THE DEGRADATION OF RESIDENT BIOSOLIDS CONTAMINANTS WITHIN AERBOIC MICROCOSMS

by

Kyle Nicolas McLaughlin

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Agronomy

West Lafayette, Indiana

August 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Michael Mashtare Jr., Chair

Department of Agronomy, Department of Environmental and Ecological Engineering

Dr. Linda S. Lee

Department of Agronomy

Dr. Ronald F. Turco

Department of Agronomy

Approved by:

Dr. Linda S. Lee

Head of the Graduate Program

Dr. Ronald F. Turco

Head of the Graduate Program

This thesis is dedicated to my fiancée, Neha Sharma, for her patience, support, and love, as well as my parents, Patrick D. McLaughlin and Alexis V. McLaughlin for teaching me lessons that cannot be found at any university.

ACKNOWLEDGMENTS

I will always be grateful to my major advisor, Dr. Michael L. Mashtare Jr., for working so hard to help me reach my potential. I also extend my gratitude to Drs. Linda S. Lee and Ronald F. Turco for providing indispensable insight and guidance as committee members and instructors.

I am thankful to all my many labmates for their camaraderie and cheer. A special thanks is owed to Dr. Rooney Kim Lazcano for always making herself available as a go-to resource. Thank you to Peyman Yousefi for the rides and the laughs. Thank you to Christina Schilling for the many engaging conversations. Thank you to Dr. Chloé de Perre for the plane ride to Lake Michigan and the many delectable treats. Countless more thanks are owed to all those I have had the privilege to know over these past few years.

Thank you to Yueyun Zhang and Dr. Arman Sabbaghi of the Purdue Statistical Consulting Service for their vital input into this research. Lastly, I would also like to thank the 2016 ESE cohort and all those that worked to make our symposium a success. It was an experience I will cherish always.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

CBZ – Carbamazepine

CDB – centrifuge-dried biosolids

C_s – concentration in solid phase

C_w – concentration in aqueous phase

DT_{50} – time to 50% dissipation

EPA – Environmental Protection Agency

EQ – exceptional quality

f_{oc} – fraction organic carbon

LBD – lagoon-