constants of NF were 0 and 0.005 d^{-1} respectively. Boxplot graphic of quinolones showing their biodegradation rate constants in August and November is presented in Figure 4.11.

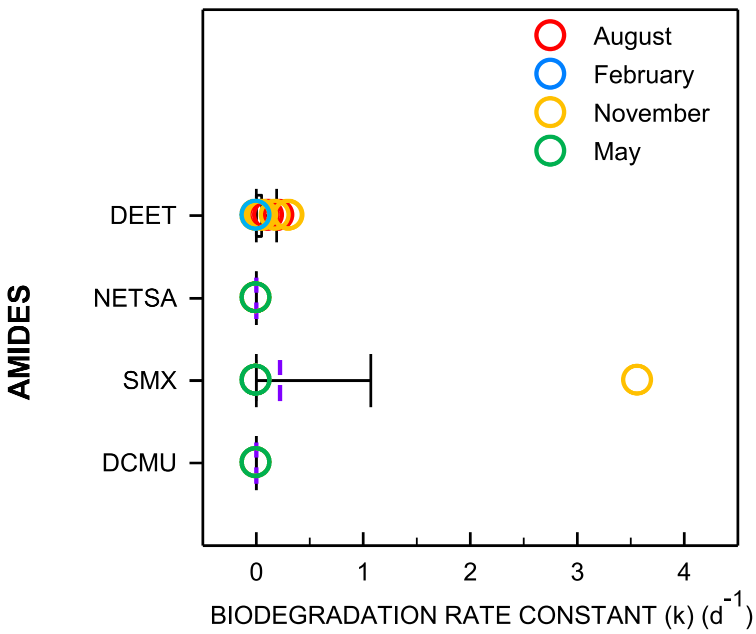


Figure 4.10. The seasonal biodegradation rate constants of amides

Target micropollutants belonging to the chemical group triazines resisted to biodegradation. Hexa(methoxymethyl)melamine (HMMM) was identified in forty-one samples and it was degraded in only six samples. The median and mean biodegradation rate constants of HMMM were 0 and 0.102 d^{-1} respectively. Terbutryn was identified only in four August samples and did not get degraded in those samples. On the other hand, atrazine-desethyl (DEA) was degraded in six of seven samples. The median and mean biodegradation rate constants of DEA was 0.050 and 1.050 d^{-1} respectively. Boxplot graphic of triazines showing their biodegradation rate constants in the four months is presented in Figure 4.12.

Tris(2-butoxyethyl) phosphate (TBEP), which belongs to the chemical group organic phosphorous compounds (OPCs), was degraded in most samples. TBEP was degraded in twenty-five samples while it was not removed in seven samples. The median and mean biodegradation rate constants of TBEP were 0.135 d^{-1} and 0.356 d^{-1} respectively. Quinalphos (QNP), which is a pesticide, was degraded very fast in seven samples where it was identified. The median and mean biodegradation rate constants of QNP were 3.565 d^{-1} and 2.246 d^{-1} respectively. On the other hand, insecticide omethoate (OMA) did not get degraded in three samples where it was detected. Boxplot graphic of organic phosphorous compounds (OPCs) showing their biodegradation rate constants in the four months is presented in Figure 4.13.

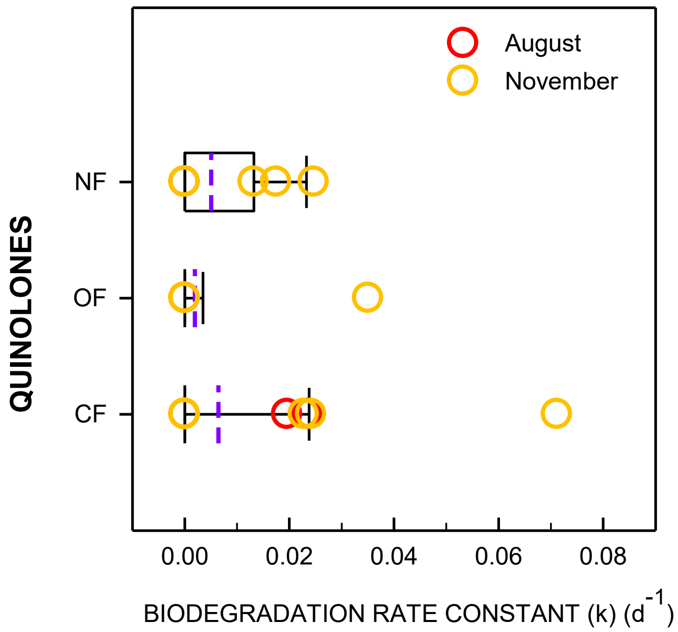


Figure 4.11. The seasonal biodegradation rate constants of quinolones

Benzophenones showed similar biodegradability. 2,4-Dihydroxybenzophenone (BP-1) was degraded in all six samples where it was detected. The median and mean biodegradation rate constants of BP-1 were 0.208 d^{-1} and 0.232 d^{-1} respectively. Oxybenzone (BP-3) was degraded in all nine samples where it was detected. The median and mean biodegradation rate constants of BP-3 were 0.276 d^{-1} and 0.223 d^{-1} respectively. In a previous study, BP-3 was reported to be degraded by activated sludge microorganisms at a slower rate in oxic conditions. Biodegradation rate constant of BP-3 was reported as 0.0647±0.0033 d^{-1} in that study (Liu et al., 2012). Boxplot graphic of benzophenones showing their biodegradation rate constants in the four months is presented in Figure 4.14.

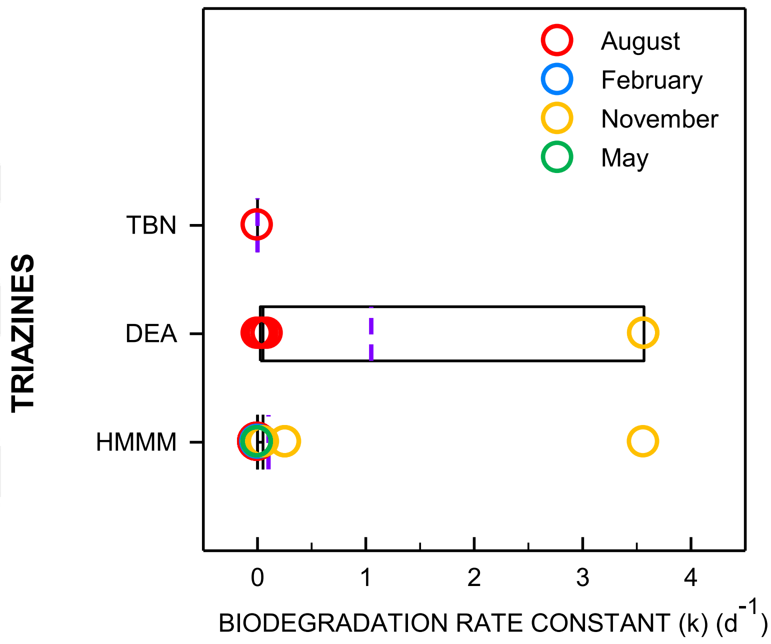


Figure 4.12. The seasonal biodegradation rate constants of triazines

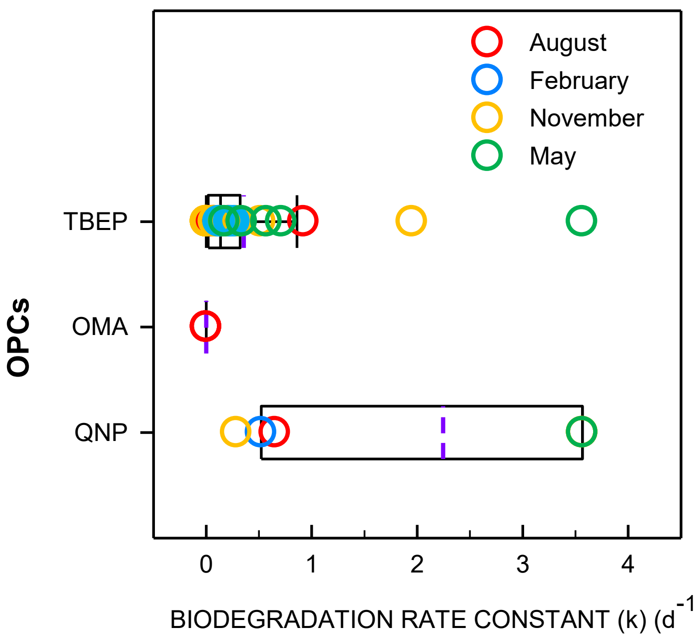


Figure 4.13. The seasonal biodegradation rate constants of organic phosphorous compounds (OPCs)

Celestolide (ADBI), galaxolide (HHCB), and phantolide (AHDI), which belong to the chemical group indanes, were susceptible to biodegradation. AHDI was degraded in six of seven samples. The median and mean biodegradation rate constants of AHDI were 0.561 d^{-1} and 1.711 d^{-1} respectively. Like AHDI, ADBI was also degraded in six of seven samples. The median and mean biodegradation rate constants of AHDI were 0.384 d^{-1} and 0.744 d^{-1} respectively. Another indane HHCB was identified in three samples and degraded in all of them. In two samples, biodegradation rate constants of HHCB were 0.230 d^{-1} and 0.250 d^{-1}. In the other sample, it was degraded faster and its biodegradation rate constant was 3.540 d^{-1}. Since volatilization is nearly negligible for AHDI and HHCB (Suárez et al., 2008, Suarez et al., 2010) but can be important for ADBI (Suarez et al., 2010), biodegradation rate constant of ADBI might be found greater than its actual value. Boxplot graphic of indanes showing their biodegradation rate constants in February, November, and May is presented in Figure 4.15.

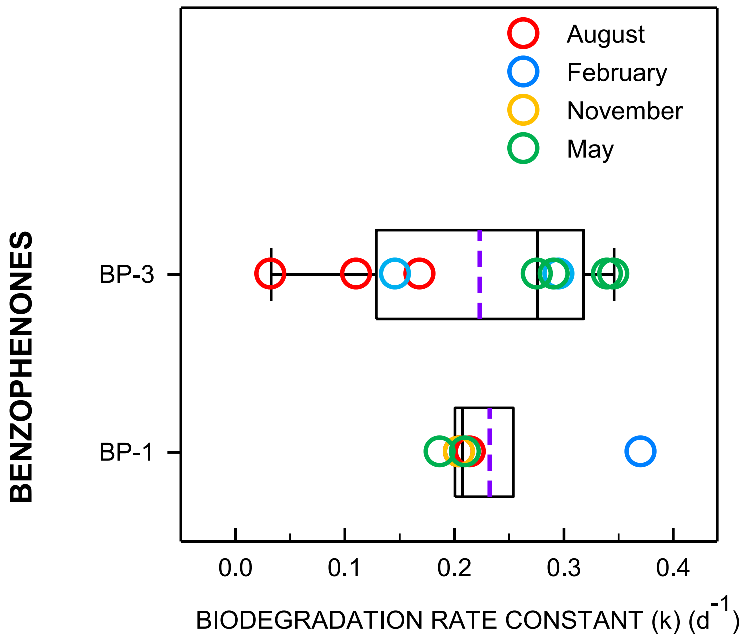


Figure 4.14. The seasonal biodegradation rate constants of benzophenones

Benzo[a]pyrene (BaP), which is a polyaromatic hydrocarbon (PAH) composed of five fuse rings, and benzanthrone (BZA), which is a PAH derivative, did not get degraded in five and seven November samples, respectively. PAHs containing more than three fused rings are generally considered resistant to biodegradation (Boethling et al., 2007), which is supported by the finding of this study about BaP.

Macrolides antibiotics azithromycin and clarithromycin were identified and degraded in one and two August samples respectively. Biodegradation rate constant of azithromycin was calculated 0.200 d^{-1}. Biodegradation rate constants of clarithromycin in two samples were calculated 0.366 d^{-1} and 0.205 d^{-1}.

Dicyclohexylamine (DCH), which is an aliphatic amine, was susceptible to biodegradation. It was degraded in twenty-three samples while stayed unchanged in two samples. The median and mean biodegradation rate constants of DCH were 0.250 d^{-1} and 0.364 d^{-1} respectively. Boxplot graphic of DCH showing their biodegradation rate constants in the four months is presented in Figure 4.16.

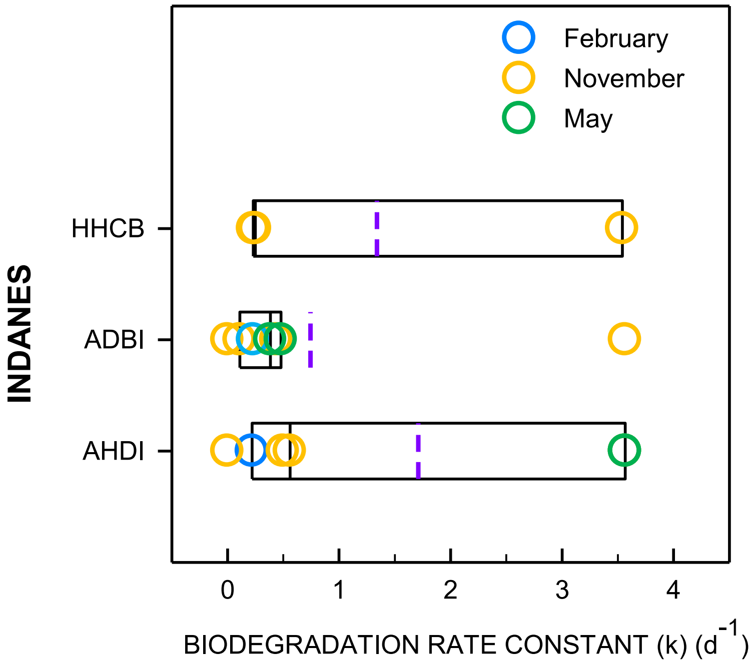


Figure 4.15. The seasonal biodegradation rate constants of indanes

Carbendazim (MBC), which is a fungicide belonging to the chemical group benzimidazoles, resisted to biodegradation. MBC was degraded in only two February samples while it was not removed in eighteen samples. Biodegradation rate constants of MBC in February samples were 0.127 d^{-1} and 0.121 d^{-1}. Boxplot graphic of MBC showing their biodegradation rate constants in the four months is presented in Figure 4.17.

Di(2-ethylhexyl) phthalate (DEHP), which belongs to the chemical group phthalates, was degraded in four of six samples where it was identified. The median and mean biodegradation rate constants of DEHP were 0.123 d^{-1} and 0.134 d^{-1} respectively. Boxplot graphic of DCH showing their biodegradation rate constants in August and November is presented in Figure 4.18.

Acetamiprid (ACMP), which is a monochloropyridine, a nitrile, and a carboxamidine, showed resistance to biodegradation. It was degraded in only one sample taken in February while it was not removed in eleven samples. Biodegradation rate constant of ACMP was 0.017 d^{-1}. Boxplot graphic of ACMP showing their biodegradation rate constants in the four months is presented in Figure 4.19.

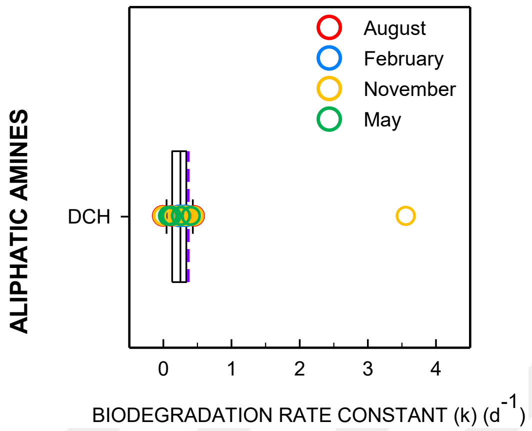


Figure 4.16. The seasonal biodegradation rate constants of dicyclohexylamine (DCH)

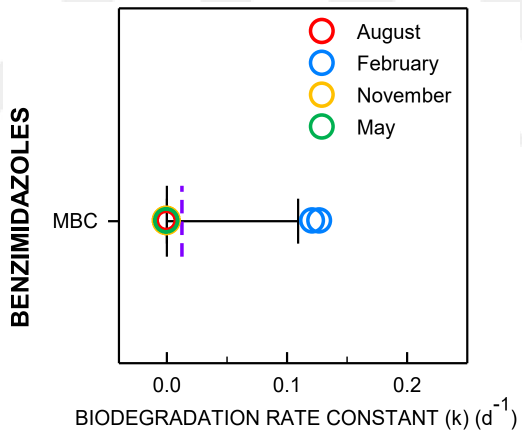


Figure 4.17. The seasonal biodegradation rate constants of carbendazim (MBC)

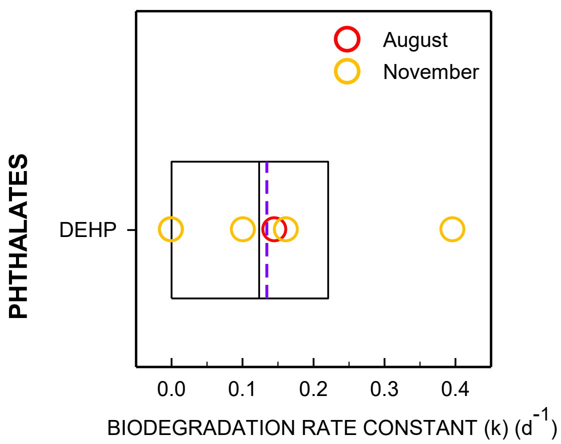


Figure 4.18. The seasonal biodegradation rate constants of di(2-ethylhexyl) phthalate (DEHP)

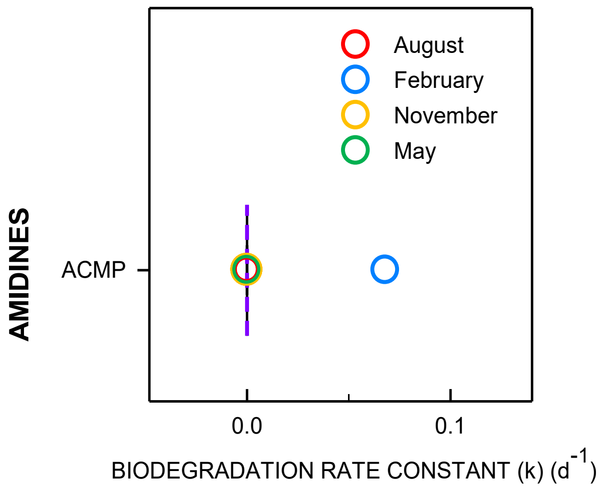


Figure 4.19. The seasonal biodegradation rate constants of acetamiprid (ACMP)

4.2.1.2. Half-lives of the target compounds. Half-lives of the target compounds in water samples were calculated using the equation below.

$$t_{1/2} = \ln 2/k \tag{1}$$

Where $t_{1/2}$ is half-life, k refers to first-order rate constant. The unit of the rate constant is day^{-1} (d^{-1}) and the half-life is expressed as days (d) (Battersby, 1990).

The predicted half-life of each compound was also calculated as suggested by Arnot and co-workers (2005) using BIOWIN3 and BIOWIN4 scores of the compounds. The predicted half-lives of all target compounds in this thesis were found by averaging the values calculated using two equations below.

$$t_{1/2} = -1.07x + 4.20 \tag{2}$$

Where $t_{1/2}$ is the compound's half-life, and x is BIOWIN3 score of the compound.

$$t_{1/2} = -1.46x + 6.51 \tag{3}$$

Where y is compound's half-life, and x is BIOWIN4 score of the compound.

Mean half-life values of the degraded target compounds were between 0.2 and 41 days (Table 4.1). Target compounds with minimum and maximum half-life were acetaminophen (APAP) and acetamiprid (ACMP), respectively. Predicted half-lives of the compounds ranged between 2.5 and 1428.6 days and were generally much higher than the experimental half-lives of the compounds. For example, the half-life of APAP ranged between 0.2 and 3.4 days in the river while its predicted half-life was 10.3 days. Similarly, the predicted half-life of hexa(methoxymethyl)melamine (HMMM) was much higher than the experimental half-life of the compound. The half-life of HMMM ranged between 0.2 and 40.3 days in the river samples while its predicted half-life was calculated 1042.2 days. On the other hand, HMMM was degraded in six samples while it was not removed in thirty samples (Table 4.1), suggesting that HMMM was mostly unsusceptible to biodegradation as consistent with the prediction. Therefore, mean half-lives were not given here as an ultimate indicator for biodegradability of the compounds. Biodegradability of the compounds was discussed by also considering the number of the samples where the compounds were degraded and were not removed.

3-Chloroaniline (3-CA) was degraded eight of eleven samples and 4-Chloraniniline (4-CA) was degraded in all thirteen samples where it was identified. Mean and median half-life of 3-CA were calculated 3.4 and 2.1 days respectively while the mean and median half-life of 4-CA were 9.7 and 2.5 days respectively, suggesting that both 3-CA and 4-CA were readily biodegradable. However, 3-CA and 4-CA were not predicted as readily biodegradable by BIOWIN^{TM} (Appendix I). The predicted half-lives of both compounds were calculated 32.1 days, which also suggests that 3-CA and 4-CA are not readily biodegradable.

4-Methyl-1H-benzotriazole (4-MBTA) and 5-Methyl-1H-benzotriazole (5-MBTA) were degraded in ten and seven samples while they were not removed in twenty and twenty-one samples respectively. Experimental and predicted half-lives of these two benzotriazoles were consistent. Mean and median half-life of 4-MBTA were calculated 17.5 and 20.1 days respectively while the mean and median half-life of 5-MBTA were 12.3 and 12.1 days respectively. The predicted half-life of both compounds was 16.5 days. 1,2,3-Benzotriazole (BTA) was also not degraded in most samples. Mean and median half-life of BTA were 22.0 and 21.9 days respectively while its predicted half-life was 12.7 days. 5-Dimethyl-1-H benzotriazole (5,6-DMBTA), on the other hand, was degraded in eight out of nine samples and its half-life ranged between 0.2 and 2.1 days whereas its predicted half-life was 22.3 days (Table 4.1). These results suggest that 4-MBTA and 5-MBTA could persist in the environment while 5,6-DMBTA is readily biodegradable.

Celestolide (ADBI) and phantolide (AHDI) were degraded in six and ten samples respectively while both compounds were not removed only in one sample. The half-life of ADBI and AHDI ranged between 0.2 and 6.3 days and 0.2 and 5.1 days respectively whereas their predicted half-life was calculated 89.5 days for both. The other musk compound galaxolide (HHCB) was degraded in all three samples where it was identified, and its half-life ranged between 0.2 and 3.0 days. On the other hand, the predicted half-life of HHCB was 92.5 days (Table 4.1). As a result, those three compounds could be considered as readily biodegradable according to their half-life in the river although they were not predicted as readily biodegradable.

Azithromycin (AZM) was identified and degraded in one sample with a half-life of 3.5 days. However, the predicted half-life of AZM was 1428.6 days suggesting that AZM was highly persistent in the environment. The other macrolide antibiotic clarithromycin (CAM) was degraded in all two samples where it was identified. The half-life of CAM in those samples were found 1.9 and 3.4 days. On the other hand, the half-life of CAM was predicted 710.5 days (Table 4.1), suggesting that CAM was also a highly recalcitrant compound. As the number of samples in which AZM and CAM were studied was small, it is difficult to comment on the biodegradability of this chemical in the river.

Benzyldimethylammonium (BDMA) and benzylmethylammonium (BMA) could not be considered as persistent in the environment. The half-life of BDMA ranged between 1.4 and 15.6 days and the half-life of BMA was between 0.2 and 4.3 days. On the other hand, the predicted half-life of BDMA and BMA were 30.6 and 11.1 days respectively (Table 4.1).

The predicted half-life of benzophenones was also higher than their half-lives in the river. Both benzophenone-1 (BP-1) and benzophenone-3 (BP-3) were degraded in all six samples where they were identified. Mean and median half-life of BP-1 were 3.1 and 3.3 days while its predicted half-life was 16.2 days. Mean and median half-life of BP-1 were 6.9 and 3.3 days whereas its predicted half-life was 18.9 days (Table 4.1). According to their half-lives in the river, BP-1 and BP-3 could be considered as readily biodegradable.

Benzyldimethyldodecylammonium (C_{12}BDMA) and benzyldimethyltetradecylammonium (C_{14}BDMA) were degraded twenty-two and eighteen samples while the compounds were not removed in fourteen and thirteen samples, respectively. Mean and median half-life of C_{12}BDMA were 2.7 and 2.0 days respectively and mean and median half-life of C_{14}BDMA were 7.6 and 4.5 respectively, suggesting that two compounds could be considered as readily biodegradable. However, the predicted half-life of C_{12}BDMA and C_{14}BDMA were 14.0 and 16.2 days respectively (Table 4.1).

Half-lives of fluoroquinolone antibiotics studied, which were ciprofloxacin (CF), norfloxacin (NF), and ofloxacin (OF) were predicted 103.3, 97.0, and 279.8 days respectively. CF was degraded in five samples out of twenty-five samples and its mean and median half-life were 26.7 and 29.5. NF was degraded in three samples while it was not removed in eight samples. Mean and median half-life of NF were 40.1 and 40.0 days respectively. Ofloxacin (OF) was degraded only in one sample out of eighteen samples with a half-life of 19.7 days (Table 4.1). Half-lives of CF, NF, and OF in river water samples suggests that those compounds could persist in the environment.

Dicyclohexylamine (DCH) was degraded in twenty-three samples while it was not removed in two samples. Mean and median half-life of DCH were 3.3 and 2.6 days respectively (Table 4.1), suggesting that DCH is readily biodegradable. On the other hand, DCH was predicted as non-readily biodegradable by BIOWIN^{TM} (Appendix I).

Desethyl-atrazine (DEA) was degraded six of seven samples and its mean and median half-life were 10.5 10.6 days respectively, suggesting that DEA could be considered as readily biodegradable in the river. On the other hand, DEA was not predicted as readily biodegradable with a half-life of 91.7 days (Table 4.1).

N,N-Diethyl-m-toluamide (DEET) was degraded in twelve samples while it was not removed in twenty-four samples. The mean and median half-life of DEET were 12.7 and 10.2 days. The predicted half-life of DEET was calculated 17.9 days (Table 4.1). Although DEET could be considered as readily biodegradable by considering its half-life in the samples where it was degraded, DEET was not degraded in most of the river samples, suggesting that DEET can resist to biodegradation.

DEHP was degraded in four of six samples and its half-life ranged between 1.7 and 6.8 days in the river suggesting that DEHP could be considered as biodegradable in the river. The compound was also predicted as readily biodegradable with a half-life of 3.8 days (Table 4.1).

Carbendazim (MBC) was degraded two samples with a half-life of 5.4 and 5.7 days while its predicted half-life was 14.6 days. On the other hand, MBC was not degraded in eighteen of twenty samples (Table 4.1), suggesting that MBC could persist in the environment.

Mepiquat (MQ) was degraded four samples and its half-life ranged between 0.2 and 12.9 days in the river. However, MQ was not removed in nine of thirteen samples suggesting that MQ was not readily biodegradable in the environment. MQ was also not predicted as readily biodegradable by BIOWIN^{TM}. On the other hand, half-life of MQ was calculated 14.8 days (Table 4.1) as suggested by Arnot and co-workers (2005), suggesting that MQ could be considered as readily biodegradable.

Nonylphenol diethoxylate (NP2EO) was degraded in all thirty samples where it was identified, and its mean and median half-life in the river were 2.5 and 2.1 days respectively suggesting that NP2EO was readily biodegradable in the environment. On the other hand, NP2EO was not predicted as readily biodegradable by BIOWIN^{TM} (Appendix I), while its estimated half-life was calculated 11.9 days (Table 4.1) as suggested by Arnot and co-workers (2005), suggesting that NP2EO could be considired as readiliy biodegradable.

Piperonyl butoxide (PBO) was degraded in all nineteen samples where it was identified, and its half-life ranged between 0.2 and 11.2 days in the river. The mean and median half-life of PBO were 2.9 and 2.7 days suggesting that PBO is readily biodegradable. On the other hand, PBO was not predicted as readily biodegradable by BIOWIN^{TM} (Appendix I), while its estimated half-life was calculated 22.2 days (Table 4.1) as suggested by Arnot and co-workers (2005), suggesting that PBO could be considered as readily biodegradable.

Quinalphos (QNP) was degraded in all seven samples where it was identified, and its half-life ranged 0.2 and 2.4 days in the river (Table 4.1), suggesting that QNP is readily biodegradable. On the other hand, QNP was not predicted as readily biodegradable by BIOWIN^{TM} (Appendix I).

Tris(2-butoxyethyl) phosphate (TBEP) was degraded twenty-five samples out of thirty-two samples. Mean and median half-life of TBEP were 6.5 and 3.2 days respectively suggesting that TBEP was readily biodegradable in the river. The compound was also predicted as readily biodegradable by BIOWIN^{TM} (Appendix I). Its estimated half-life was 2.5 days (Table 4.1) calculated as suggested by Arnot and co-workers (2005).

The predicted half-life of the compounds that did not get degraded in any water samples which were benzanthrone (BZA), benzo[a]pyrene(BaP), diuron (DCMU), N-Ethyl-p-toluenesulfonamide (NETSA), omethoate (OMA), prochloraz (PCZ), and terbutryn (TBN) were 24.0, 225.3, 66.2, 23.6, 8.7, 222.7, and 119.9 days respectively (Table 4.1). Although these compounds were also predicted as non-readily biodegradable by BIOWIN^{TM} (Appendix I), BZA, NETSA, and OMA could be considered as readily biodegradable according to their half-lives calculated as suggested by Arnot and co-workers (2015).

Table 4.1. Experimental and predicted half-lives of the target compounds

Compounds	Number of Degraded Samples	Number of Non-degraded Samples	Minimum Half-Life (Days)	Maximum Half-Life (Days)	Mean Half Life (Days)	Median Half-Life (Days)	Predicted Half Life (Days)
3-CA	8	3	0.2	9.7	3.4	2.1	32.1
4-CA	13	0	0.2	58.7	9.7	2.5	32.1
4-MBTA	10	20	0.2	46.2	17.5	20.1	16.8
5,6-DMBTA	8	1	0.2	2.1	0.4	0.2	22.3
5-MBTA	7	21	0.2	30.4	12.3	12.1	16.8
ACF	1	0	0.2	0.2	0.2	0.2	87.3
ACMP	1	8	41.0	41.0	41.0	41.0	49.7
ADBI	6	1	0.2	6.3	2.4	1.7	89.5
AHDI	10	1	0.2	5.1	2.0	1.7	89.5
APAP	44	0	0.2	3.4	0.7	0.2	10.3
AZM	1	0	3.5	3.5	3.5	3.5	1428.6
BaP	0	5	-	-	-	-	225.3

Table 4.1. Continue

Compounds	Number of Degraded Samples	Number of Non-degraded Samples	Minimum Half-Life (Days)	Maximum Half-Life (Days)	Mean Half Life (Days)	Median Half-Life (Days)	Predicted Half Life (Days)
BDMA	12	3	1.4	15.6	5.6	3.2	30.6
BMA	7	0	0.2	4.3	1.6	1.6	11.1
BP-1	6	0	1.9	3.7	3.1	3.3	16.2
BP-3	6	0	0.2	40.3	6.9	3.3	18.9
BTA	4	22	14.6	29.4	22.0	21.9	12.7
BTMA	8	0	0.2	27.8	7.2	5.1	14.5
BZA	0	7	-	-	-	-	24.0
C_{12}BDMA	22	14	0.4	12.0	2.7	2.0	14.0
C_{14}BDMA	18	13	1.0	40.3	7.6	4.5	16.2
CAM	2	0	1.9	3.4	2.6	2.6	710.5
CF	5	20	9.7	35.4	26.7	29.5	103.3
DCH	23	2	0.2	8.9	3.3	2.6	15.7

Table 4.1. Continue

Compounds	Number of Degraded Samples	Number of Non-degraded Samples	Minimum Half-Life (Days)	Maximum Half-Life (Days)	Mean Half Life (Days)	Median Half-Life (Days)	Predicted Half Life (Days)
DCMU	0	21	-	-	-	-	66.2
DEA	6	1	0.2	26.6	10.5	10.6	91.7
DEET	12	24	2.2	34.1	12.7	10.2	17.9
DEHP	4	2	1.7	6.8	4.4	4.5	3.8
HHCB	3	0	0.2	3.0	2.0	2.8	92.5
HMMM	6	35	0.2	40.3	14.6	7.2	1042.2
MBC	2	18	5.4	5.7	5.6	5.6	14.6
MQ	4	9	0.2	12.9	3.9	1.3	14.8
NP2EO	30	0	0.2	13.6	2.5	2.1	11.9
NETSA	0	19	-	-	-	-	23.6
NF	3	8	28.1	52.5	40.1	40.0	97.0
OF	1	17	19.7	19.7	19.7	19.7	279.8

Table 4.1. Continue

Compounds	Number of Degraded Samples	Number of Non-degraded Samples	Minimum Half-Life (Days)	Maximum Half-Life (Days)	Mean Half Life (Days)	Median Half-Life (Days)	Predicted Half Life (Days)
OMA	0	3	-	-	-	-	8.7
PBO	19	0	0.2	11.2	2.9	2.7	22.2
PCZ	0	1	-	-	-	-	222.7
QNP	7	0	0.2	2.4	0.8	0.2	13.9
SMX	1	15	0.2	0.2	0.2	0.2	47.4
TBEP	25	7	0.2	40.5	6.5	3.2	2.5
TBN	0	4	-	-	-	-	119.9

4.3. Hexa(methoxymethyl)melamine (HMMM) Biodegradability Experiments with the Cultures of Bacterial Strains

During 30 days of incubation period, none of the strains were able to utilize HMMM as a carbon source. On the other hand, HMMM was consumed totally by *Pseudomonas* sp. BIOMIG1 in the medium containing 10 mg/L (25 µM) HMMM as the sole nitrogen source whereas *Nocardia*, *Nubsella*, *Rhodococcus*, *Flavobacterium*, *Sphingobium*, and other *Pseudomonas* strains did not consume HMMM.

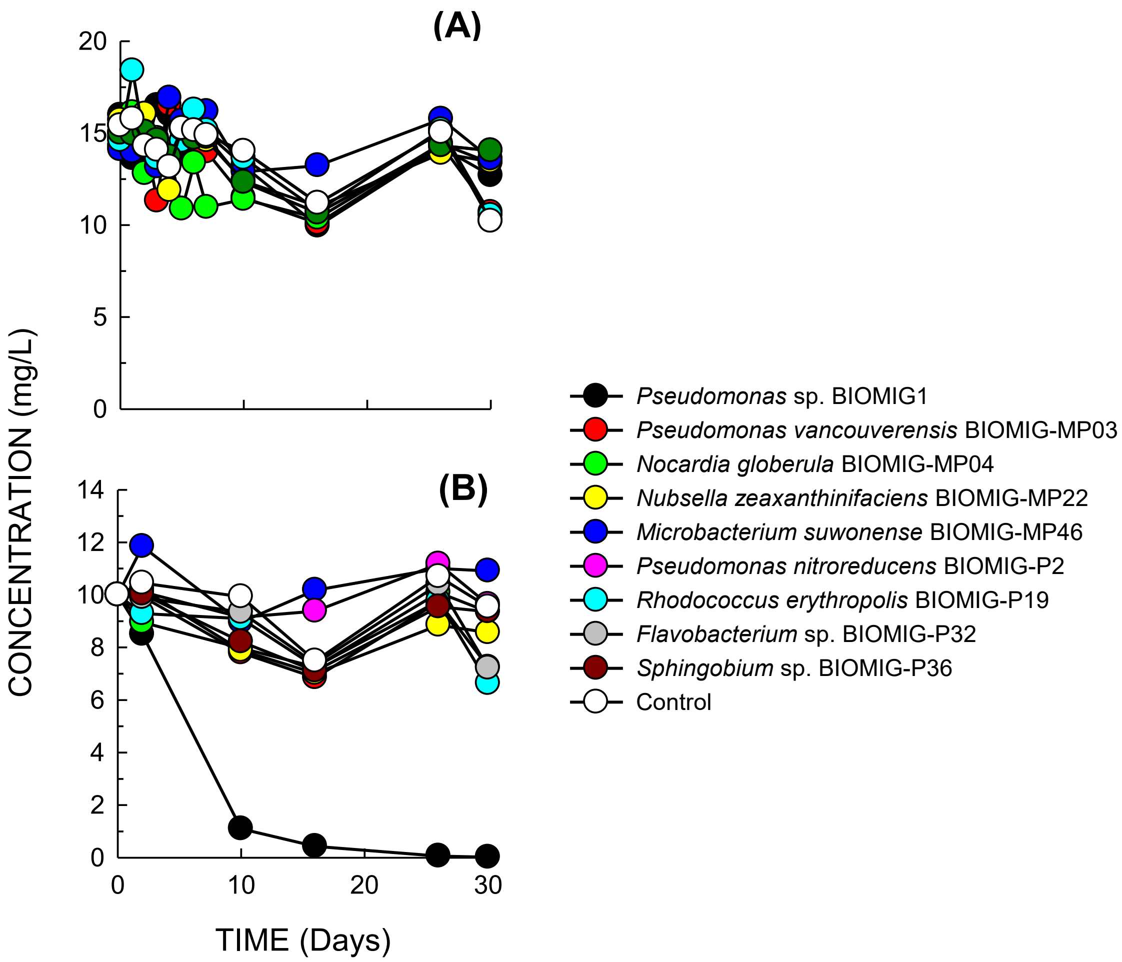


Figure 4.20. Profile of HMMM utilization by nine bacterial strains in the salt medium free of (A) additional carbon or (B) nitrogen source

Pseudomonas species are known to utilize s-triazines as a nitrogen source. For example, atrazine, which is the most widely studies s-triazine herbicide, is utilized as a nitrogen source by *Pseudomonas* sp. ADP in the presence of succinate or phenol as the carbon and energy source by a sequential hydrolysis of functional groups, i.e. chlorine, ethyl amine and isopropyl amine, attached to the carbons on the triazine ring (Mandelbaum et al., 1995, Neumann et al., 2004). After the hydrolysis of functional groups, cyanuric acid (CYA) is formed which is utilized as a nitrogen source along with ethyl and isopropyl amine during the growth. In case another nitrogen source such as ammonium or nitrate is present, on the other hand, *Pseudomonas* sp. ADP is not able to degrade atrazine (Govantes et al., 2010). For testing if BIOMIG1 utilizes HMMM following a mechanism similar to atrazine degradation by ADP, BIOMIG1 was incubated in a salt medium containing 25 μM HMMM and about 75 mM (as acetate equivalent) mixed carbon source composed of glucose, succinate and acetate. No nitrogenous compounds other than HMMM were added to the flasks. HMMM as well as MEL and CYA were monitored during the 45 days of incubation. While a minor loss of HMMM was observed in control flask, 75% of the HMMM was consumed by BIOMIG1 within 14 days. While neither MEL nor CYA was not identified over this period, 3 μM MEL was measured in flask at the 19th day. MEL concentration increased to 18 μM, that is, about 72% of HMMM that was initially amended into the flask inoculated with BIOMIG1, was converted to MEL within 45 days (Figure 4.22). Considering the minor loss of HMMM in the control flask, almost all of the HMMM was converted to MEL by BIOMIG1 in 45 days. CYA, which is a common intermediate in biodegradation of various s-triazines (Cook, 1987), was not detected in the flasks. As a result, BIOMIG1 could not liberate any free nitrogen from HMMM while converting it to M.

When a mixture of different carbon sources is available in the medium, organisms either metabolize the carbons together or prefer one of them over others (Karishma et al., 2015) and pseudomonads generally favor organic acids over glucose (Collier et al., 1996). However, during the biotransformation of HMMM to MEL, *Pseudomonas* sp. BIOMIG1 consumed only glucose while it didn't utilize neither succinic acid nor acetic acid (Figure 4.22), suggesting that MEL was not utilized as a sole nitrogen source and did not promote the cellular growth of BIOMIG1 during the incubation period. In order to further test if MEL can serve as a nitrogen source for BIOMIG1, BIOMIG1 was incubated in mineral salt medium containing mixed carbon sources and MEL as the only nitrogen source. For 30 days of incubation, MEL was not consumed by BIOMIG1. On the other hand, BIOMIG1 consumed almost all the CYA amended in a flask containing mixed carbon sources (Figure 4.22). Several bacterial strains capable of mineralizing MEL and CYA as a nitrogen source were reported (Hatakeyama et al., 2015). Melamine biodegradation was considered to occur via successive deamination resulted in cyanuric acid, which in turn was degraded to ammonia and carbon dioxide via decarboxylation and deamination reactions following a hydrolytic ring cleavage (Dodge et al., 2012, Umamaheswari et al.,2016). However, BIOMIG1 is not capable of degrading MEL due to the lack of enzymes attacking the bond between triazine-carbon and amine-nitrogen which are amidohydolayses (Dodge et al., 2012).

Although BIOMIG1 can utilize both acetate and succinate as carbon sources, it consumed neither of them when incubated without a nitrogen source other than HMMM. Carbon skeleton of glutamate and aspartate family amino acids such as 2-oxoglutarate and oxaloacetate, is generated during the tricarboxylic acid cycle (TCA) in which acetate and succinate are involved. Carbon processing in the TCA cycle and synthesis of 2-oxoglutarate and oxaloacetate are co-regulated with nitrogen anabolism (Udaondo and Ramos, 2018). Cells recognize the external nitrogen availability using the internal concentration of glutamine and regulates nitrogen anabolism and synthesis of amino acids using their nitrogen regulatory (Ntr) system (Huergo and Dixon, 2015). Bacteria generally prefer free inorganic nitrogen such as ammonium over complex organics containing nitrogen as a nitrogen source (Wang et al., 2016). In case there is no free nitrogen available, Ntr system stops the nitrogen assimilation and amino acid synthesis which result in accumulation of 2-oxoglutarate in the cell. As result of accumulation, TCA cycle is switched off (Huergo and Dixon, 2015). Succinate directly goes into the TCA whereas acetate should be transformed to acetyl-coA. HMMM was not recognized as a nitrogen source by BIOMIG1, therefore TCA cycle was not initiated. As a result, acetate and succinate were not consumed by BIOMIG1.

As opposed to acetate and succinate, only carbon source that was degraded by BIOMIG1 in the course of experiment under nitrogen deficiency was glucose. However, it was not mineralized as a carbon source, instead converted to acetate. Bacteria that belong to *Pseudomonas* genus uses Entner-Doudoroff pathway for carbohydrate metabolism to save energy especially under nitrogen and phosphorus limited conditions (Peekhaus et al., 1998). Glucose is converted to first 2-keto-3-deoxy-6-P-gluconate (KDPG) then to pyruvate with a sequence of reactions in the Entner-Doudoroff pathway. Pyruvate produced at the end of the carbohydrate metabolism is generally converted to acetyl-CoA and enters the TCA cycle (Udaondo and Ramos, 2018). In this experiment, since TCA cycle was turned off due the nitrogen deficiency in the medium, BIOMIG1 prefer to convert pyruvate to acetate via fermentation. The overall metabolism initially consumes 1 ATP and finally produces 2 ATPs. Multiple enzymes belonging to hydrolase, isomerase, oxidoreductase, lyase and transferase enzyme families including KDPG aldolase and pyruvate formate lyase are involved in the conversion of glucose to acetate in *Pseudomonas* spp. Given the fact that HMMM biotransformation to MEL was happening during the glucose conversion to acetate when nitrogen was not present, either one of the enzymes that involves in the Entner-Doudoroff pathway or Pfl may catalyze a sequence of a same kind of reactions serendipitously and transform HMMM to MEL, or metabolites of Entner-Doudoroff pathway may promote the expression of a specific enzyme. In both cases, an enzyme is responsible for sequential elimination of methoxymethyl groups from HMMM and subsequent n-methoxymethl melamines, , which are penta(methoxymethyl)melamine (PMMM), tetra(methoxymethyl)melamine

(TMMM), tri(methoxymethyl)melamine (TriMMM), di(methoxymethyl)melamine (DMMM), and mono(methoxymethyl)melamine (MMMM) respectively and formation of melamine (Figure 4.23).

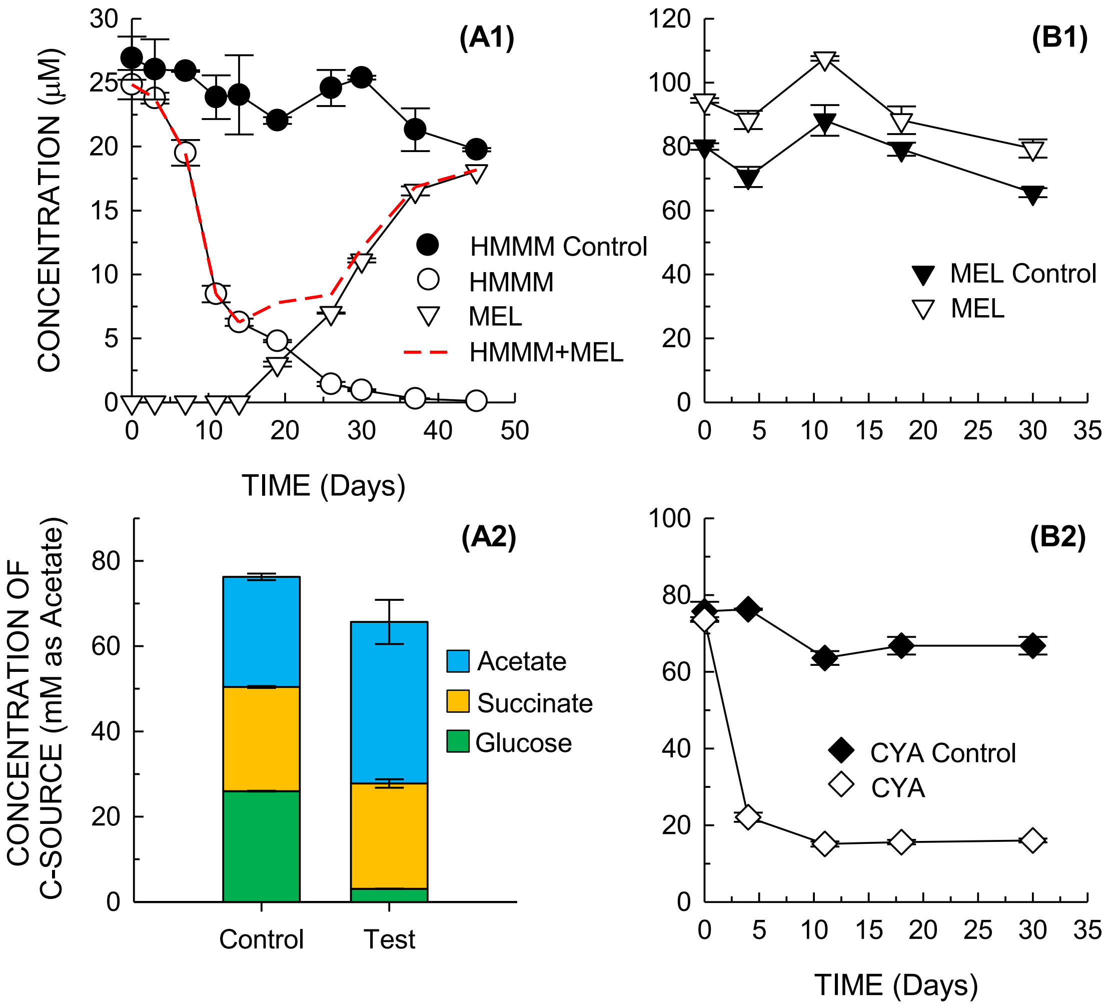


Figure 4.21. Profile of (A1) HMMM and MEL, and (A2) glucose, succinate and acetate during the HMMM biotransformation by *Pseudomonas* sp. BIOMIG1 in the N-free salt medium containing a total of 80 mM mixed carbon source and 25 μM HMMM. Utilization of (B1) MEL and (B2) CYA by *Pseudomonas* sp. BIOMIG1 in a N-free salt medium containing a total of 80 mM mixed carbon source.

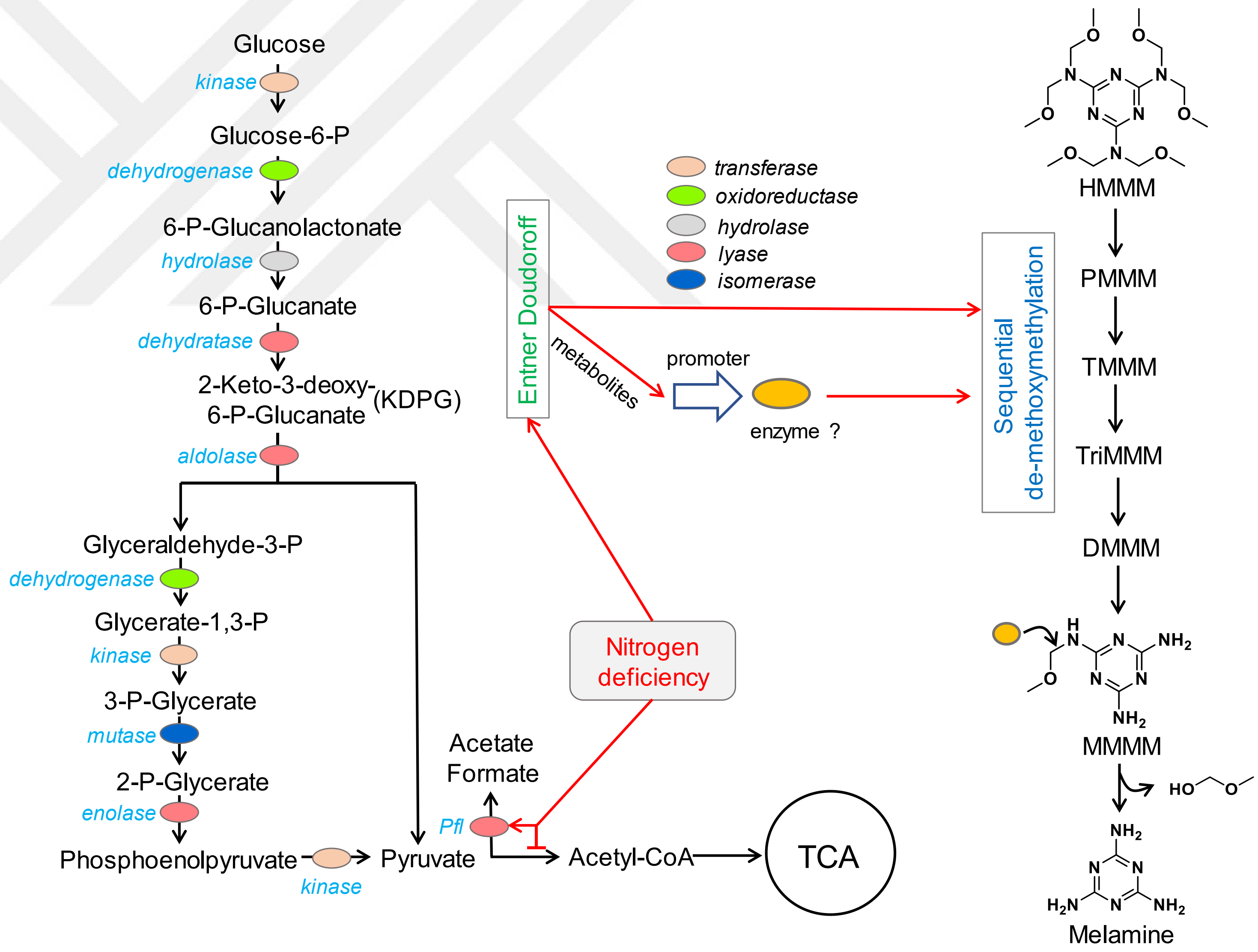


Figure 4.22. Proposed pathway of HMMM conversion to MEL by *Pseudomonas* sp. BIOMIG1 in the nitrogen-free medium with glucose as the energy source

5. CONCLUSION

The Ergene River suffers from severe organic and metal pollution. Micropollutants, many of which are emerging contaminants that are potentially toxic and endocrine-disrupting chemicals and play a role in antibiotic resistance, are present in Ergene River. In this study, aerobic biodegradation of forty-three micropollutants belonging to various chemical groups such as industrial chemicals i.e. plasticizers, UV blockers, surfactants etc., antibiotics, pesticides and personal care products i.e. fragrances, pharmaceuticals etc. was investigated in forty-four river water samples taken in August 2017, November 2017, February 2018, and May 2018 in shake-flasks using ultra-high-performance liquid chromatography and tandem mass spectrometry (UHPLC-MS^n). One thousand and eight hundred samples were run using UHPLC-MS^n and forty-three micropollutants were screened in each run. In total, more than seventy-seven thousand manual reads were performed.

Reference chemical acetaminophen got degraded in all river water samples within four days while only one-fourth of the compound got lost in a control flask at most which were free of any sources of inoculum, which proved that acetaminophen was mostly degraded by microbial activity in river water samples. Out of forty-three target micropollutants, thirty-six were degraded at different rates at least in one sample while seven compounds, which are benzo[a]pyrene, benzanthrone, diuron, N-Ethyl-p-toluenesulfonamide, omethoate, prochloraz, and terbutryn did not get degraded in any samples. Biodegradation rate constants and corresponding half-lives of the compounds were calculated using the first-order decay kinetics model. Biodegradation rate constants and half-lives of the micropollutants degraded in water samples ranged between 0.017 and 3.565 d^{-1} and 0.2 days to 41.0 days, respectively. Half-lives of the compounds were also predicted using their BIOWIN3 and BIOWIN4 scores. Predicted half-lives of the compounds were generally much greater than their half-lives in river water. Pesticides such as diuron, omethoate, and terbutryn, antibiotics such as ciprofloxacin, norfloxacin, ofloxacin, and polyaromatic hydrocarbons generally persisted in water samples whereas fragrances, surfactants, and UV filters got mostly degraded.

Heatmap graphics of the target compounds clustered based on their occurrence and biodegradation rate constants revealed that frequently detected pollutants such as hexa(methoxymethyl)melamine, 4-Methylbenzotriazole, benzyldimethyltetradecylammonium, and N,N-Diethyl-m-toluamide were either non-degradable or slowly degradable. These graphics also revealed spatiotemporal variations in biodegradation in the river, that is, biodegradation of the compounds varied among sampling points and the months in which they were collected. Biodegradation of the compounds such as acetaminophen, 5,6-Dimethylbenzotriazole, and quinalphos was generally faster in the samples with heavy contamination compared to the samples that are less contaminated with target micropollutants, which could be a result of the high amount and variety of nutrients that promotes microbial diversity and adaptation of microorganisms in more contaminated samples. In addition, some compounds such as tris (2-butoxyethyl phosphate), piperonyl butoxide, 4-Methylbenzotriazole, and 5methylbenzotriazole got degraded faster in spring samples, which could be a result of warmer temperatures that promotes microbial growth in that season.

The effects of the functional groups on biodegradability of micropollutants were assessed plotting biodegradation rate constant of the compounds that share the same moieties in four seasons. These graphics revealed that even though chemicals had similar structures, their biodegradability could vary greatly. Methyl substitutions on benzotriazole ring, and hydroxyl and amide groups on anilines such as on acetaminophen increased biodegradation rates of the benzotriazoles and anilines at a great extent respectively. Chloro groups on anilines such on 3-Chloraniline and 4-Chlororaniline, on the other hand, reduced biodegradation rates. Increased length of the alkyl chains in benzalkonium chlorides also resulted in reduced biodegradation rates. Similarly, branching of the nitrogen atom in benzylamines decreased biodegradation rates. Synesthetic musk compounds, which were celestolide and phantolide containing keto groups that are susceptible to enzymatic hydrolysis, and galaxolide containing an ether group, got degraded at most samples in a few days. However, target compounds having amide groups which are also susceptible to enzymatic hydrolysis on their structure, such as diuron and sulfamethoxazole which were reported to be degraded by microorganisms in several studies, and N-Ethyl-p-toluenesulfonamide, persisted in the samples, which may result from the river water lacking those degraders.

Utilization of hexa(methoxymethyl)melamine, one of the most abundant micropollutants in the river, was investigated using nine bacterial strains which are micropollutant degraders. While hexa(methoxymethyl)melamine was not utilized by any of the strains in the medium free of additional carbon sources, one strain named *Pseudomonas* sp. BIOMIG1 converted almost all hexa(methoxymethyl)melamine into melamine in a salt medium containing a total of 80 mM mixed carbon source composed of glucose, succinate, and acetate, and 25 µM hexa(methoxymethyl)melamine which was the only nitrogenous compound in the medium within 45 days. During the biotransformation of hexa(methoxymethyl)melamine into melamine by BIOMIG1, only glucose but neither succinate nor acetate was consumed since BIOMIG1 did not liberate free nitrogen from melamine, which was also tested incubating BIOMIG1 in a mineral salt medium containing melamine as the sole nitrogen source and a total of 80 mM mixed carbon source composed of glucose, succinate, and acetate. Hexa(methoxymethyl)melamine was not able to utilize melamine in this medium while cyanuric acid, a common intermediate in biodegradation of various s-triazines that was amended in a flask containing mixed carbon sources, was consumed almost totally by BIOMIG1. These results confirmed that glucose was utilized as an energy source through Entner-Doudoroff Pathway, which is a glycolytic pathway *Pseudomonas* genus uses for carbohydrate metabolism to save energy, especially under nitrogen and phosphorus limited conditions, during the conversion of hexa(methoxymethyl)melamine to melamine by BIOMIG1. An increase in acetate concentration during the transformation of hexa(methoxymethyl)melamine to melamine also suggested the involvement of pyruvate formate-lyase in glucose metabolism of BIOMIG1, which is an enzyme yielding format and acetate from pyruvate under nitrogen deficiency. As a result, a series of enzymes involved in Entner-Doudoroff Pathway or a specific enzyme activated by the metabolites formed during Entner-Doudoroff Pathway was proposed to be responsible for the conversion of hexa(methoxymethyl)melamine to melamine via a sequential de-methoxymethylation pathway in *Pseudomonas* sp. BIOMIG1. This study is the first to show biotransformation of HMMM by a strain of bacteria.

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APPENDIX A: LOCATIONS AND CONVENTIONAL PARAMETERS OF THE SAMPLES

Table A1. Locations and conventional parameters of August samples

Sampling Point	Location		pH	Temperature (ºC)
	Latitude	Longitude		
4	41°09'49.2"	27°29'35.7"	7.88	29.30
8	41°20'34.1"	27°27'24.8"	6.19	30.80
28	41°12'11.3"	27°28'37.1"	7.92	28.30
48	41°10'17.0"	27°47'41.6"	8.83	30.60
50	41°12'26.8"	27°51'50.8"	8.47	33.80

Table A2. Locations and conventional parameters of November samples

Sampling Point	Location		pH	Temperature (ºC)
	Latitude	Longitude		
4	41°09'49.2"	27°29'35.7"	8.21	16.80
5	41°11'42.6"	27°28'36.8"	7.54	16.60
6	41°15'42.9"	27°32'53.2"	8.85	26.50
7	41°17'38.9"	27°28'03.2"	7.78	14.20
8	41°20'34.1"	27°27'24.8"	7.19	19.40
9	41°21'27.1"	27°23'14.7"	7.36	14.80
10	41°21'06.2"	27°19'11.5"	7.87	13.50
11	41°20'25.1"	27°16'03.5"	8.45	12.30
12	41°22'11.6"	27°10'33.6"	7.42	12.70
13	41°21'08.7"	27°09'37.1"	8.39	12.90
14	41°22'03.9"	27°08'24.4"	7.47	13.90
15	41°21'01.7"	27°06'33.4"	7.54	11.80
16	41°20'27.4"	27°04'56.3"	8.20	13.10
17	41°04'41.3"	27°04'35.5"	7.87	14.00
18	41°21'52.9"	27°03'42.5"	8.03	12.90
19	41°24'23.4"	26°55'44.2"	8.30	14.90

Table A2. Continue

Sampling Point	Location		pH	Temperature (°C)
	Latitude	Longitude		
20	41°22'56.9"	26°54'42.7"	7.98	14.40
21	41°20'54.9"	26°53'11.1"	8.09	14.90
22	41°18'30.9"	26°47'23.2"	8.31	14.40
23	41°16'30.7"	26°40'54.6"	7.81	13.90
24	41°15'02.3"	26°38'41.8"	7.75	12.80
25	41°12'23.9"	26°36'28.6"	8.60	13.30
26	41°05'09.1"	26°32'50.2"	7.88	14.00
28	41°12'11.3"	27°28'37.1"	8.25	16.90

Table A2. Continue

Sampling Point	Location		pH	Temperature (°C)
	Latitude	Longitude		
48	41°10'17.0"	27°47'41.6"	8.66	27.60
50	41°12'26.8"	27°51'50.8"	8.66	29.00
54	41°13'46.5"	27°38'35.7"	8.45	20.00
55	41°15'51.1"	27°38'20.6"	8.99	28.00
65	41°20'28.2"	27°41'42.2"	8.14	12.30

Table A3. Locations and conventional parameters of the February samples

Sampling Point	Location		pH	Temperature (°C)
	Latitude	Longitude		
4	41°09'49.2"	27°29'35.7"	8.03	7.00
8	41°20'34.1"	27°27'24.8"	7.88	8.20
28	41°12'11.3"	27°28'37.1"	8.01	6.60
48	41°10'17.0"	27°47'41.6"	9.00	8.80
50	41°12'26.8"	27°51'50.8"	9.01	10.90

Table A4. Locations and conventional parameters of May samples

Sampling Point	Location		pH	Temperature (°C)
	Latitude	Longitude		
4	41°09'49.2"	27°29'35.7"	7.88	21.50
8	41°20'34.1"	27°27'24.8"	7.72	22.40
28	41°12'11.3"	27°28'37.1"	7.73	22.50
48	41°10'17.0"	27°47'41.6"	9.10	23.60
50	41°12'26.8"	27°51'50.8"	7.66	26.20

APPENDIX B: PHYSICOCHEMICAL PROPERTIES OF TARGET COMPOUNDS

Table B1. Physicochemical properties of target compounds

Compound	Solubility in water at 25°C (mg/L)	LogP
1,2,3-Benzotriazole	20000	1.34
2,4-Dihydroxybenzophenone	413.40	3.17
3-Chloroaniline	5400	1.81
4-Chloroaniline	3900	1.76
4-Methyl-1H-benzotriazole	3069	1.80
5,6-Dimethyl-1H-benzotriazole	914.20	2.26
5-Methyl-1H-benzotriazole (5-Tolytriazole)	3069	1.80
7H-Benzo(de)anthracen-7-one (Benzanthrone)	0.18	4.81
Acetaminophen (Paracetamol)	14000	0.46
Acetamiprid	4250	0.80
Aclonifen	6.95	4.04
Atrazine-desethyl	3200	1.51
Azithromycin	2.37	3.33
Benzo[a]pyrene	0.01	5.99
Benzyldimethyldodecylammonium	1000000	2.93
Benzyldimethyltetradecylammonium	1000	3.91
Benzyltrimethylammonium chloride	1000000	-2.47
Carbendazim	8.00	1.52
Celestolide	0.22	5.51
Ciprofloxacin	11480	0.28
Clarithromycin	1.69	3.16
Cyanuric acid	2000	1.95
Di(2- ethylhexyl)phthalate (DEHP)	0.27	7.40
Dicyclohexylamine	800	3.69
Diuron	42.00	2.68
Galaxolide	1.75	6.23
Hexa(methoxymethyl)melamine	149.30	3.07
Melamine	3230	-1.37
Mepiquat chloride	529000	-2.82
N-Benzyldimethylamine	12000	1.98
N-Benzylmethylamine	1000000	1.52
N-Ethyl-P-toluenesulfonamide	1106	1.96
N,N-Diethyl-m-toluamide	666	2.18
Nonylphenol diethoxylate	1.05	5.30
Norfloxacin	177900	-1.03
Ofloxacin	10800	-0.39
Omethoate	1.01	-0.74

Table B1. Continue

Compound	Solubility in water at 25^{o}C (mg/L)	LogP
Oxybenzone	69.00	3.64
Phantolide	0.75	5.80
Piperonylbutoxide	28.90	4.75
Prochloraz	34.00	4.10
Quinalphos	22.00	4.44
Sulfamethoxazole	610	0.89
Terbutryn	25.00	3.75

APPENDIX C: MRM TRANSITIONS AND OPTIMIZED MS/MS PARAMETERS

Table C1. MRM transitions and optimized MS/MS parameters of the compounds

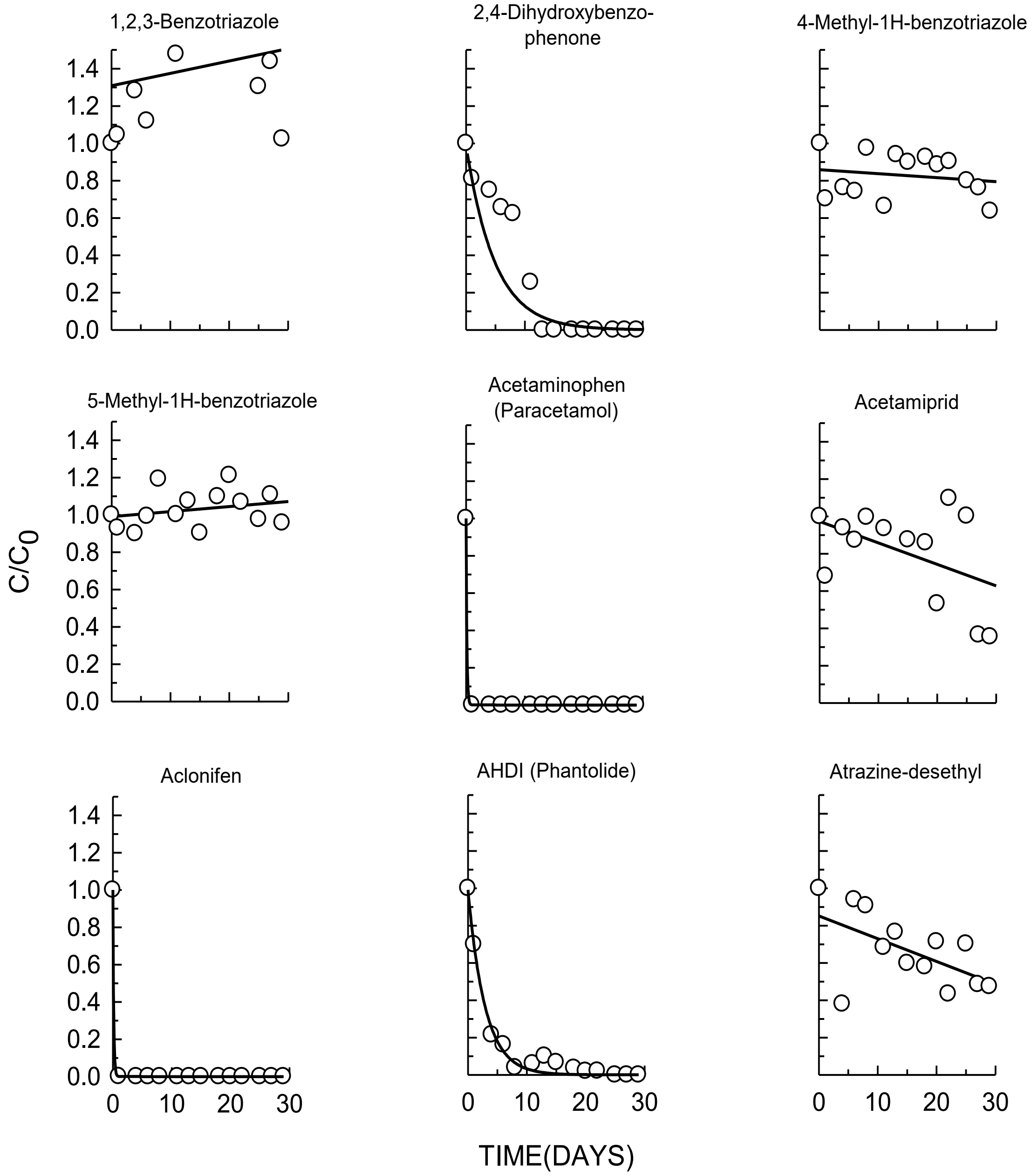
Chemical Name	Q1	Q3	t_R (min)	DP (V)	EP (V)	CE (V)	CXP (V)	LOD	LOQ	R^2
3-CA	128.0	92.9 / 74.9	2.83	51	10	25 / 43	8 / 6	0.0229	0.0762	0.998
4-CA	128.0	93.0 / 74.8	2.83	56	10	25 / 43	8 / 6	0.0152	0.0508	0.997
4-MBTA	134.0	76.9 / 78.9	3.09	46	10	37 / 27	8 / 6	0.0145	0.0482	0.999
5,6-DMBTA	148.1	76.9 / 92.9	3.34	81	10	39/ 27	6 / 8	0.0055	0.0182	0.997
5-MBTA	134.0	77.0 / 79.0	3.08	56	10	33 / 27	6 / 6	0.0224	0.0747	0.997
ACF	265.0	248.0 / 182.1	6.16	61	10	25 / 39	8 / 12	0.0569	0.1896	0.998
ACMP	223.1	126.0 / 99.0	2.99	76	10	31 / 55	11 / 9	0.0252	0.0841	0.994
ADBI	245.2	189.1 / 131.0	7.39	66	10	19 / 29	8 / 6	0.0114	0.0380	0.998
AHDI	245.2	175.1 / 119.0	7.33	71	10	25 / 47	8 / 6	0.0668	0.2227	0.999
APAP	152.0	110.0 / 65.0	2.65	56	10	23 / 41	8 / 6	0.0455	0.1516	0.998
AZM	749.5	591.3 / 82.8	2.80	86	10	43 / 89	6 / 6	0.0106	0.0352	0.997
BaP	253.1	252.1 / 250.1	7.97	80.0	10	50 / 80	16 / 10	0.0041	0.0138	0.995
BDMA	136.1	91.0 / 65.0	0.51	41	10	25 / 47	6 / 6	0.0102	0.0341	0.999
BMA	122.2	90.8 / 64.9	0.51	36	10	23 / 41	8 / 16	0.0371	0.1237	0.988
BP-1	215.0	136.9 / 80.9	4.56	61	10	27 / 51	8 / 6	0.0039	0.0129	0.999
BP-3	229.1	151.0 / 104.8	6.01	61	10	27 / 25	8 / 12	0.0059	0.0196	0.997
BTA	120.0	65.0 / 92.0	2.87	61	10	29 / 23	8 / 6	0.0831	0.2769	0.998
BTMA	150.1	91.0 / 64.9	0.50	66	10	31 / 51	6 / 6	0.0067	0.0224	1.000
BZA	230.8	202.0 / 150.0	6.43	40.0	10	45 / 89	14 / 5.	0.0377	0.1257	0.996
C_{12}BDMA	304.3	90.9 / 212.2	5.75	11	10	53 / 29	8 / 4	0.0059	0.0198	0.999
C_{14}BDMA	332.3	90.7 / 240.1	6.39	96	10	47 / 31	6 / 10	0.0032	0.0107	0.996

Table C1. Continue

Chemical Name	Q1	Q3	t_R (min)	DP (V)	EP (V)	CE (V)	CXP (V)	LOD	LOQ	R^2
CYA	128.0	42.0 / 84.7	0.70	-50	-10	-28 / -14	-5 / -9	0.3397	1.0295	0.999
CAM	748.4	158.0 / 82.8	3.75	46	10	37 / 91	10 / 8	0.0037	0.0124	0.999
CF	332.2	314.0 / 230. 9	2.72	76	10	29 / 51	12 / 10	0.3077	1.0258	0.995
DCH	182.2	83.0 / 100.0	2.78	61	10	29 / 27	6 / 6	0.0129	0.0431	0.998
DCMU	233.0	71.9 / 159.9	4.22	61	10	41 / 37	6 / 8	0.0218	0.0726	0.999
DEA	188.2 /190.1	146. 0 / 148.0	3.09	56 / 46	10	25 / 25	11 / 13	0.0201	0.0669	0.996
DEET	192.1	118.9 / 90.9	4.10	71	10	25 / 43	6 / 6	0.0042	0.0139	0.993
DEHP	391.3	120.8 / 148.9	8.36	61	10	69 / 35	8 / 8	2.6823	8.9409	0.991
HHCB	257.1	227.1 / 114.9	7.54	91	10	41 / 99	10 / 8	0.0648	0.2161	0.978
HMMM	391.0	177.1 / 283.1	5.23	26	10	39 / 19	6 / 8	0.0036	0.0109	0.989
INT	280.1	183.0 / 152.0	3.82	96	10	61 / 91	10 / 8			
M	127.0	84.9 / 67.9	0.88	56.0	10	25 / 41	8 / 6	0.0038	0.0114	0.9900
MBC	192.0	160.0 /131.9	2.73	71	10	23 / 39	8 / 10	0.0248	0.0828	0.995
MQ	113.9	58.1 / 98.1	0.50	46	10	29 / 29	6 / 10	0.0732	0.2439	0.997
NETSA	200.0	90.9 / 155.0	3.29	46	10	37 / 15	8 / 8	0.0243	0.0812	0.994
NF	320.2	302.0 / 231.0	2.71	71	10	29 / 55	12 / 10	0.4866	1.6220	0.737
NP2EO	326.2	183.1 / 121.0	7.37	61	10	15 / 31	10 / 6	0.0175	0.0582	0.993
OF	362.2	318.0 / 261.0	2.70	46	10	27 / 37	6 / 10	0.0178	0.0593	0.969
OMA	213.9	124.9 / 182.9	2.66	51	10	27 / 13	10 / 14	0.0870	0.2901	0.994
PBO	356.2	177.0 / 118.9	6.99	16	10	17 / 47	8 / 12	0.0042	0.0141	0.997
QNP	299.0	147.0 / 163.0	6.13	61 / 51	10	29 / 29	14 / 15	0.0382	0.1274	0.995
SMX	254.0	91.9 / 107.8	2.85	46	10	35 / 35	6 / 8	0.0340	0.1133	0.999
TBEP	399.2	299.1 / 198.8	6.89	26	10	19 / 23	4 / 8	0.0256	0.0853	0.999
TBN	242.0	186.0 / 68.0	4.09	61	10	27 / 63	8 / 6	0.0038	0.0127	0.999

APPENDIX D: BIODEGRADATION GRAPHICS OF AUGUST 2017 SAMPLES

SP_4



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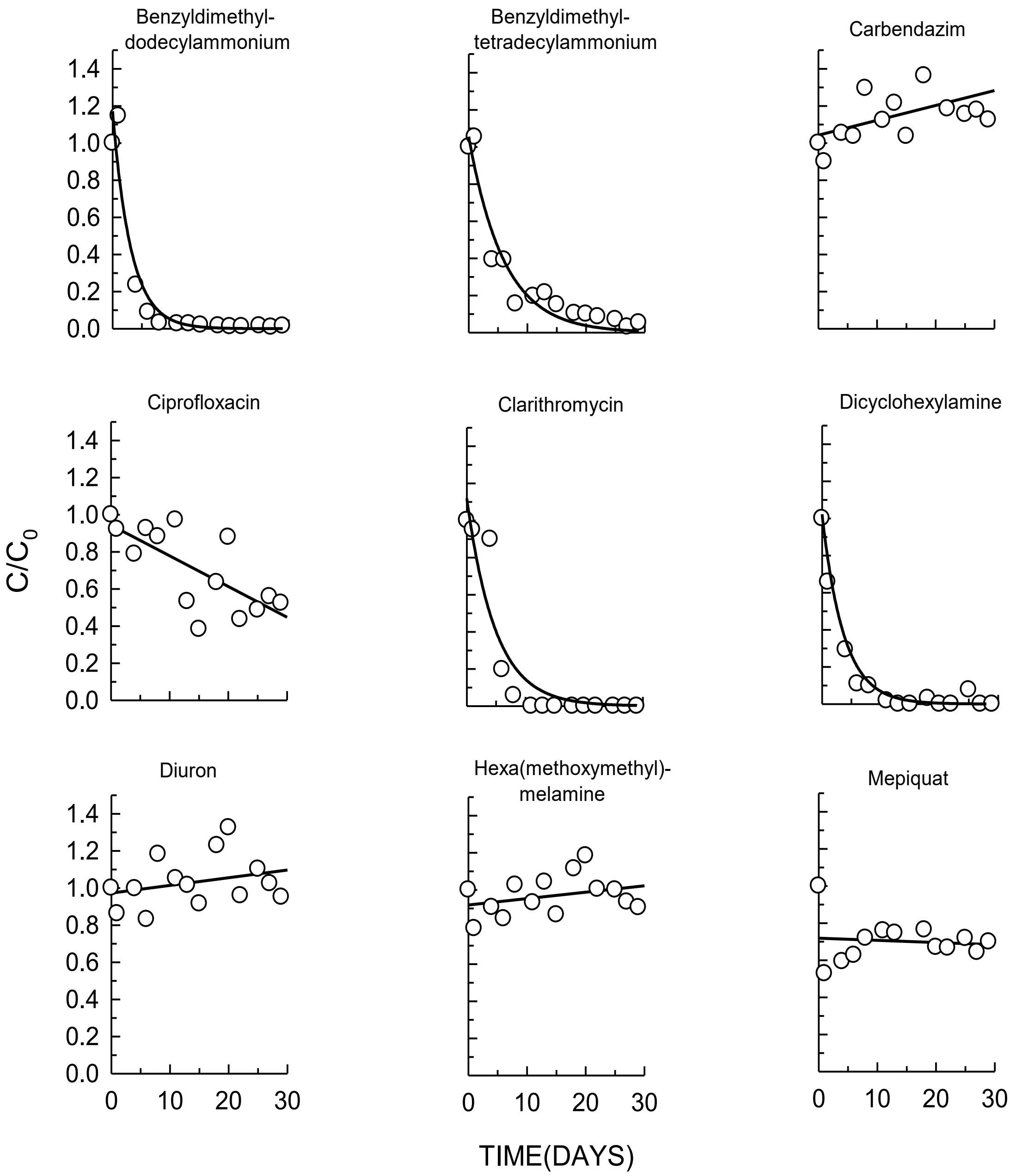

Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Carbendazim
Ciprofloxacin
Clarithromycin
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
Mepiquat
C/C0
TIME(DAYS)
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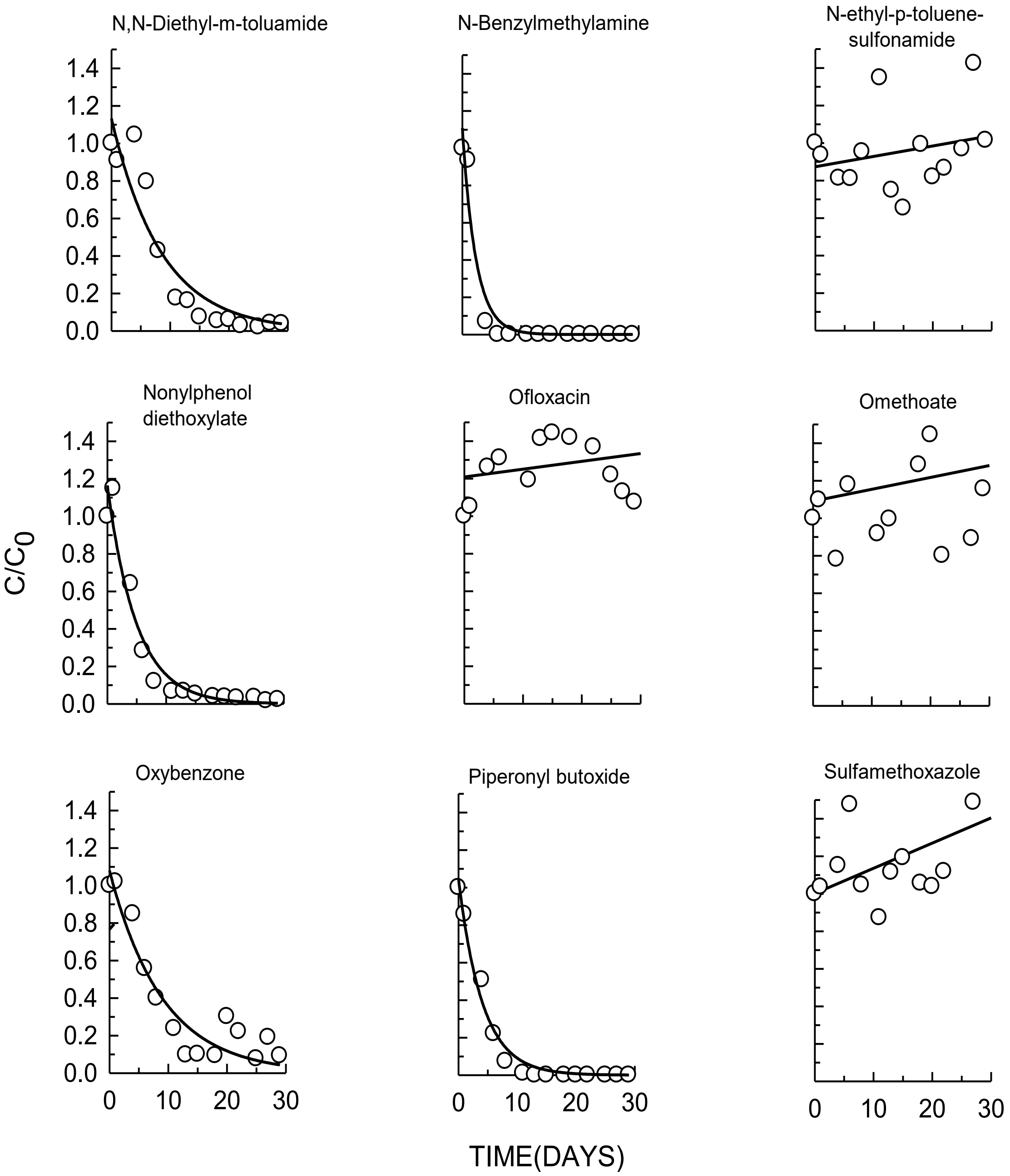
0 10 20 30

SP_4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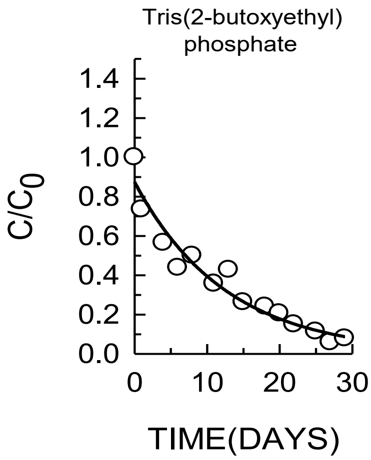
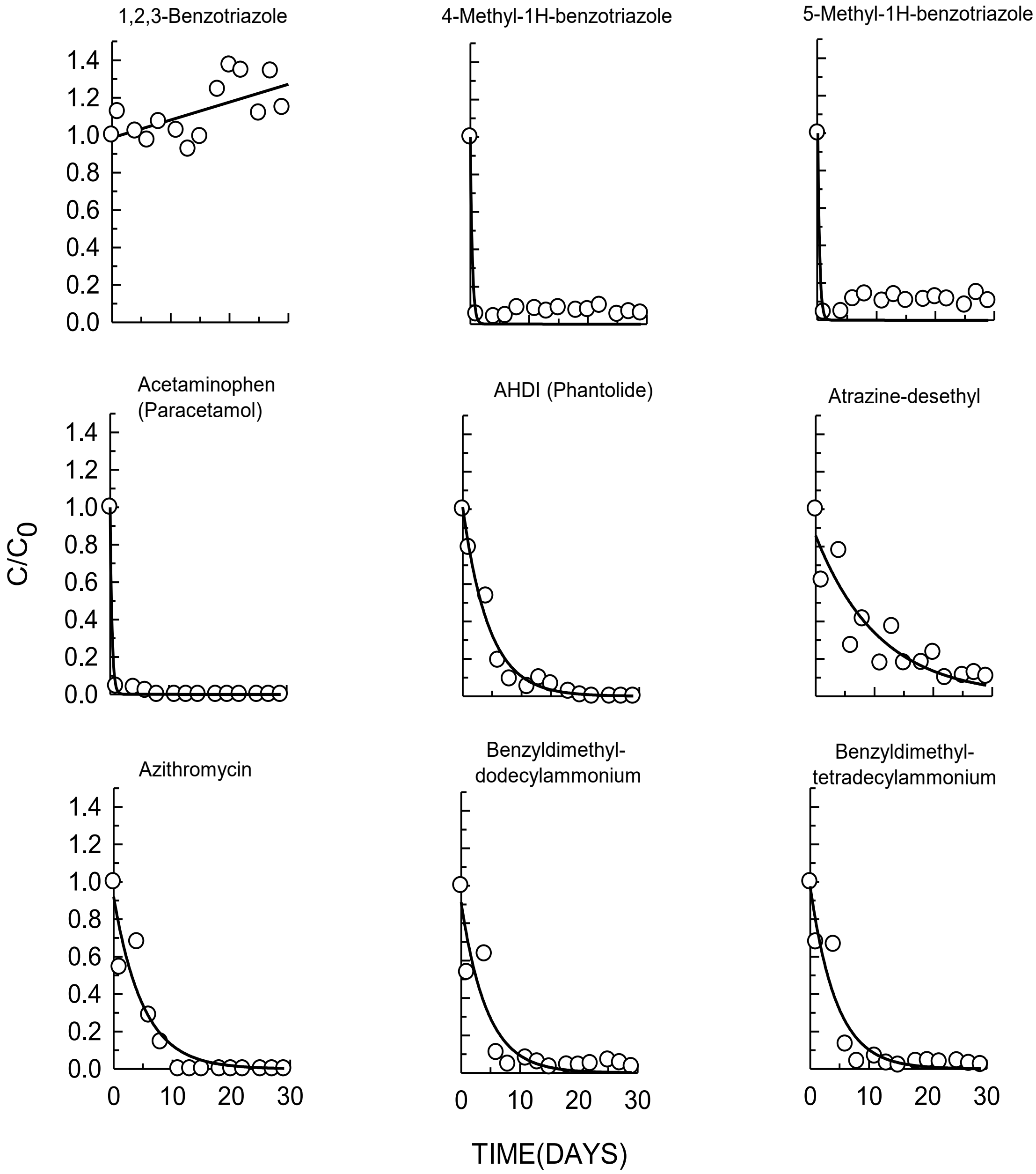
N-Benzylmethylamine
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Ofloxacin
Omethoate
Oxybenzone
Piperonyl butoxide
Sulfamethoxazole
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_4


Tris(2-butoxyethyl)
phosphate
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C/C0
0
10
20
30
TIME(DAYS)

SP_8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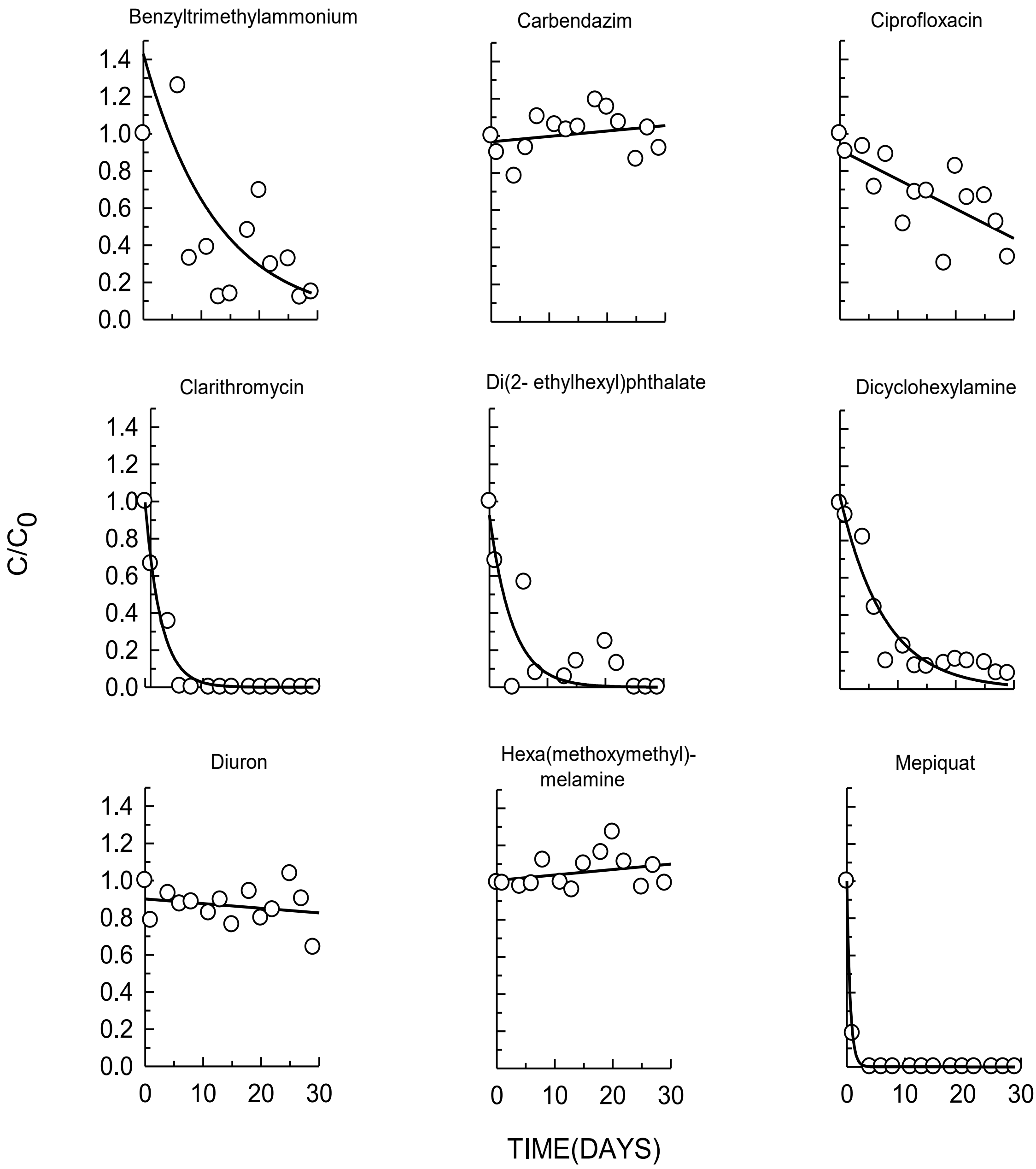
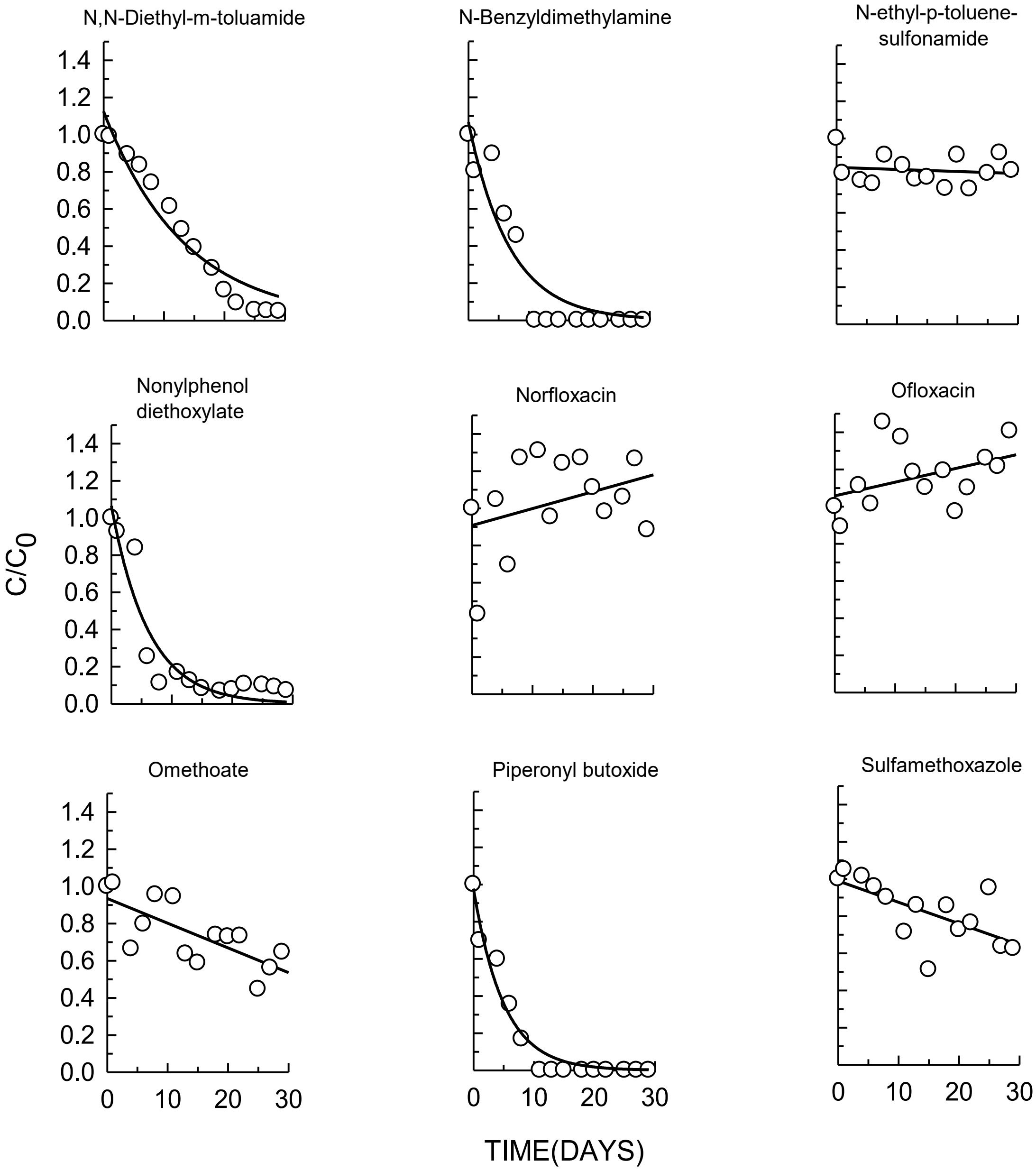
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
Acetaminophen (Paracetamol)
AHDI (Phantolide)
Atrazine-desethyl
Azithromycin
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_8



Benzyltrimethylammonium
Carbendazim
Ciprofloxacin
Clarithromycin
Di(2- ethylhexyl)phthalate
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
Mepiquat
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_8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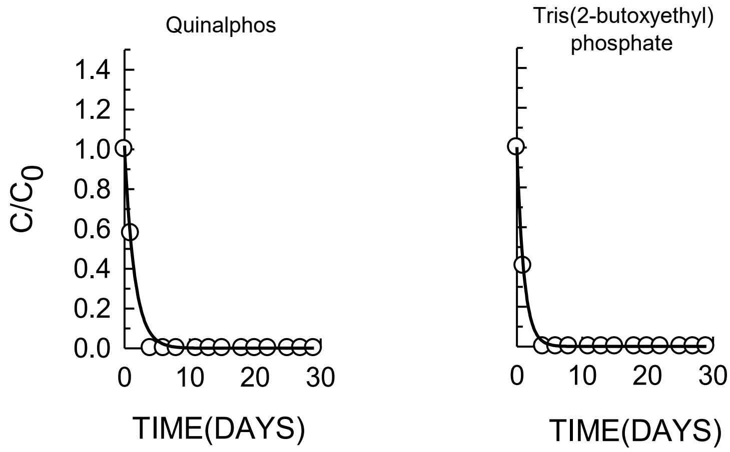
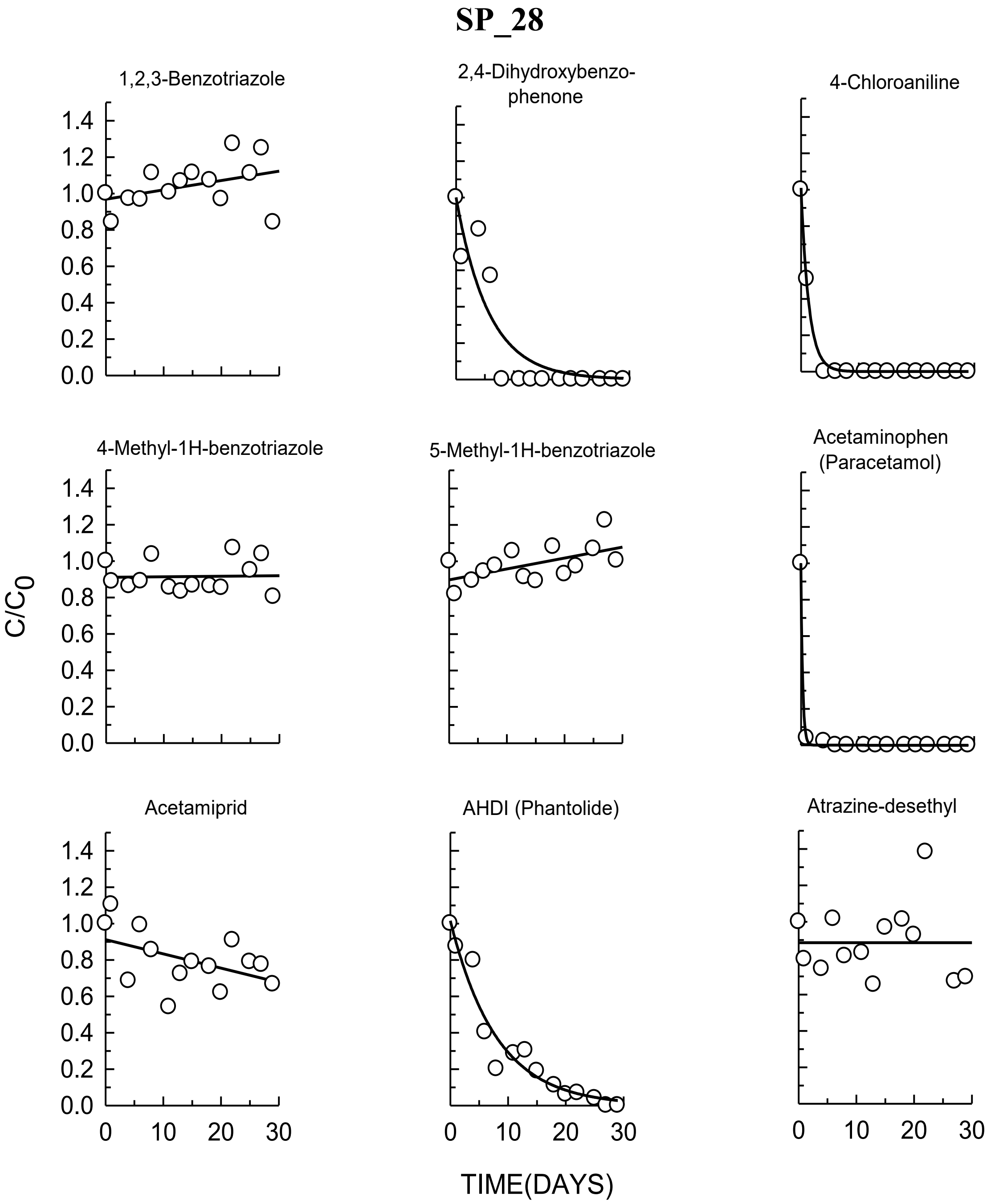
N-Benzyldimethylamine
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Omethoate
Piperonyl butoxide
Sulfamethoxazole
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_8


Quinalphos
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
TIME(DAYS)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2,4-Dihydroxybenzo-
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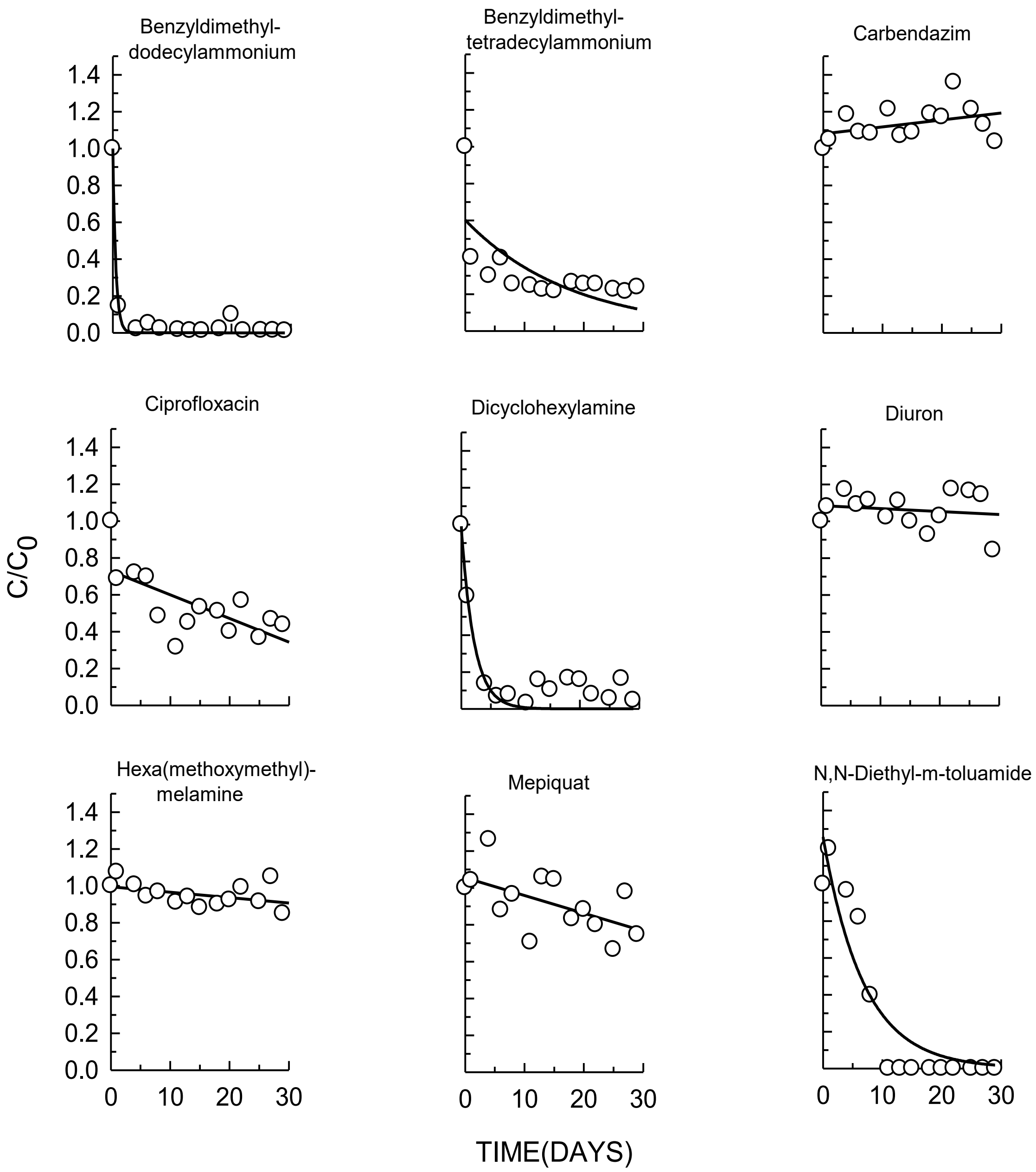
4-Chloroaniline
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
Acetaminophen
(Paracetamol)
Acetamiprid
AHDI (Phantolide)
Atrazine-desethyl
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_28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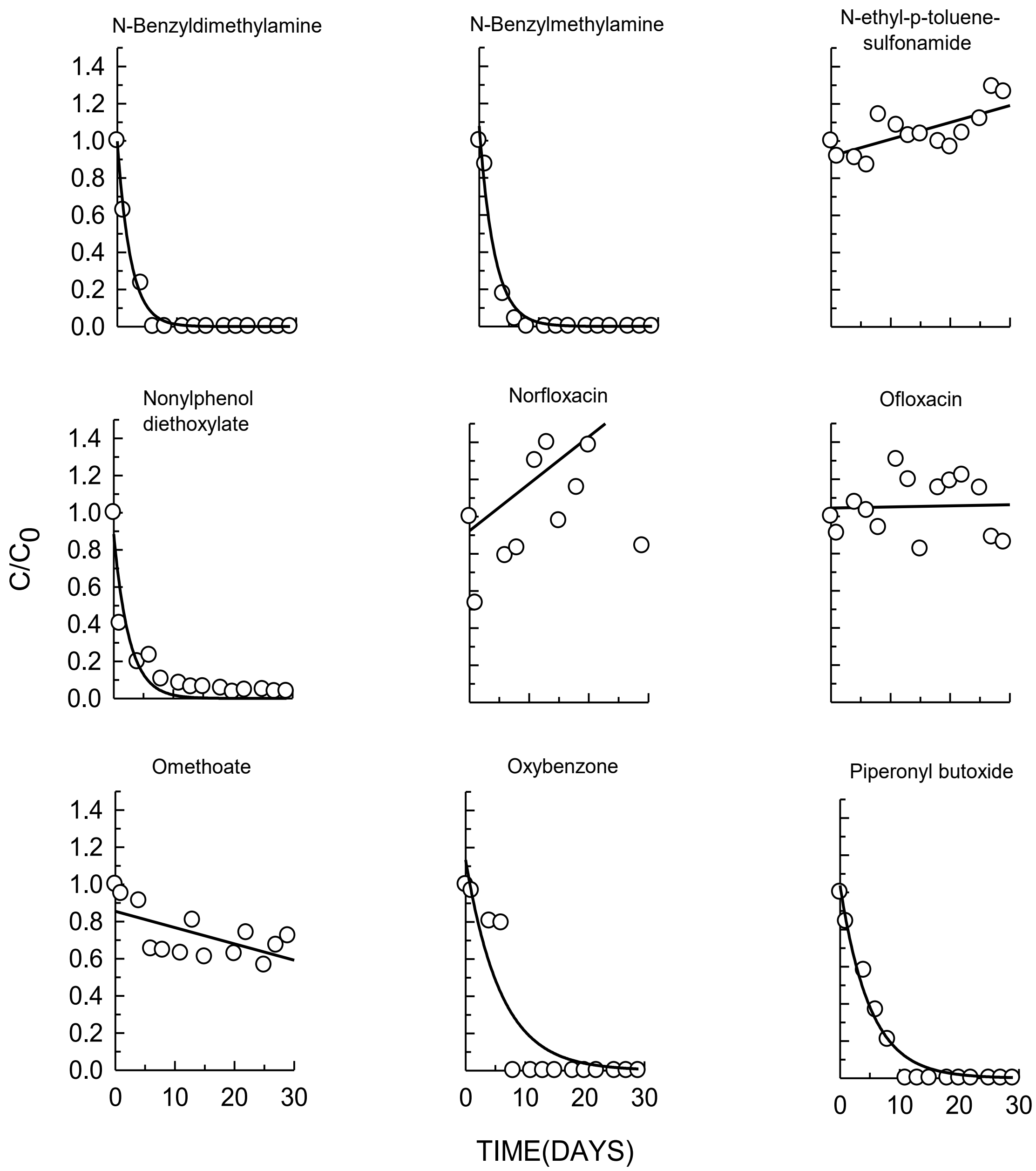
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Carbendazim
Ciprofloxacin
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
Mepiquat
N,N-Diethyl-m-toluamide
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_28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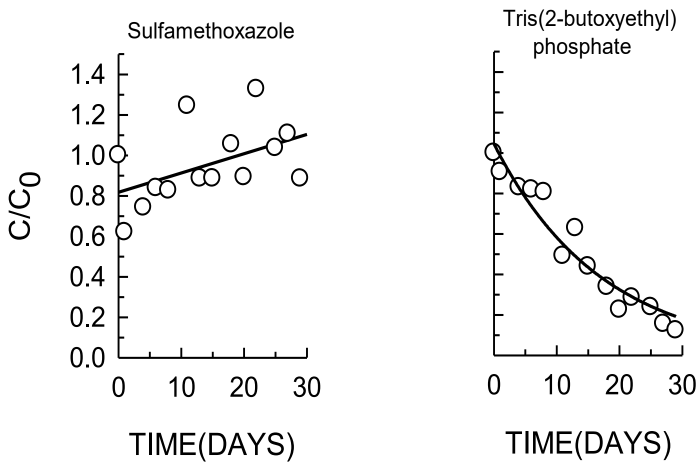
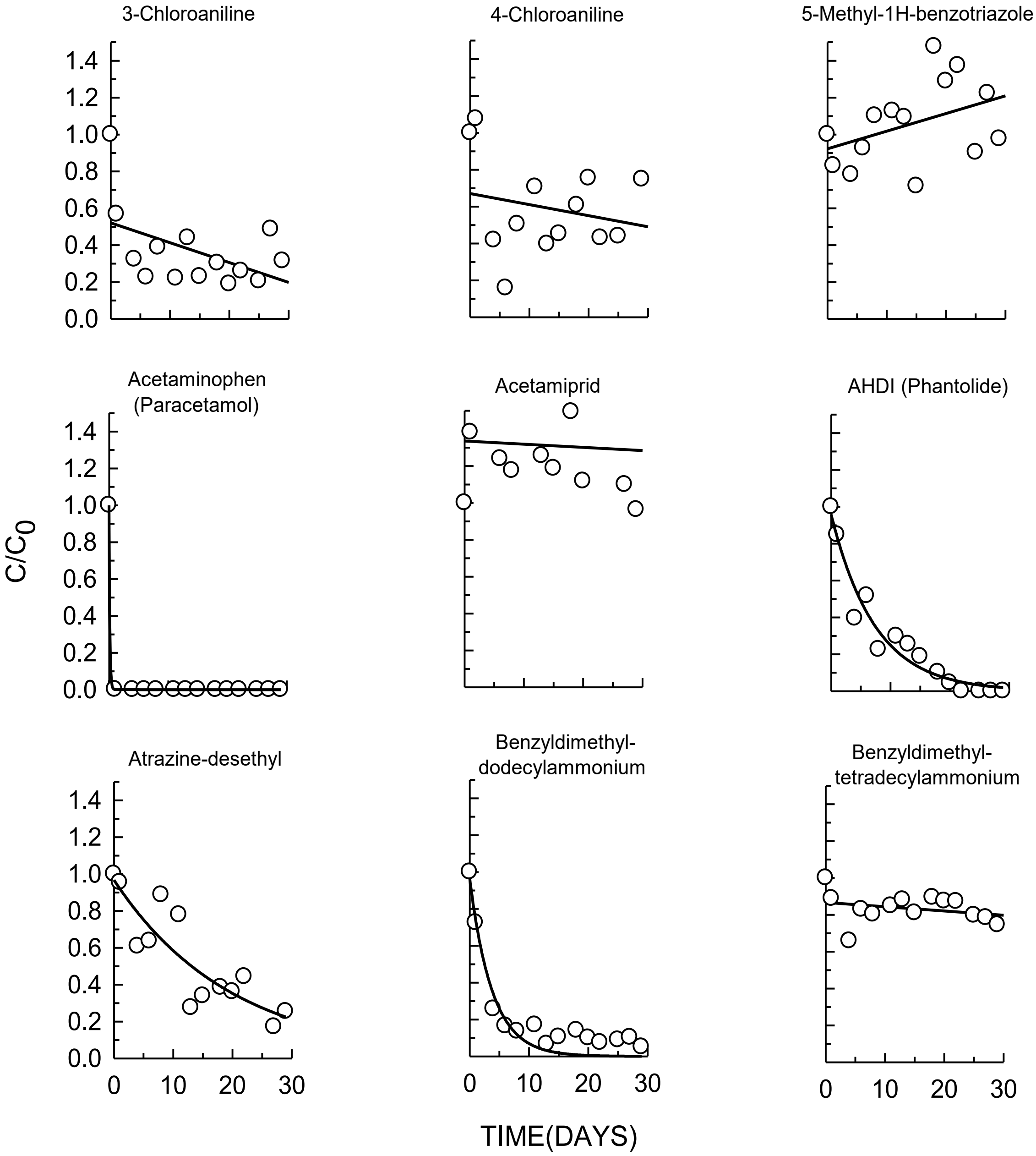
N-Benzylmethylamine
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Omethoate
Oxybenzone
Piperonyl butoxide
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

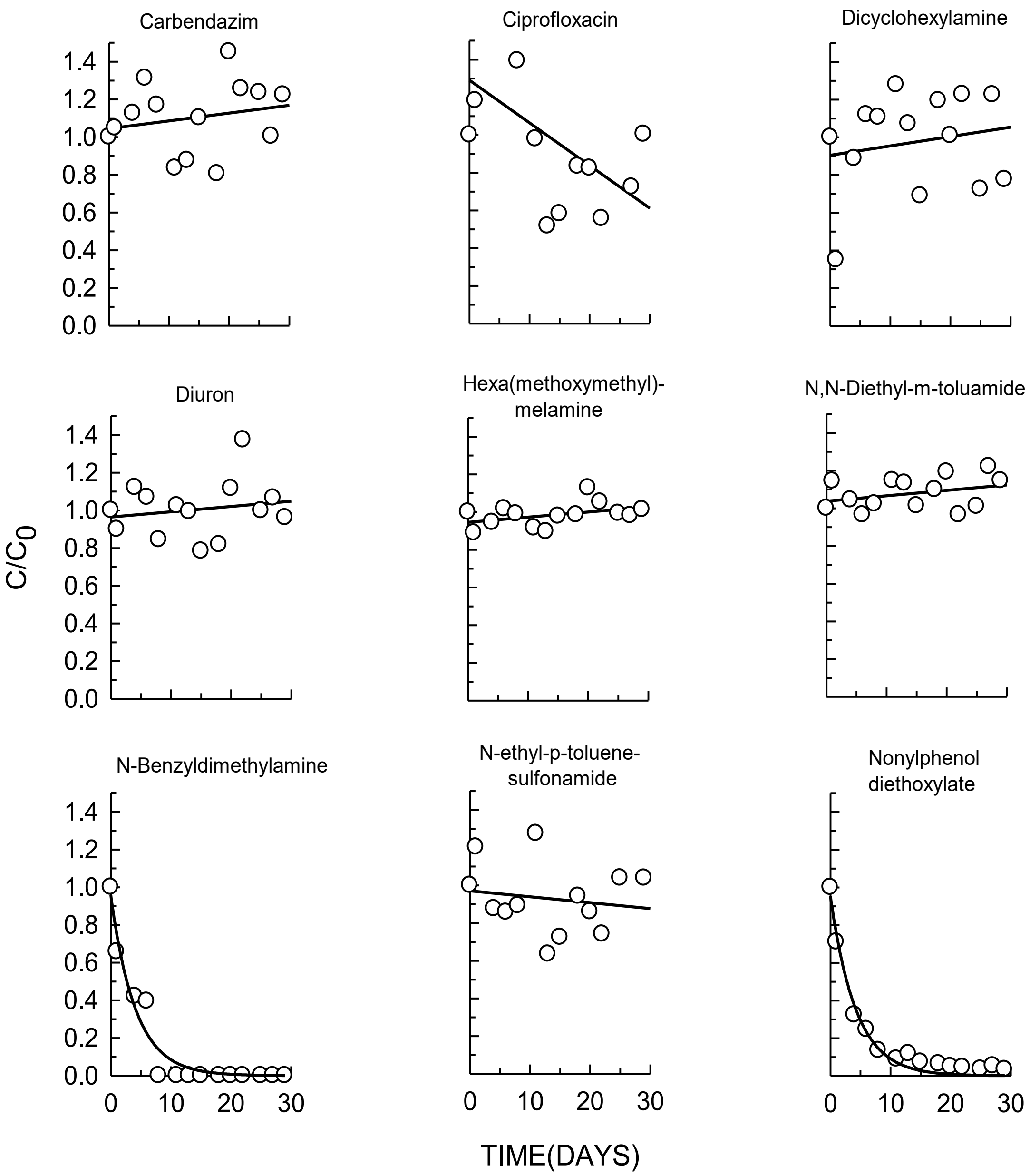
SP_28


Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
TIME(DAYS)

SP_48

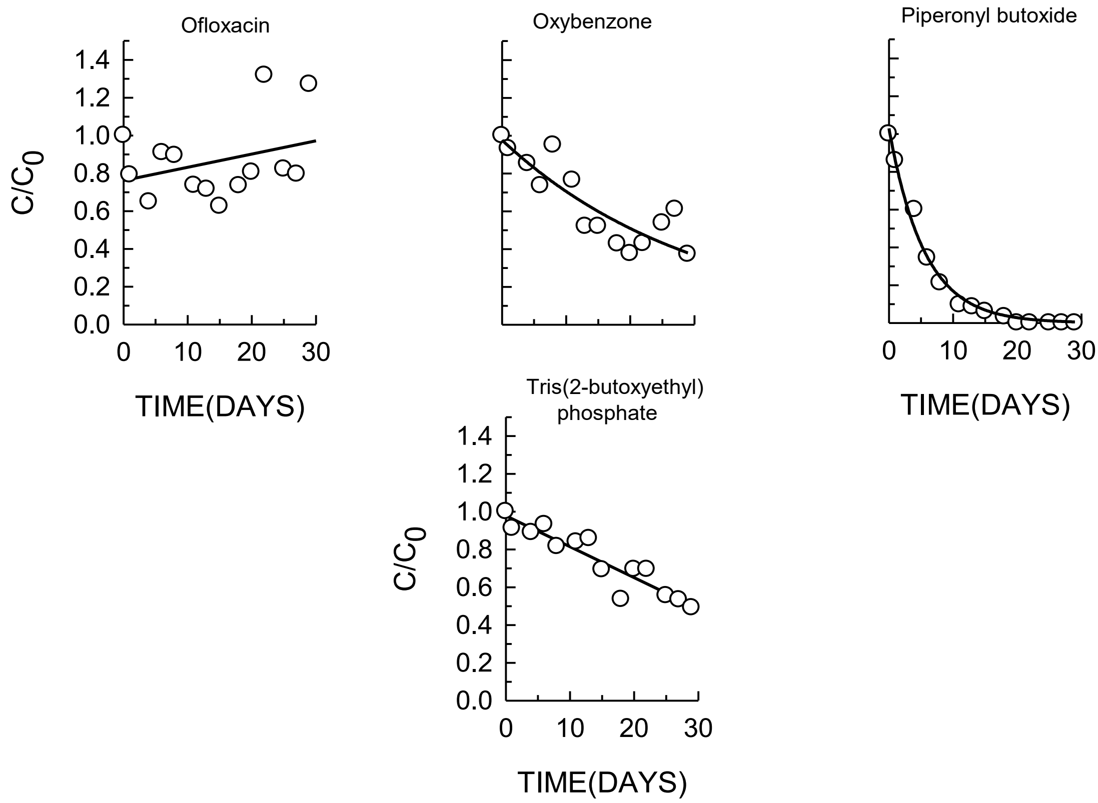

3-Chloroaniline
4-Chloroaniline
5-Methyl-1H-benzotriazole
Acetaminophen (Paracetamol)
Acetamiprid
AHDI (Phantolide)
Atrazine-desethyl
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_48



Carbendazim
Ciprofloxacin
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_48


Ofloxacin
Oxybenzone
Piperonyl butoxide
Tris(2-butoxyethyl) phosphate
C/C0
TIME(DAYS)
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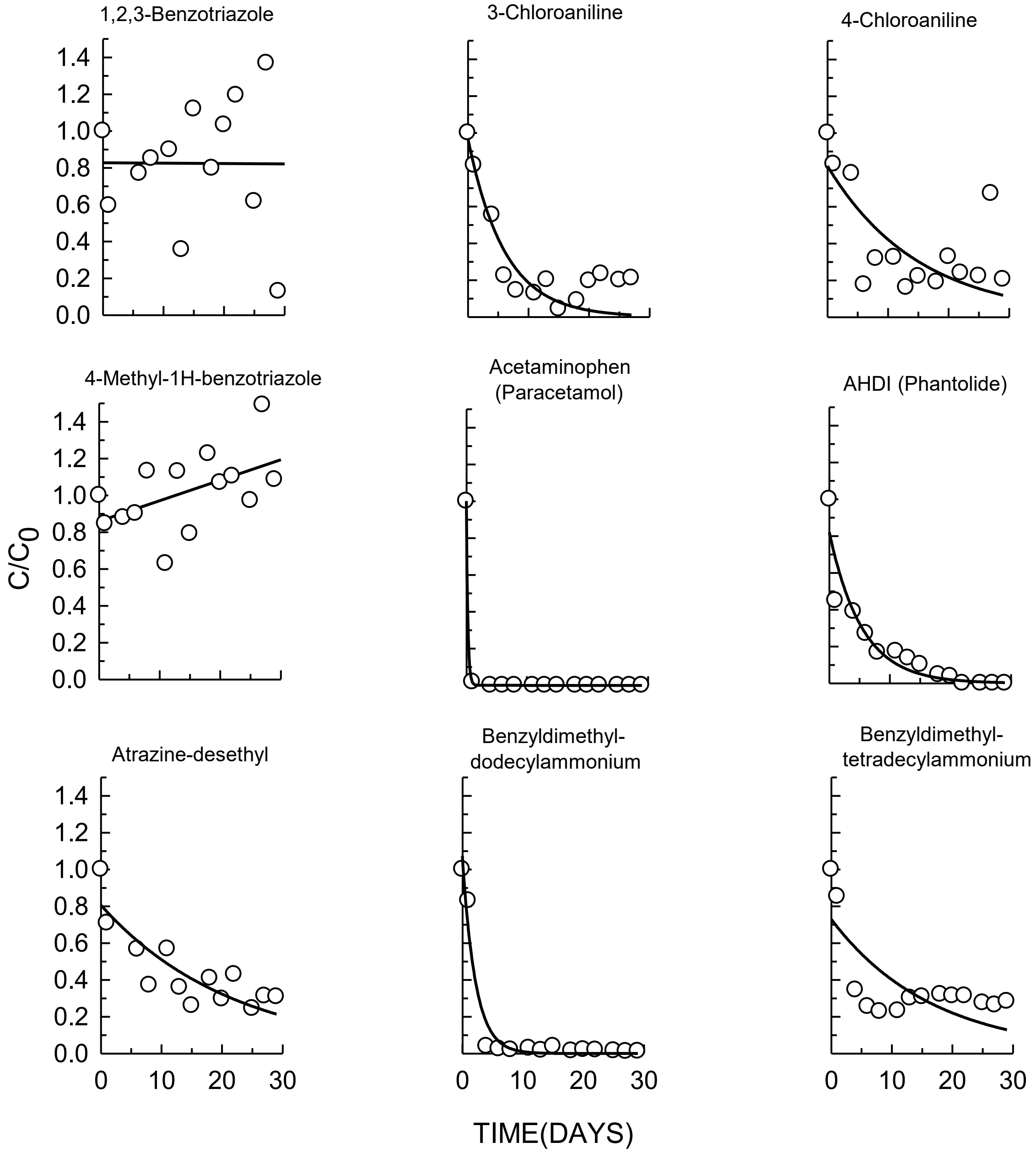
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30

SP_50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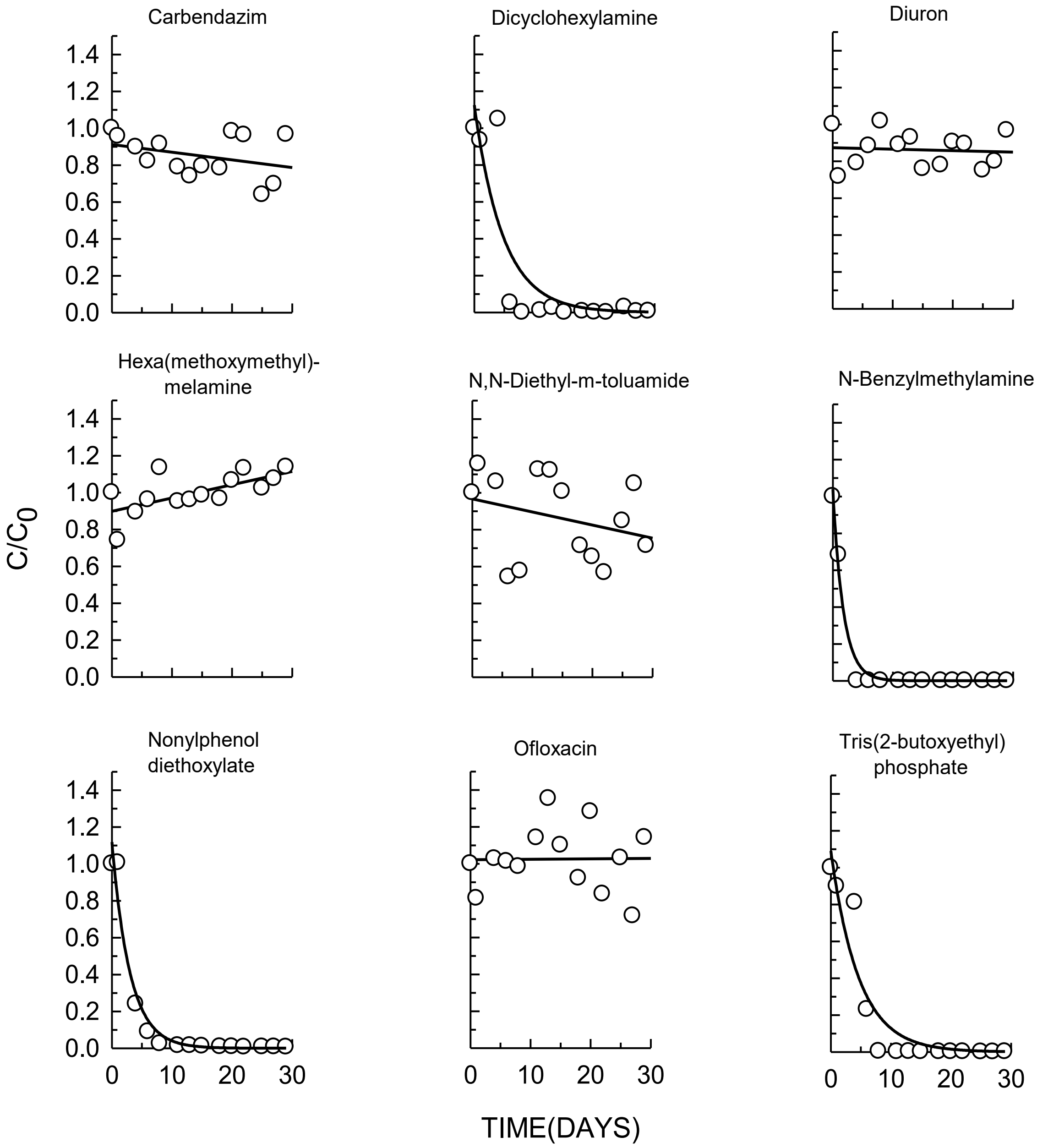
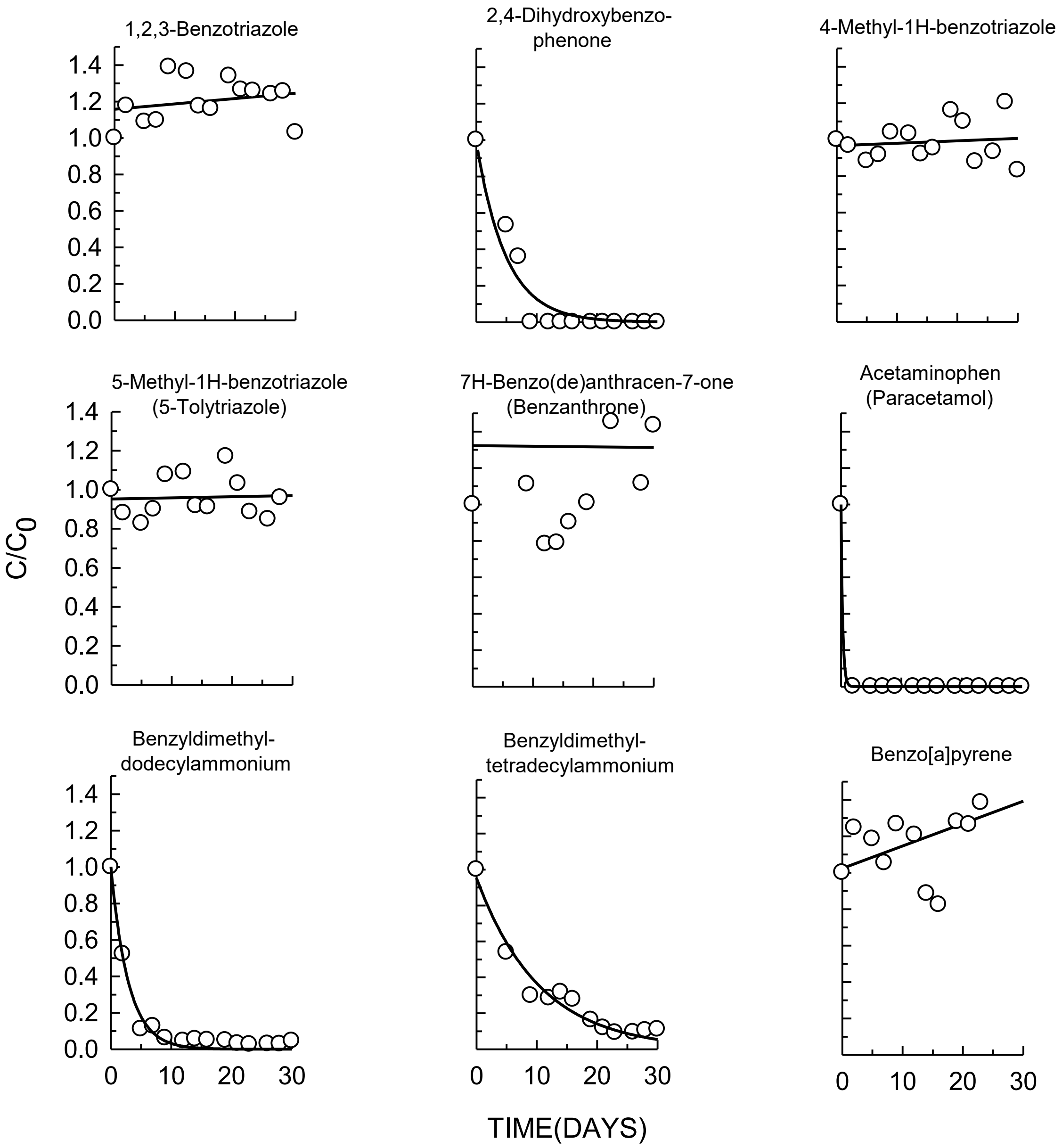
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
Acetaminophen
(Paracetamol)
AHDI (Phantolide)
Atrazine-desethyl
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_50

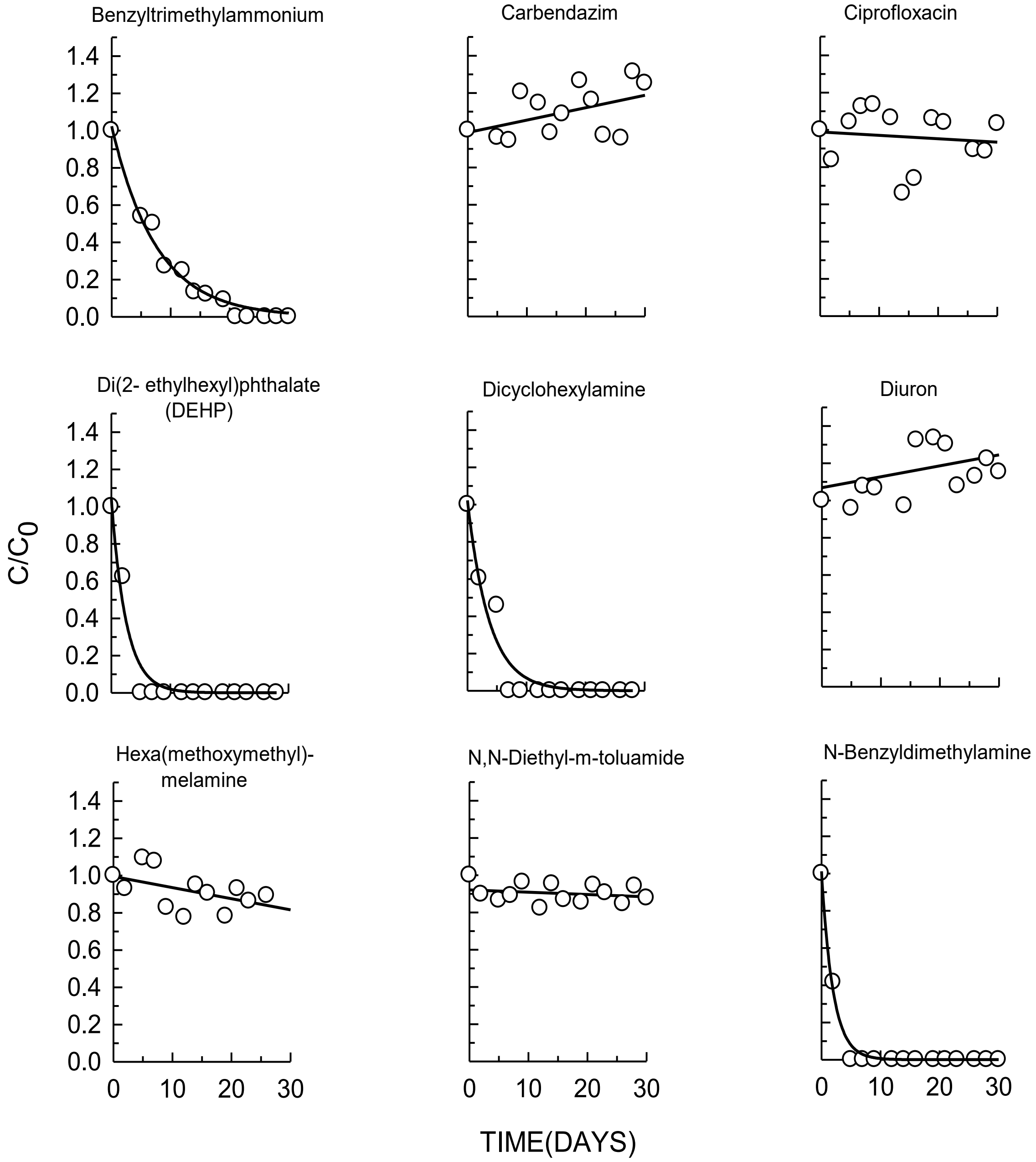

Carbendazim
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
N-Benzylmethylamine
Nonylphenol diethoxylate
Ofloxacin
Tris(2-butoxyethyl) phosphate
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

APPENDIX E: BIODEGRADATION GRAPHICS OF NOVEMBER 2017 SAMPLES

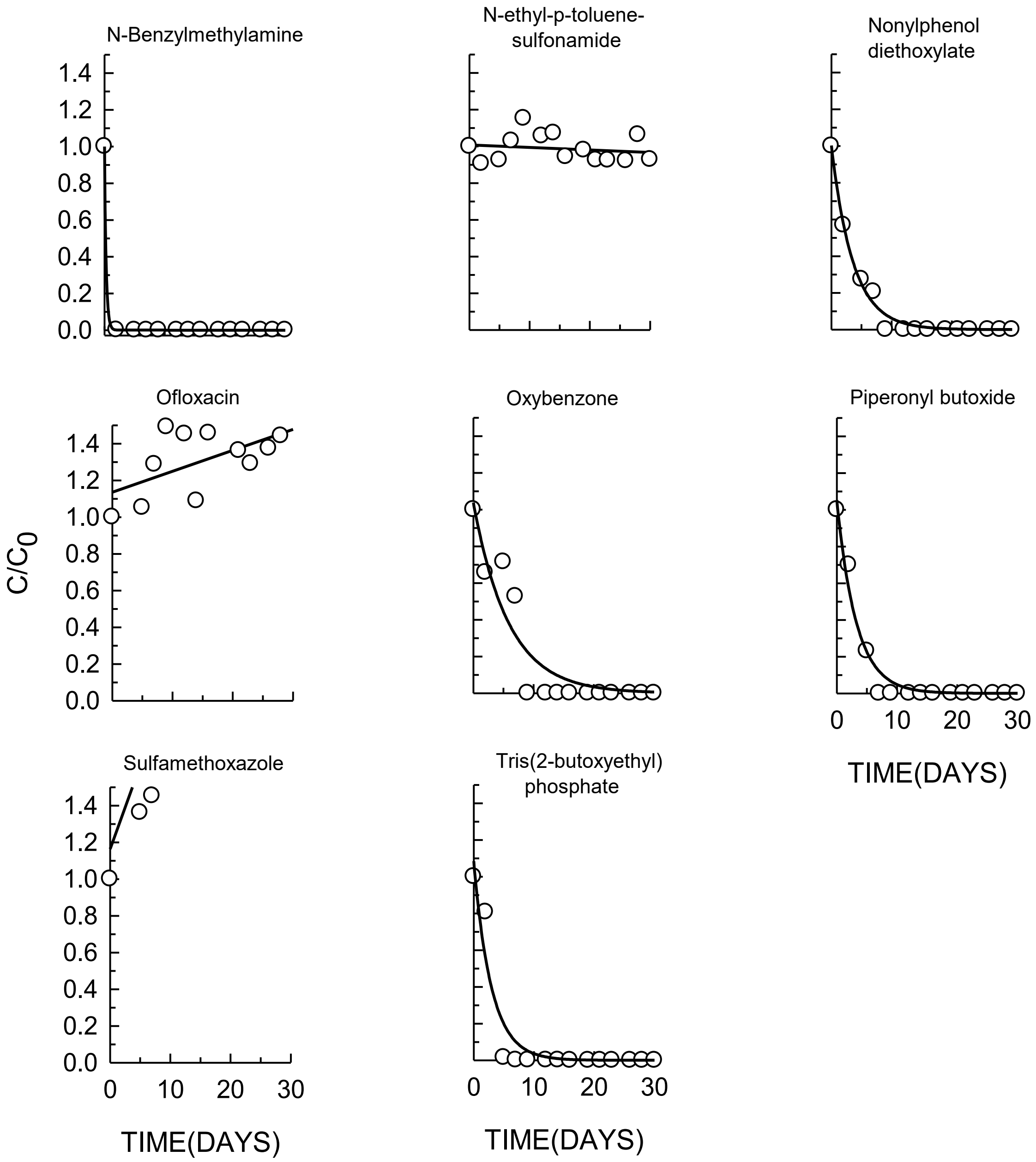
SP_4

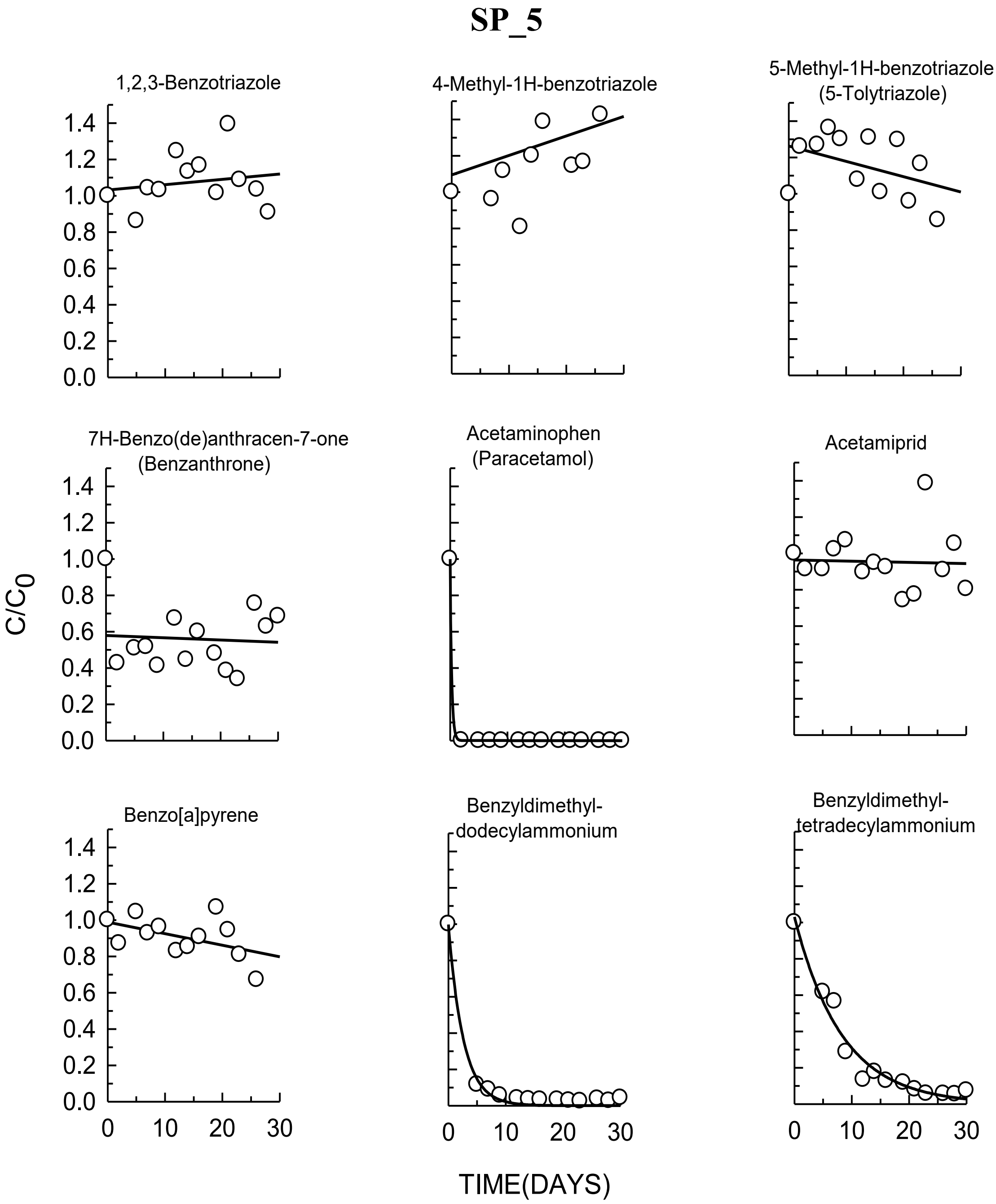


SP_4

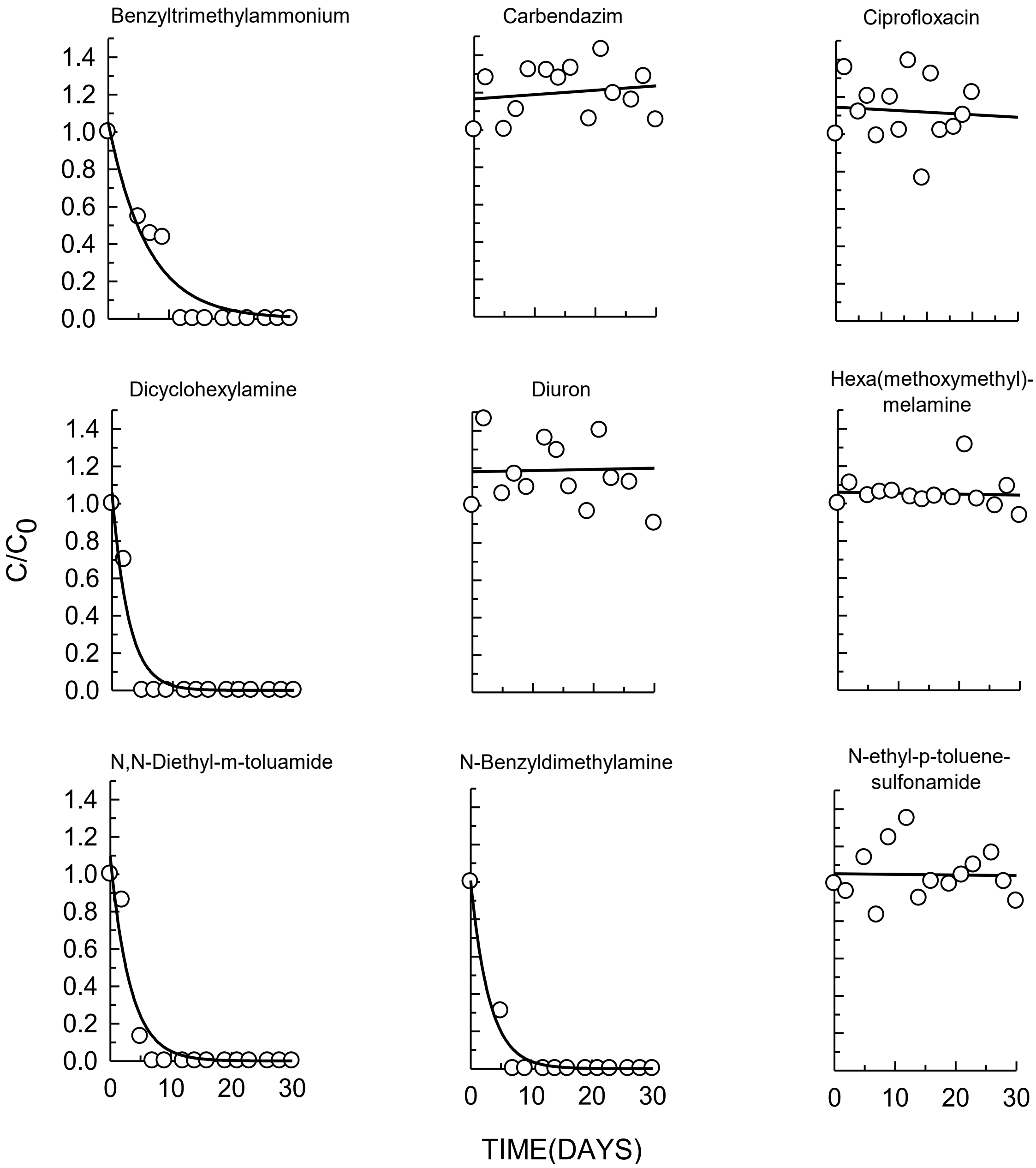

Benzyltrimethylammonium
Carbendazim
Ciprofloxacin
Di(2- ethylhexyl)phthalate (DEHP)
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

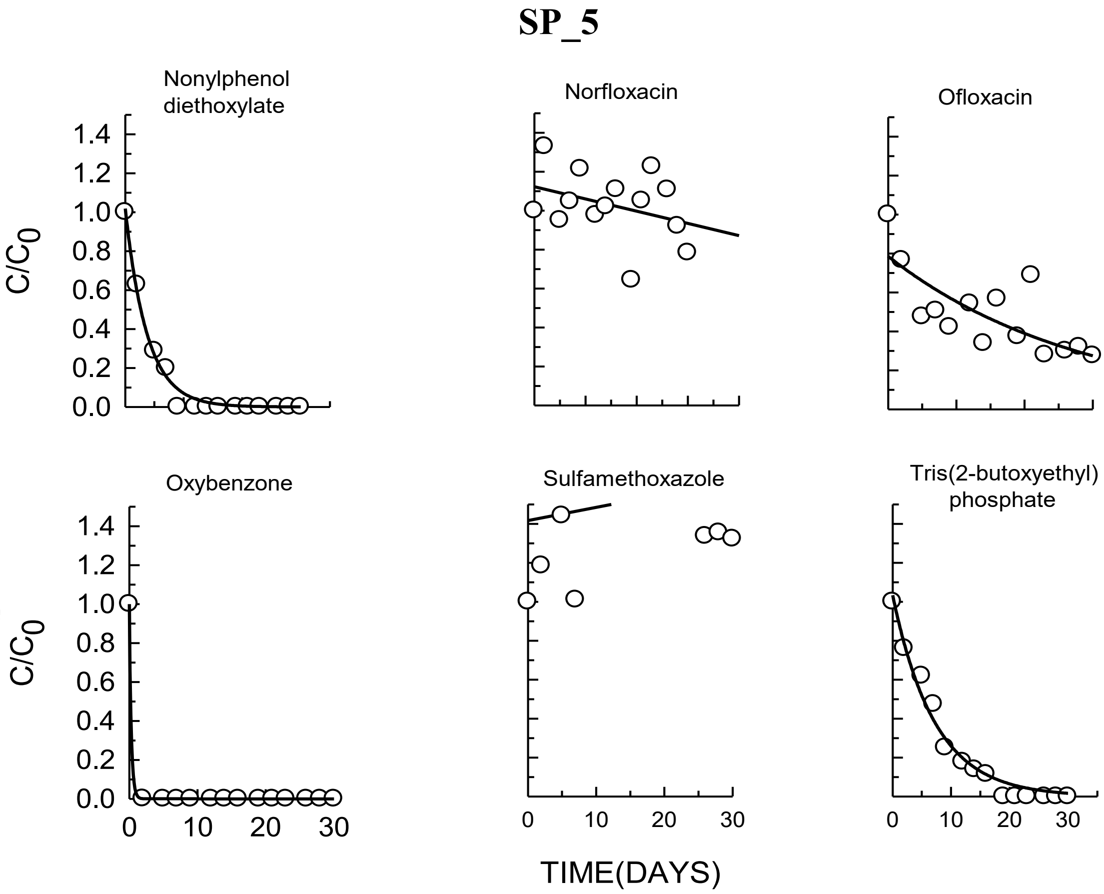
SP_4


N-Benzylmethylamine
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Ofloxacin
Oxybenzone
Piperonyl butoxide
Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

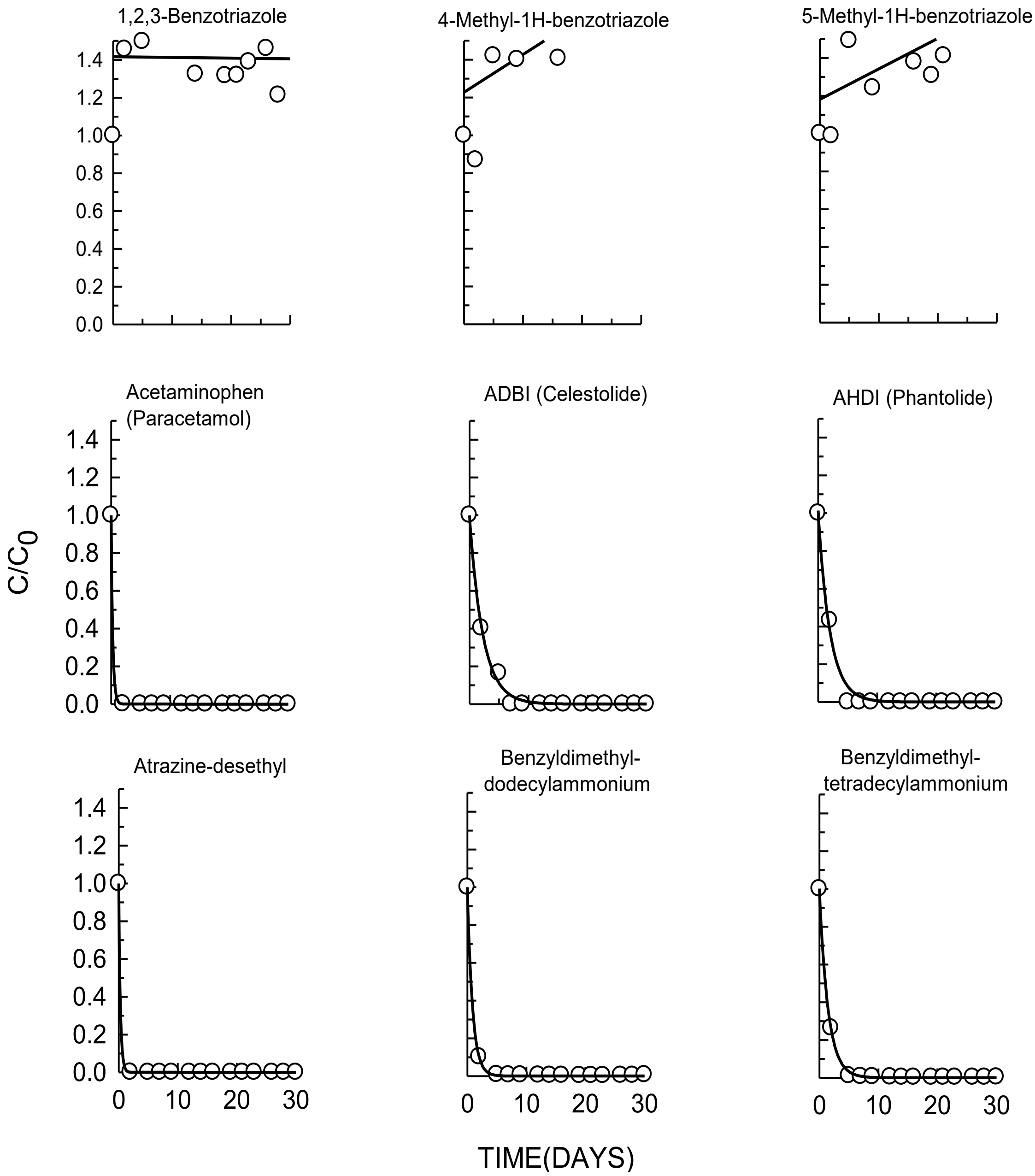

SP_5
1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
7H-Benzo(de)anthracen-7-one (Benzanthrone)
Acetaminophen (Paracetamol)
Acetamiprid
Benzo[a]pyrene
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
C/C0
TIME(DAYS)

SP_5

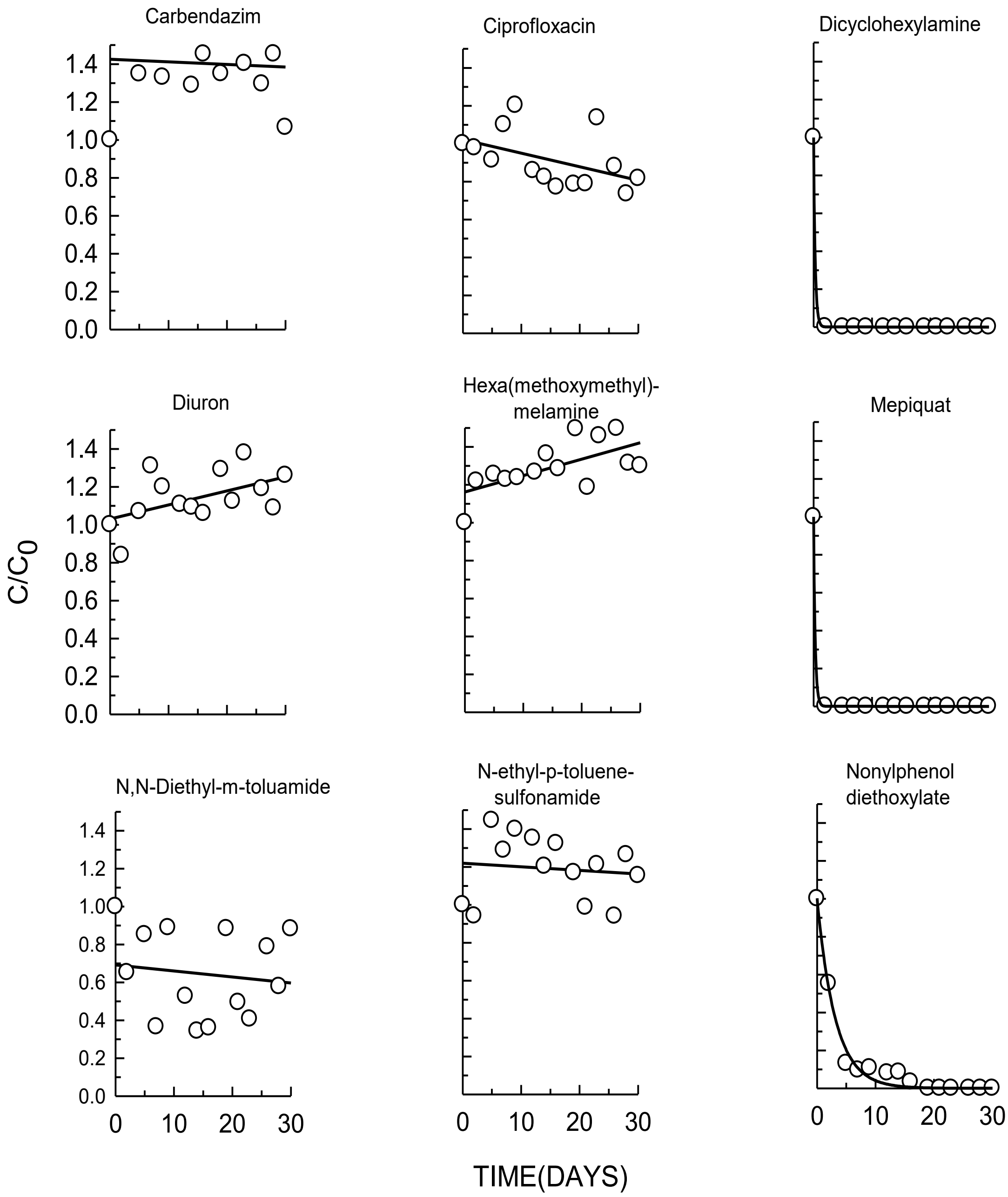

Benzyltrimethylammonium
Carbendazim
Ciprofloxacin
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
N-ethyl-p-toluene-
sulfonamide
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)


SP_5
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Oxybenzone
Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_6

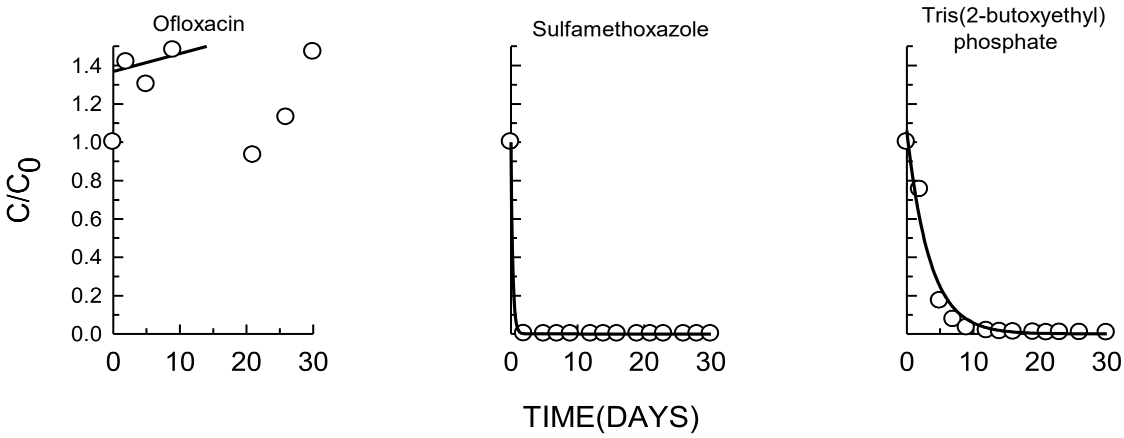

1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
Acetaminophen (Paracetamol)
ADBI (Celestolide)
AHDI (Phantolide)
Atrazine-desethyl
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_6

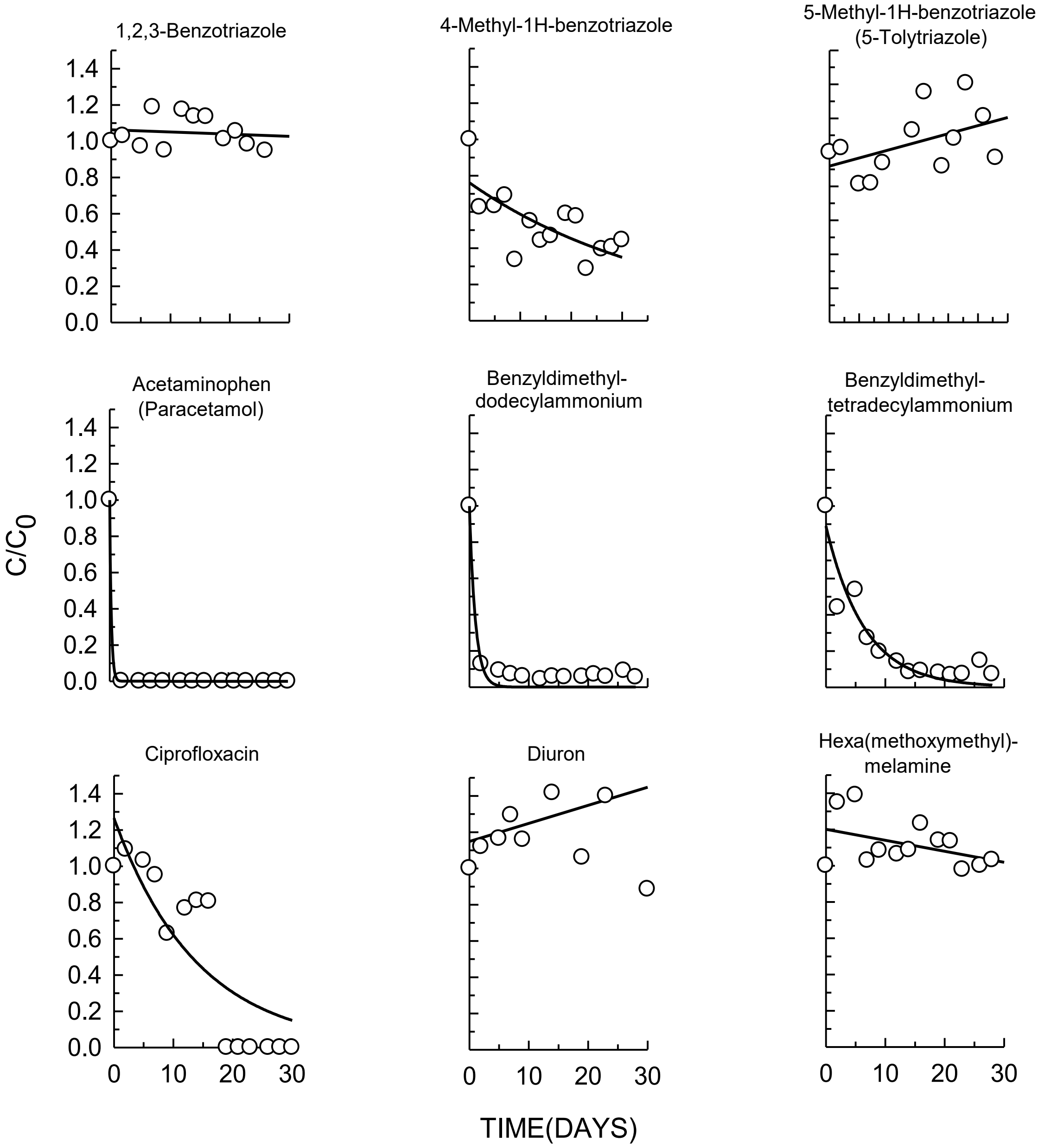


Carbendazim
Ciprofloxacin
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
Mepiquat
N,N-Diethyl-m-toluamide
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

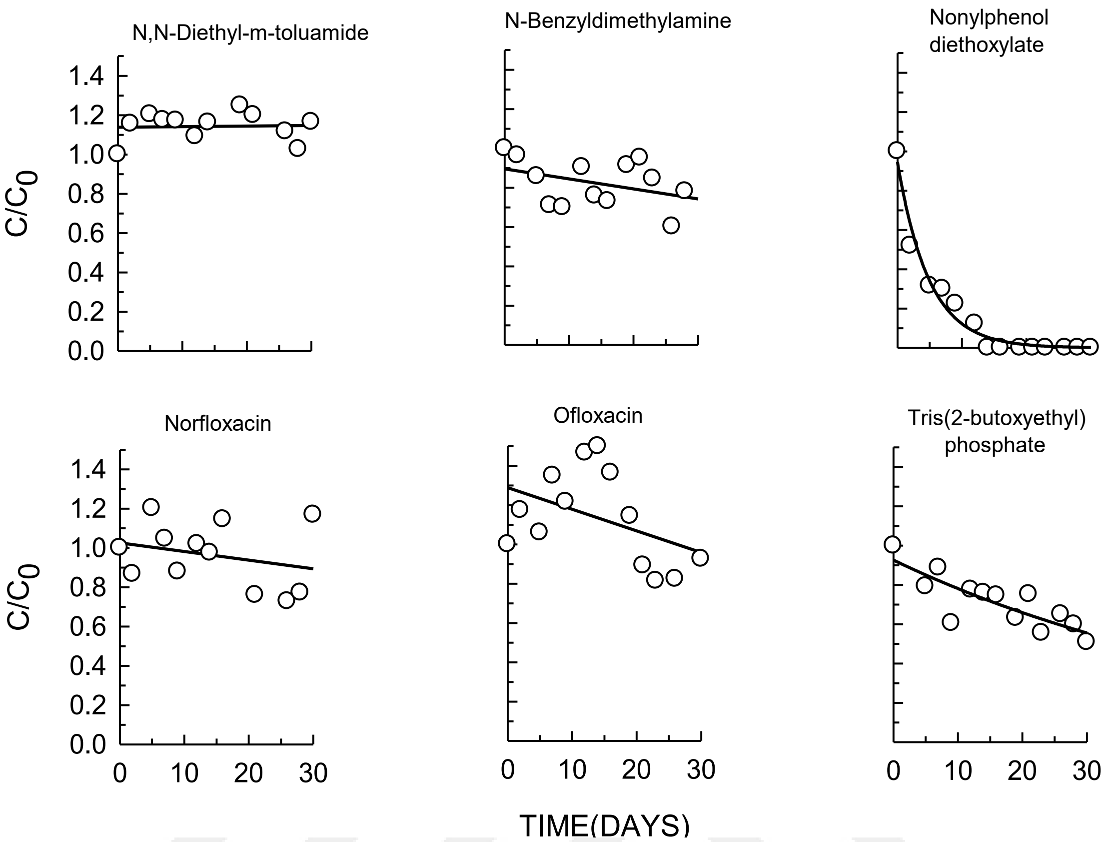
SP_6


Ofloxacin
Sulfamethoxazole
Tris(2-butoxyethyl)
phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

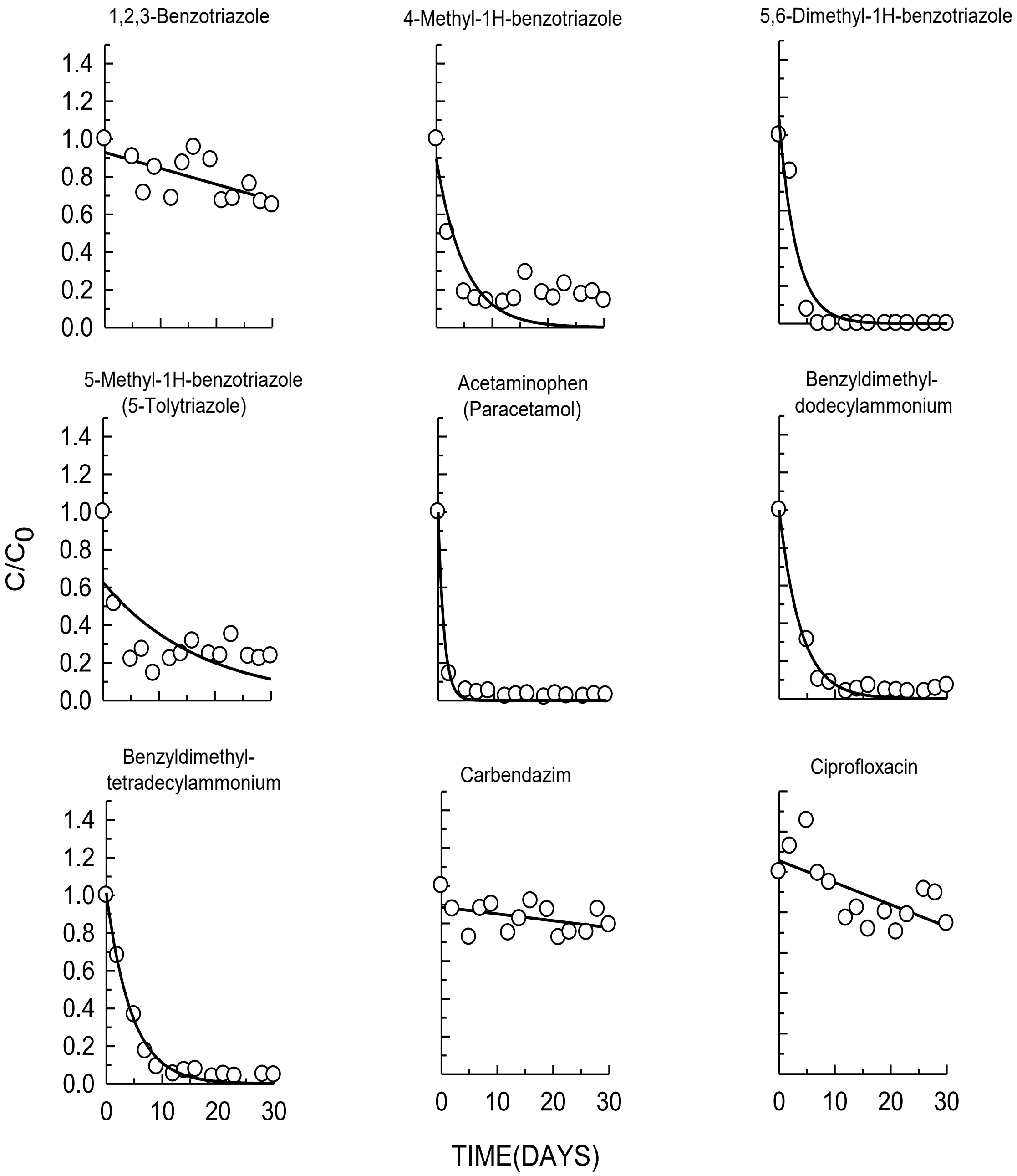
SP_7


1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Ciprofloxacin
Diuron
Hexa(methoxymethyl)-
melamine
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_7

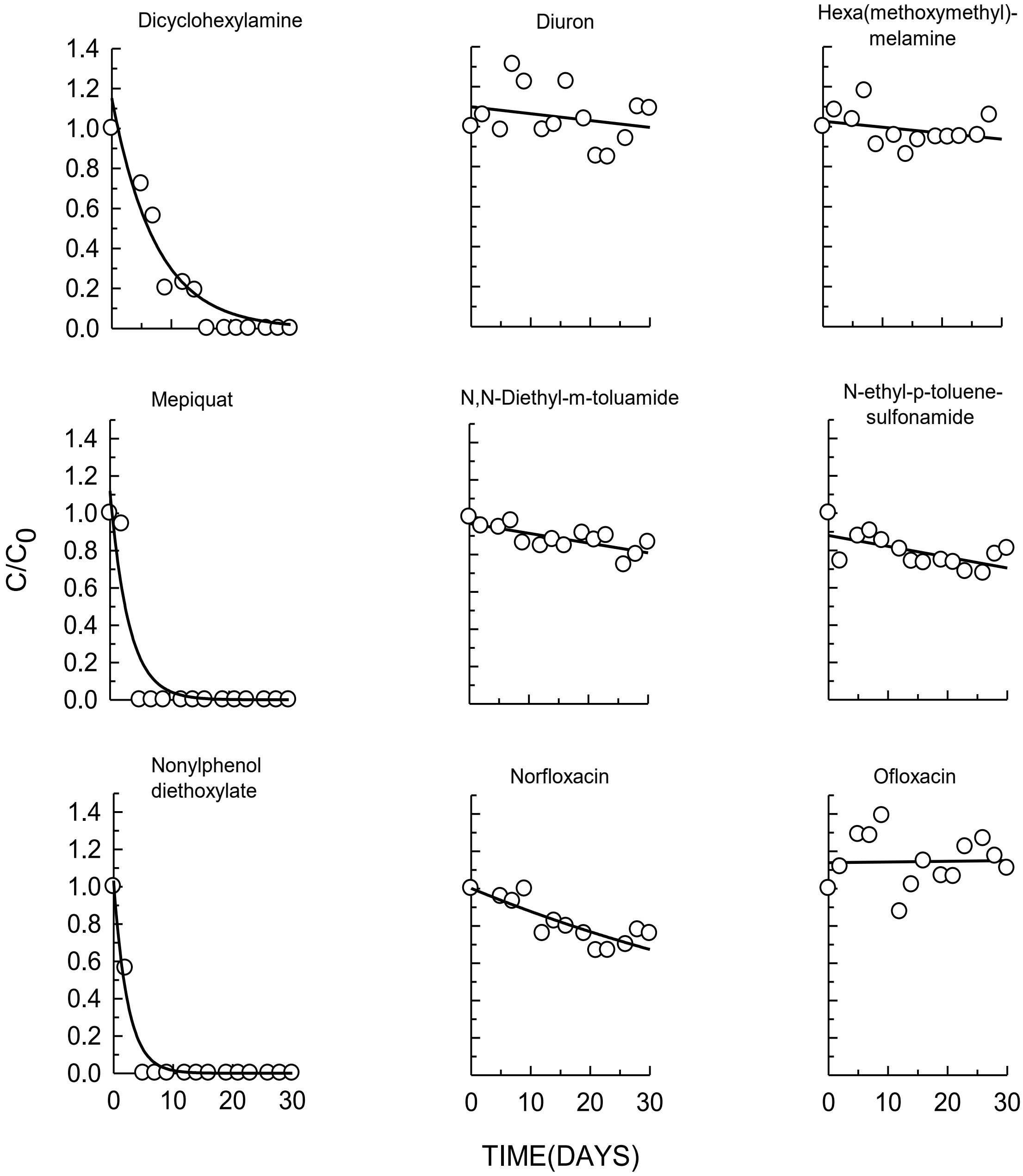

N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_8

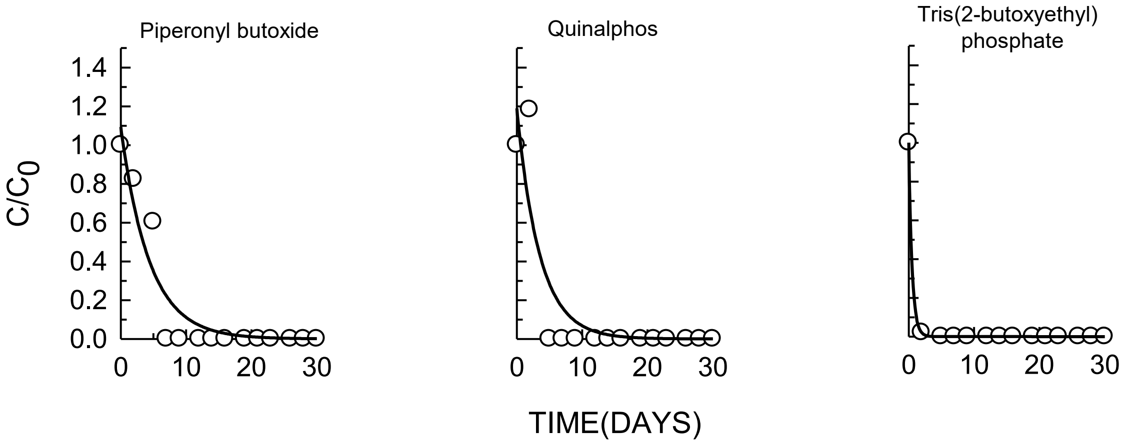


1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Carbendazim
Ciprofloxacin
C/C0
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4
0 10 20 30
TIME(DAYS)

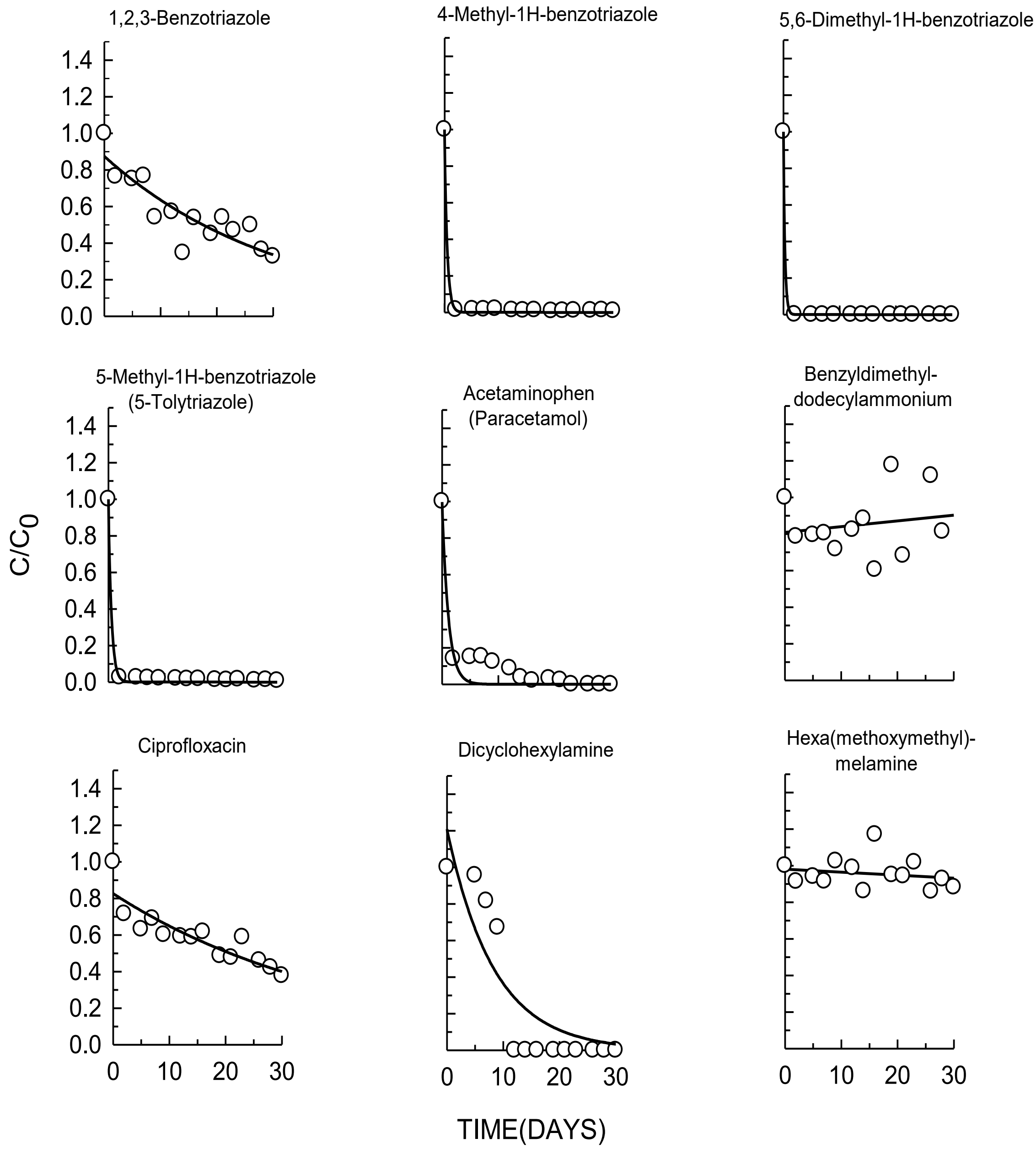
SP_8


Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
Mepiquat
N,N-Diethyl-m-toluamide
N-ethyl-p-toluene-
sulfonamide
Nonylphenol
diethoxylate
Norfloxacin
Ofloxacin
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C/C0
0
10
20
30
TIME(DAYS)

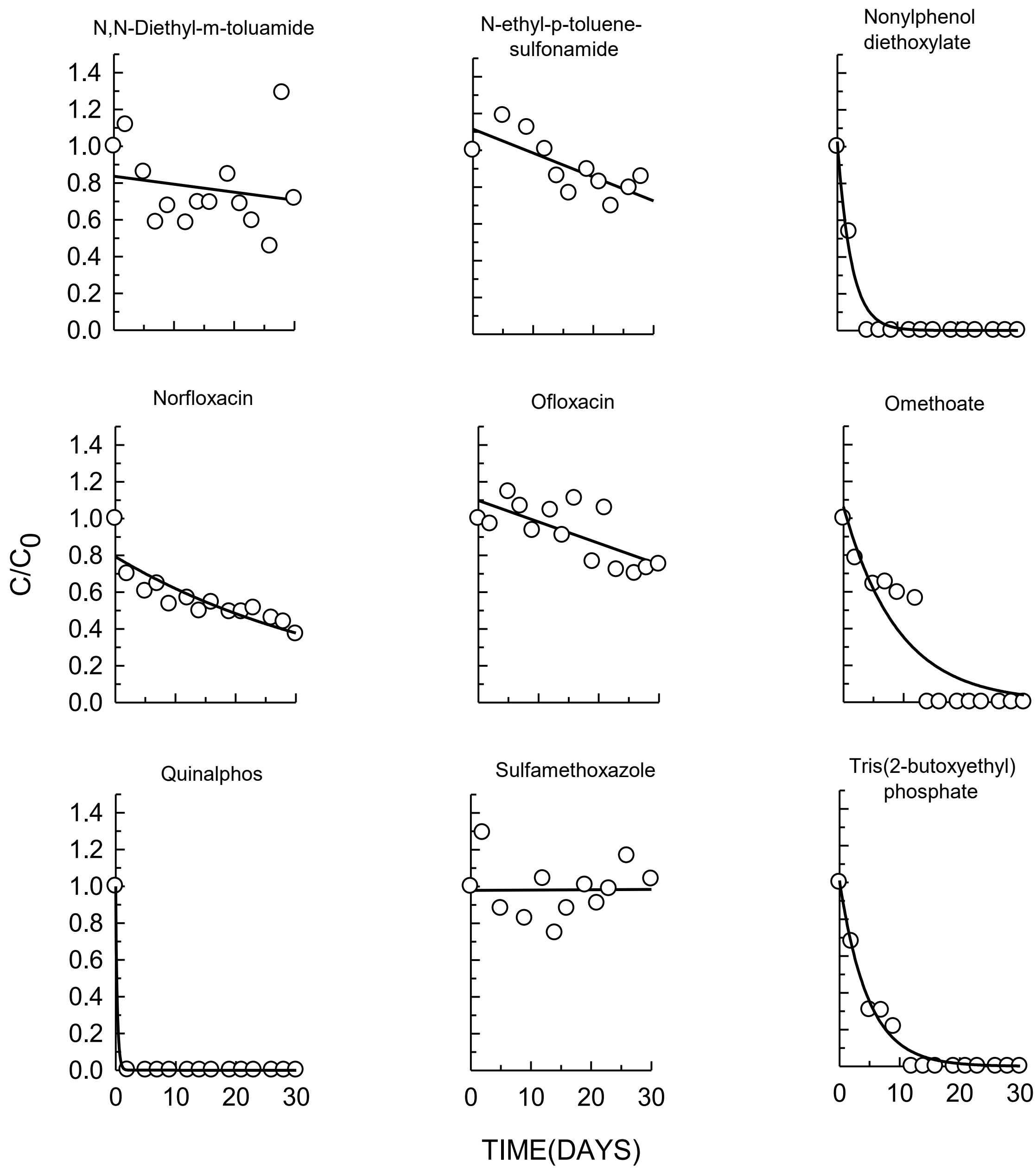
SP_8


Piperonyl butoxide
Quinalphos
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

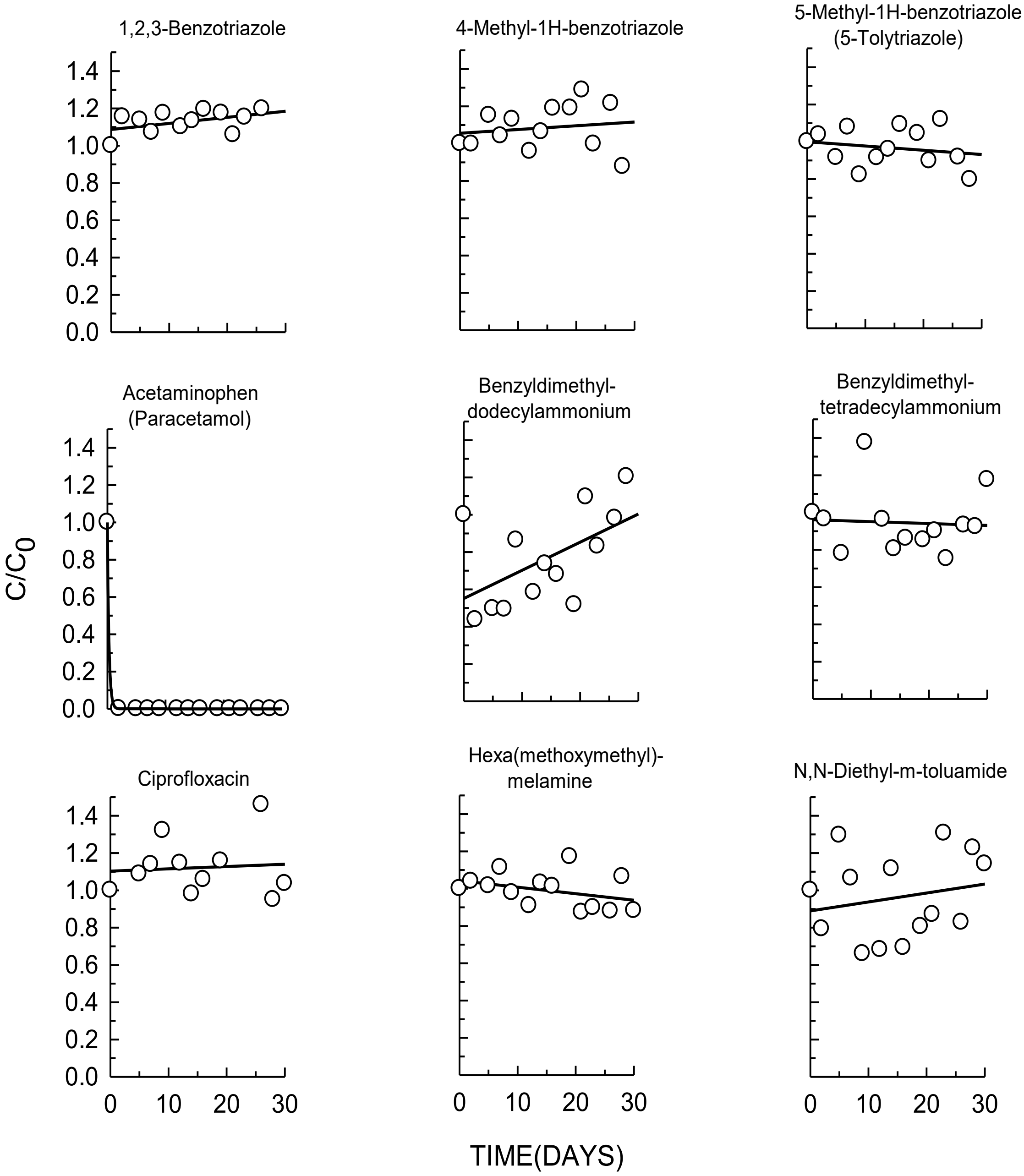
SP_9


1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Ciprofloxacin
Dicyclohexylamine
Hexa(methoxymethyl)-melamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

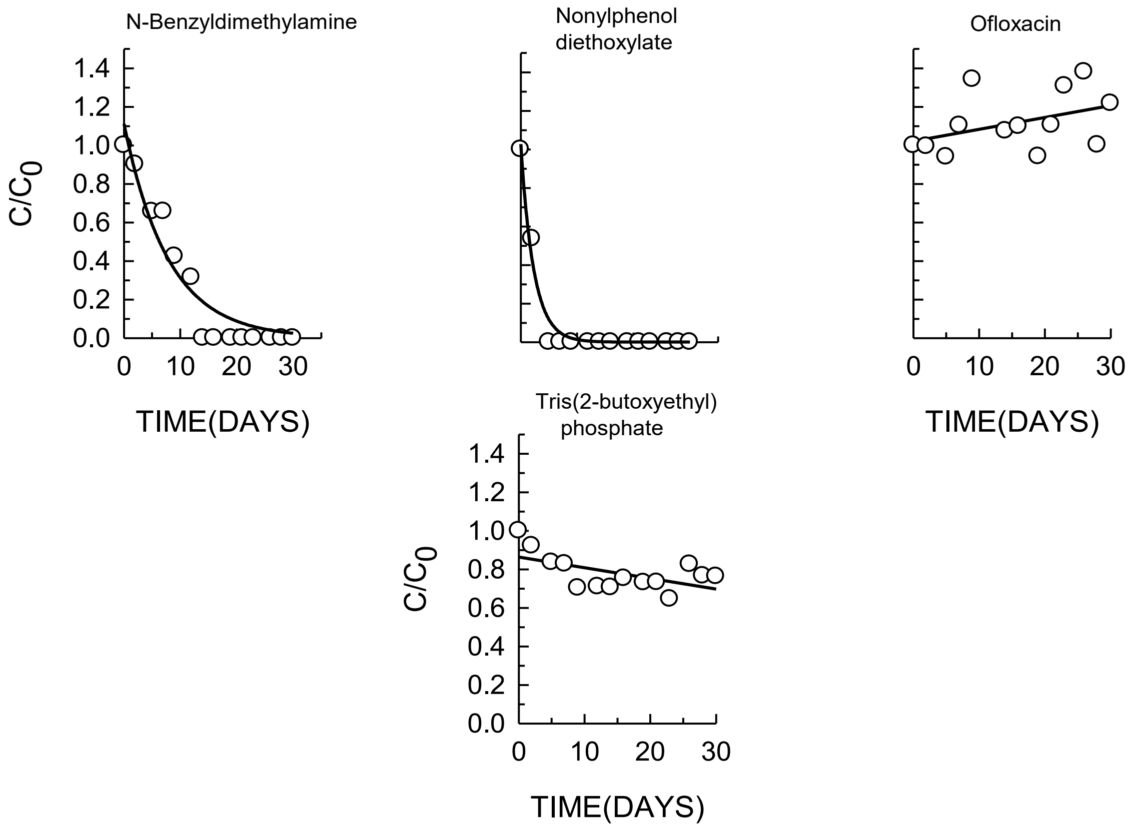
SP_9


N,N-Diethyl-m-toluamide
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Omethoate
Quinalphos
Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

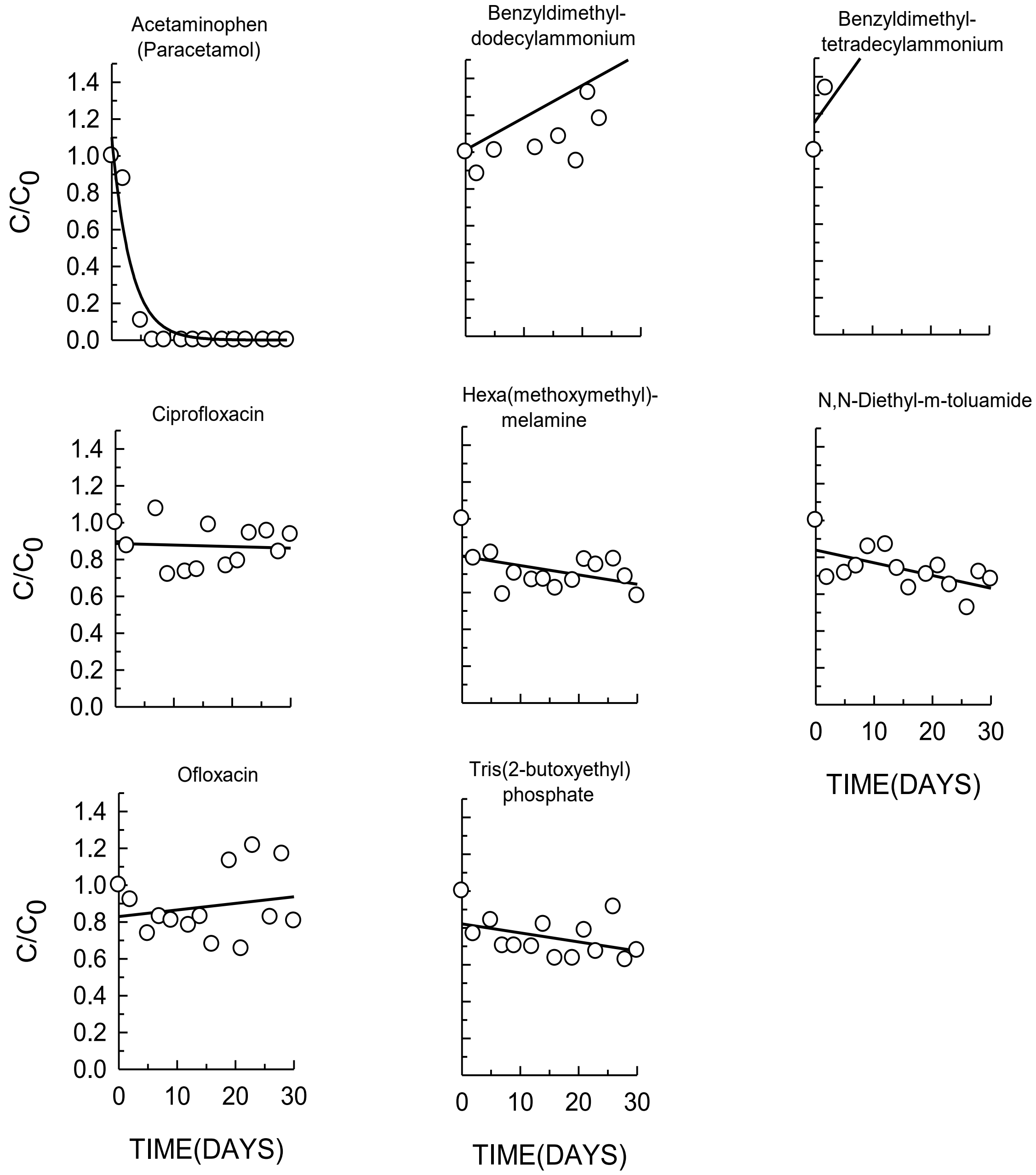
SP_10


1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Ciprofloxacin
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

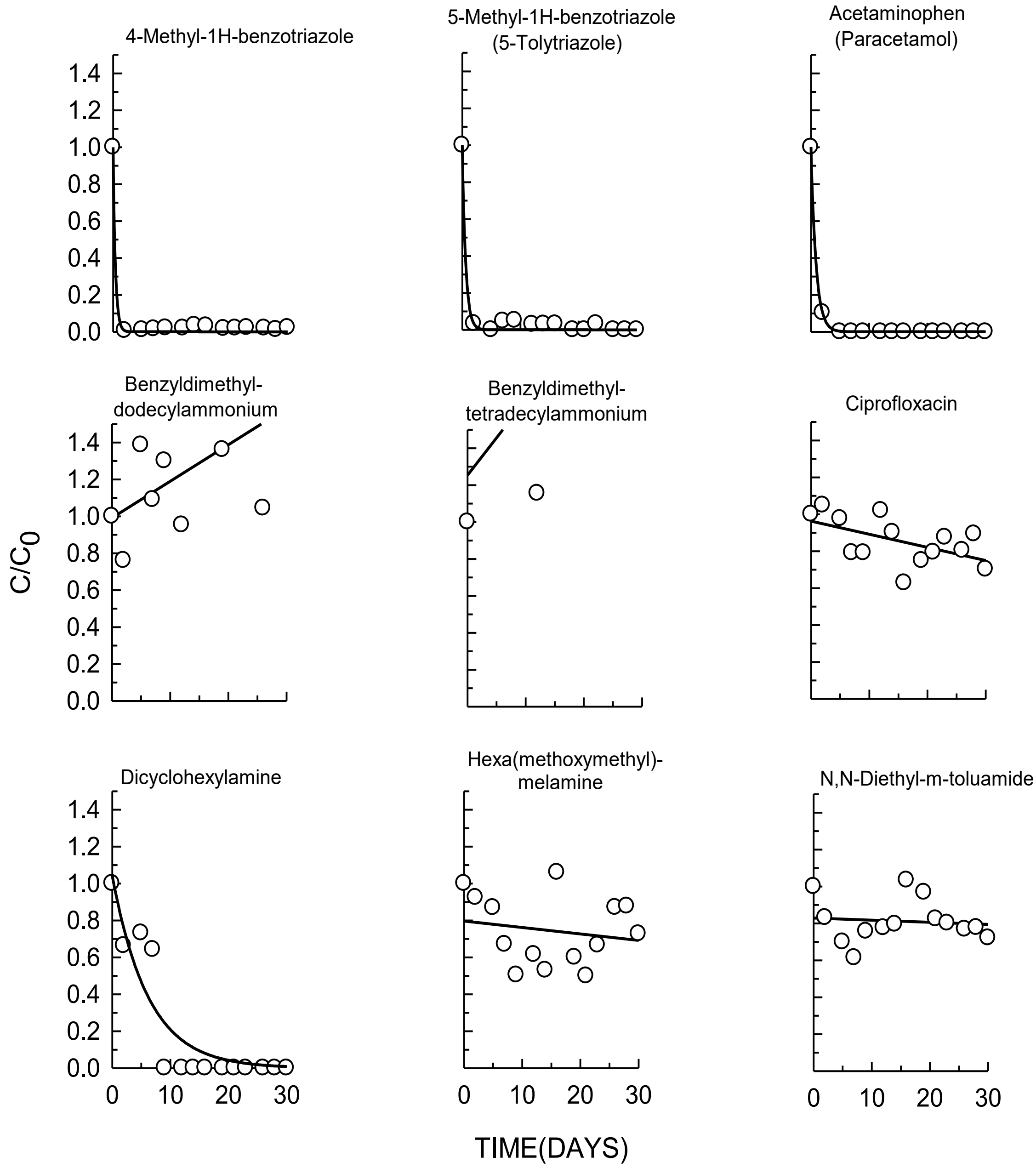
SP_10


N-Benzyldimethylamine
Nonylphenol diethoxylate
Ofloxacin
Tris(2-butoxyethyl) phosphate
C/C0
TIME(DAYS)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30

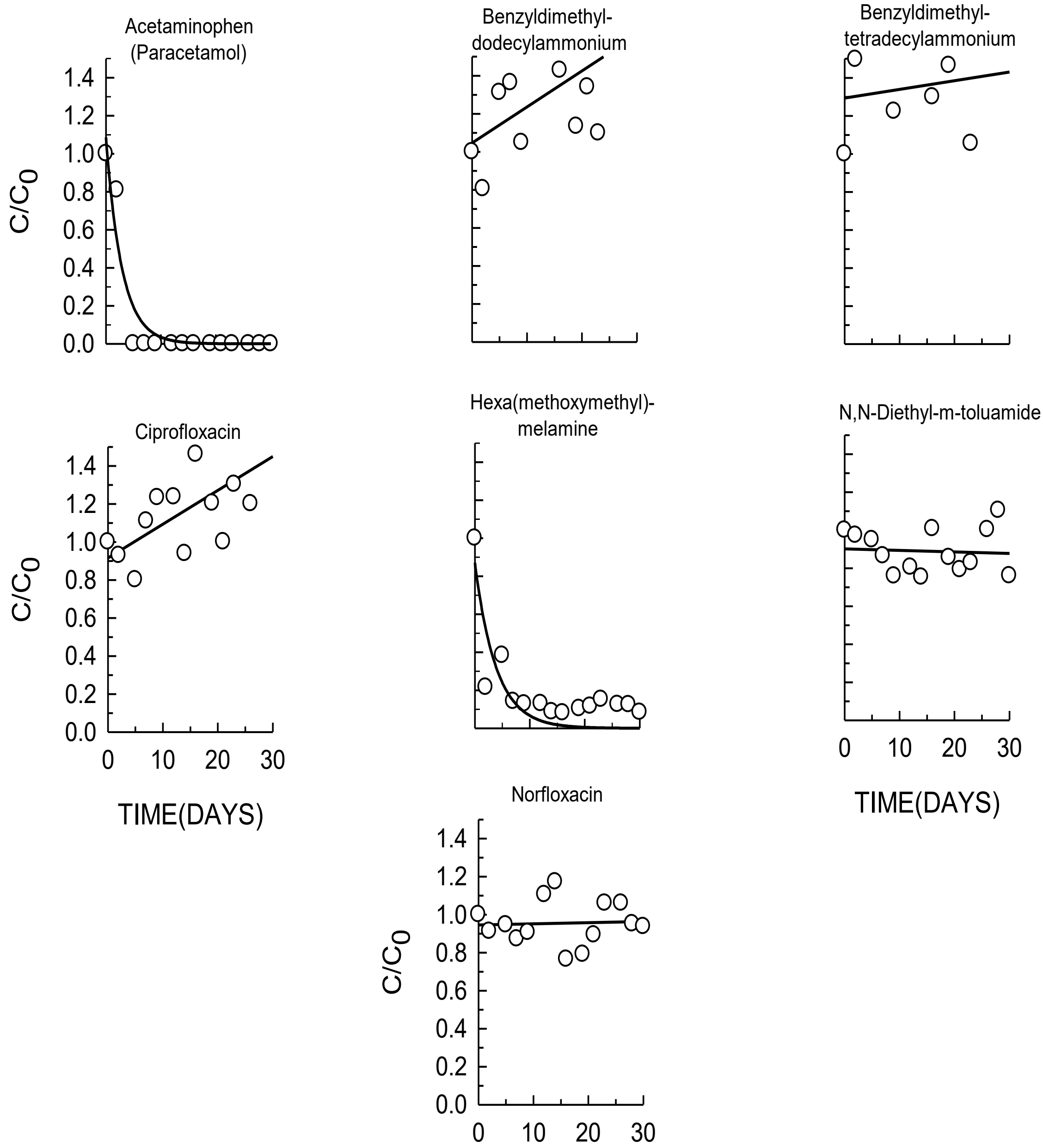
SP_11


Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Ciprofloxacin
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
Ofloxacin
Tris(2-butoxyethyl)
phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

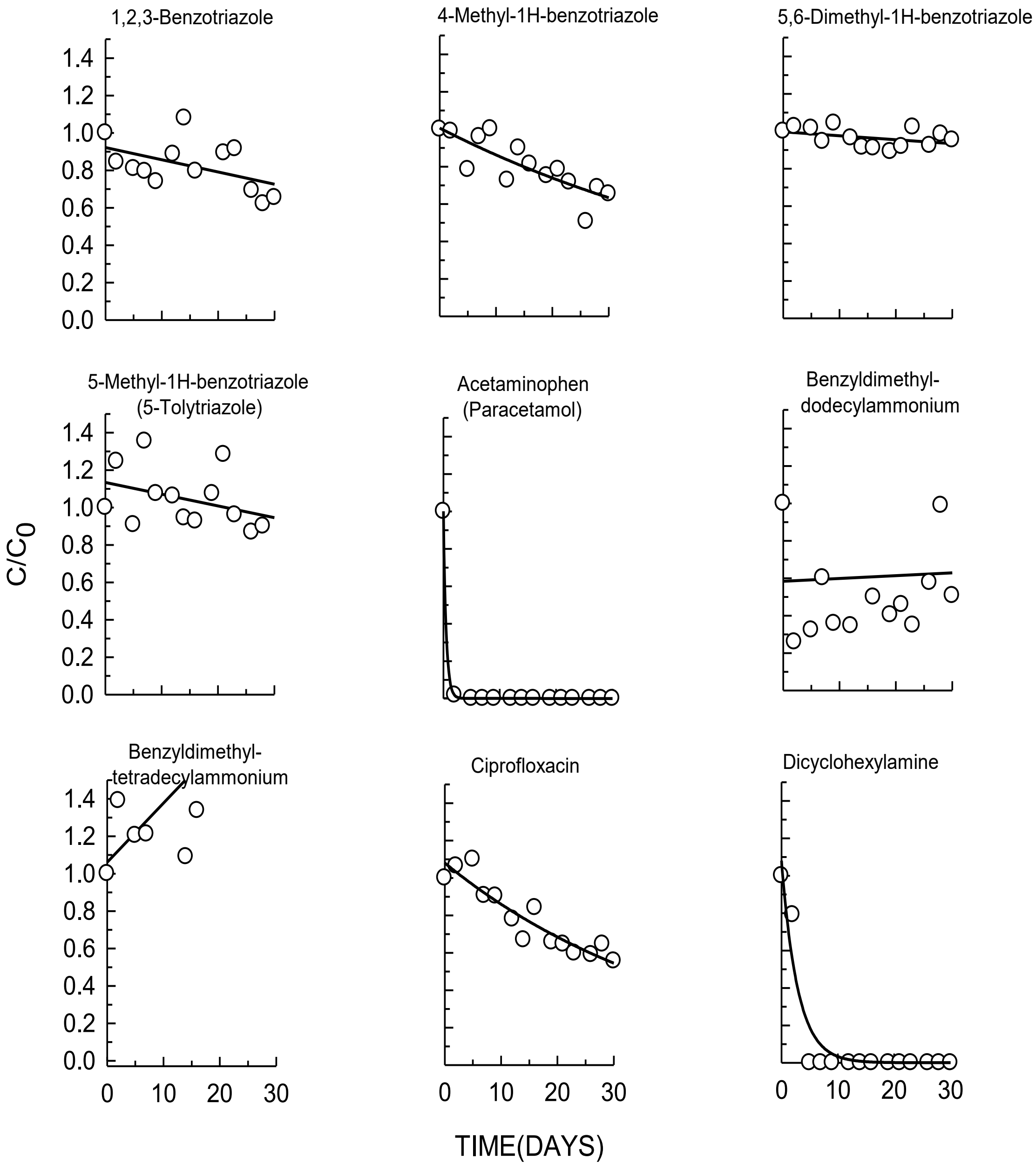
SP_12


4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Ciprofloxacin
Dicyclohexylamine
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

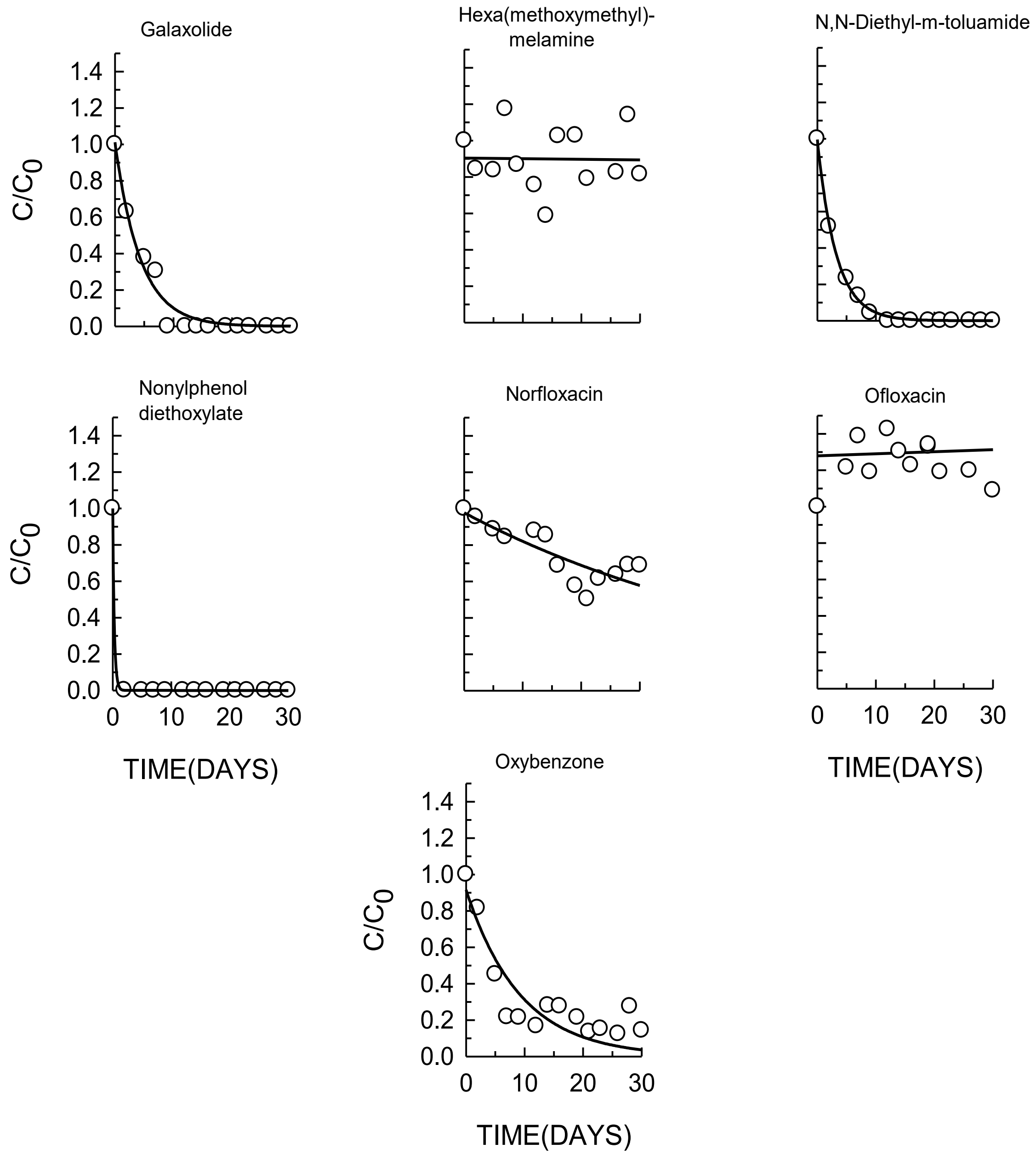
SP_13


Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Ciprofloxacin
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
Norfloxacin
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_14

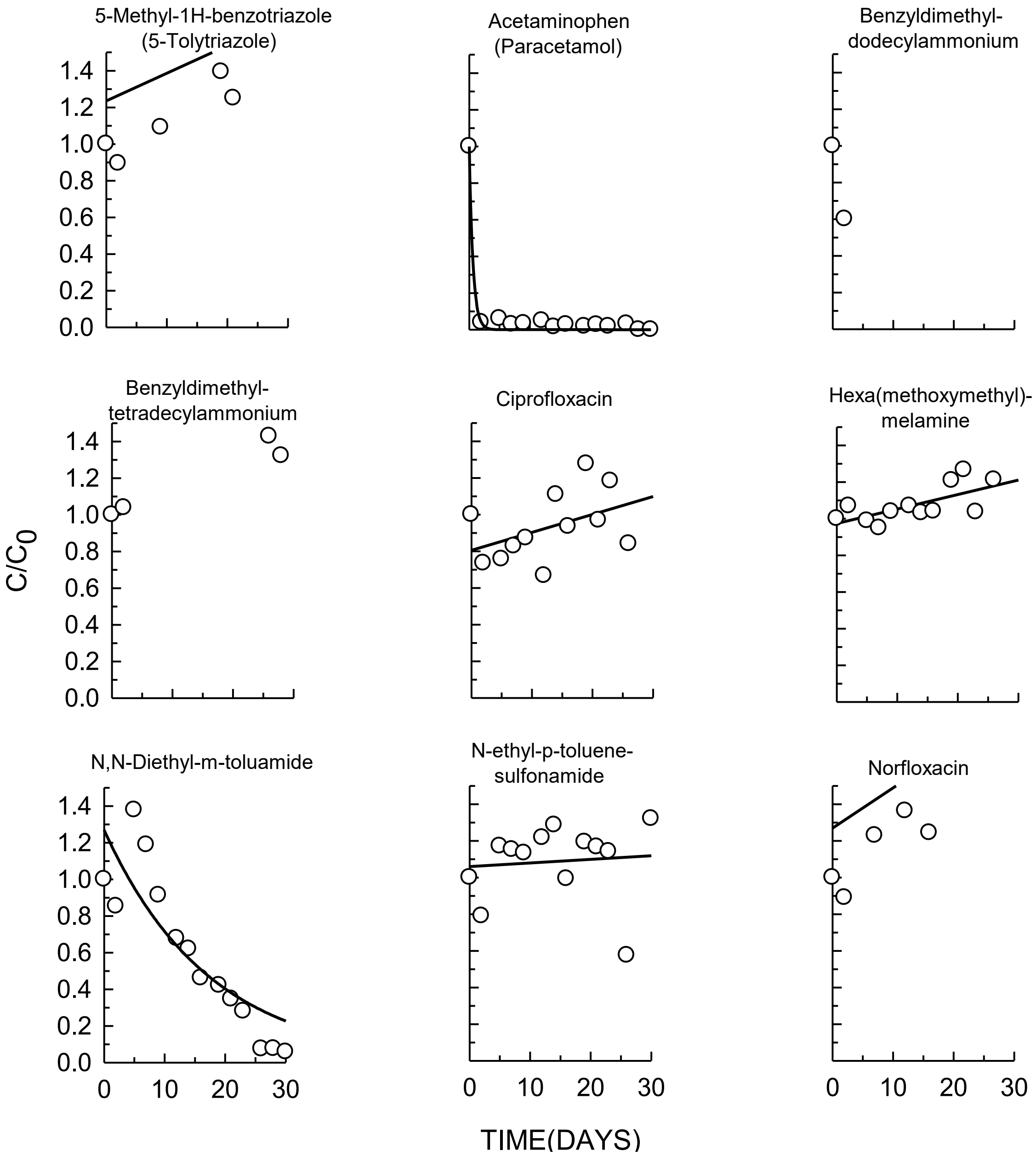

1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Ciprofloxacin
Dicyclohexylamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_14

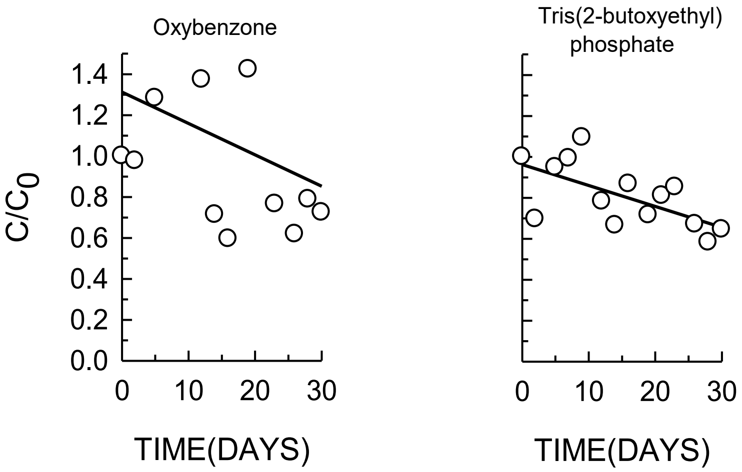


Galaxolide
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Oxybenzone
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

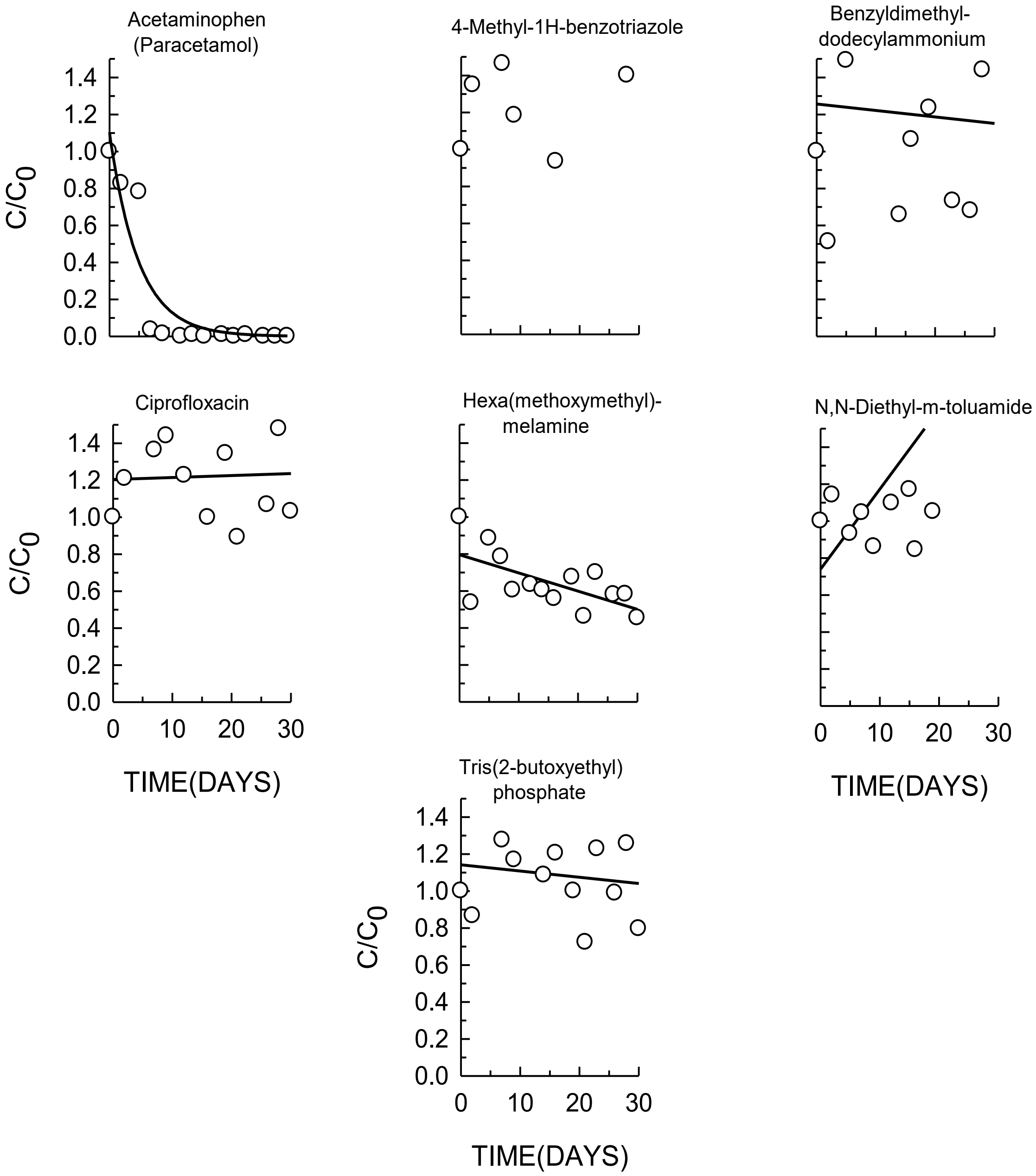
SP_15


5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Ciprofloxacin
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
N-ethyl-p-toluene-sulfonamide
Norfloxacin
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

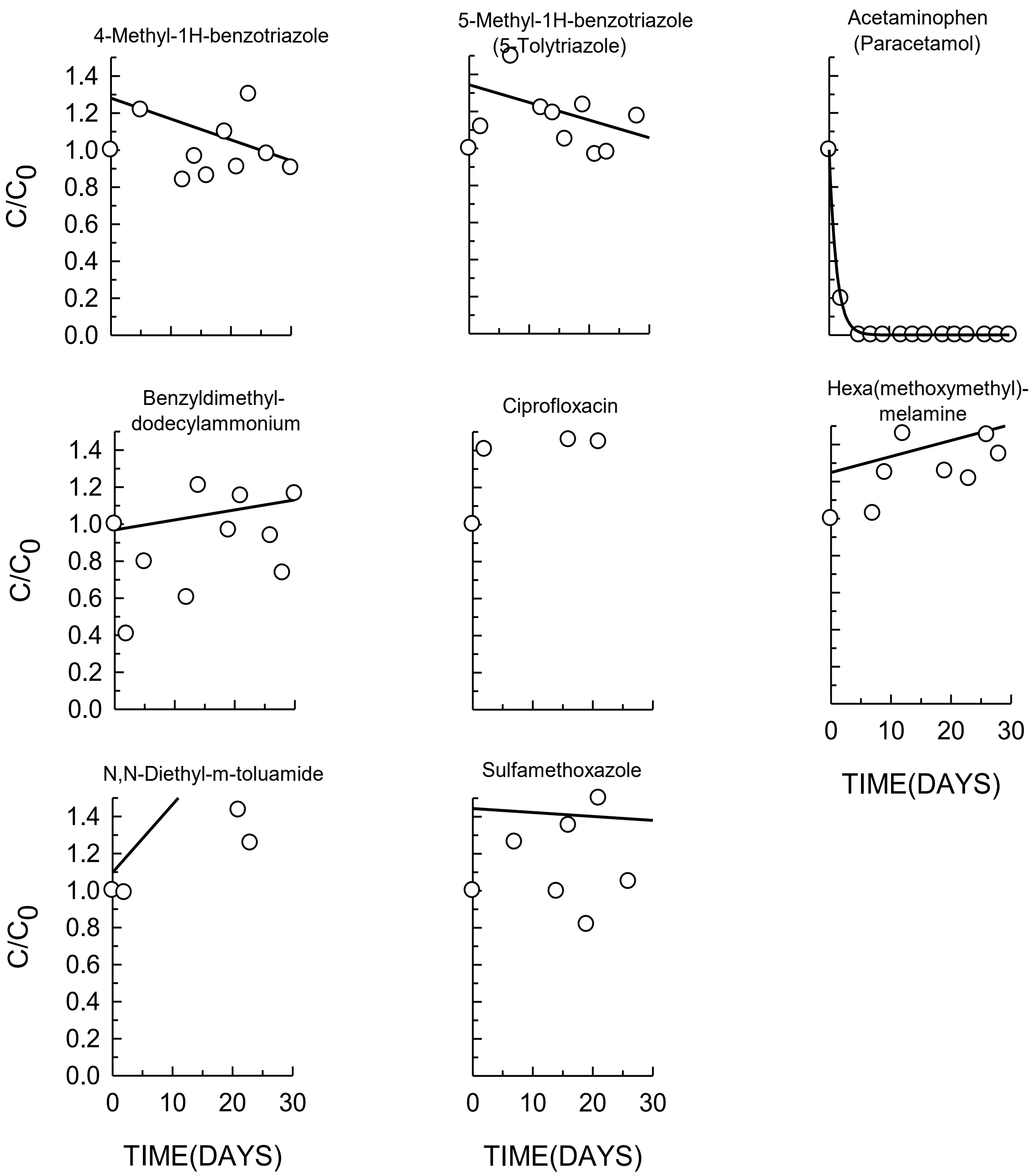
SP_15


Oxybenzone
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
TIME(DAYS)

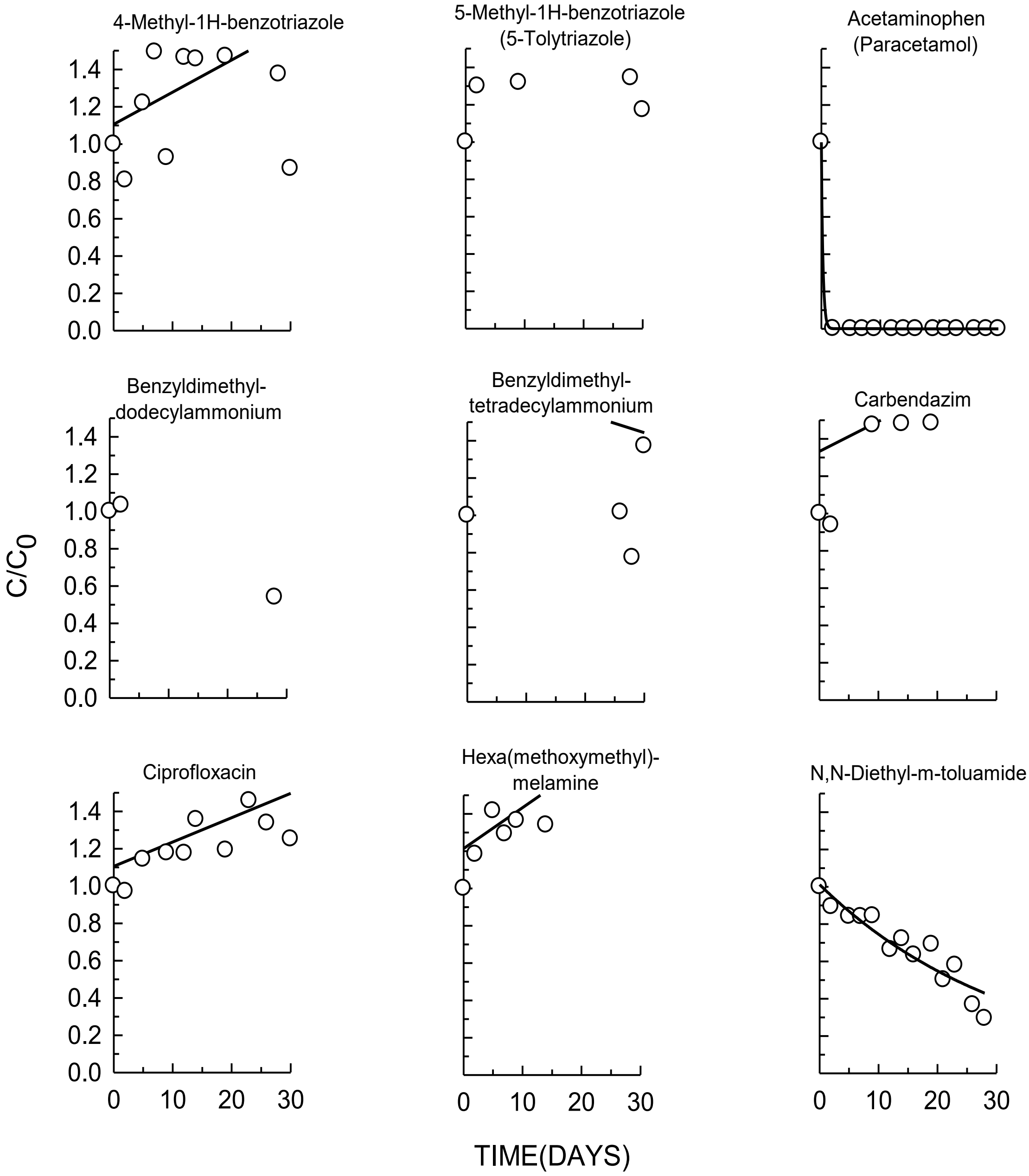
SP_16


Acetaminophen
(Paracetamol)
4-Methyl-1H-benzotriazole
Benzyldimethyl-
dodecylammonium
Ciprofloxacin
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
Tris(2-butoxyethyl)
phosphate
C/C0
TIME(DAYS)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30

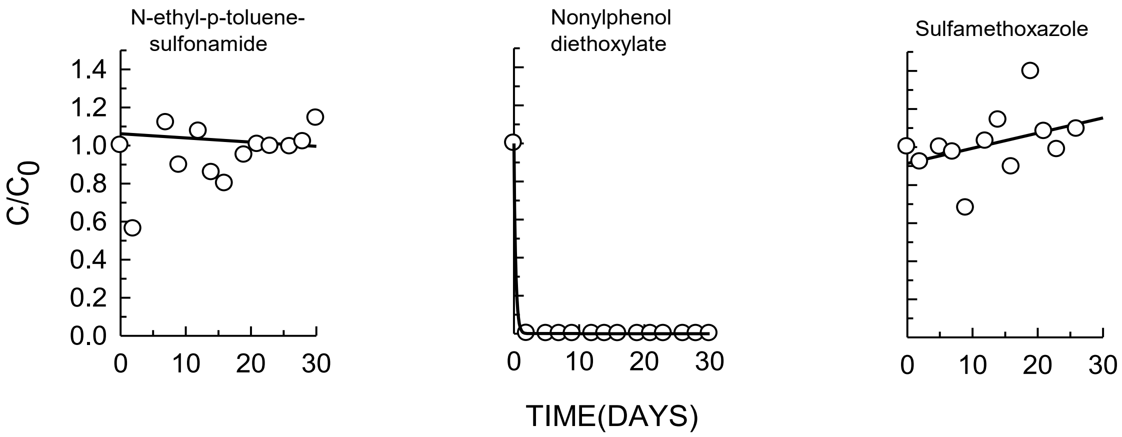
SP_17


4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Ciprofloxacin
Hexa(methoxymethyl)-melamine
N,N-Diethyl-m-toluamide
Sulfamethoxazole
C/C0
TIME(DAYS)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30

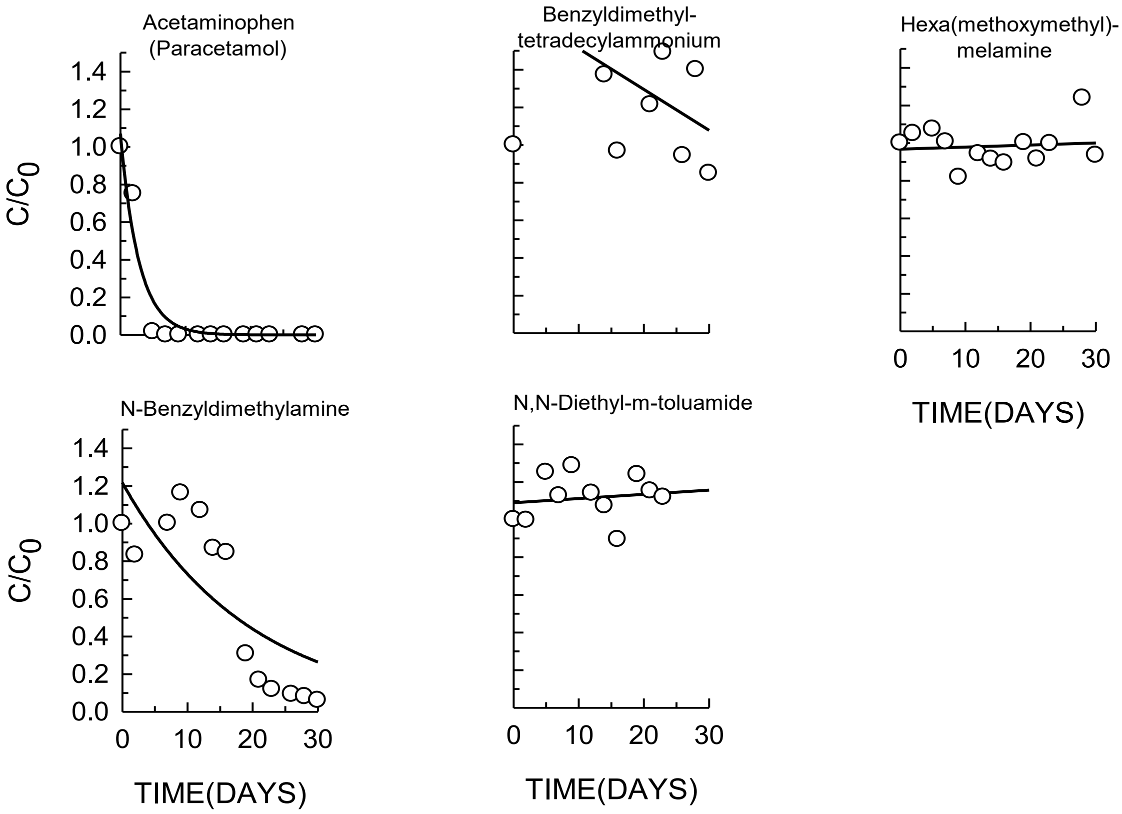
SP_18


4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Carbendazim
Ciprofloxacin
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

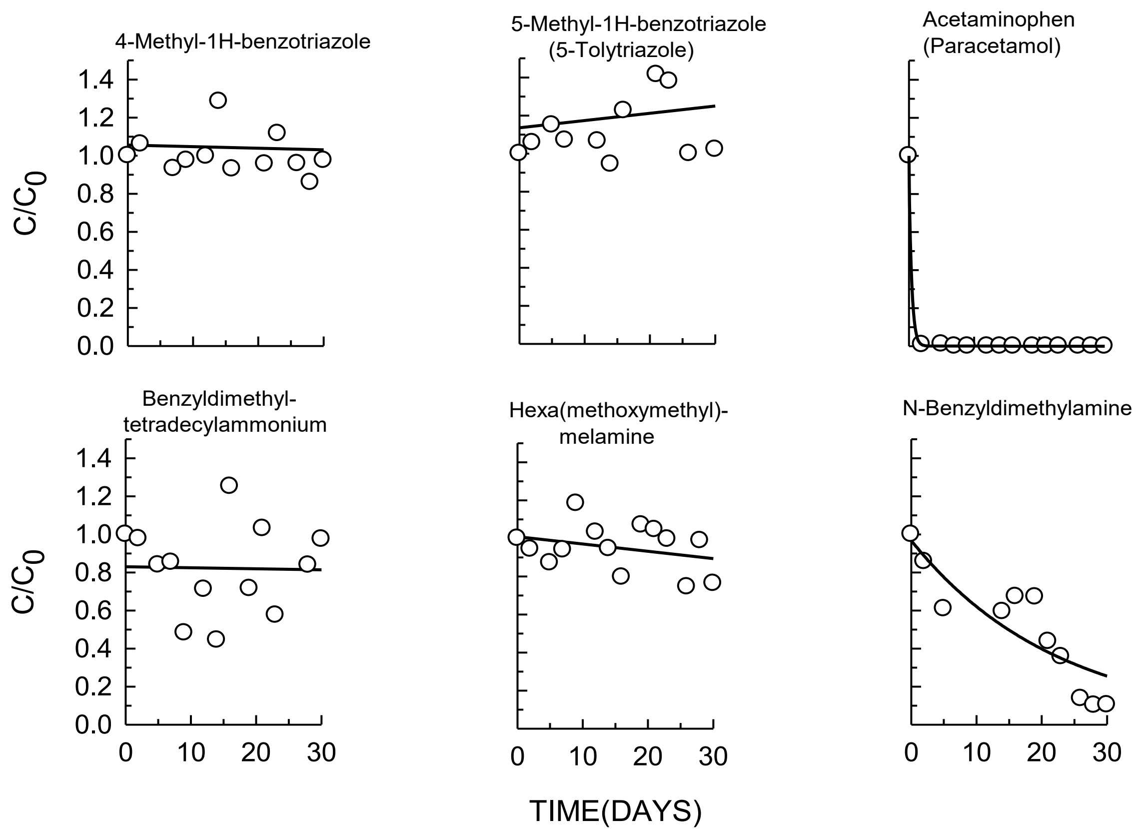
SP_18


N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Sulfamethoxazole
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

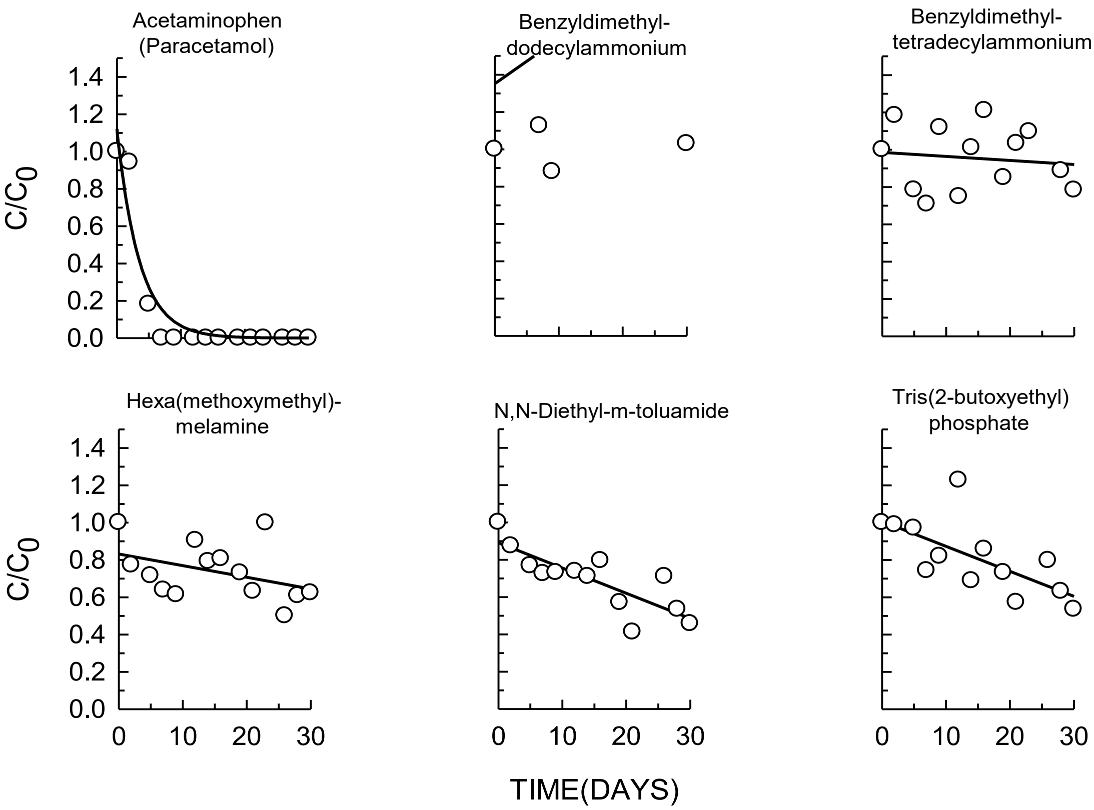
SP_19


Acetaminophen
(Paracetamol)
Benzyldimethyl-
tetradecylammonium
Hexa(methoxymethyl)-
melamine
N-Benzyldimethylamine
N,N-Diethyl-m-toluamide
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

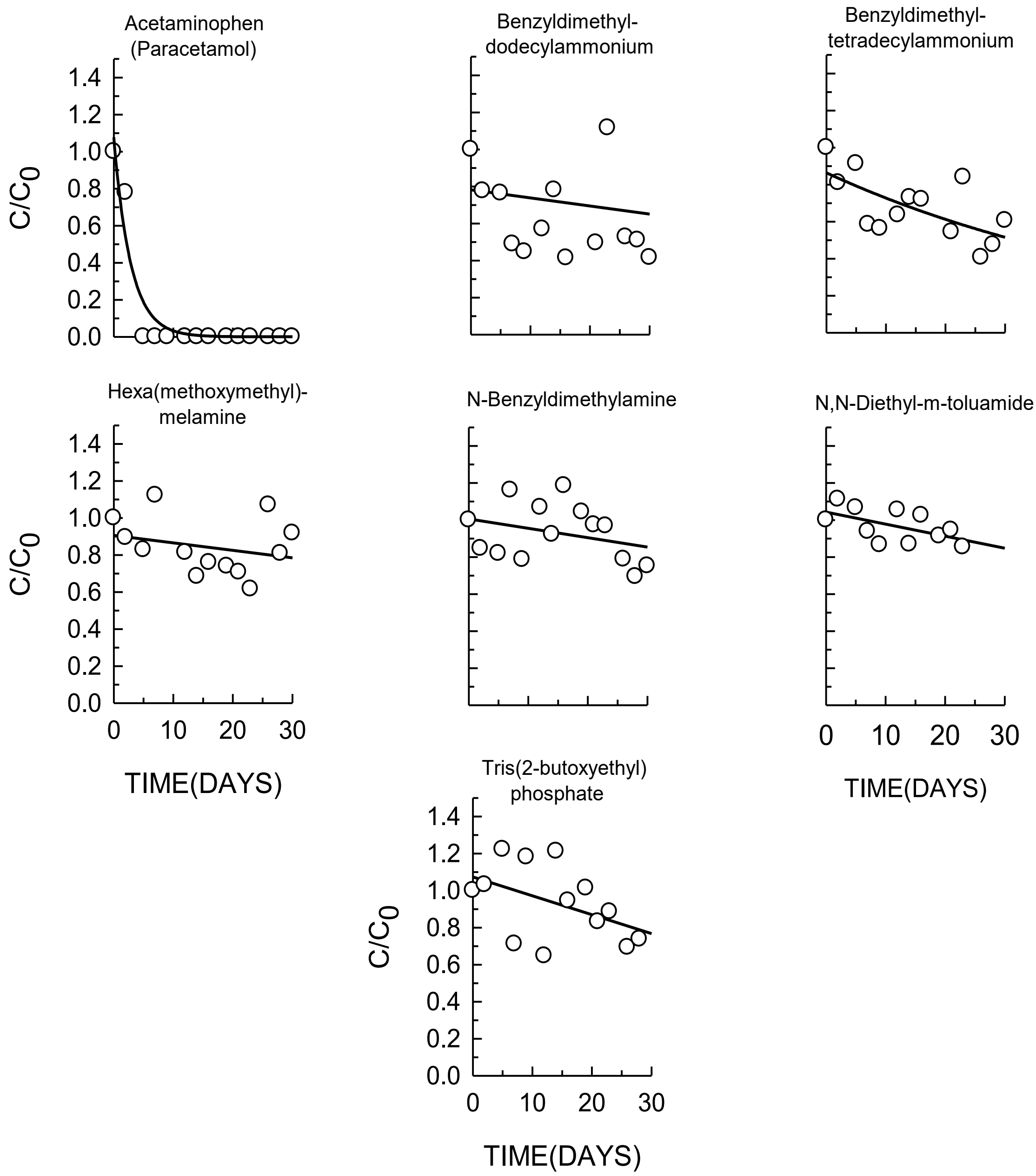
SP_20


4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
Benzyldimethyl-
tetradecylammonium
Hexa(methoxymethyl)-
melamine
N-Benzyldimethylamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

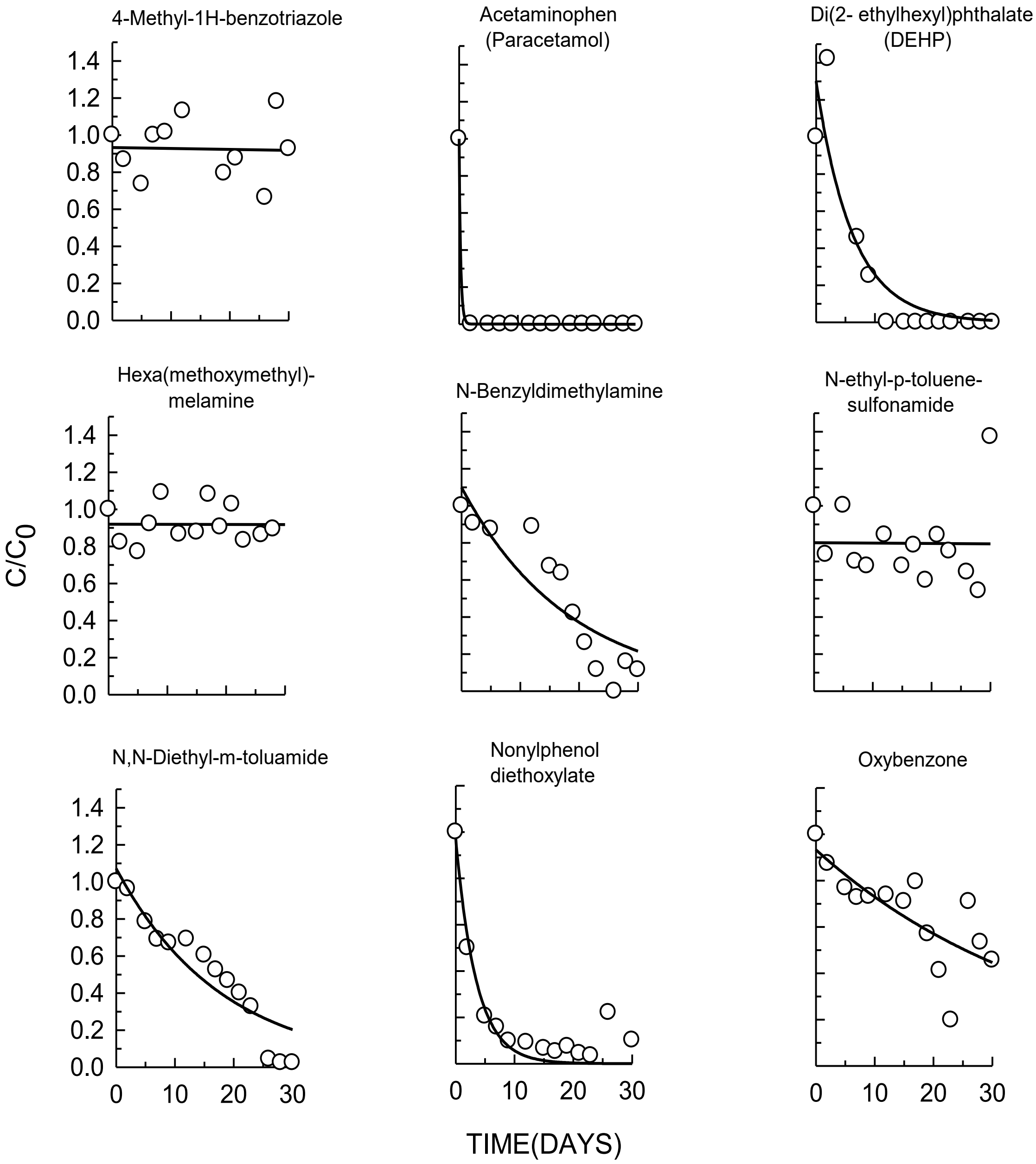
SP_21


Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
Tris(2-butoxyethyl)
phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

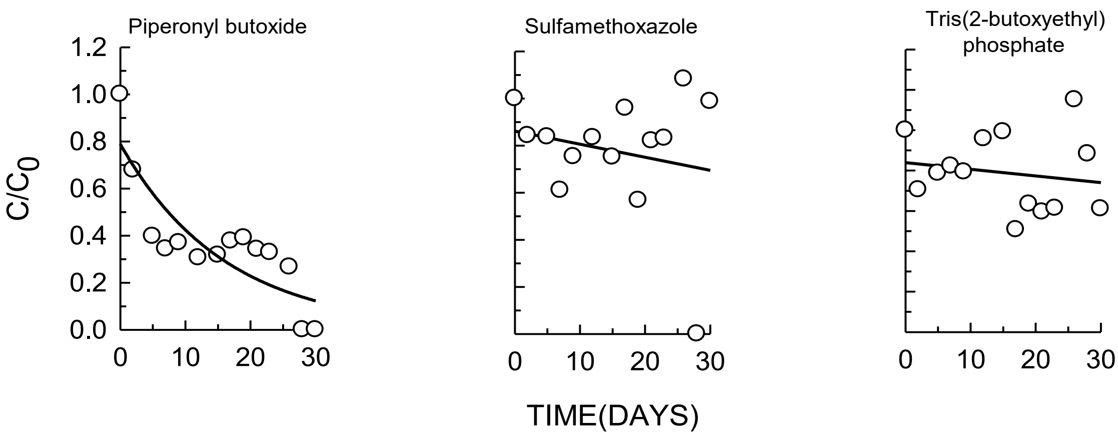
SP_22


Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Hexa(methoxymethyl)-
melamine
N-Benzyldimethylamine
N,N-Diethyl-m-toluamide
Tris(2-butoxyethyl)
phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

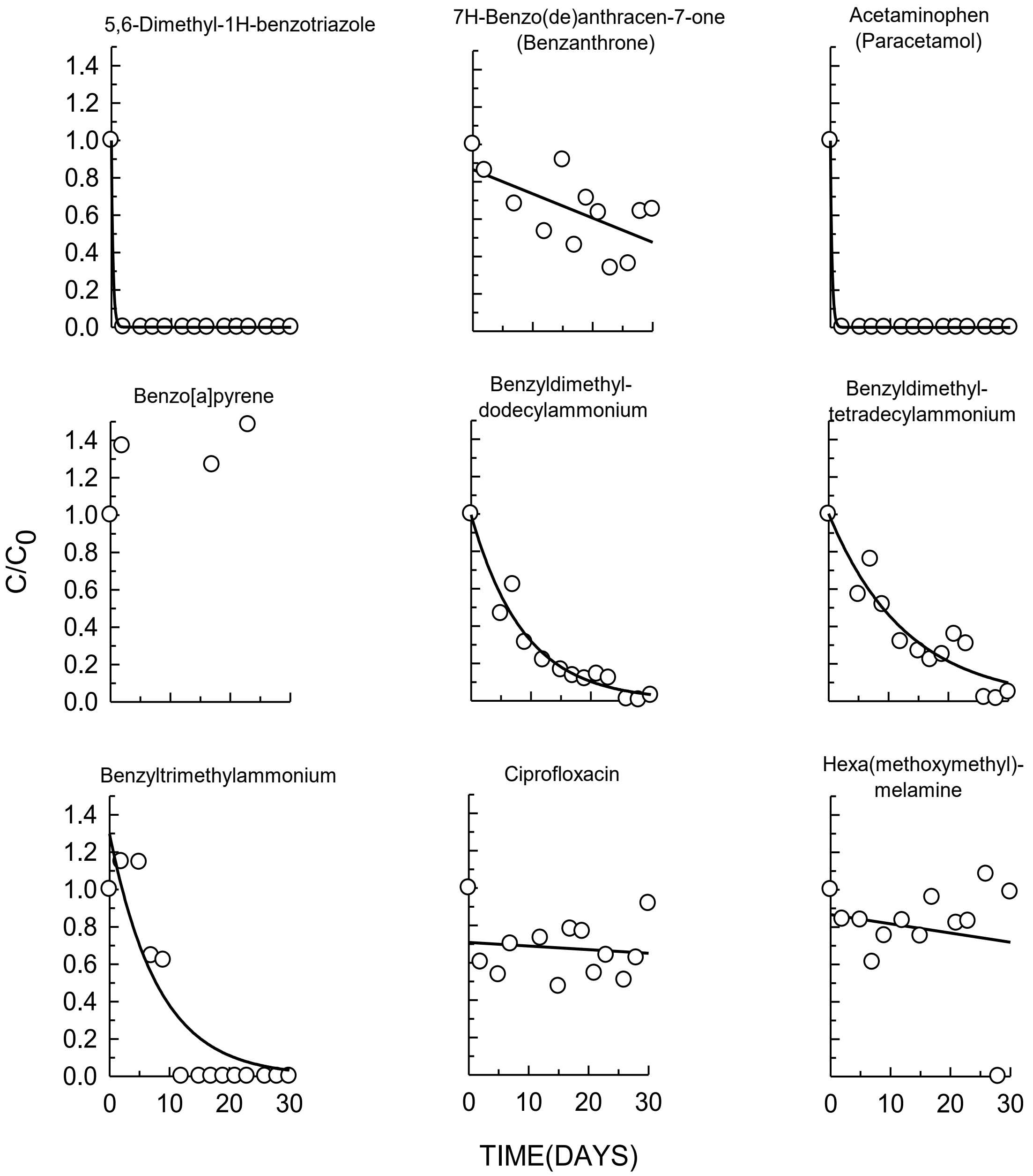
SP_23


4-Methyl-1H-benzotriazole
Acetaminophen (Paracetamol)
Di(2- ethylhexyl)phthalate (DEHP)
Hexa(methoxymethyl)-melamine
N-Benzyldimethylamine
N-ethyl-p-toluene-sulfonamide
N,N-Diethyl-m-toluamide
Nonylphenol diethoxylate
Oxybenzone
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
C/C0
TIME(DAYS)

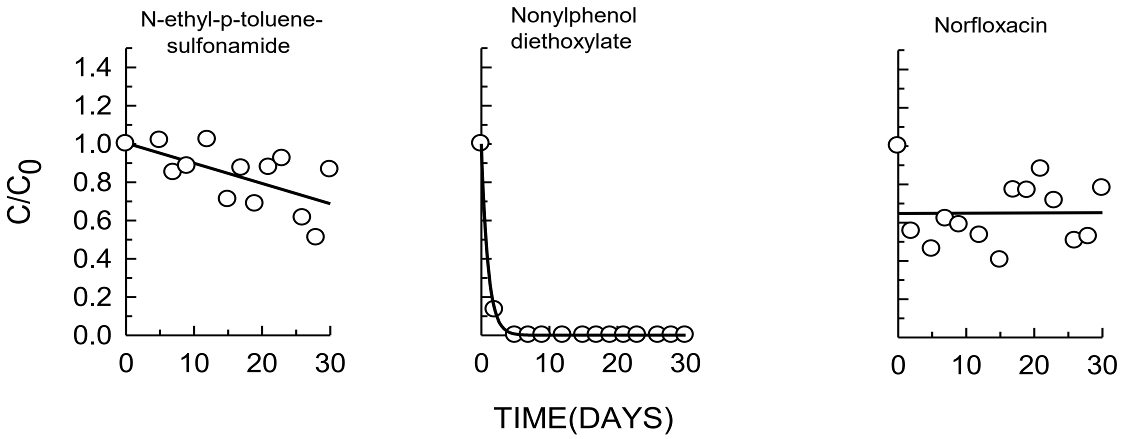
SP_23


Piperonyl butoxide
Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

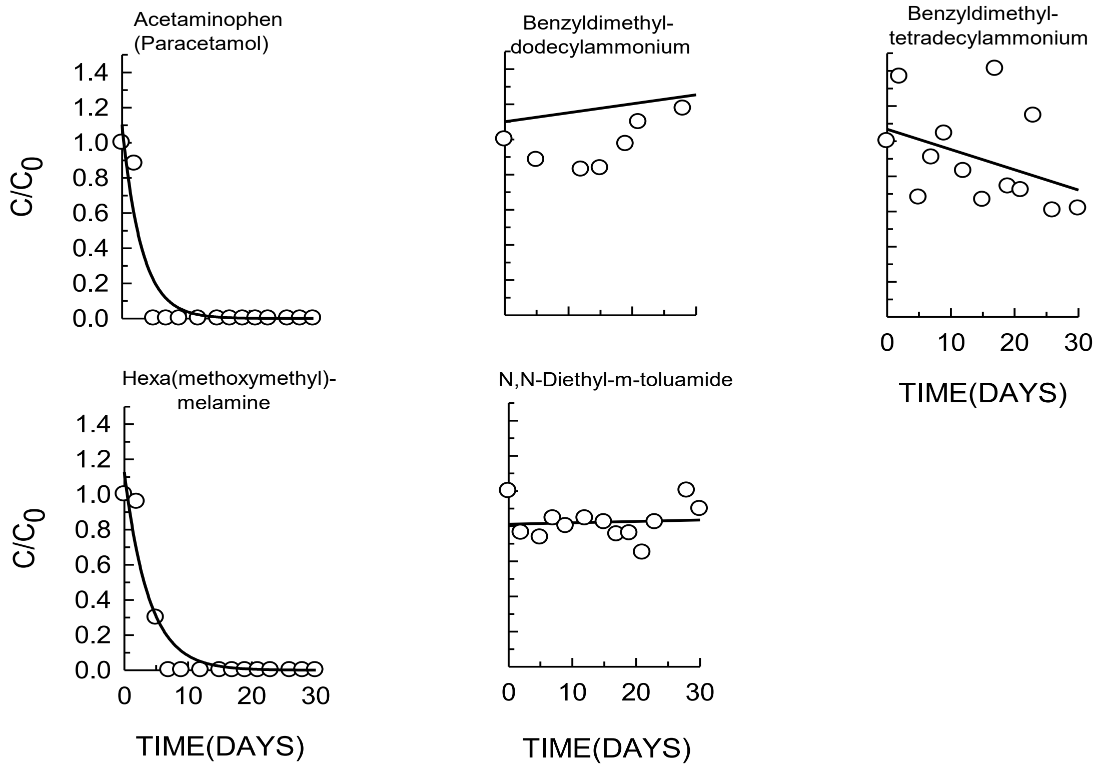
SP_24


5,6-Dimethyl-1H-benzotriazole
7H-Benzo(de)anthracen-7-one
(Benzanthrone)
Acetaminophen
(Paracetamol)
Benzo[a]pyrene
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Benzyltrimethylammonium
Ciprofloxacin
Hexa(methoxymethyl)-
melamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

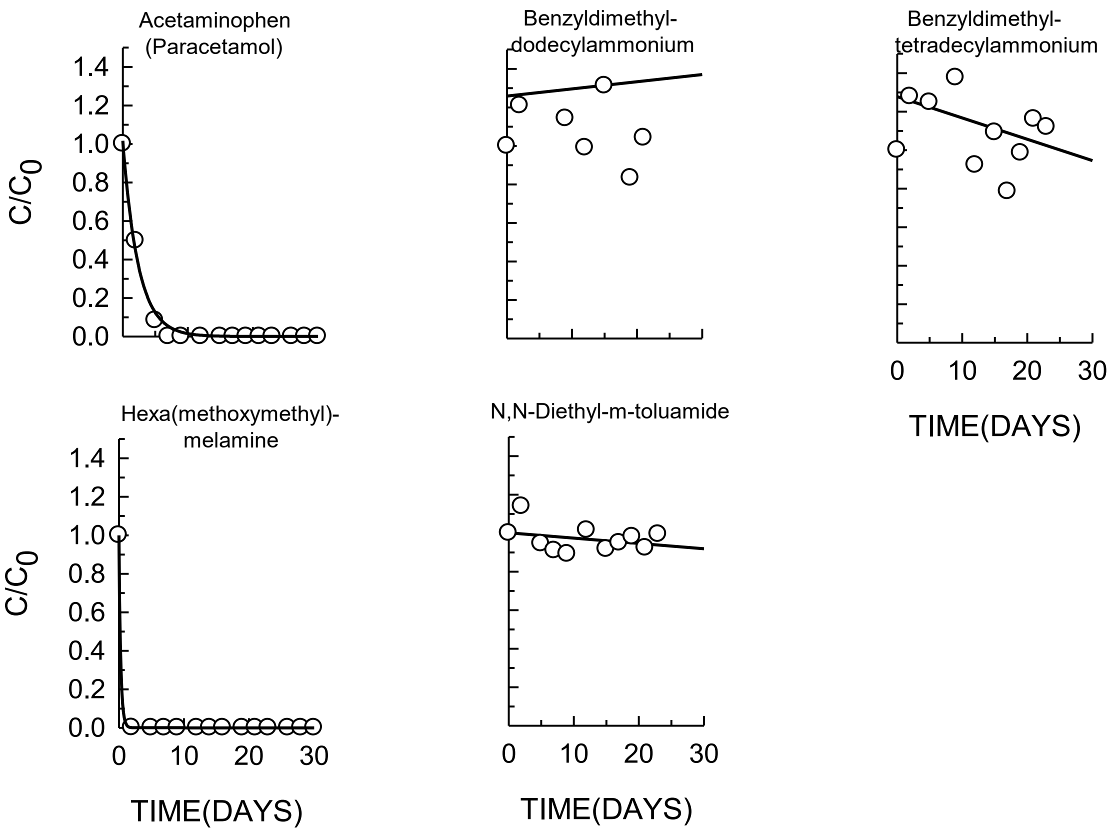
SP_24


N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Norfloxacin
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

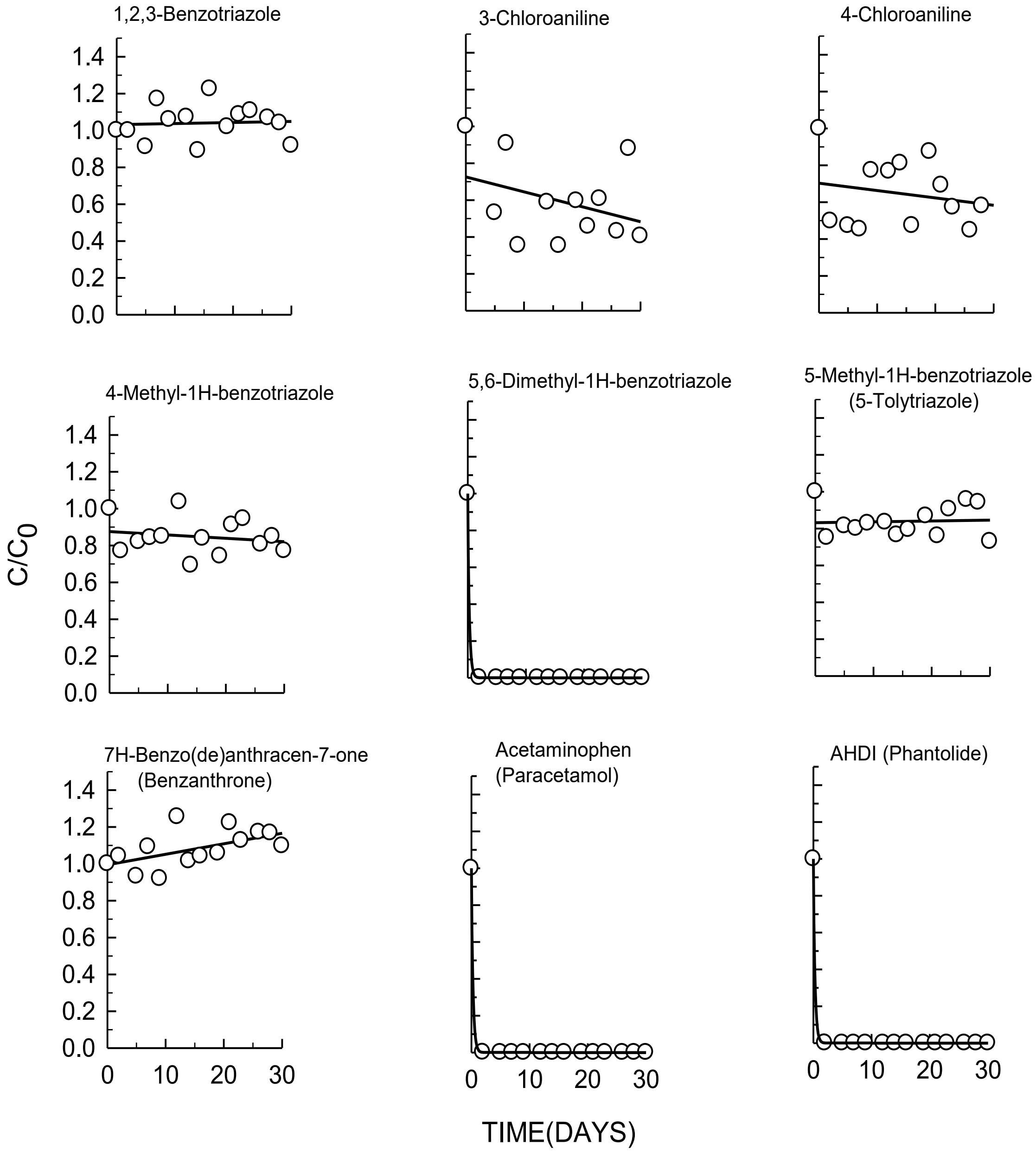
SP_25


Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

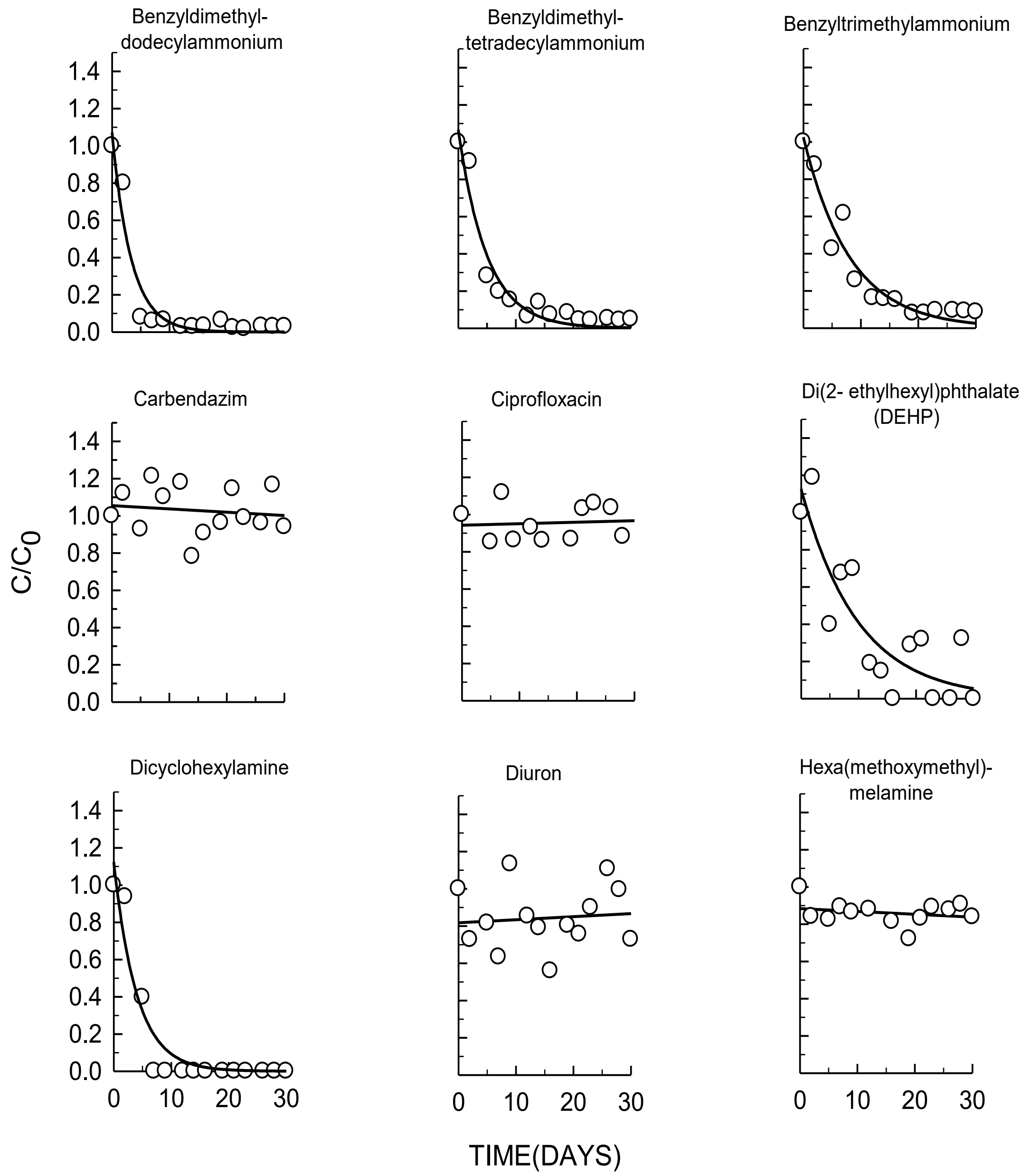
SP_26


Acetaminophen
(Paracetamol)
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

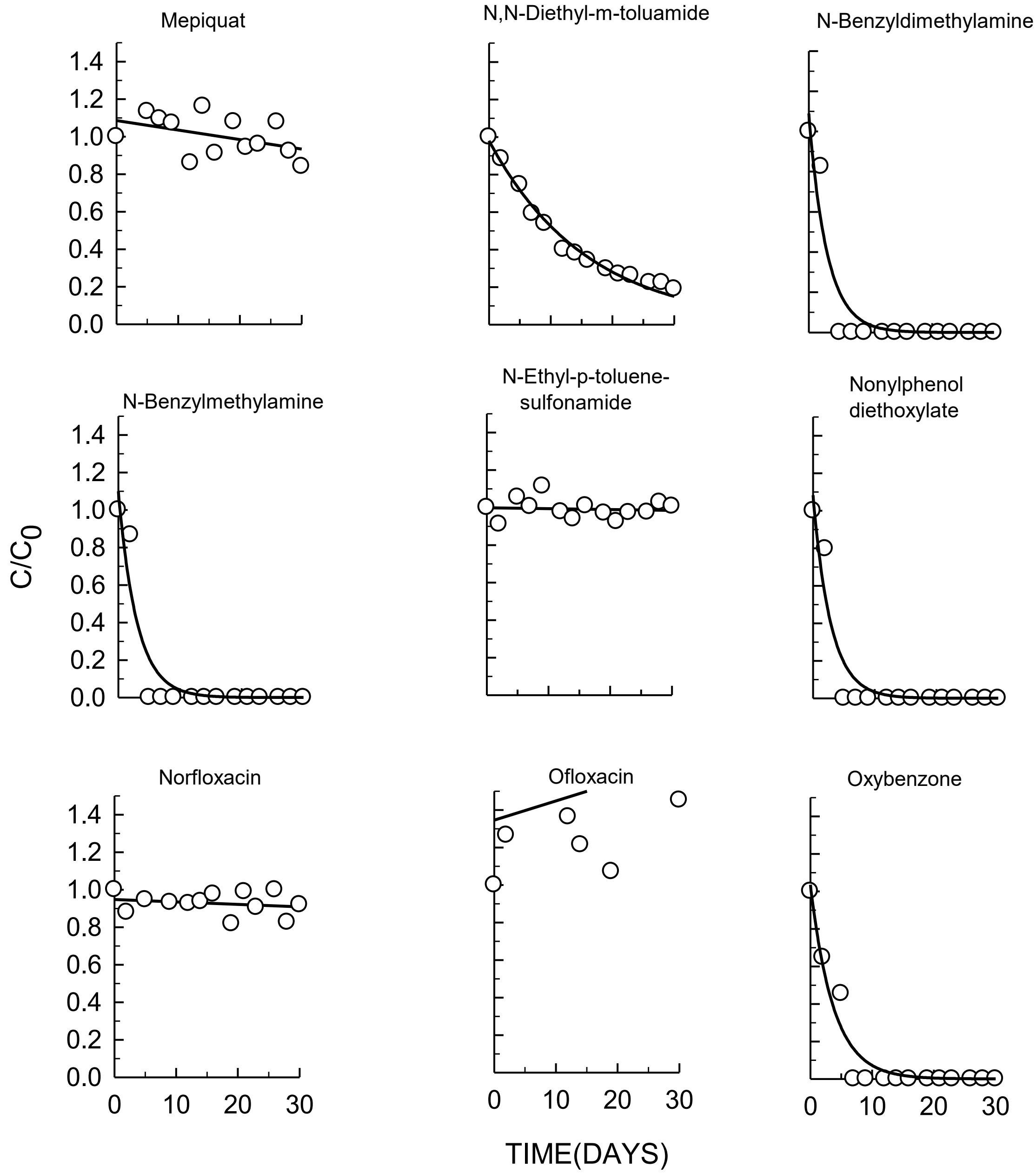
SP_28


1,2,3-Benzotriazole
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
7H-Benzo(de)anthracen-7-one
(Benzanthrone)
Acetaminophen
(Paracetamol)
AHDI (Phantolide)
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

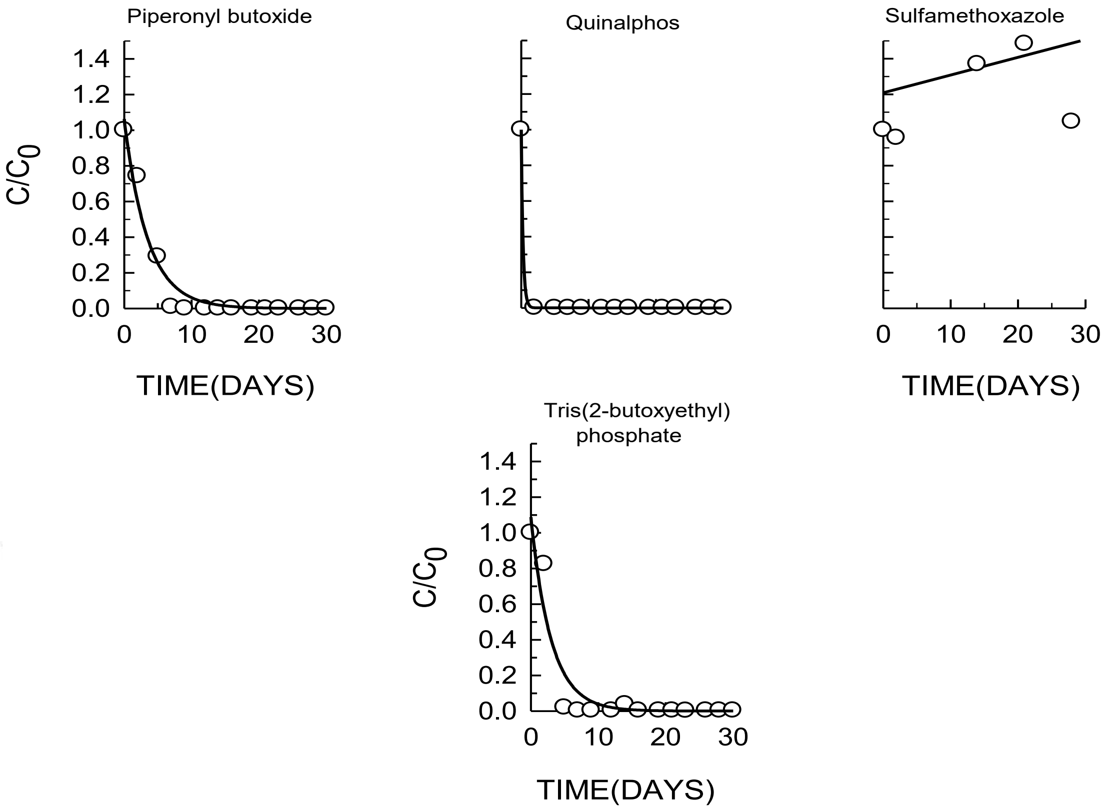
SP_28


Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Benzyltrimethylammonium
Carbendazim
Ciprofloxacin
Di(2- ethylhexyl)phthalate
(DEHP)
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

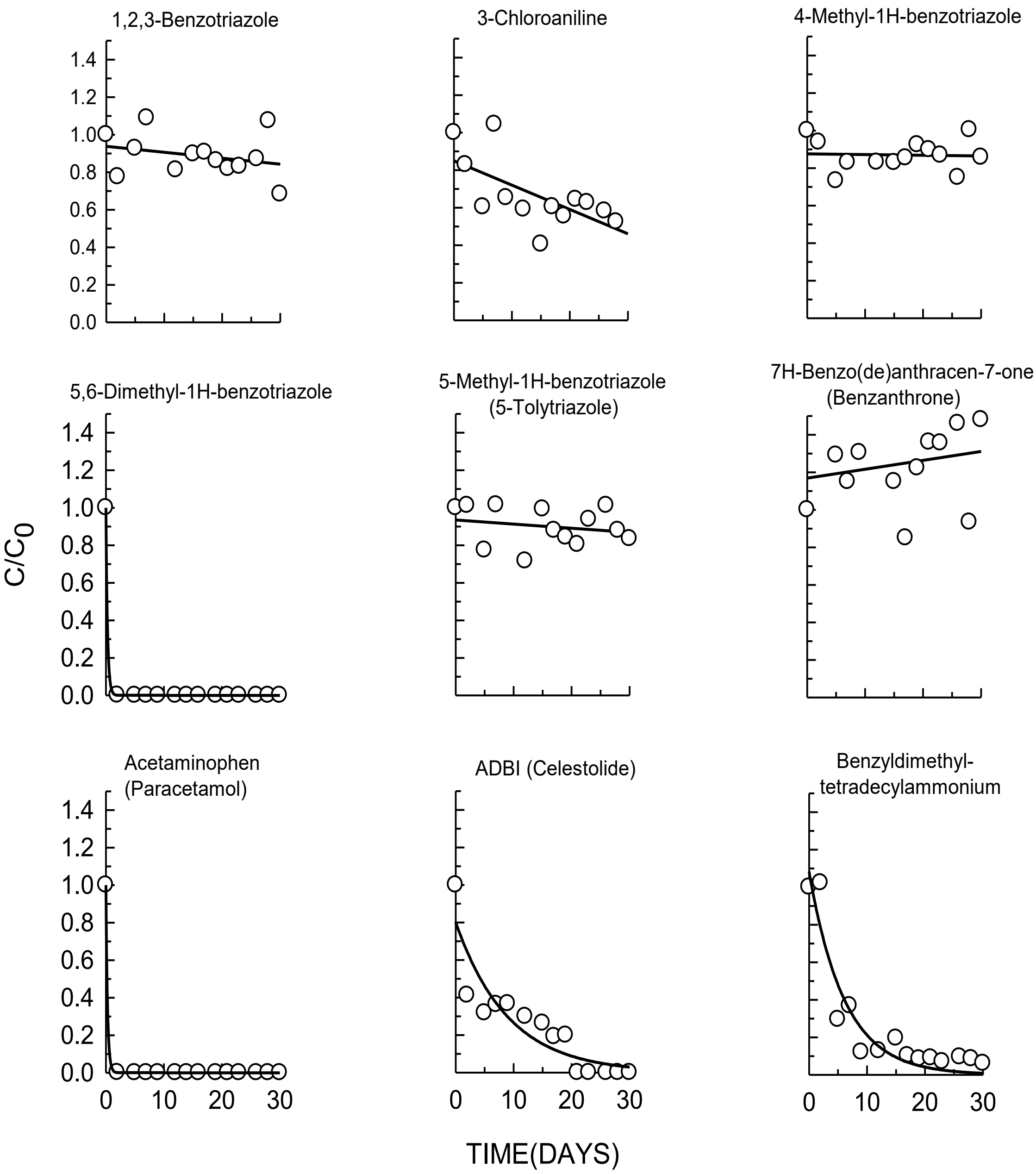
SP_28


Mepiquat
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
N-Benzylmethylamine
N-Ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Norfloxacin
Ofloxacin
Oxybenzone
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

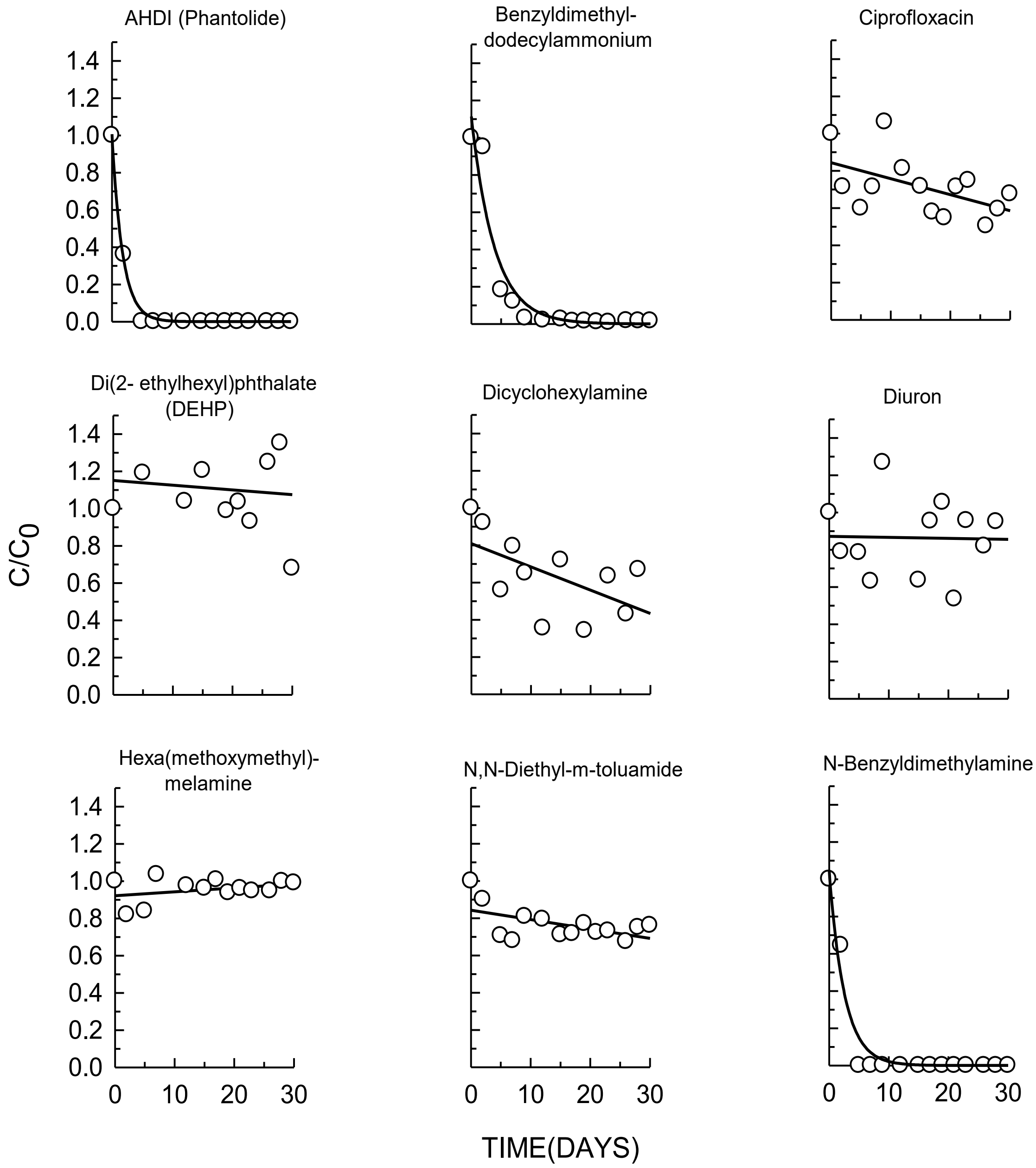
SP_28

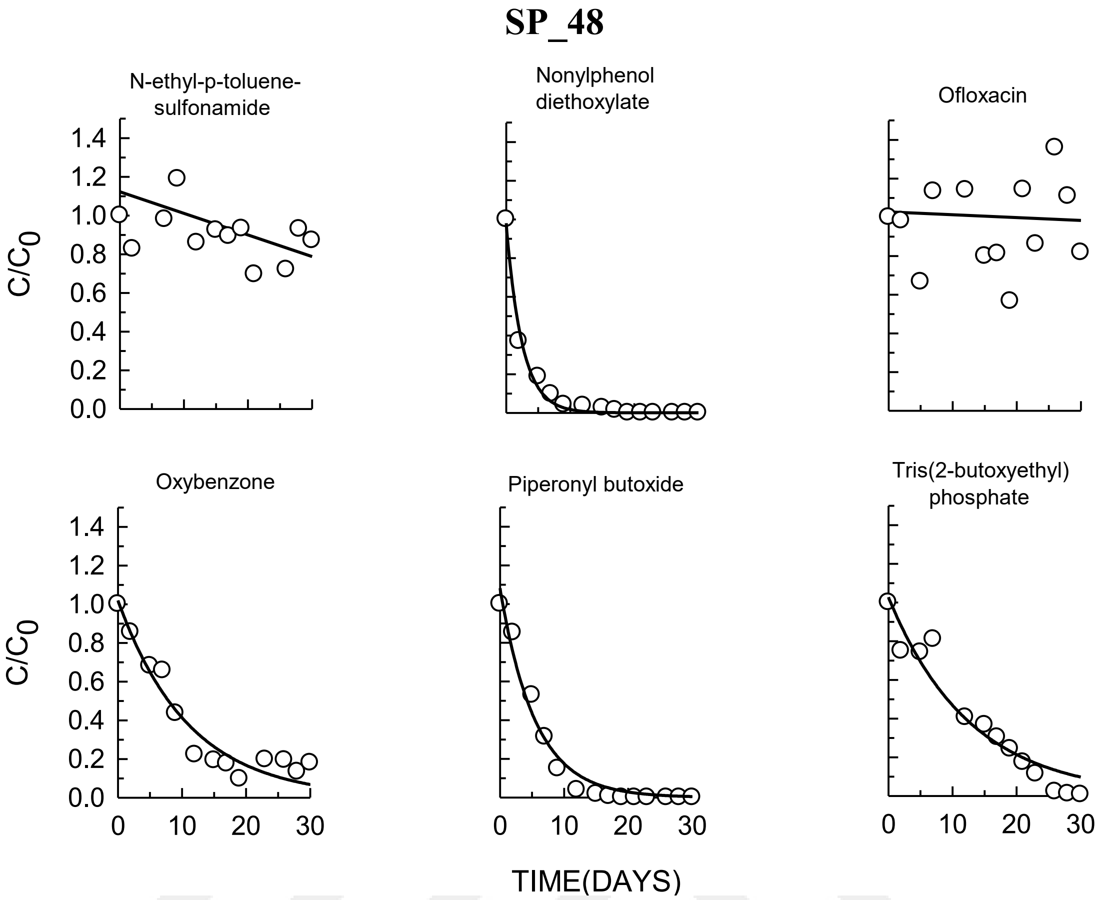

Piperonyl butoxide
Quinalphos
Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_48

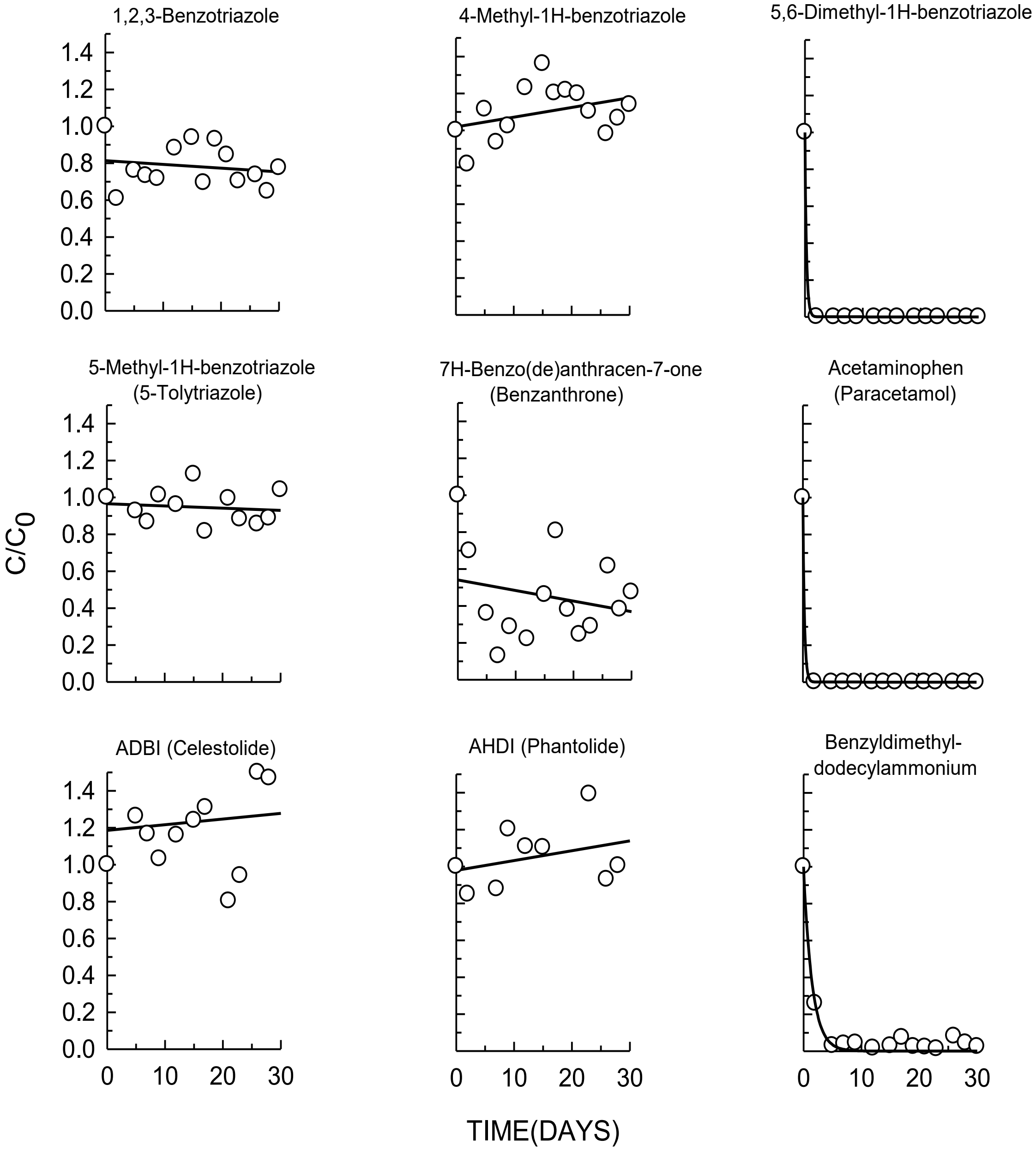

1,2,3-Benzotriazole
3-Chloroaniline
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
7H-Benzo(de)anthracen-7-one (Benzanthrone)
Acetaminophen (Paracetamol)
ADBI (Celestolide)
Benzyldimethyl-tetradecylammonium
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_48

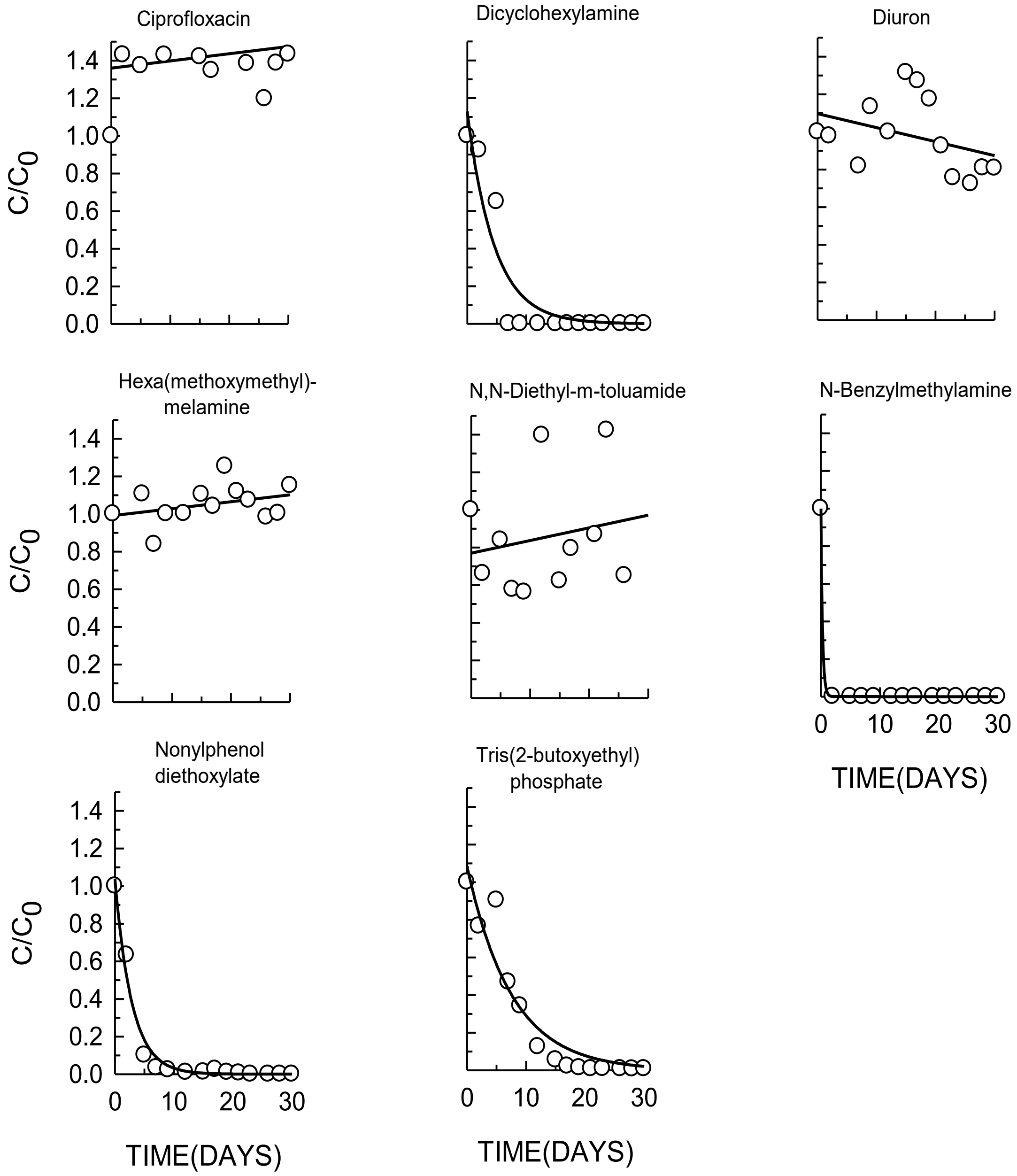

AHDI (Phantolide)
Benzyldimethyl-
dodecylammonium
Ciprofloxacin
Di(2- ethylhexyl)phthalate
(DEHP)
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)


SP_48
N-ethyl-p-toluene-sulfonamide
Nonylphenol diethoxylate
Ofloxacin
Oxybenzone
Piperonyl butoxide
Tris(2-butoxyethyl) phosphate
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

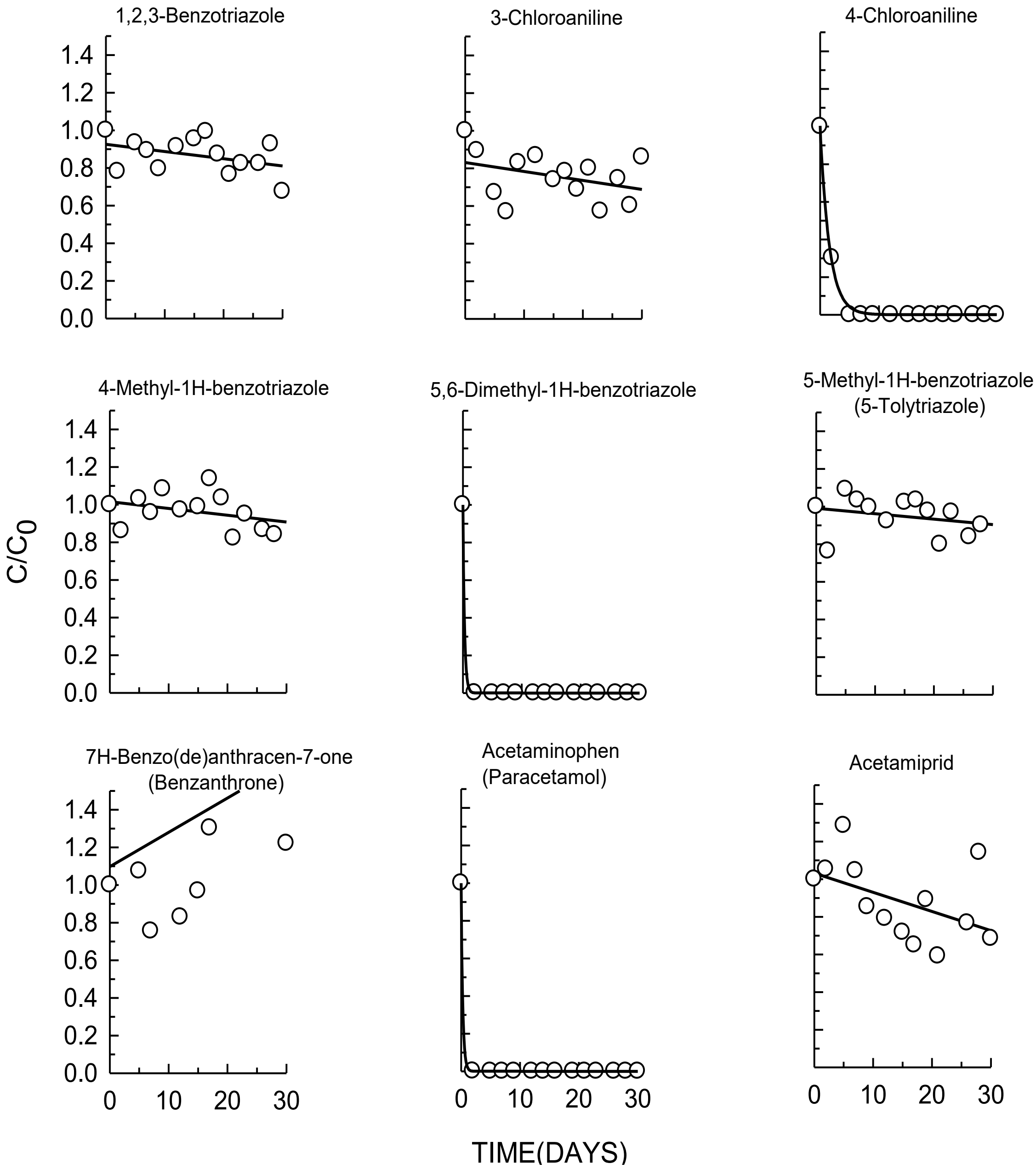
SP_50


1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
7H-Benzo(de)anthracen-7-one
(Benzanthrone)
Acetaminophen
(Paracetamol)
ADBI (Celestolide)
AHDI (Phantolide)
Benzyldimethyl-
dodecylammonium
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

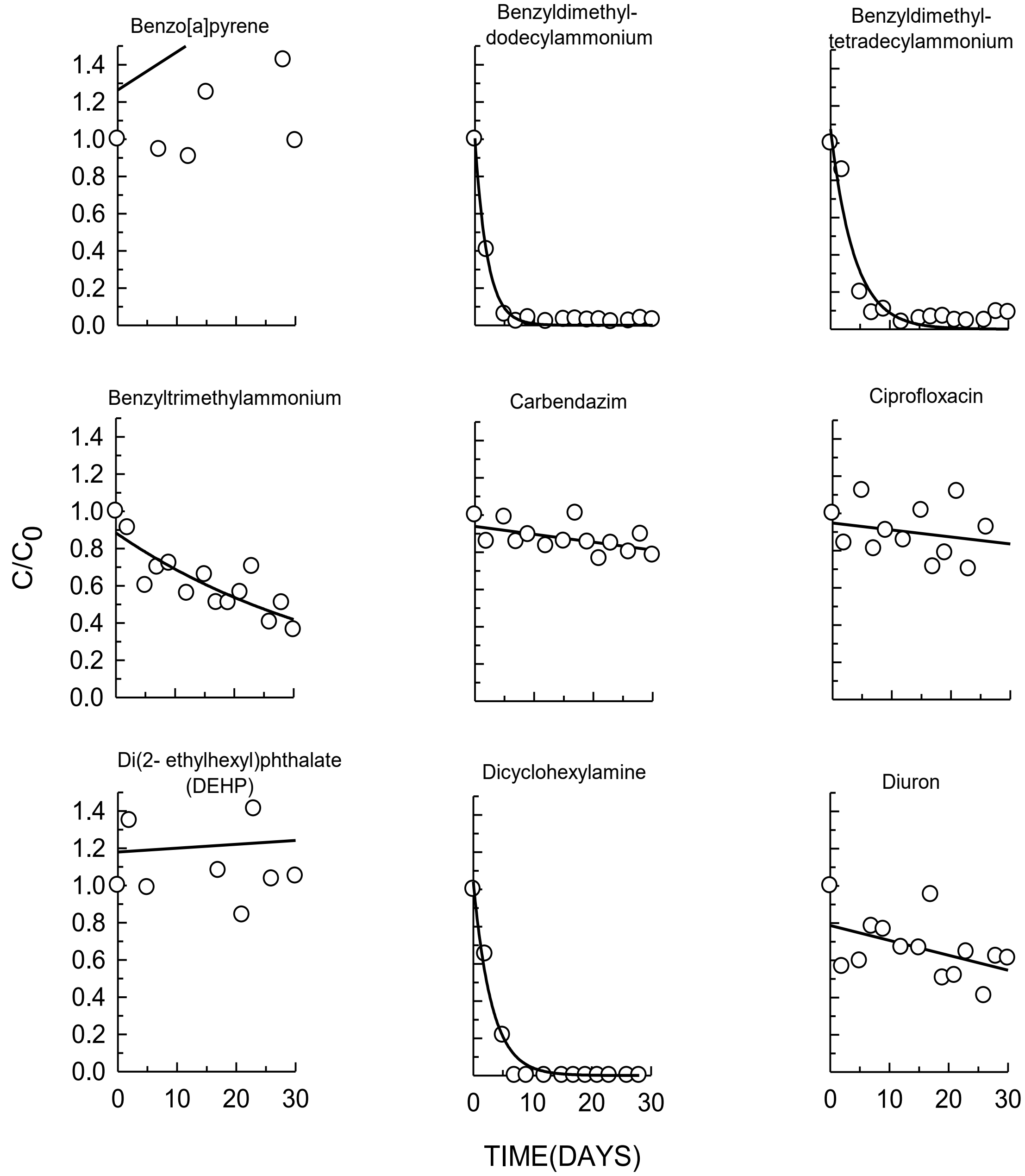
SP_50


Ciprofloxacin
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
N-Benzylmethylamine
Nonylphenol
diethoxylate
Tris(2-butoxyethyl)
phosphate
C/C0
TIME(DAYS)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30

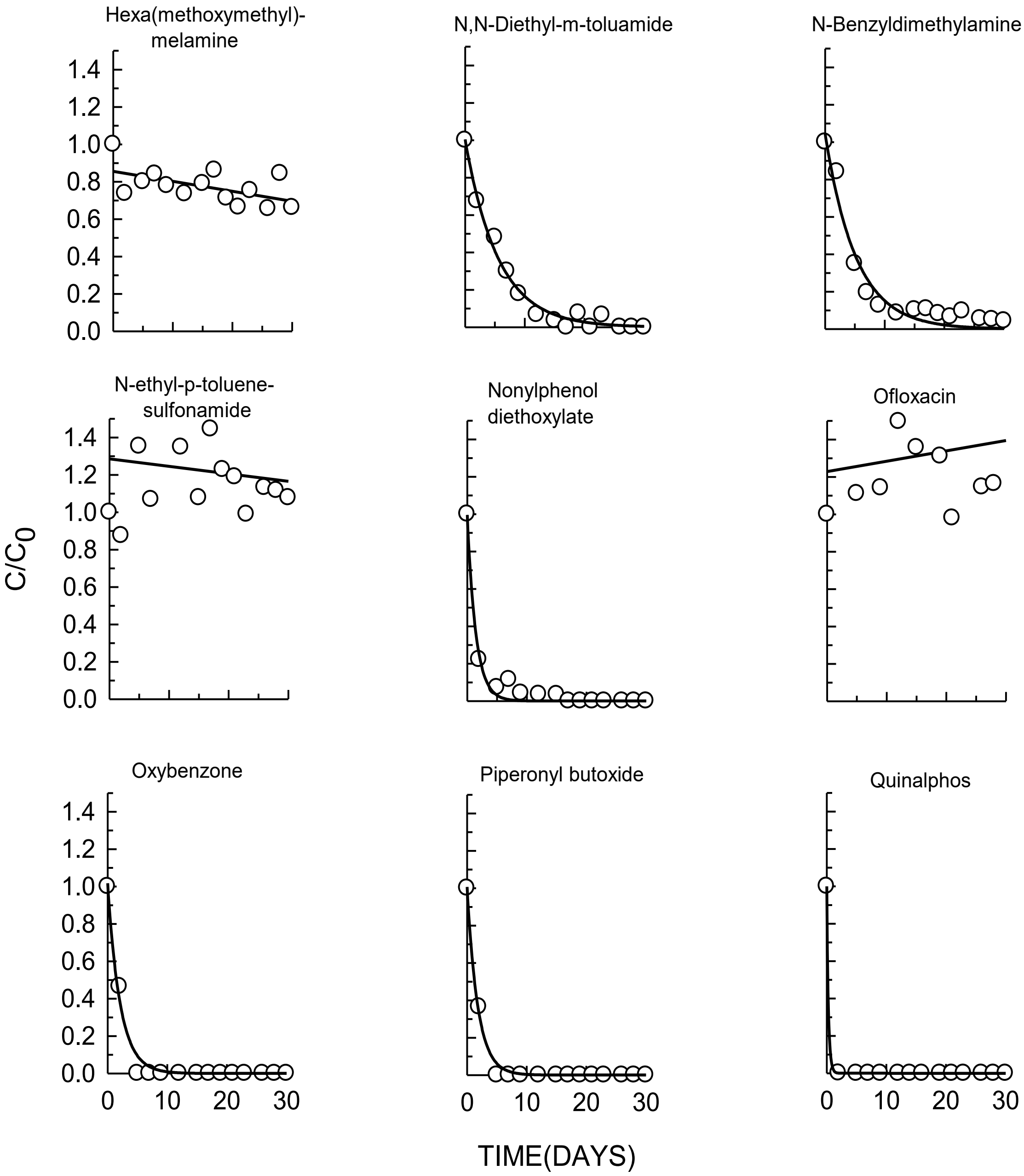
SP_54


1,2,3-Benzotriazole
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
7H-Benzo(de)anthracen-7-one (Benzanthrone)
Acetaminophen (Paracetamol)
Acetamiprid
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

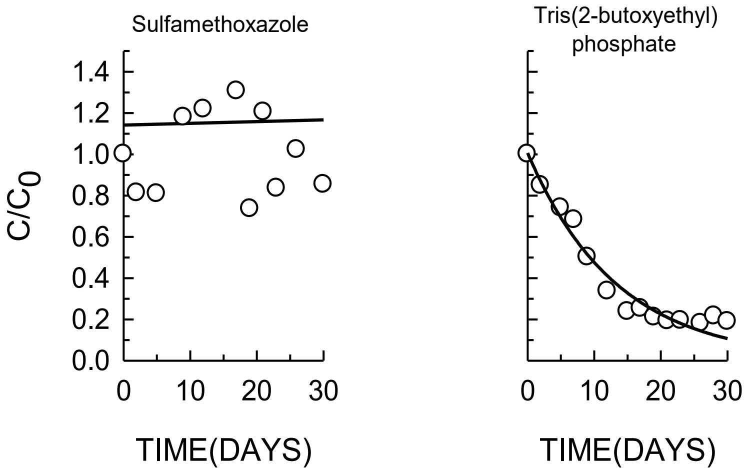
SP_54


Benzo[a]pyrene
Benzyldimethyl-
dodecylammonium
Benzyldimethyl-
tetradecylammonium
Benzyltrimethylammonium
Carbendazim
Ciprofloxacin
Di(2- ethylhexyl)phthalate
(DEHP)
Dicyclohexylamine
Diuron
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

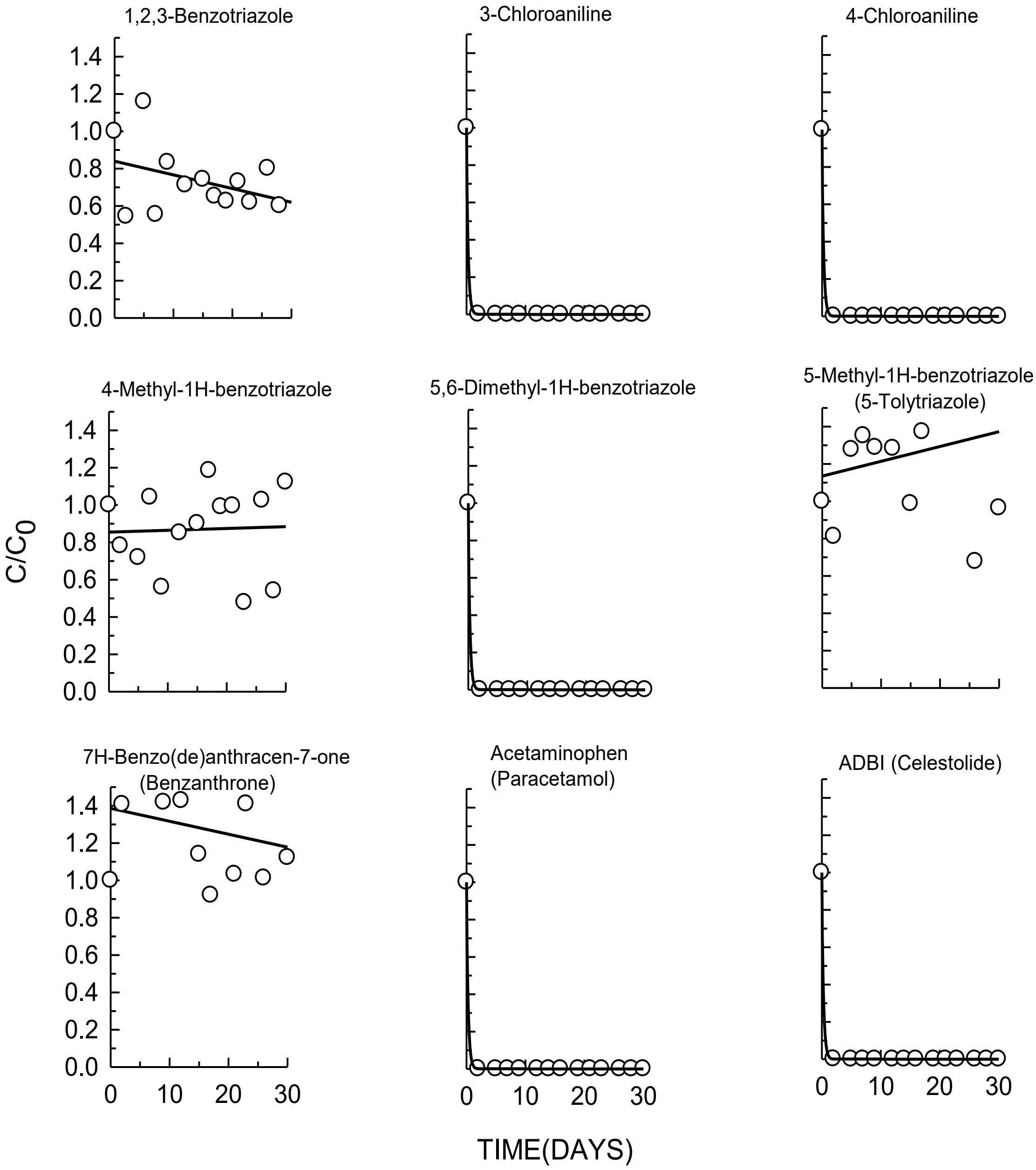
SP_54


Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
N-ethyl-p-toluene-
sulfonamide
Nonylphenol
diethoxylate
Ofloxacin
Oxybenzone
Piperonyl butoxide
Quinalphos
C/C_0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

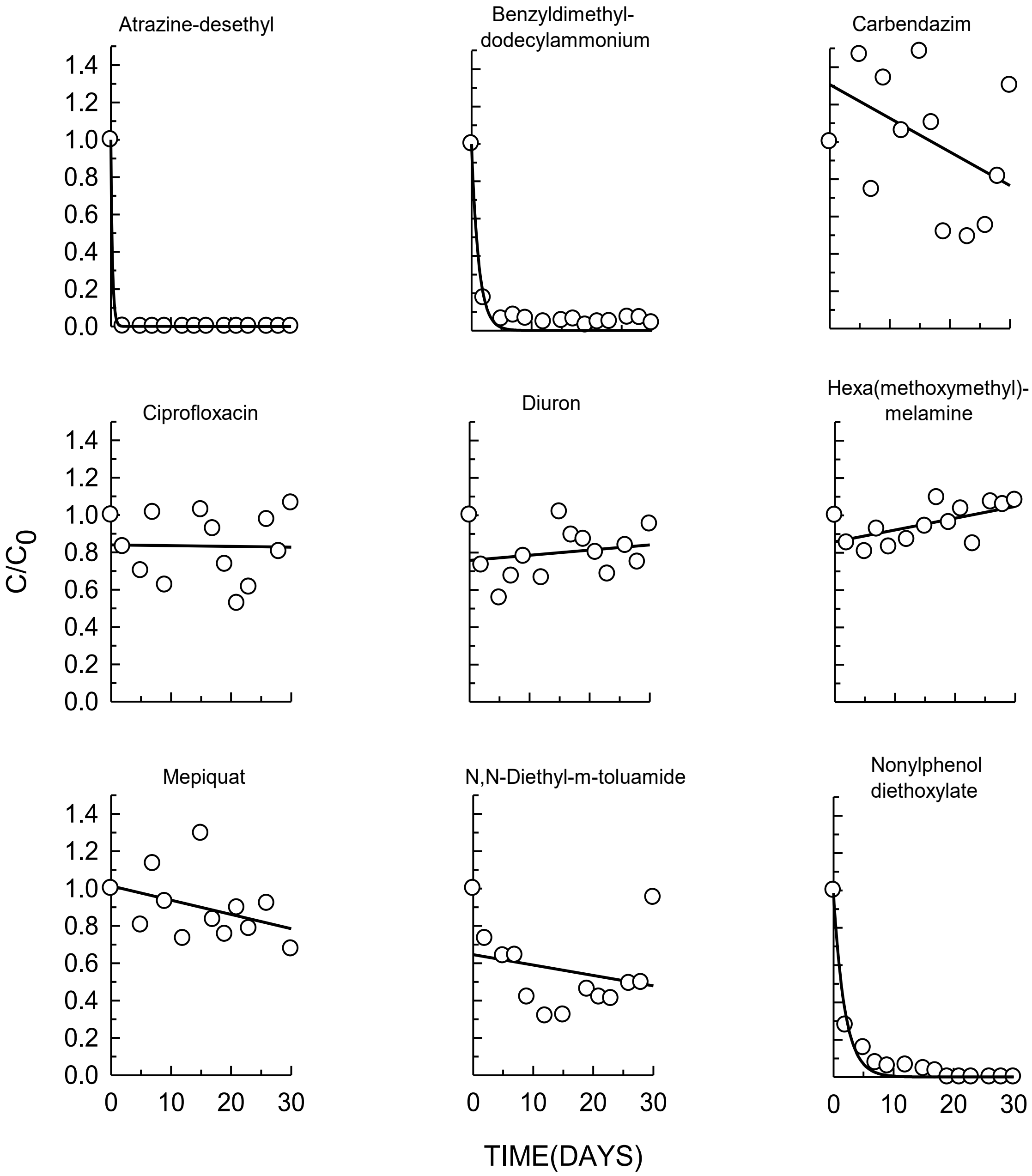
SP_54


Sulfamethoxazole
Tris(2-butoxyethyl) phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
TIME(DAYS)

SP_55

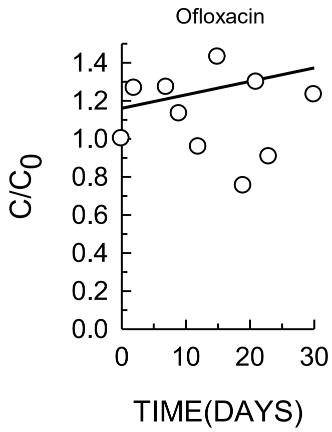

1,2,3-Benzotriazole
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5,6-Dimethyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
7H-Benzo(de)anthracen-7-one (Benzanthrone)
Acetaminophen (Paracetamol)
ADBI (Celestolide)
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_55

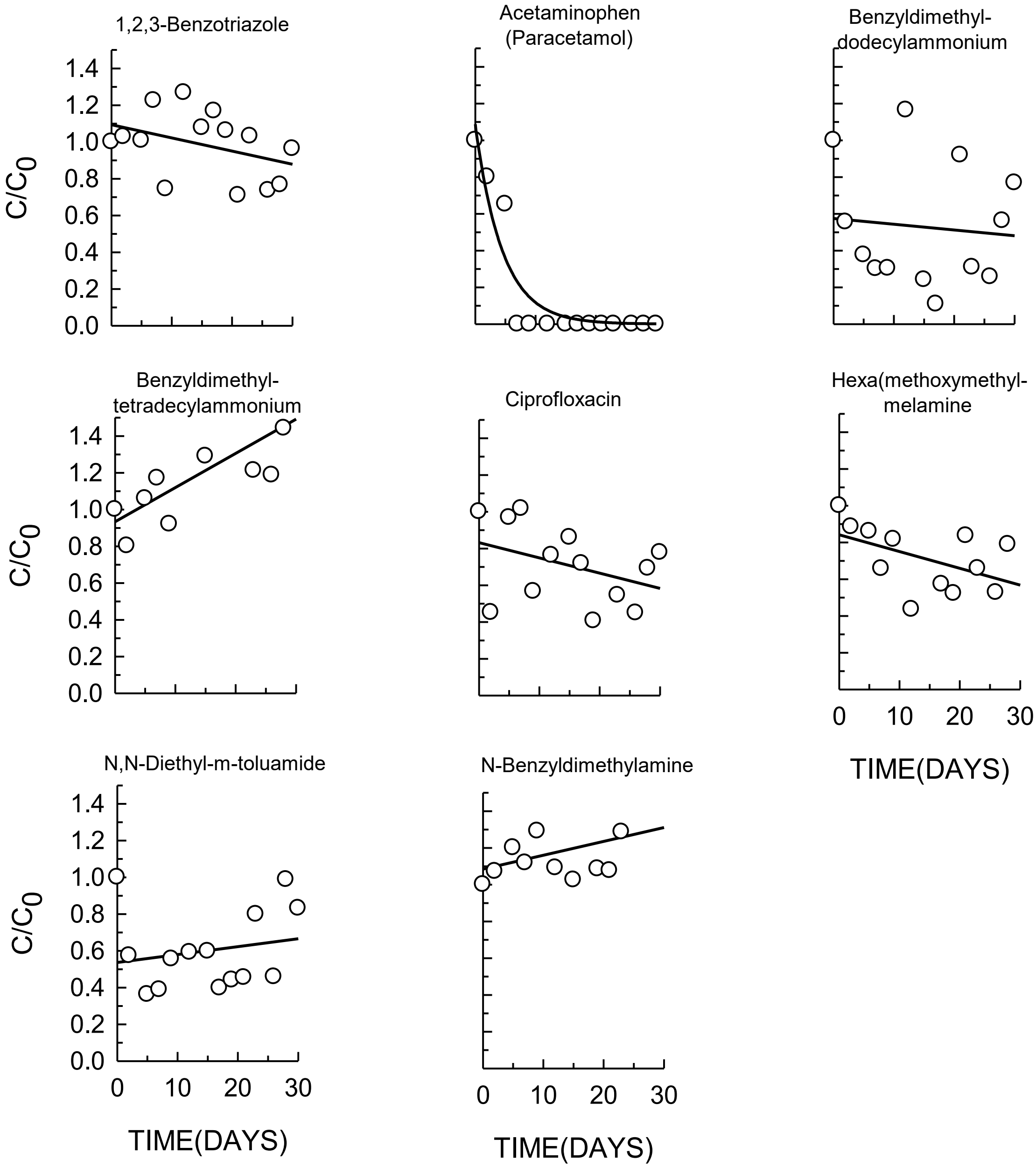


Atrazine-desethyl
Benzyldimethyl-dodecylammonium
Carbendazim
Ciprofloxacin
Diuron
Hexa(methoxymethyl)-melamine
Mepiquat
N,N-Diethyl-m-toluamide
Nonylphenol diethoxylate
C/C0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

SP_55

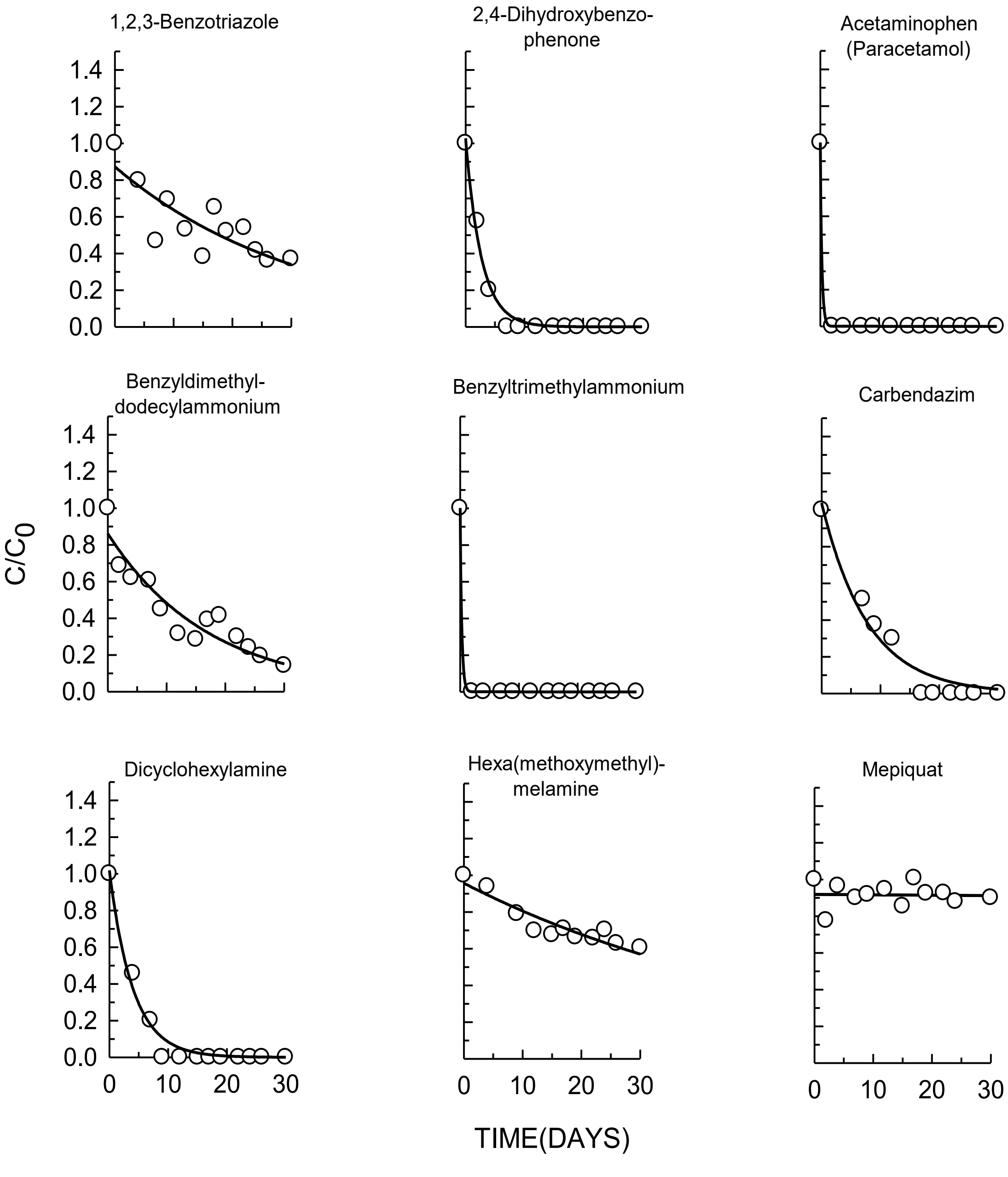


SP_65

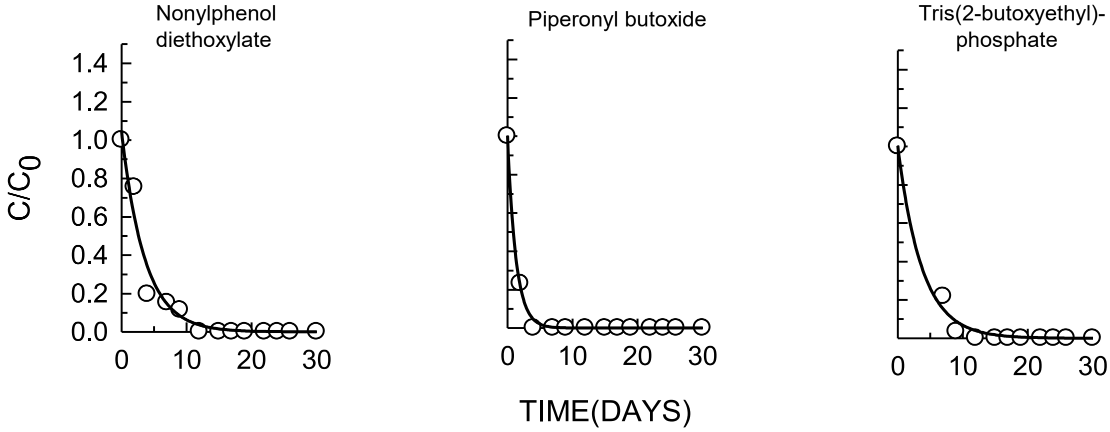

1,2,3-Benzotriazole
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Ciprofloxacin
Hexa(methoxymethyl-melamine
N,N-Diethyl-m-toluamide
N-Benzyldimethylamine
C/C_0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

APPENDIX F: BIODEGRADATION GRAPHICS OF FEBRUARY 2018 SAMPLES

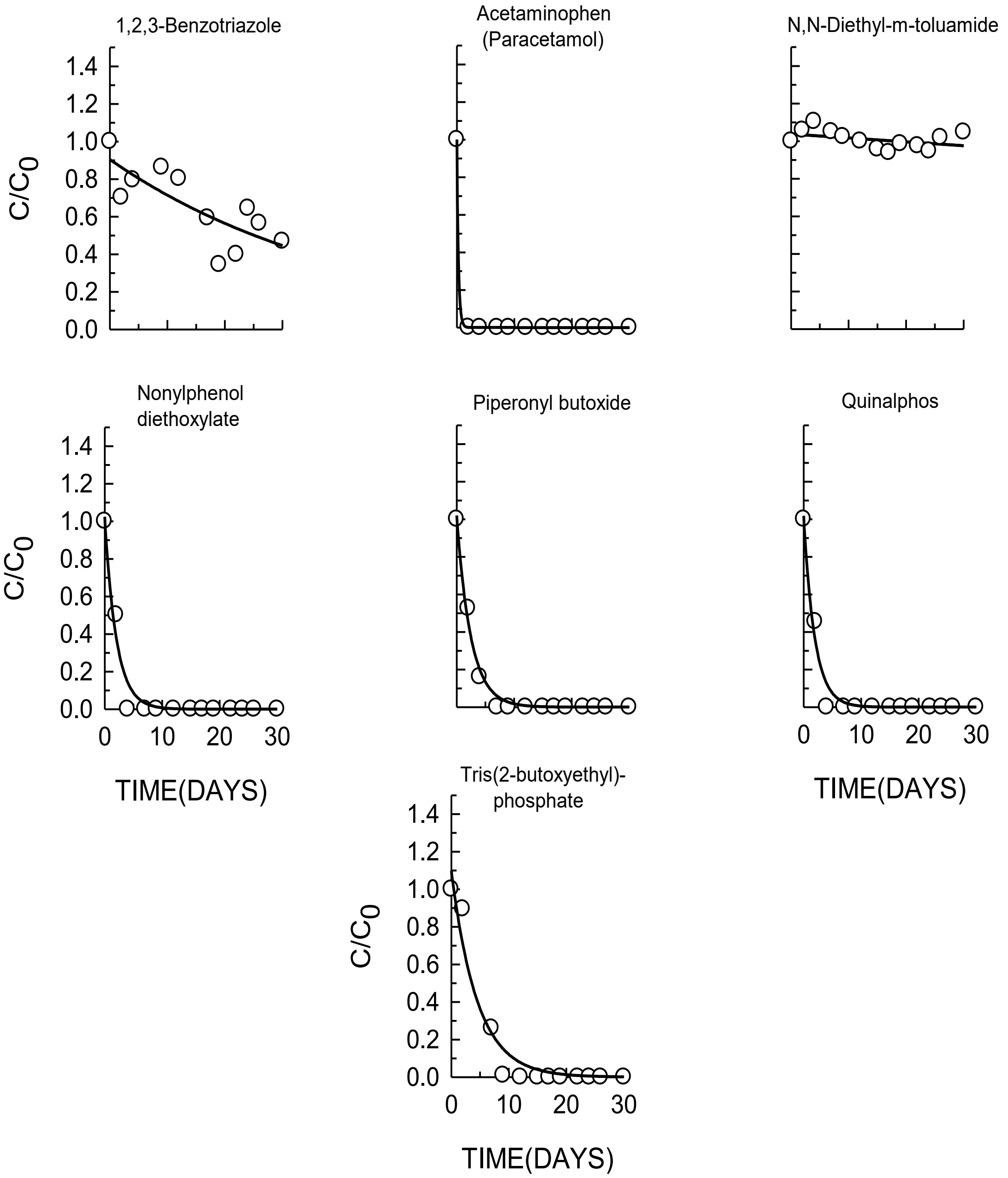
SP_4



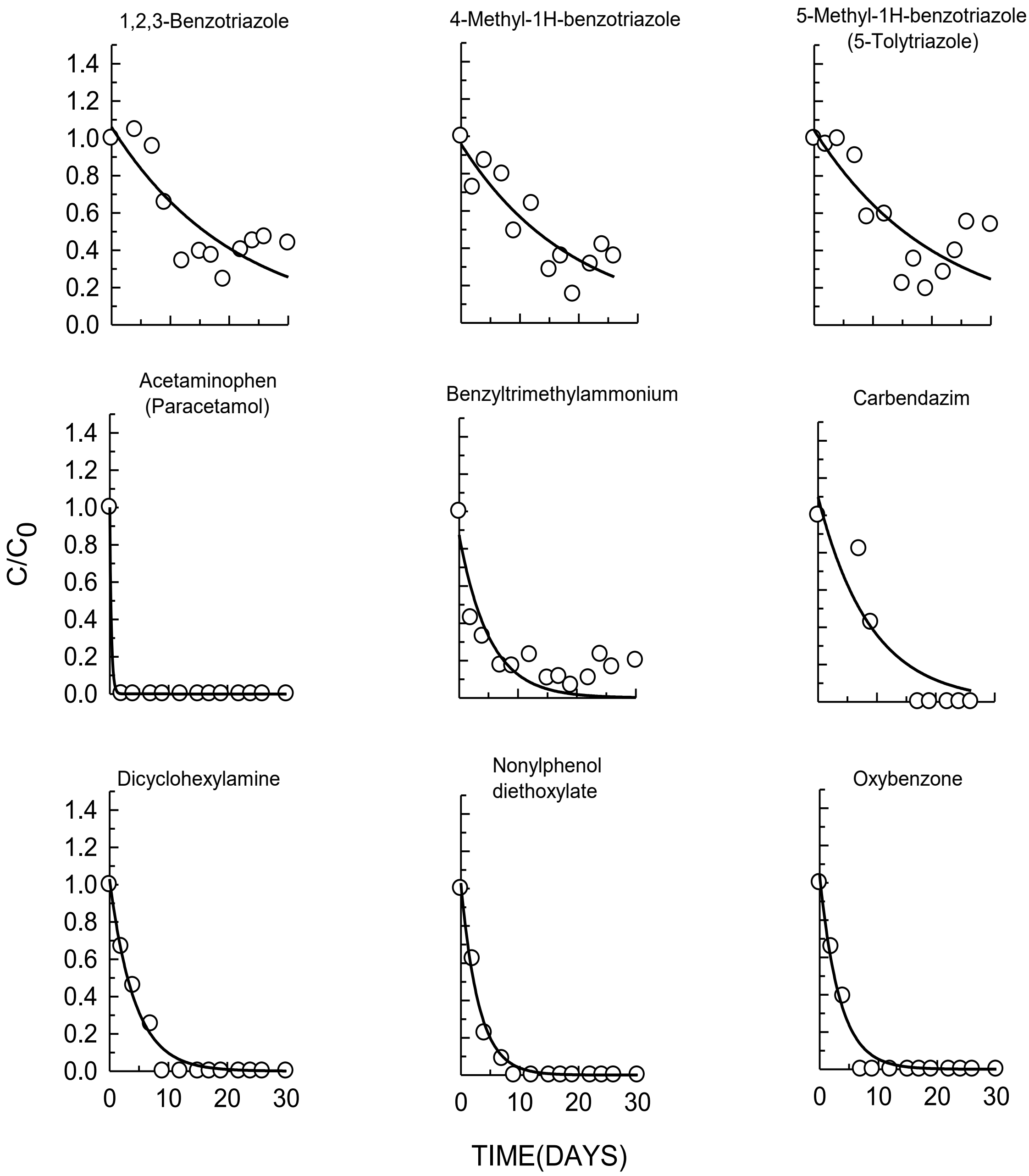
SP_4


Nonylphenol diethoxylate
Piperonyl butoxide
Tris(2-butoxyethyl)-phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_8

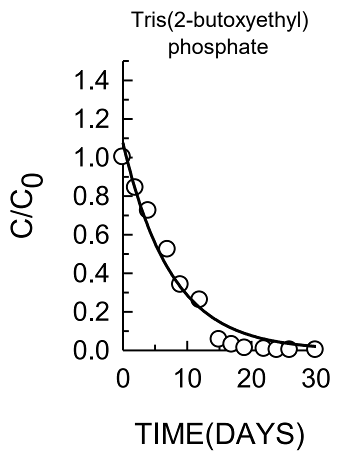

1,2,3-Benzotriazole
Acetaminophen
(Paracetamol)
N,N-Diethyl-m-toluamide
Nonylphenol
diethoxylate
Piperonyl butoxide
Quinalphos
Tris(2-butoxyethyl)-
phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_28

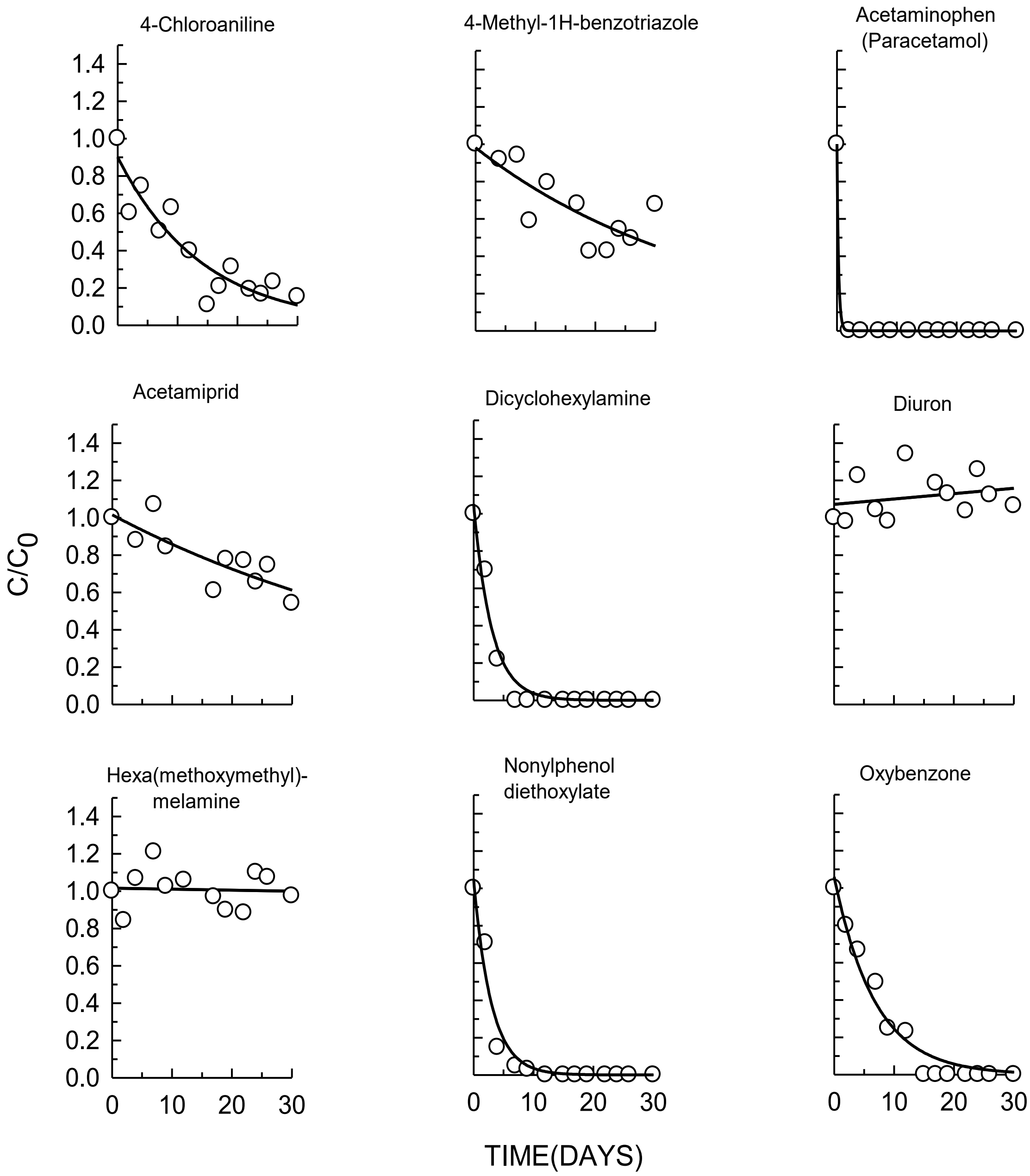


1,2,3-Benzotriazole
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
Benzyltrimethylammonium
Carbendazim
Dicyclohexylamine
Nonylphenol
diethoxylate
Oxybenzone
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

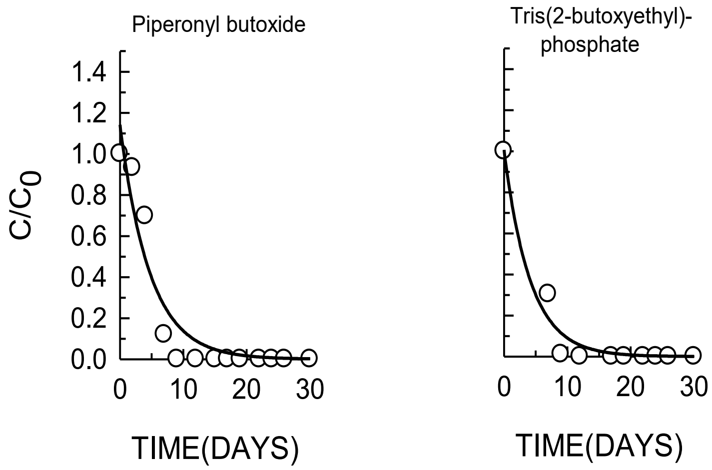
SP_28


Tris(2-butoxyethyl)
phosphate
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C/C0
0
10
20
30
TIME(DAYS)

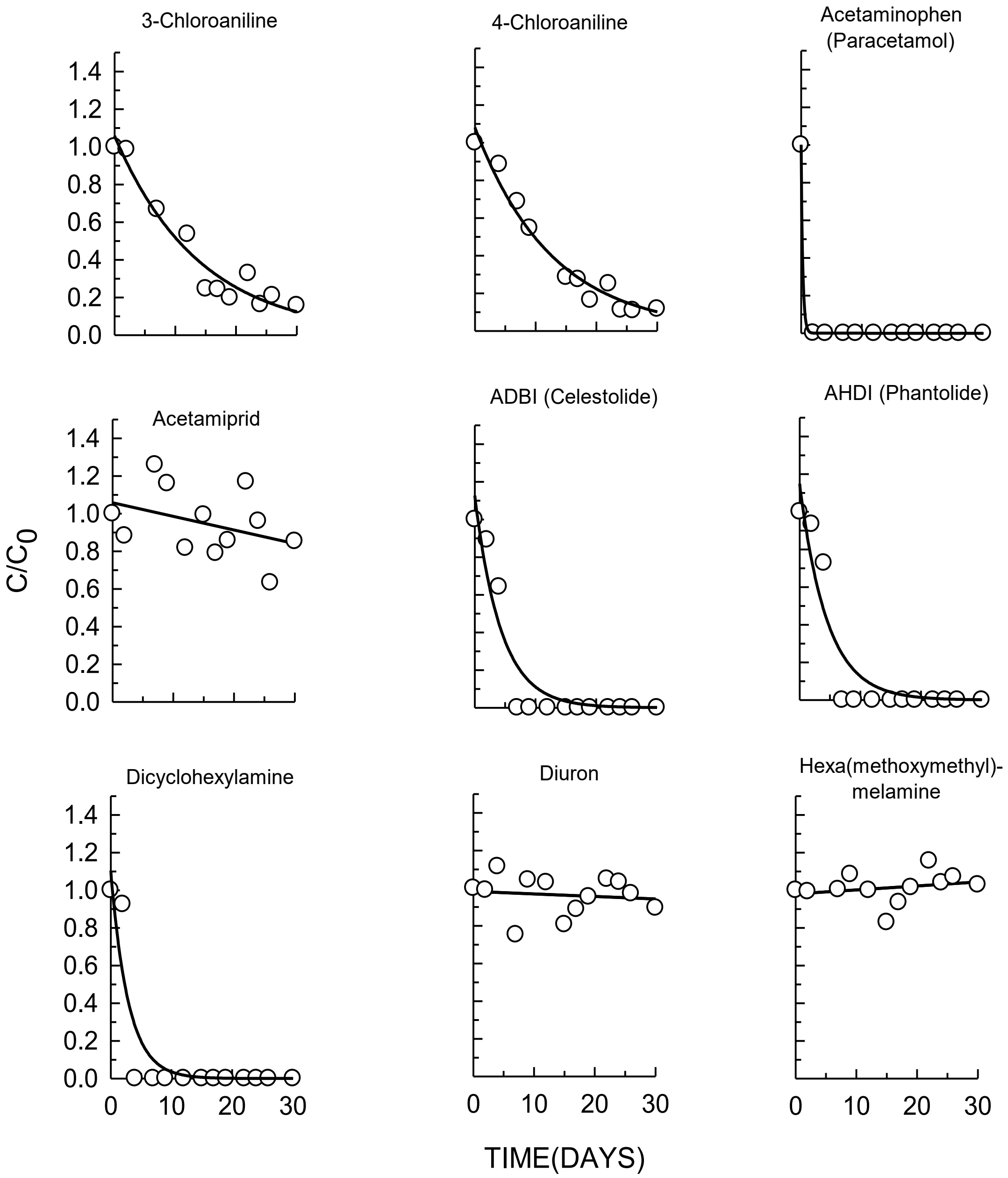
SP_48

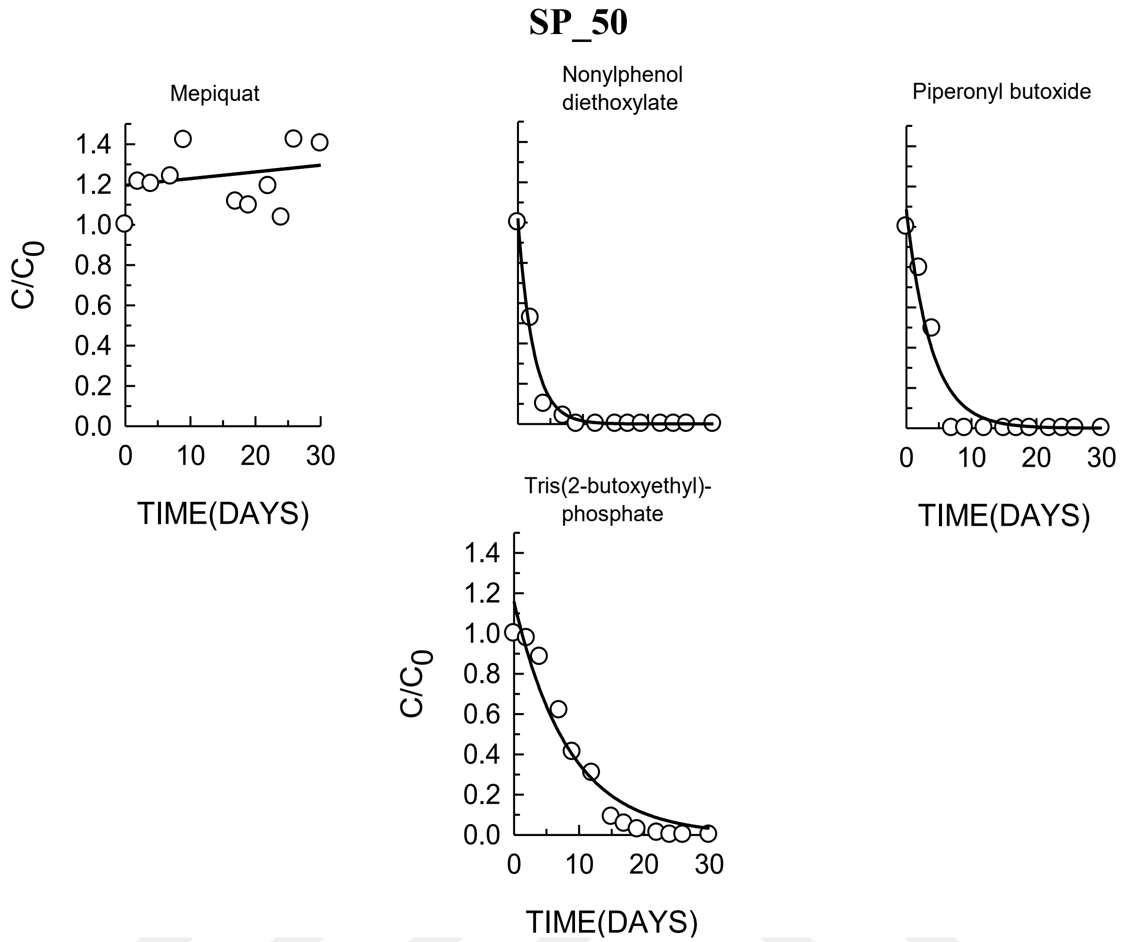

4-Chloroaniline
4-Methyl-1H-benzotriazole
Acetaminophen
(Paracetamol)
Acetamiprid
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
Nonylphenol
diethoxylate
Oxybenzone
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_48


Piperonyl butoxide
Tris(2-butoxyethyl)-phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
TIME(DAYS)

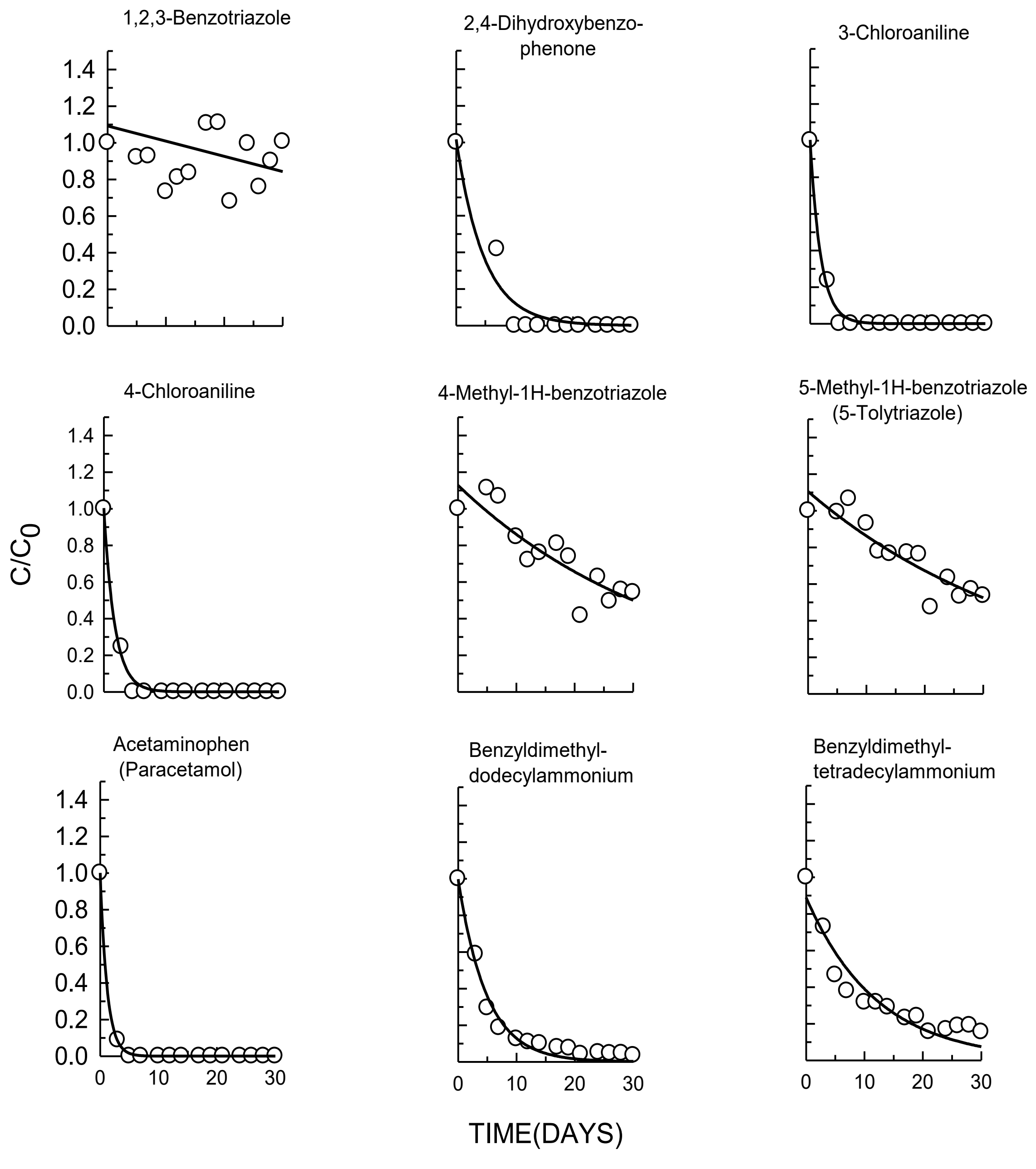
SP_50


3-Chloroaniline
4-Chloroaniline
Acetaminophen (Paracetamol)
Acetamiprid
ADBI (Celestolide)
AHDI (Phantolide)
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)


SP_50
Mepiquat
Nonylphenol diethoxylate
Piperonyl butoxide
Tris(2-butoxyethyl)-phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

APPENDIX G: BIODEGRADATION GRAPHICS OF MAY 2018 SAMPLES

SP_4



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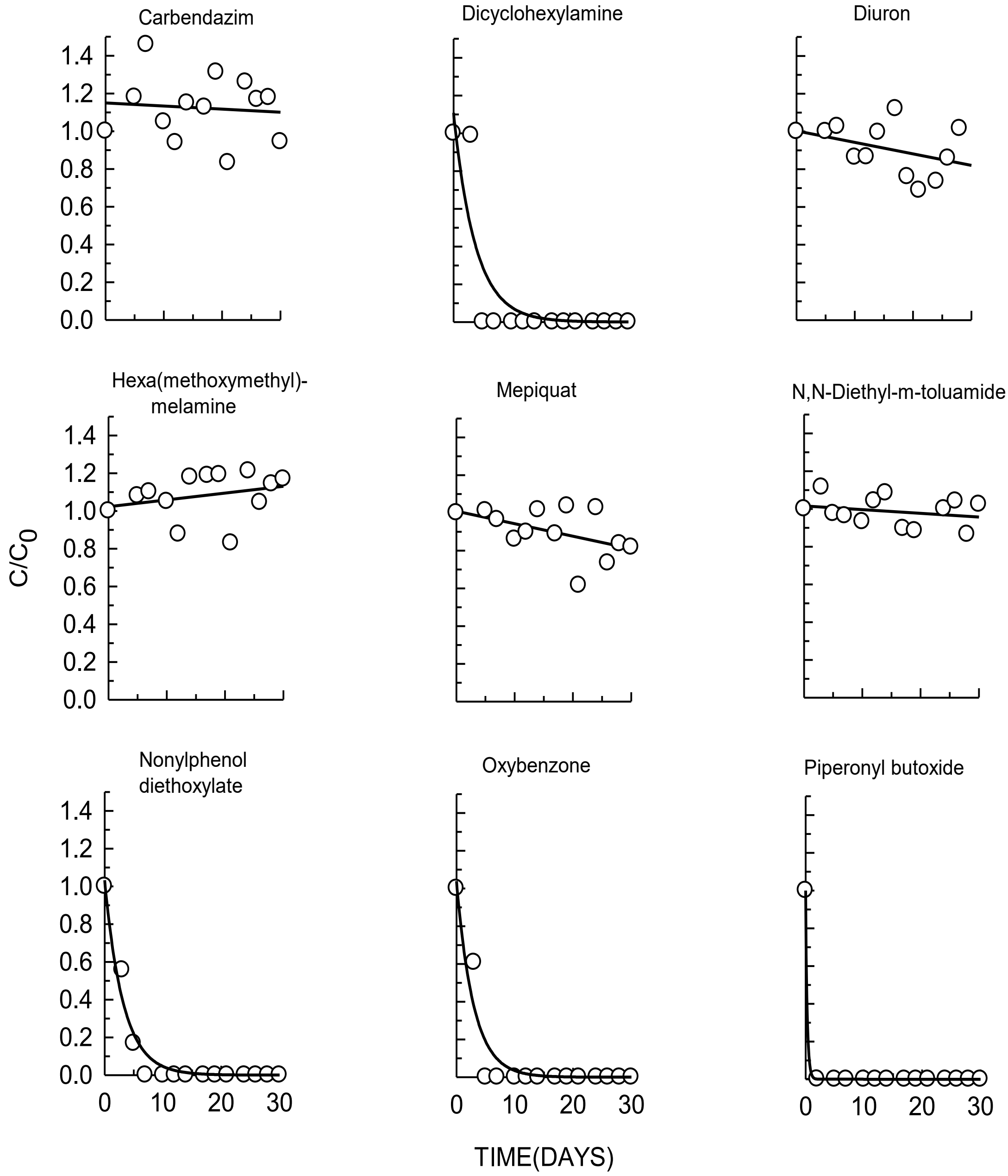
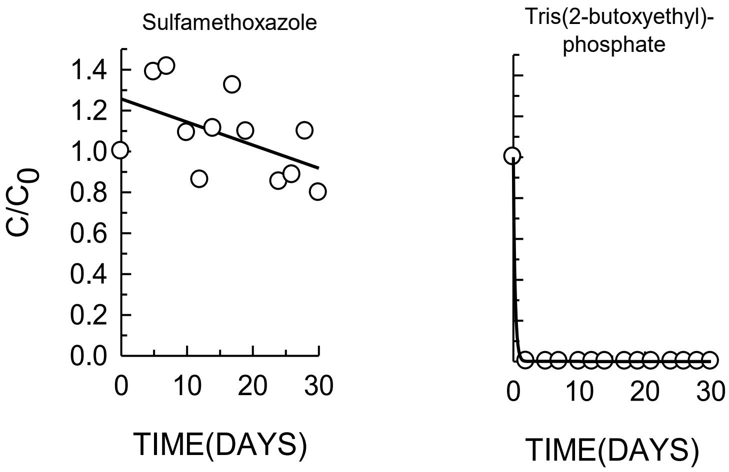
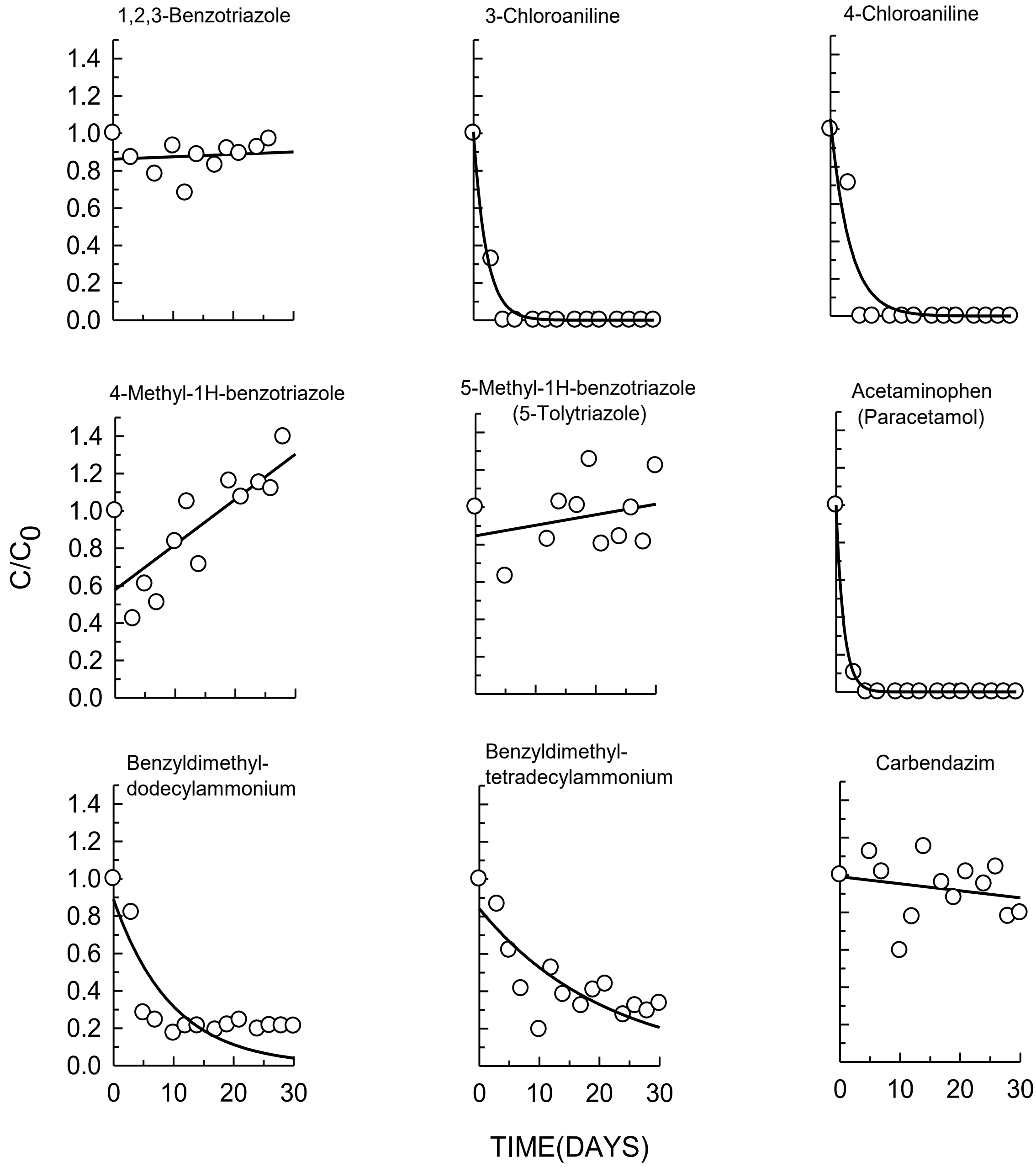

Carbendazim
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
Mepiquat
N,N-Diethyl-m-toluamide
Nonylphenol diethoxylate
Oxybenzone
Piperonyl butoxide
C/C0
TIME(DAYS)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30

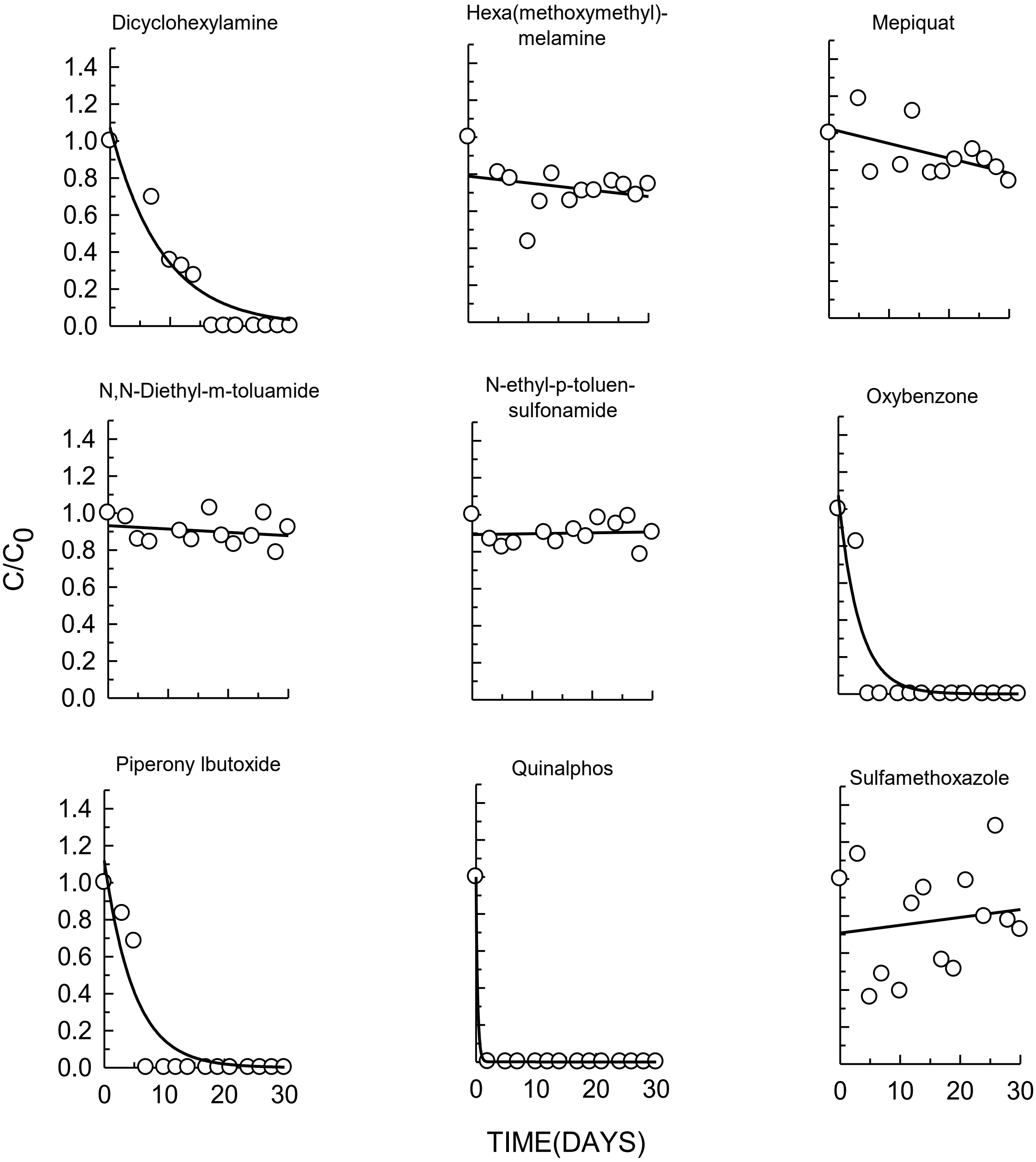
SP_4


Sulfamethoxazole
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
Tris(2-butoxyethyl)-
phosphate
0
10
20
30
TIME(DAYS)

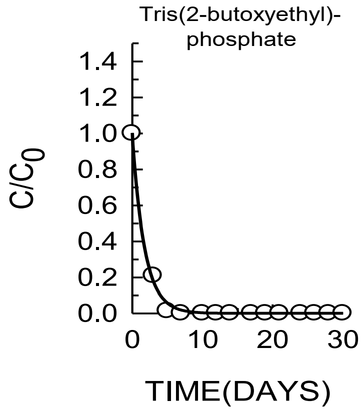
SP_8


1,2,3-Benzotriazole
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Carbendazim
C/C_0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
TIME(DAYS)

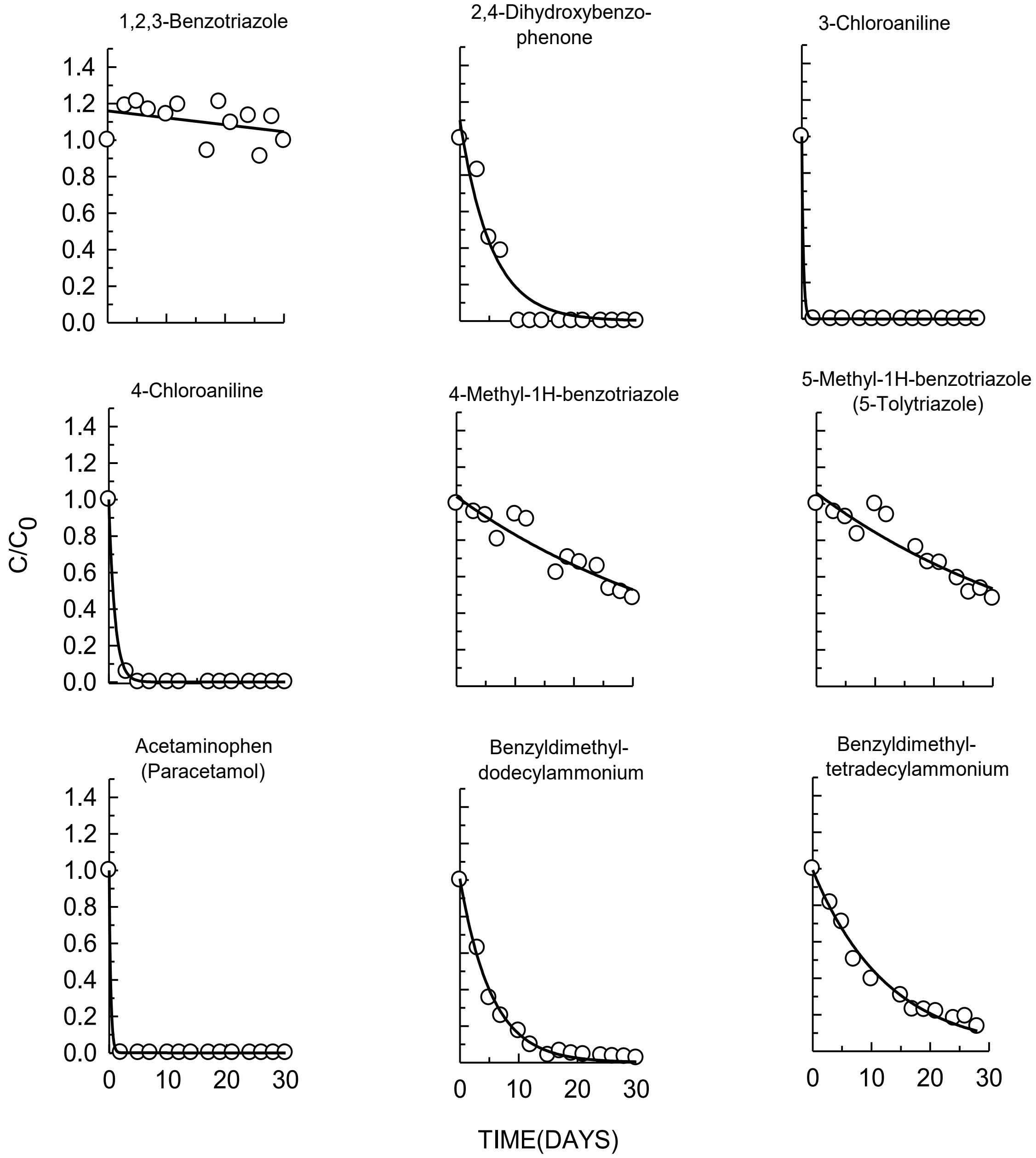
SP_8


Dicyclohexylamine
Hexa(methoxymethyl)-melamine
Mepiquat
N,N-Diethyl-m-toluamide
N-ethyl-p-toluen-sulfonamide
Oxybenzone
Piperony lbutoxide
Quinalphos
Sulfamethoxazole
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_8


Tris(2-butoxyethyl)-
phosphate
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C/C0
0
10
20
30
TIME(DAYS)

SP_28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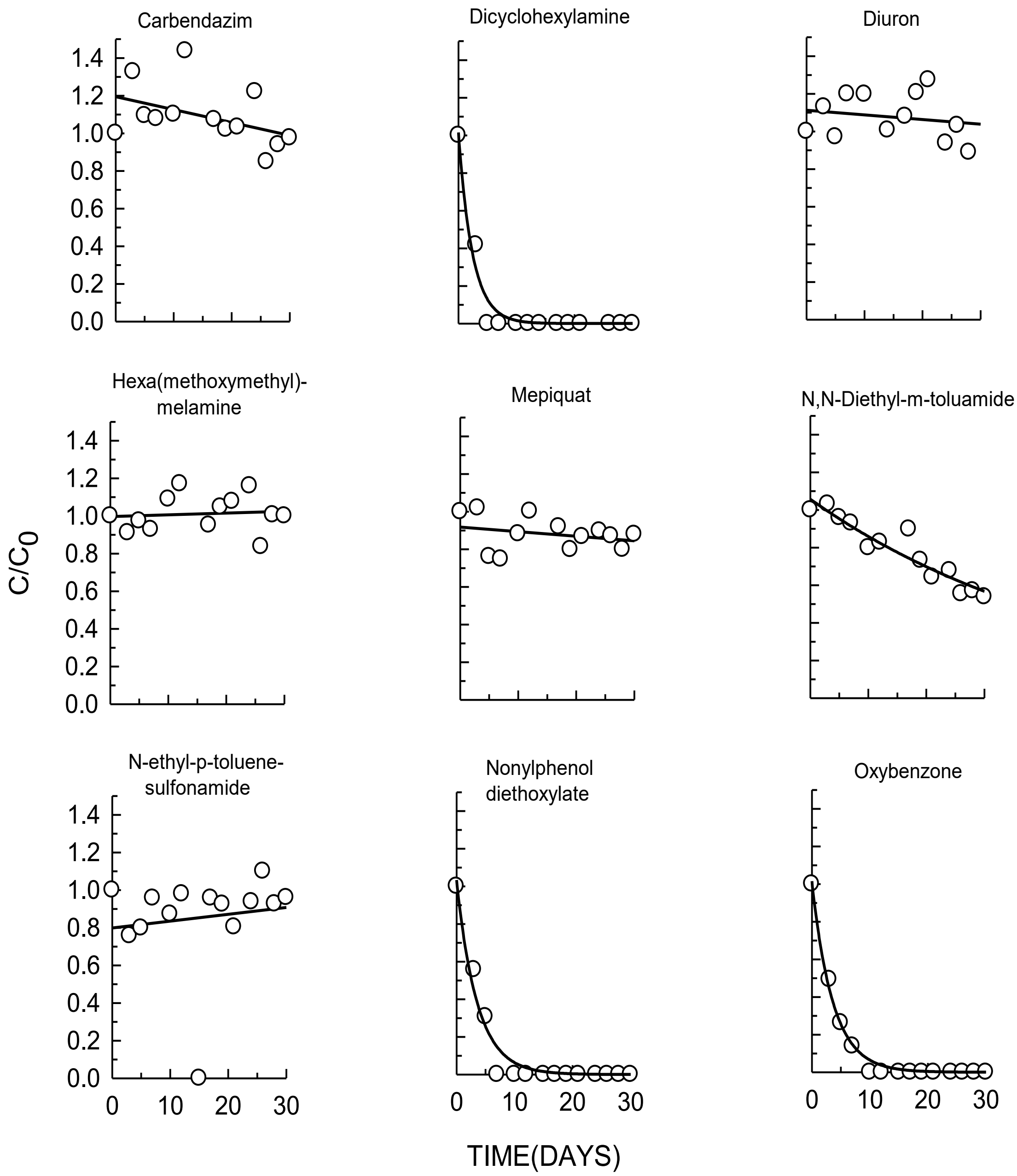
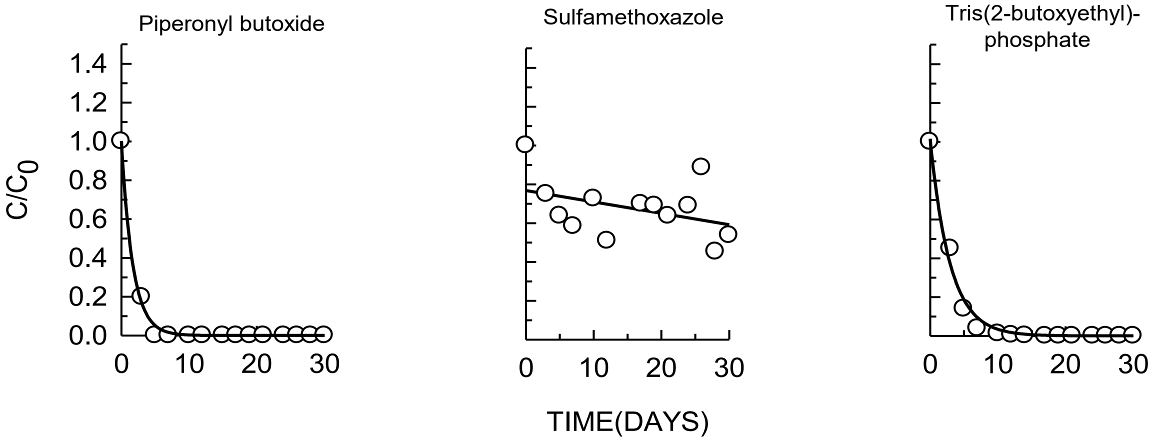
2,4-Dihydroxybenzo-phenone
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole (5-Tolytriazole)
Acetaminophen (Paracetamol)
Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

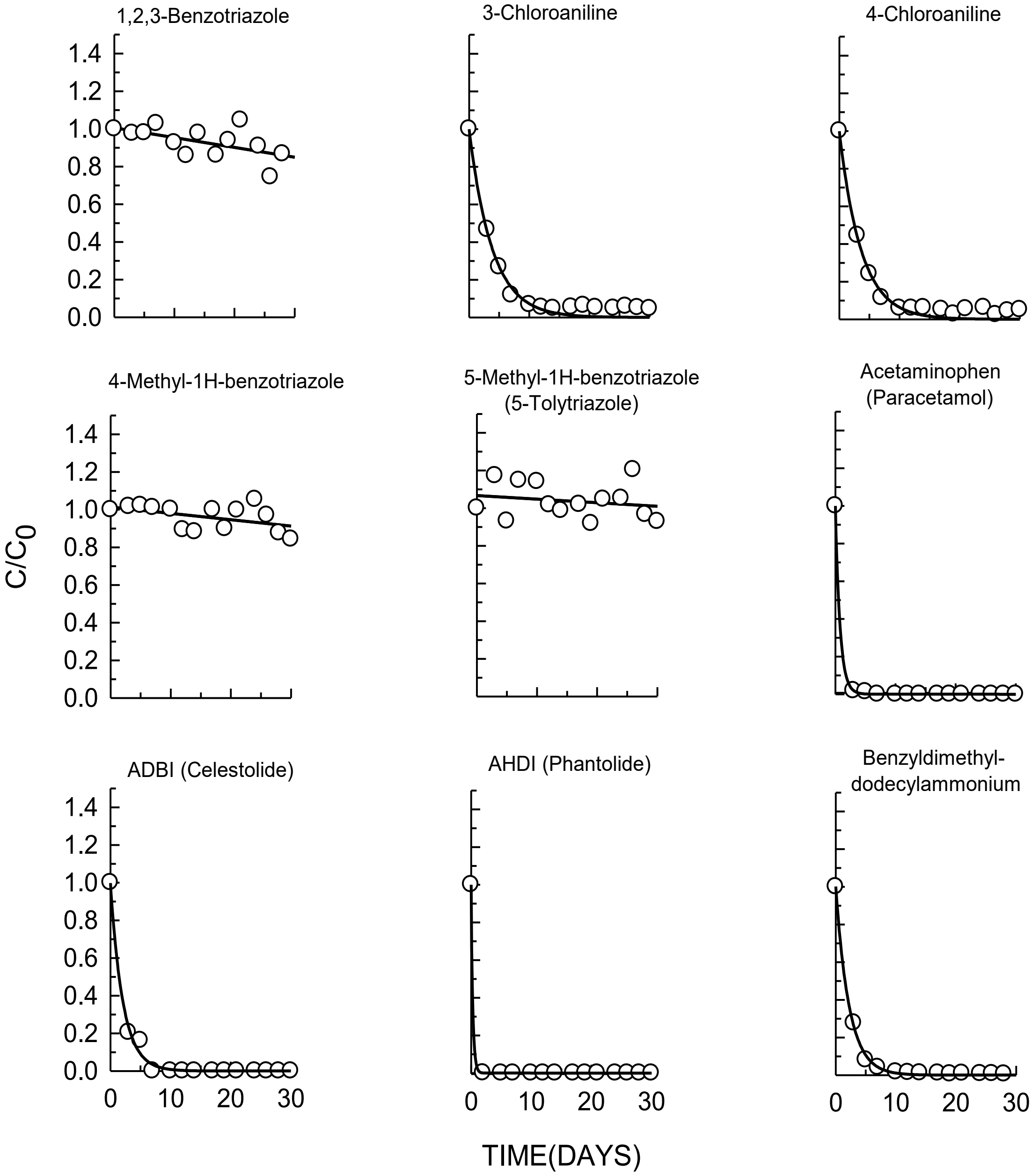
SP_28


Carbendazim
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
Mepiquat
N,N-Diethyl-m-toluamide
N-ethyl-p-toluene-
sulfonamide
Nonylphenol
diethoxylate
Oxybenzone
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

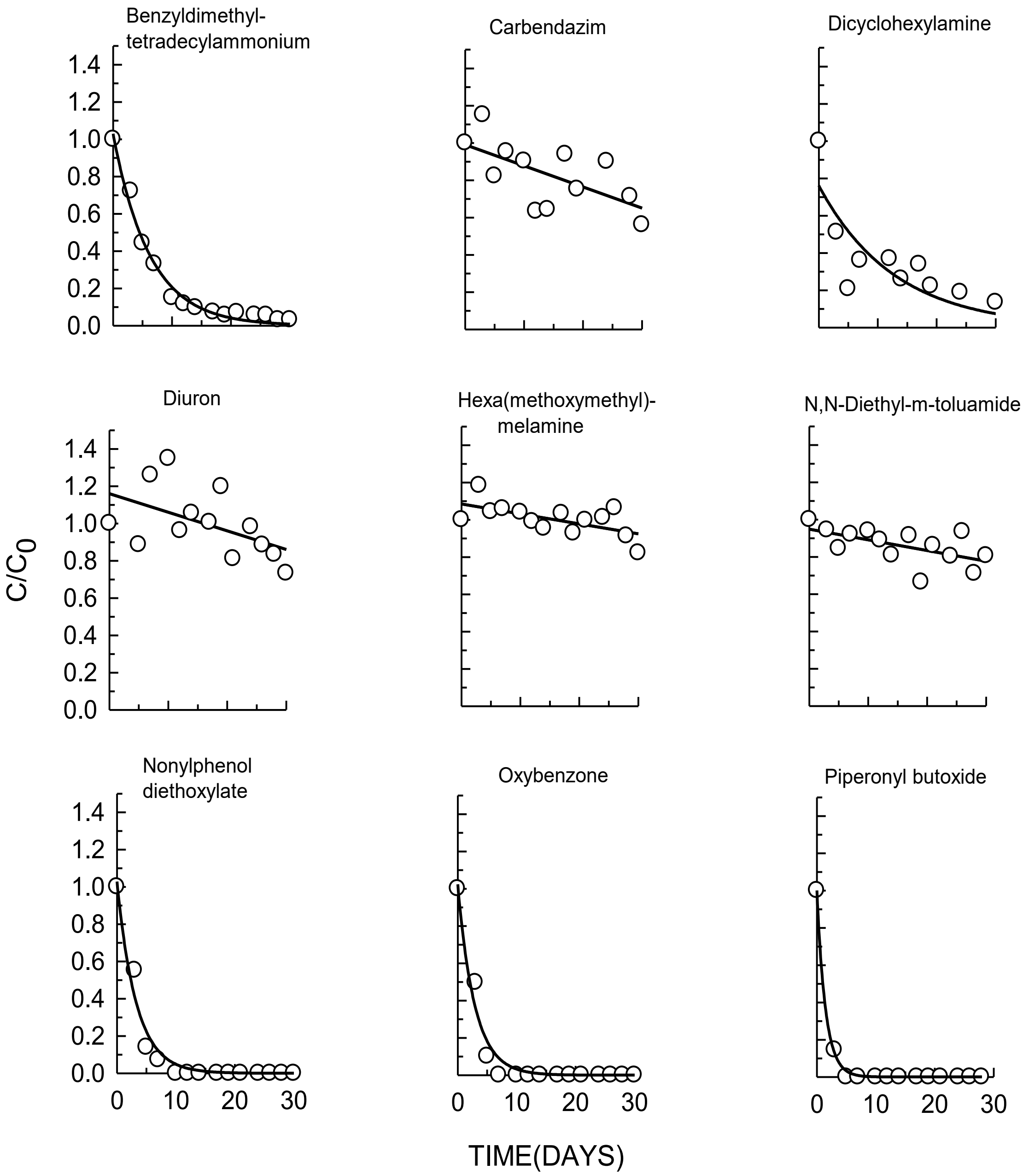
SP_28


Piperonyl butoxide
Sulfamethoxazole
Tris(2-butoxyethyl)-phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

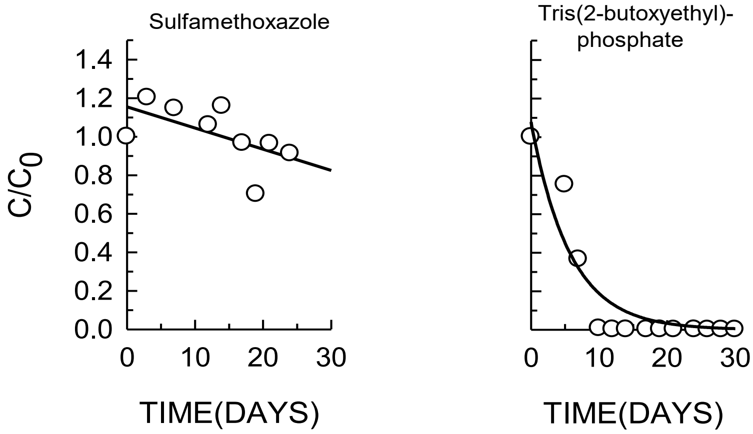
SP_48


1,2,3-Benzotriazole
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
ADBI (Celestolide)
AHDI (Phantolide)
Benzyldimethyl-
dodecylammonium
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

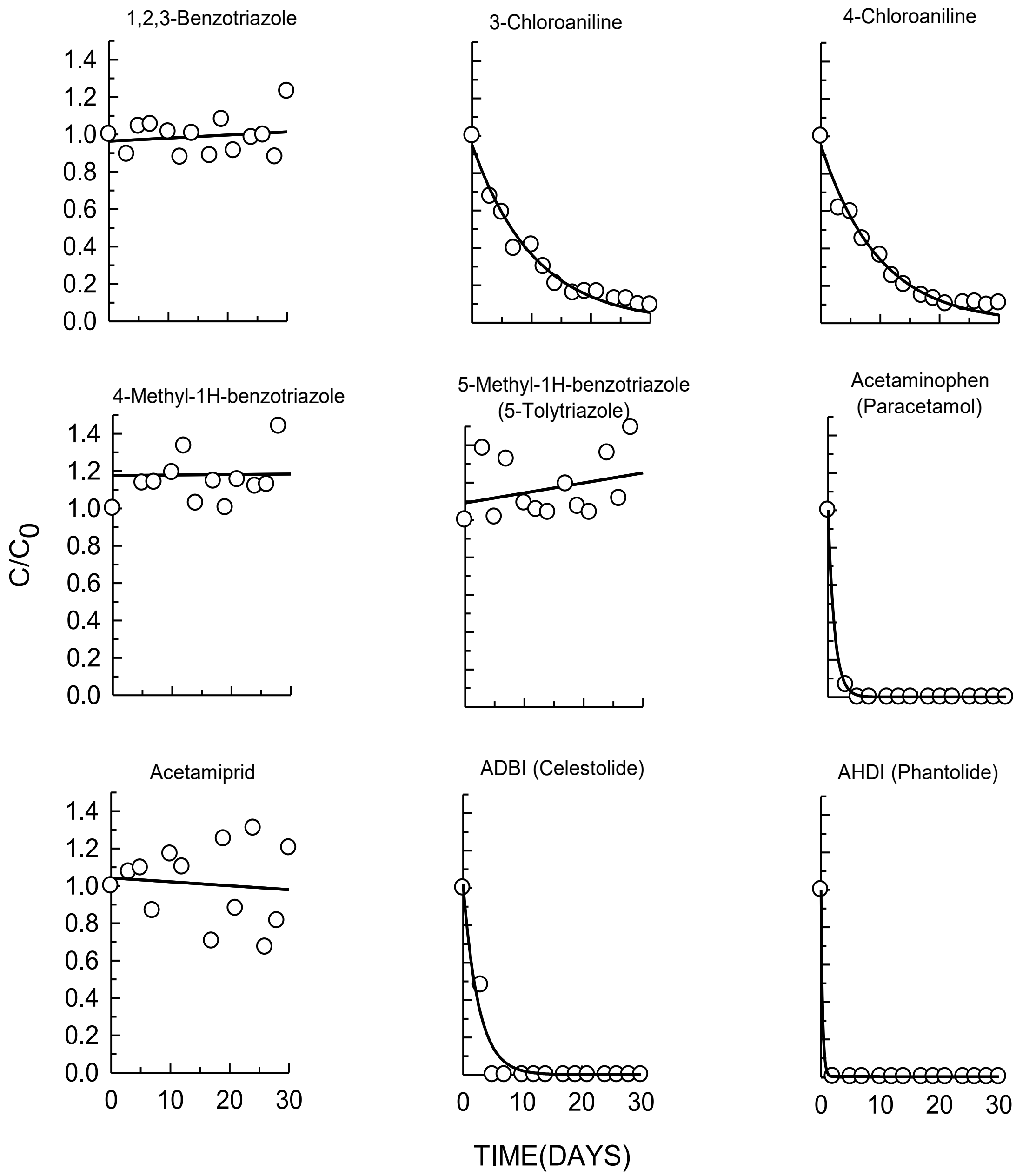
SP_48


Benzyldimethyl-
tetradecylammonium
Carbendazim
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-
melamine
N,N-Diethyl-m-toluamide
Nonylphenol
diethoxylate
Oxybenzone
Piperonyl butoxide
C/C_0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

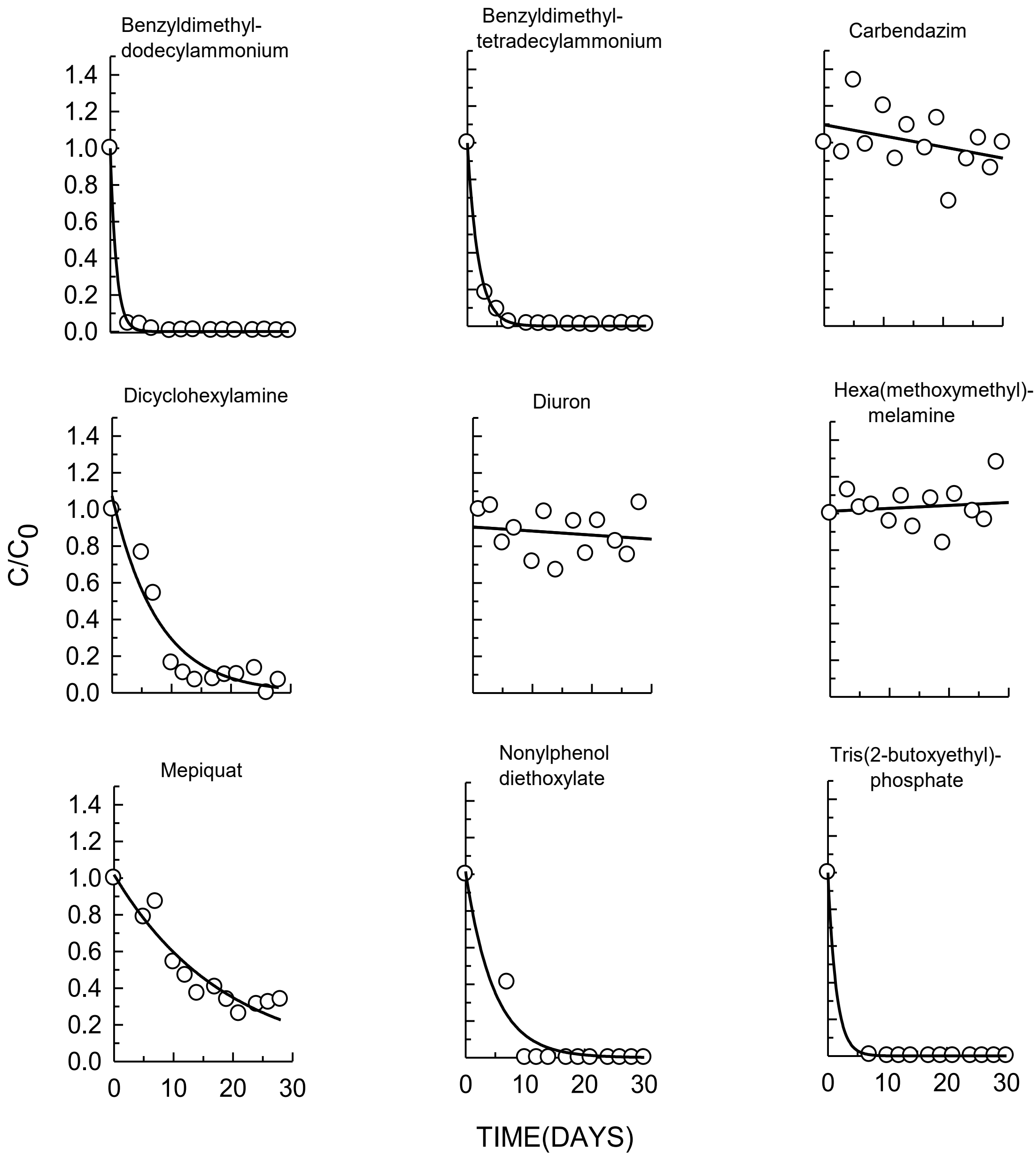
SP_48


Sulfamethoxazole
Tris(2-butoxyethyl)-phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)
TIME(DAYS)

SP_50


1,2,3-Benzotriazole
3-Chloroaniline
4-Chloroaniline
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole
(5-Tolytriazole)
Acetaminophen
(Paracetamol)
Acetamiprid
ADBI (Celestolide)
AHDI (Phantolide)
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

SP_50


Benzyldimethyl-dodecylammonium
Benzyldimethyl-tetradecylammonium
Carbendazim
Dicyclohexylamine
Diuron
Hexa(methoxymethyl)-melamine
Mepiquat
Nonylphenol diethoxylate
Tris(2-butoxyethyl)-phosphate
C/C0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
TIME(DAYS)

APPENDIX H: INITIAL CONCENTRATIONS, BIODEGRADATION RATE CONSTANTS AND HALF-LIFE OF THE COMPOUNDS

Table H1. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 4 in August

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-CA	0.41	0.585	0.985	0.060	1.2
4-MBTA	15.74	0.000	-	-	-
5-MBTA	6.85	0.000	-	-	-
ACF	0.19	3.565	1.000	0.000	0.2
ACMP	0.04	0.000	-	-	-
AHDI	0.53	0.342	0.984	0.028	2.0
APAP	12.82	3.565	1.000	0.000	0.2
BMA	0.21	0.431	0.948	0.076	1.6
BP-1	0.47	0.205	0.930	0.030	3.4
BP-3	0.25	0.111	0.902	0.015	6.3
BTA	5.72	0.000	-	-	-
C_{12}BDMA	23.46	0.377	0.997	0.013	1.8
C_{14}BDMA	6.51	0.155	0.932	0.017	4.5
CAM	0.39	0.205	0.880	0.042	3.4
CF	1.65	0.000	-	-	-
DCH	0.38	0.271	0.942	0.040	2.6
DCMU	1.06	0.000	-	-	-
DEA	0.03	0.026	0.763	0.005	26.6
DEET	1.35	0.117	0.883	0.019	5.9
HMMM	32046.35	0.000	-	-	-
MBC	3.51	0.000	-	-	-
MQ	0.24	0.000	-	-	-
NETSA	0.09	0.000	-	-	-
NP2EO	4.57	0.195	0.958	0.019	3.6
OF	0.82	0.000	-	-	-
OMA	0.13	0.000	-	-	-
PBO	0.47	0.245	0.981	0.020	2.8
SMX	0.31	0.000	-	-	-
TBEP	24.22	0.080	0.941	0.007	8.7
TBN	0.03	0.000	-	-	-

Table H2. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 8 in August

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	25.36	2.920	0.924	1.332	0.2
5-MBTA	10.77	3.168	0.797	2.692	0.2
ACMP	0.61	0.000	-	-	-
AHDI	1.06	0.225	0.978	0.019	3.1
APAP	28.12	3.128	0.998	0.274	0.2
AZM	3.56	0.200	0.896	0.037	3.5
BDMA	3.65	0.147	0.880	0.027	4.7
BTA	4.57	0.000	-	-	-
BTMA	0.14	0.139	0.769	0.0318	5.0
C_{12}BDMA	2.24	0.228	0.870	0.048	3.0
C_{14}BDMA	1.19	0.348	0.990	0.031	2.0
CAM	2.74	0.368	0.976	0.040	1.9
CF	6.33	0.024	0.697	0.005	29.5
DCH	1.34	0.131	0.911	0.017	5.3
DCMU	0.68	0.000	-	-	-
DEA	9.56	0.093	0.825	0.016	7.5
DEET	1.99	0.0746	0.931	0.008	9.3
DEHP	8.04	0.146	0.866	0.034	4.8
HMMM	2482.03	0.000	-	-	-
MBC	1.83	0.000	-	-	-
MQ	0.16	1.717	1.000	0.002	0.4
NETSA	0.52	0.000	-	-	-
NF	15.32	0.000	-	-	-
NP2EO	0.54	0.1626	0.898	0.026	4.3
OF	2.32	0.000	-	-	-
OMA	0.43	0.000	-	-	-
PBO	0.15	0.2002	0.957	0.0233	3.5
QNP	1.03	0.653	0.993	0.048	1.1
SMX	0.83	0.000	-	-	-
TBEP	7.21	0.9232	0.999	0.020	0.8
TBN	0.01	0.000	-	-	-

Table H3. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 28 in August

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-CA	0.65	0.736	0.997	0.036	0.9
4-MBTA	26.94	0.000	-	-	-
5-MBTA	14.14	0.000	-	-	-
ACMP	0.29	0.000	-	-	-
AHDI	0.84	0.125	0.944	0.014	5.6
APAP	26.39	3.232	1.000	0.160	0.2
BDMA	0.10	0.445	0.992	0.031	1.6
BMA	0.27	0.391	0.976	0.044	1.8
BP-1	0.67	0.215	0.893	0.048	3.2
BP-3	0.07	0.169	0.843	0.038	4.1
BTA	9.38	0.000	-	-	-
C_{12}BDMA	8.98	1.933	0.983	0.247	0.4
CF	1.43	0.020	0.621	0.005	35.4
DCH	1.26	0.4606	0.865	0.117	1.5
DCMU	2.64	0.000	-	-	-
DEA	0.08	0.000	-	-	-
DEET	1.80	0.214	0.930	0.033	3.2
HMMM	29883.62	0.000	-	-	-
MBC	3.55	0.000	-	-	-
MQ	0.52	0.000	-	-	-
NETSA	0.24	0.000	-	-	-
NF	2.13	0.000	-	-	-
NP2EO	1.45	0.384	0.887	0.088	1.8
OF	1.00	0.000	-	-	-
OMA	0.11	0.000	-	-	-
PBO	0.17	0.199	0.974	0.018	3.5
SMX	1.15	0.000	-	-	-
TBEP	38.96	0.058	0.934	0.006	11.9
TBN	0.03	0.000	-	-	-

Table H4. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 48 in August

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
5-MBTA	3.49	0.000	-	-	-
ACMP	0.18	0.000	-	-	-
AHDI	2.84	0.135	0.947	0.015	5.1
APAP	34.52	3.565	1.000	0.000	0.2
BDMA	0.07	0.242	0.948	0.033	2.9
BMA	0.37	0.159	0.911	0.027	4.3
BP-3	0.18	0.033	0.757	0.006	21.3
C_{12}BDMA	1.90	0.263	0.918	0.041	2.6
C_{14}BDMA	0.60	0.000	-	-	-
DCH	0.12	0.000	-	-	-
DCMU	1.85	0.000	-	-	-
DEA	0.19	0.051	0.755	0.010	13.7
DEET	0.61	0.000	-	-	-
HMMM	329166.95	0.000	-	-	-
MBC	1.01	0.000	-	-	-
NETSA	0.04	0.000	-	-	-
NP2EO	1.40	0.235	0.978	0.019	2.9
OF	2.35	0.000	-	-	-
PBO	0.20	0.181	0.990	0.010	3.8
TBEP	270.17	0.022	0.856	0.003	31.9
TBN	0.03	0.000	-	-	-

Table H5. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 50 in August

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	1.49	0.165	0.819	0.033	4.2
4-CA	1.36	0.093	0.731	0.020	7.5
4-MBTA	2.52	0.000	-	-	-
AHDI	3.44	0.186	0.898	0.032	3.7
APAP	10.00	3.565	1.000	0.000	0.2
BMA	0.09	0.567	0.982	0.064	1.2
BTA	4.44	0.000	-	-	-
C_{12}BDMA	10.08	0.463	0.963	0.069	1.5

Table H5. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
C_{14}BDMA	4.42	0.060	0.493	0.017	11.6
DCH	5.13	0.318	0.973	0.054	2.2
DCMU	1.10	0.000	-	-	-
DEA	0.02	0.046	0.728	0.009	15.1
DEET	0.03	0.000	-	-	-
HMMM	113433.08	0.000	-	-	-
MBC	0.55	0.000	-	-	-
NP2EO	8.29	0.333	0.960	0.045	2.1
OF	0.25	0.000	-	-	-
TBEP	207.37	0.214	0.900	0.041	3.2

Table H6. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 4 in August

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	6.34	0.000	-	-	-
5-MBTA	4.33	0.000	-	-	-
APAP	15.77	3.565	1.000	0.000	0.2
BaP	0.37	0.000	-	-	-
BDMA	0.07	0.511	0.990	0.036	1.4
BMA	0.21	3.565	1.000	0.000	0.2
BP-1	0.74	0.205	0.930	0.030	3.4
BP-3	0.14	0.170	0.869	0.033	4.1
BTA	10.94	0.000	-	-	-
BTMA	0.50	0.132	0.979	0.008	5.3
BZA	3.99	0.000	-		-
C_{12}BDMA	5.08	0.344	0.981	0.028	2.0
C_{14}BDMA	1.49	0.096	0.959	0.007	7.3
CF	4.39	0.000	-	-	-
DCH	0.61	0.271	0.942	0.040	2.6
DCMU	0.72	0.000	-	-	-
DEET	0.17	0.000	-	-	-
DEHP	6.88	0.397	0.958	0.056	1.7
HHCM	1.95	3.565	1.000	0.000	0.2
HMMM	31921.38	0.000	-	-	-
MBC	0.77	0.000	-	-	-

Table H6. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
NETSA	0.13	0.000	-	-	-
NP2EO	0.78	0.278	1.000	0.000	2.5
OF	1.74	0.000	-	-	-
PBO	11.61	0.308	0.969	0.033	2.2
SMX	0.37	0.000	-	-	-
TBEP	208.73	0.514	0.994	0.029	1.3

Table H7. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 5 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	10.83	0.000	-	-	-
5-MBTA	5.03	0.000	-	-	-
ACMP	0.41	0.000	-	-	-
APAP	14.09	3.565	1.000	0.000	0.2
BaP	0.30	0.000	-	-	-
BDMA	0.02	0.325	0.973	0.037	2.1
BP-3	0.04	3.565	1.000	0.000	0.2
BTA	3.09	0.000	-	-	-
BTMA	0.06	0.154	0.918	0.021	4.5
BZA	1.95	0.000	-	-	-
C_{12}BDMA	14.69	0.383	0.987	0.034	1.8
C_{14}BDMA	3.67	0.121	0.963	0.009	5.7
CF	3.42	0.000	-	-	-
DCH	0.49	0.370	0.940	0.060	1.9
DCMU	0.80	0.000	-	-	-
DEET	0.29	0.303	0.923	0.052	2.3
HMMM	7942.08	0.000	-	-	-
MBC	1.77	0.000	-	-	-
NETSA	0.29	0.000	-	-	-
NF	1.39	0.000	-	-	-
NP2EO	0.59	0.267	0.988	0.017	2.6
OF	0.32	0.035	0.562	0.009	19.7
SMX	0.47	0.000	-	-	-
TBEP	2.27	0.138	0.977	0.010	5.0

Table H8. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 6 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	3.29	0.000	-	-	-
5-MBTA	1.97	0.000	-	-	-
ADBI	0.82	0.439	0.995	0.022	1.6
AHDI	2.52	0.499	0.989	0.037	1.4
APAP	10.00	3.565	1.000	0.000	0.2
BTA	9.22	0.000	-	-	-
C_{12}BDMA	110.42	1.140	1.000	0.020	0.6
C_{14}BDMA	40.54	0.681	0.999	0.014	1.0
CF	4.06	0.000	-	-	-
DCH	0.05	3.565	1.000	0.000	0.2
DCMU	1.18	0.000	-	-	-
DEA	0.12	3.565	1.000	0.000	0.2
DEET	0.02	0.000	-	-	-
HMMM	155934.96	0.000	-	-	-
MBC	0.32	0.000	-	-	-
MQ	0.42	3.565	1.000	0.000	0.2
NETSA	0.02	0.000	-	-	-
NP2EO	4.59	0.320	0.983	0.025	2.2
OF	0.03	0.000	-	-	-
SMX	0.56	3.565	1.000	0.000	0.2
TBEP	39.15	0.292	0.967	0.032	2.4

Table H9. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 7 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	0.95	0.026	0.495	0.007	26.76
5-MBTA	0.15	0.000	-		-
APAP	10.64	3.565	1.000	0.000	0.19
BDMA	0.03	0.000	-	-	-
BTA	9.88	0.000	-	-	-
C_{12}BDMA	3.90	0.983	0.943	0.231	0.71
C_{14}BDMA	1.43	0.154	0.887	0.024	4.50
CF	2.50	0.071	0.722	0.017	9.74
DCMU	0.31	0.000	-	-	-
DEET	0.04	0.000	-	-	-

Table H9. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
HMMM	298.49	0.000	-	-	-
NF	0.77	0.000	-	-	-
NP2EO	0.41	0.204	0.970	0.019	3.39
OF	1.49	0.000	-	-	-
TBEP	0.64	0.017	0.672	0.004	40.53

Table H10. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 8 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	8.24	0.199	0.577	0.057	3.5
5,6-DMBTA	6.45	0.325	0.928	0.055	2.1
5-MBTA	3.70	0.057	0.388	0.019	12.1
APAP	14.80	0.948	1.000	0.110	0.7
BTA	2.93	0.000	-	-	-
C_{12}BDMA	2.09	0.259	0.975	0.022	2.7
C_{14}BDMA	1.02	0.221	0.985	0.014	3.1
CF	15.83	0.000	-	-	-
DCH	0.54	0.136	0.933	0.017	5.1
DCMU	0.81	0.000	-	-	-
DEET	0.29	0.000	-	-	-
HMMM	774.04	0.000	-	-	-
MBC	0.85	0.000	-	-	-
MQ	1.16	0.318	0.872	0.074	2.2
NETSA	0.22	0.000	-	-	-
NF	9.08	0.013	0.703	0.003	52.5
NP2EO	0.31	0.421	0.970	0.049	1.6
OF	3.77	0.000	-	-	-
PBO	0.13	0.229	0.897	0.042	3.0
QNP	0.56	0.286	0.799	0.085	2.4
TBEP	33.64	1.951	1.000	0.000	0.4

Table H11. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 9 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	73.70	2.082	0.998	0.411	0.3
5,6-MBTA	118.29	3.565	1.000	0.000	0.2
5-MBTA	33.03	1.801	0.996	0.305	0.4
APAP	19.09	0.879	1.000	0.220	0.8
BTA	12.50	0.032	0.773	0.005	21.7
C_{12}BDMA	0.39	0.000	-	-	-
CF	21.32	0.024	0.776	0.004	28.6
DCH	0.23	0.120	0.772	0.028	5.8
DEET	0.04	0.000	-	-	-
HMMM	327.47	0.000	-	-	-
NETSA	0.36	0.000	-	-	-
NF	25.31	0.025	0.736	0.004	28.1
NP2EO	0.14	0.434	0.975	0.047	1.6
OF	2.49	0.000	-	-	-
OMA	0.91	0.110	0.847	0.021	6.3
QNP	0.36	3.565	1.000	0.000	0.2
SMX	1.60	0.000	-	-	-
TBEP	0.89	0.211	0.982	0.015	3.3

Table H12. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 10 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	1.99	0.000	-	-	-
5-MBTA	1.20	0.000	-	-	-
APAP	11.31	3.565	1.000	0.000	0.2
BDMA	0.12	0.127	0.920	0.017	5.5
BTA	18.72	0.000	-	-	-
C_{12}BDMA	0.22	0.000	-	-	-
C_{14}BDMA	0.21	0.000	-	-	-
CF	2.27	0.000	-	-	-
DEET	0.06	0.000	-	-	-
HMMM	318.49	0.000	-	-	-
NP2EO	0.32	0.434	0.975	0.047	1.6
OF	0.15	0.000	-	-	-

Table H12. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
TBEP	1.71	0.000	-	-	-

Table H13. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 11 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.306	0.915	0.056	2.26
C_{12}BDMA	0.15	0.000	-	-	-
C_{14}BDMA	0.12	0.000	-	-	-
CF	3.59	0.000	-	-	-
HMMM	201.29	0.000	-	-	-
DEET	0.03	0.000	-	-	-
OF	0.29	0.000	-	-	-
TBEP	1.15	0.000	-	-	-

Table H14. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 12 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	27.15	2.491	0.994	1.563	0.3
5-MBTA	11.12	1.700	0.990	0.402	0.4
APAP	11.59	1.134	1.000	0.005	0.6
C_{12}BDMA	0.09	0.000	-	-	-
C_{14}BDMA	0.06	0.000	-	-	-
CF	0.84	0.000	-	-	-
DCH	0.09	0.161	0.839	0.035	4.3
DEET	0.04	0.000	-	-	-
HMMM	59.65	0.000	-	-	-

Table H15. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 13 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.343	0.911	0.074	2.0
C_{12}BDMA	0.08	0.000	-	-	-
C_{14}BDMA	0.05	0.000	-	-	-
CF	2.37	0.000	-	-	-
DEET	0.04	0.000	-	-	-
HMMM	66.71	0.257	0.698	0.067	2.7
NF	2.31	0.000	-	-	-

Table H16. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 14 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	19.94	0.015	0.670	0.003	1.1
5,6-DMBTA	5.91	0.000	-		-
5-MBTA	8.70	0.000	-		-
APAP	64.91	2.030	1.000	0.000	0.3
BP-3	0.16	0.108	0.789	0.019	6.4
BTA	2.04	0.000	-		-
C_{12}BDMA	0.27	0.000	-		-
C_{14}BDMA	0.17	0.000	-		-
CF	15.52	0.023	0.866	0.003	30.4
DCH	0.03	0.347	0.916	0.066	2.0
DEET	0.64	0.309	0.998	0.009	2.2
HHCB	2.96	0.231	0.970	0.022	3.0
HMMM	74.90	0.000	-		-
NF	4.53	0.018	0.703	0.003	39.6
NP2EO	0.23	3.565	1.000	0.000	0.2
OF	0.77	0.000	-		-

Table H17. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 15 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
5-MBTA	1.38	0.000	-		-
APAP	12.30	1.602	0.987	0.377	0.4
BP-3	0.04	0.000	-	-	-
C_{12}BDMA	0.26	0.000	-		-
C_{14}BDMA	0.23	0.000	-		-
CF	11.38	0.000	-		-
DEET	0.69	0.057	0.750	0.012	12.1
HMMM	2034.94	0.000	-		-
NETSA	0.08	0.000	-	-	-
NF	9.35	0.000	-	-	-
TBEP	0.58	0.000	-	-	-

Table H18. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 16 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	0.04	0.000	-	-	-
APAP	10.00	0.203	0.854	0.044	3.4
C_{12}BDMA	0.11	0.000	-	-	-
CF	2.78	0.000	-	-	-
DEET	0.03	0.000	-	-	-
HMMM	152.68	0.000	-	-	-
TBEP	0.10	0.000	-	-	-

Table H19. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 17 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	0.71	0.000	-	-	-
5-MBTA	0.22	0.000	-	-	-
APAP	10.00	0.823	1.000	0.013	0.84
C_{12}BDMA	0.10	0.000	-	-	-

Table H19. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
CF	4.76	0.000	-	-	-
DEET	0.05	0.000	-	-	-
HMMM	58.28	0.000	-	-	-
SMX	0.14	0.000	-	-	-

Table H20. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 18 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	0.89	0.000	-	-	-
5-MBTA	0.72	0.000	-	-	-
APAP	10.00	3.565	1.000	0.000	0.2
C_{12}BDMA	0.11	0.000	-		-
C_{14}BDMA	0.07	0.000	-		-
CF	2.93	0.000	-	-	-
DEET	0.08	0.031	0.874	0.005	28.5
HMMM	131.50	0.000	-	-	-
MBC	1.27	0.000	-		-
NETSA	0.04	0.000	-	-	-
NP2EO	0.46	3.565	1.000	0.000	0.2
SMX	0.23	0.000	-	-	-

Table H21. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 19 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.353	0.930	0.063	2.0
BDMA	0.02	0.051	0.609	0.015	13.6
C_{14}BDMA	0.22	0.000	-	-	-
DEET	0.02	0.000	-	-	-
HMMM	61.96	0.000	-	-	-

Table H22. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 20 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	1.74	0.000	-	-	-
5-MBTA	0.71	0.000	-	-	-
APAP	10.00	2.429	1.000	0.188	0.3
BDMA	0.04	0.045	0.758	0.009	15.6
C_{14}BDMA	0.12	0.000	-	-	-
HMMM	367.75	0.000	-	-	-

Table H23. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 21 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.283	0.906	0.053	2.5
C_{12}BDMA	0.12	0.000	-	-	-
C_{14}BDMA	0.08	0.000	-	-	-
DEET	0.07	0.020	0.682	0.004	34.1
HMMM	205.34	0.000	-	-	-
TBEP	1.40	0.000	-	-	-

Table H24. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 22 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.350	0.919	0.006	2.0
BDMA	0.03	0.000	-	-	-
C_{12}BDMA	0.18	0.000	-		-
C_{14}BDMA	0.17	0.017	0.425	0.065	40.3
DEET	0.06	0.000	-	-	-
HMMM	116.72	0.000	-	-	-
TBEP	0.99	0.000	-	-	-

Table H25. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 23 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	0.23	0.000	-	-	-
APAP	16.46	3.565	1.000	0.000	0.19
BDMA	0.02	0.054	0.778	0.011	12.77
BP-3	0.05	0.017	0.577	0.006	40.30
DEET	0.36	0.056	0.861	0.008	12.47
DEHP	26.32	0.162	0.847	0.038	4.28
HMMM	328.11	0.000	-	-	-
NETSA	0.04	0.000	-		-
NP2EO	5.42	0.285	0.909	0.047	2.43
PBO	0.08	0.062	0.706	0.013	11.18
SMX	0.35	0.000	-	-	-
TBEP	1.10	0.000	-	-	-

Table H26. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 24 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
5,6-DMBTA	1.63	3.565	1.000	0.000	0.2
APAP	10.00	3.565	1.000	0.000	0.2
BaP	0.17	0.000	-	-	-
BTMA	0.03	0.123	0.816	0.027	5.6
BZA	4.95	0.000	-	-	-
C_{12}BDMA	9.07	0.114	0.948	0.010	6.1
C_{14}BDMA	1.52	0.078	0.878	0.010	8.9
CF	9.38	0.000	-	-	-
HMMM	166.95	0.000	-	-	-
NETSA	0.05	0.000	-	-	-
NF	7.78	0.000	-	-	-
NP2EO	0.40	1.015	1.000	0.007	0.7

Table H27. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 25 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.328	0.890	0.071	2.1
C_{12}BDMA	0.11	0.000	-	-	-
C_{14}BDMA	0.08	0.000	-	-	-
DEET	0.05	0.000	-	-	-
HMMM	59.49	0.261	0.910	0.047	2.7

Table H28. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 26 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.421	0.992	0.025	1.6
C_{12}BDMA	0.10	0.000	-	-	-
C_{14}BDMA	0.06	0.000	-	-	-
DEET	0.06	0.000	-	-	-
HMMM	13.81	3.565	1.000	0.000	0.2

Table H29. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 28 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.43	0.000	-	-	-
4-CA	0.35	0.000	-	-	-
4-MBTA	18.75	0.000	-	-	-
5,6-DMBTA	7.53	3.565	1.000	0.000	0.2
5-MBTA	9.65	0.000	-	-	-
AHDI	0.53	3.565	1.000	0.000	0.2
APAP	19.85	3.565	1.000	0.000	0.2
BDMA	0.20	0.339	0.906	0.068	2.0
BMA	0.20	0.331	0.894	0.070	2.1
BP-3	0.11	0.266	0.944	0.037	2.6
BTA	11.45	0.000	-	-	-
BTMA	0.91	0.123	0.937	0.013	5.6

Table H29. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
BZA	0.32	0.000	-	-	-
C_{12}BDMA	3.97	0.301	0.932	0.047	2.3
C_{14}BDMA	1.16	0.201	0.944	0.024	3.5
CF	10.95	0.000	-	-	-
DCH	0.97	0.249	0.913	0.043	2.8
DCMU	1.19	0.000	-	-	-
DEET	0.23	0.063	0.983	0.003	11.1
DEHP	40.37	0.101	0.757	0.023	6.8
HMMM	39419.84	0.000	-	-	-
MBC	2.07	0.000	-	-	-
MQ	0.52	0.000	-	-	-
NETSA	0.21	0.000	-	-	-
NF	2.61	0.000	-	-	-
NP2EO	0.51	0.346	0.914	0.066	2.0
OF	2.82	0.000	-	-	-
PBO	14.33	0.286	0.961	0.034	2.4
QNP	0.89	3.565	1.000	0.000	0.2
SMX	0.62	0.000	-	-	-
TBEP	185.87	0.332	0.910	0.064	2.1

Table H30. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 48 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	1.26	0.000	-	-	-
4-MBTA	19.40	0.000	-	-	-
5,6-DMBTA	5.35	3.565	1.000	0.000	0.2
5-MBTA	8.53	0.000	-	-	-
ADBI	1.25	0.111	0.822	0.021	6.3
AHDI	4.01	0.561	0.995	0.030	1.2
APAP	49.27	3.565	1.000	0.000	0.2
BDMA	8.48	0.388	0.953	0.056	1.8
BP-3	0.14	0.090	0.935	0.009	7.7
BTA	23.00	0.000	-	-	-
BZA	1.61	0.000	-	-	-
C_{12}BDMA	9.48	0.256	0.921	0.042	2.7
C_{14}BDMA	3.46	0.163	0.894	0.025	4.2

Table H30. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
CF	25.13	0.000	-	-	-
DCH	0.47	0.000	-	-	-
DCMU	1.23	0.000	-	-	-
DEET	0.14	0.000	-	-	-
DEHP	100.65	0.000	-	-	-
HHCB	6.89	0.251	0.981	0.020	2.8
HMMM	304783.95	0.000	-	-	-
NETSA	0.04	0.000	-	-	-
NP2EO	3.79	0.403	0.988	0.028	1.7
OF	1.81	0.000	-	-	-
PBO	4.38	0.182	0.972	0.016	3.8
TBEP	164.01	0.079	0.923	0.009	8.8

Table H31. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 50 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	13.86	0.000	-	-	-
5,6-DMBTA	1.82	3.565	1.000	0.000	0.2
5-MBTA	7.89	0.000	-	-	-
ADBI	0.53	0.000	-	-	-
AHDI	1.61	0.000	-	-	-
APAP	10.00	3.565	1.000	0.000	0.2
BDMA	0.32	3.565	1.000	0.000	0.2
BTA	6.60	0.000	-	-	-
BZA	1.15	0.000	-	-	-
C_{12}BDMA	12.47	0.669	0.978	0.074	1.0
CF	4.16	0.000	-	-	-
4-MBTA	13.86	0.000	-	-	-
DCH	0.22	0.217	0.877	0.044	3.2
DCMU	1.04	0.000	-	-	-
DEET	0.01	0.000	-	-	-
HMMM	79261.08	0.000	-	-	-
NP2EO	18.40	0.348	0.978	0.032	2.0
TBEP	59.51	0.132	0.903	0.020	5.3

Table H32. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 54 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.25	0.000	-	-	-
4-CA	0.27	0.631	0.998	0.024	1.1
4-MBTA	3.88	0.000	-	-	-
5,6-DMBTA	0.79	3.565	1.000	0.000	0.2
5-MBTA	2.31	0.000	-	-	-
ACMP	0.22	0.000	-	-	-
APAP	10.00	3.565	1.000	0.000	0.2
BaP	0.11	0.000	-	-	-
BDMA	0.16	0.193	0.954	0.020	3.6
BP-3	0.04	0.475	0.985	0.040	1.5
BTA	7.55	0.000	-	-	-
BTMA	0.08	0.025	0.694	0.005	27.8
BZA	1.15	0.000	-	-	-
C_{12}BDMA	5.01	0.477	0.990	0.032	1.5
C_{14}BDMA	1.91	0.250	0.923	0.038	2.8
CF	2.66	0.000	-	-	-
DCH	0.67	0.322	0.976	0.031	2.2
DCMU	0.84	0.000	-	-	-
DEET	0.21	0.180	0.986	0.011	3.8
DEHO	31.63	0.000	-	-	-
HMMM	7625.49	0.000	-	-	-
MBC	1.04	0.000	-	-	-
NETSA	0.17	0.000	-	-	-
NP2EO	1.11	0.695	0.980	0.076	1.0
OF	0.31	0.000	-	-	-
PBO	0.03	0.557	0.995	0.030	1.2
QNP	0.21	3.565	1.000	0.000	0.2
SMX	0.21	0.000	-	-	-
TBEP	1.19	0.075	0.960	0.005	9.3

Table H33. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 55 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.51	3.565	1.000	0.000	0.2
4-CA	0.62	3.565	1.000	0.000	0.2
4-MBTA	0.42	0.000	-	-	-

Table H33. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
5-MBTA	0.33	0.000	-	-	-
5,6-DMBTA	0.30	3.565	1.000	0.000	0.2
ADBI	0.44	3.565	1.000	0.000	0.2
APAP	13.78	3.565	1.000	0.000	0.2
BaP	0.17	0.000	-	-	-
BTA	4.61	0.000	-	-	-
BZA	4.71	0.000	-	-	-
C_{12}BDMA	3.96	0.832	0.955	0.147	0.8
CF	3.21	0.000	-	-	-
DCMU	0.67	0.000	-	-	-
DEA	0.03	3.565	1.000	0.000	0.2
DEET	0.01	0.000	-	-	-
HMMM	33558.38	0.000	-	-	-
MBC	0.13	0.000	-	-	-
MQ	0.22	0.000	-	-	-
NP2EO	5.59	0.529	0.974	0.060	1.3
OF	0.31	0.000	-	-	-

Table H34. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 65 in November

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.00	0.223	0.886	0.044	3.1
BDMA	0.05	0.000	-	-	-
BTA	1.06	0.000	-	-	-
C_{12}BDMA	0.10	0.000	-	-	-
C_{14}BDMA	0.04	0.000	-	-	-
CF	2.41	0.000	-	-	-
DEET	0.02	0.000	-	-	-
HMMM	147.11	0.000	-	-	-

Table H35. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 4 in February

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.5	3.565	1.000	0.000	0.2
BP-1	0.11	0.371	0.985	0.029	1.9
BTA	0.49	0.031	0.671	0.007	22.1
BTA	0.13	3.565	1.000	0.000	0.2
C_{12}BDMA	3.88	0.058	0.885	0.007	12.0
DCH	0.16	0.249	0.978	0.022	2.8
HMMM	1021.51	0.017	0.862	0.002	40.3
MBC	0.08	0.127	0.953	0.015	5.4
MQ	0.10	0.000	-	-	-
NP2EO	1.95	0.281	0.960	0.034	2.5
PBO	0.06	0.775	0.997	0.033	0.9
TBEP	6.42	0.270	0.990	0.023	2.6

Table H36. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 8 in February

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
APAP	10.3	3.565	1.000	0.000	0.2
BTA	0.47	0.024	0.602	0.007	29.4
DEET	0.08	-	-	-	-
NP2EO	0.21	0.492	0.966	0.062	1.4
PBO	0.12	0.405	0.989	0.027	1.7
QNP	0.12	0.522	0.975	0.058	1.3
TBEP	90.26	0.221	0.950	0.034	3.1

Table H37. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 28 in February

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-MBTA	0.25	0.047	0.862	0.007	14.7
5-MBTA	0.16	0.048	0.662	0.011	14.4
APAP	10.1	3.566	1.000	0.000	0.2
BP-3	0.03	0.296	0.971	0.031	2.3
BTA	0.46	0.047	0.686	0.010	14.6
BTMA	0.10	0.194	0.709	0.051	3.6

Table H37. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
DCH	0.07	0.236	0.978	0.021	2.9
MBC	0.06	0.121	0.843	0.027	5.7
NP2EO	0.73	0.330	0.987	0.023	2.1
PBO	0.01	0.295	0.966	0.034	2.4
TBEP	3.61	0.133	0.967	0.012	5.2

Table H38. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 48 in February

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-CA	0.07	0.071	0.853	0.011	9.8
4-MBTA	1.56	0.026	0.634	0.006	27.1
ACMP	0.15	0.017	0.687	0.004	41.0
APAP	14	3.566	1.000	0.000	0.2
BP-3	0.05	0.146	0.964	0.015	4.7
DCH	0.18	0.333	0.965	0.040	2.1
DCMU	0.35	0.000	-	-	-
HMMM	3436.34	0.000	-	-	-
NP2EO	1.26	0.338	0.959	0.044	2.1
PBO	0.11	0.211	0.912	0.038	3.3
TBEP	7.76	0.241	0.969	0.033	2.9

Table H39. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 50 in February

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.13	0.071	0.946	0.007	9.7
4-CA	0.11	0.079	0.966	0.006	8.8
ACMP	0.25	0.000	-	-	-
ADBI	0.17	0.232	0.904	0.044	3.0
AHDI	0.53	0.221	0.883	0.047	3.1
APAP	10.1	3.566	1.000	0.000	0.2
DCH	0.12	0.343	0.849	0.091	2.0
DCMU	1.37	0.000	-	-	-

Table H39. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
HMMM	4605.35	0.000	-	-	-
MQ	0.07	0.000	-	-	-
NP2EO	1.81	0.423	0.984	0.035	1.6
PBO	0.01	0.259	0.941	0.039	2.7
PCZ	0.33	0.000	-	-	-
TBEP	5.70	0.119	0.941	0.014	5.8

Table H40. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 4 in May

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.652	0.549	0.993	0.035	1.3
4-CA	0.537	0.538	0.992	0.035	1.3
4-MBTA	2.679	0.027	0.775	0.005	25.6
5-MBTA	1.722	0.025	0.829	0.004	27.9
APAP	10.000	0.831	1.000	0.019	0.8
BP-1	0.156	0.210	0.940	0.032	3.3
BP-3	0.057	0.340	0.913	0.059	2.0
BTA	1.596	0.000	-	-	-
C_{12}BDMA	8.959	0.205	0.976	0.015	3.4
C_{14}BDMA	1.520	0.083	0.900	0.009	8.4
DCH	0.192	0.266	0.778	0.075	2.6
DCMU	0.461	0.000	-	-	-
DEET	0.068	0.000	-	-	-
HMMM	10524.069	0.000	-	-	-
MBC	0.616	0.000	-	-	-
MQ	0.511	0.000	-	-	-
NP2EO	0.468	0.311	0.962	0.034	2.2
PBO	0.150	3.565	1.000	0.000	0.2
SMX	0.167	0.000	-	-	-
TBEP	36.904	3.565	1.000	0.000	0.2

Table H41. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 8 in May

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.20	0.468	0.982	0.042	1.5
4-CA	0.18	0.313	0.878	0.064	2.2
4-MBTA	0.98	0.000	-	-	-
5-MBTA	0.42	0.000	-	-	-
APAP	10.000	0.782	1.000	0.021	0.9
BP-3	0.02	0.291	0.838	0.069	2.4
BTA	0.64	0.000	-	-	-
C_{12}BDMA	0.14	0.104	0.666	0.023	6.7
C_{14}BDMA	0.07	0.047	0.640	0.010	14.7
DCH	0.20	0.115	0.904	0.016	6.0
DEET	0.12	0.000	-	-	-
HMMM	527.91	0.000	-	-	-
MBC	0.17	0.000	-	-	-
MQ	0.30	0.000	-	-	-
NETSA	0.05	0.000	-	-	-
PBO	0.04	0.202	0.853	0.042	3.4
QNP	0.41	3.565	1.000	0.000	0.2
SMX	0.09	0.000	-	-	-
TBEP	3.77	0.572	0.997	0.027	1.2

Table H42. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 28 in May

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	0.74	3.565	1.000	0.000	0.2
4-CA	0.85	0.971	1.000	0.015	0.7
4-MBTA	3.04	0.023	0.870	0.003	30.8
5-MBTA	1.75	0.023	0.860	0.003	30.4
APAP	11.25	3.565	1.000	0.000	0.2
BP-1	0.24	0.187	0.928	0.026	3.7
BP-3	0.07	0.276	0.992	0.013	2.5
BTA	2.09	0.000	-	-	-
C_{12}BDMA	11.65	0.186	0.991	0.008	3.7
C_{14}BDMA	2.02	0.079	0.980	0.004	8.8
DCH	0.25	0.413	0.965	0.051	1.7
DCMU	0.67	0.000	-	-	-

Table H42. Continue

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
DEET	0.09	0.021	0.889	0.002	33.5
HMMM	13036.08	0.000	-	-	-
MBC	0.72	0.000	-	-	-
MQ	0.42	0.000	-	-	-
NETSA	0.05	0.000	-	-	-
NP2EO	0.59	0.279	0.963	0.029	2.5
PBO	0.15	0.595	0.996	0.031	1.2
SMX	0.24	0.000	-	-	-
TBEP	30.67	0.340	0.987	0.022	2.0

Table H43. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 48 in May

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
3-CA	1.90	0.261	0.978	0.019	2.7
4-CA	1.89	0.275	0.981	0.019	2.5
4-MBTA	3.88	0.000	-	-	-
5-MBTA	2.33	0.000	-	-	-
ADBI	0.55	0.480	0.992	0.029	1.4
AHDI	1.34	3.565	1.000	0.000	0.2
APAP	26.72	1.298	1.000	0.053	0.5
BP-3	0.10	0.346	0.968	0.036	2.0
BTA	14.00	0.000	-	-	-
C12BDMA	22.48	0.449	0.998	0.012	1.5
C14BDMA	3.52	0.161	0.984	0.009	4.3
DCH	0.12	0.078	0.643	0.023	8.9
DCMU	0.38	0.000	-	-	-
DEET	0.05	0.000	-	-	-
HMMM	94068.28	0.000	-	-	-
MBC	0.09	0.000	-	-	-
NP2EO	1.02	0.305	0.970	0.029	2.3
PBO	0.23	0.683	0.999	0.027	1.0
SMX	0.29	0.000	-	-	-
TBEP	227.62	0.174	0.879	0.031	4.0

Table H44. Initial concentrations, biodegradation rate constants and half-life of the compounds in Sample 50 in May

Compounds	Initial Concentration (µg/L)	Rate Constant (k) (d^{-1})	R^2	Standard Error	Half Life ($t_{1/2}$) (d)
4-CA	5.428	0.096	0.974	0.006	7.2
4-CA	5.839	0.103	0.978	0.006	6.7
4-MBTA	1.012	0.000	-	-	-
5-MBTA	0.569	0.000	-	-	-
ACMP	0.169	0.000	-	-	-
ADBI	0.490	0.384	0.951	0.052	1.8
AHDI	0.898	3.565	1.000	0.000	0.2
APAP	10.000	0.921	1.000	0.016	0.8
BTA	2.672	0.000	-	-	-
C_{12}BDMA	8.106	0.984	0.998	0.077	0.7
C_{14}BDMA	2.098	0.540	0.998	0.018	1.3
DCH	0.460	0.130	0.902	0.018	5.3
DCMU	0.534	0.000	-	-	-
HMMM	153642.178	0.000	-	-	-
MBC	0.302	0.000	-	-	-
MQ	0.343	0.054	0.892	0.006	12.9
NP2EO	3.238	0.212	0.942	0.032	3.3
TBEP	565.224	0.713	1.000	0.010	1.0

APPENDIX I: BIODEGRADABILTY PREDICTION SCORES OF THE TARGET COMPOUNDS

Table I1. Biodegradability scores of the target compounds estimated using BIOWIN^{TM} v 4.11

Compound Name	Linear Model (BIOWIN1)	Non-Linear Model (BIOWIN2)	Ultimate Survey Model (BIOWIN3)	Primary Survey Model (BIOWIN4)	Miti Linear Model (BIOWIN5)	Miti Non-Linear Model (BIOWIN6)	Ready Biodegradability
BTA	0.691	0.789	2.936	3.676	0.391	0.394	NO
BP-1	1.012	0.958	2.838	3.601	0.387	0.292	NO
3-CA	0.271	0.062	2.576	3.390	0.214	0.107	NO
4-CA	0.271	0.062	2.576	3.390	0.214	0.107	NO
4-MBTA	0.739	0.845	2.830	3.587	0.382	0.343	NO
5,6-DMBTA	0.787	0.888	2.724	3.498	0.374	0.296	NO
5-MBTA	0.739	0.845	2.830	3.587	0.382	0.343	NO
BZA	0.645	0.329	2.668	3.493	0.227	0.110	NO
APAP	1.002	0.989	2.867	3.875	0.487	0.509	NO
ACMO	0.305	0.022	2.286	3.342	-0.008	0.013	NO
ACF	0.160	0.042	2.067	3.166	-0.162	0.001	NO
ADBI	0.3248	0.0222	2.1376	3.0975	0.221	0.081	NO
AHDI	0.325	0.022	2.138	3.098	0.311	0.086	NO
DEA	0.018	0.000	2.062	3.137	-0.088	0.000	NO
AZM	-1.658	0.000	0.975	2.299	-0.328	0.000	NO
BaP	-0.030	0.000	1.842	2.782	0.060	0.035	NO

Table I1. Continue

Compound Name	Linear Model (BIOWIN1)	Non-Linear Model (BIOWIN2)	Ultimate Survey Model (BIOWIN3)	Primary Survey Model (BIOWIN4)	Miti Linear Model (BIOWIN5)	Miti Non-Linear Model (BIOWIN6)	Ready Biodegradability
C12BDMA	0.839	0.910	2.844	3.681	0.324	0.314	NO
C14BDMA	0.825	0.872	2.782	3.640	0.339	0.325	NO
BTMA	0.804	0.935	2.887	3.634	0.240	0.259	NO
MBC	0.736	0.786	2.730	3.766	0.133	0.112	NO
CF	-0.397	0.000	1.917	3.214	0.060	0.000	NO
CAM	-1.793	0.000	1.201	2.557	-0.140	0.000	NO
DEHP	1.127	1.000	3.213	4.280	0.665	0.689	YES
DCH	0.815	0.824	2.823	3.629	0.428	0.292	NO
DCMU	0.272	0.013	2.271	3.181	0.057	0.014	NO
HHCB	-0.036	0.0009	2.1204	3.0898	0.0657	0.026	NO
HMMM	-2.129	0.000	1.274	2.305	-0.279	0.000	NO
MQ	0.676	0.708	2.868	3.632	0.357	0.421	NO
DEET	0.921	0.972	2.647	3.709	0.444	0.395	NO
BDMA	0.606	0.660	2.668	3.370	0.211	0.154	NO
BMA	0.972	0.985	2.978	3.721	0.371	0.318	NO
NETSA	0.707	0.681	2.684	3.492	0.244	0.120	NO
NP2EO	0.707	0.728	2.834	3.800	0.674	0.719	YES
NF	-0.392	0.000	1.944	3.231	0.093	0.000	NO
OF	-0.639	0.000	1.513	2.916	0.020	0.000	NO
OMA	1.170	1.000	2.828	4.211	0.410	0.245	NO
BP-3	1.022	0.986	2.693	3.618	0.476	0.392	NO

Table H1. Continue

Compound Name	Linear Model (BIOWIN1)	Non-Linear Model (BIOWIN2)	Ultimate Survey Model (BIOWIN3)	Primary Survey Model (BIOWIN4)	Miti Linear Model (BIOWIN5)	Miti Non-Linear Model (BIOWIN6)	Ready Biodegradability
PBO	-0.029	0.006	2.533	3.685	0.421	0.228	NO
PCZ	0.153	0.002	1.689	2.885	-0.105	0.002	NO
QNP	0.920	1.000	2.694	3.883	0.120	0.031	NO
SMX	0.441	0.108	2.399	3.285	-0.158	0.004	NO
TBN	-0.009	0.000	1.938	3.072	-0.085	0.000	NO
TBEP	0.155	1.000	3.341	4.516	0.428	0.159	NO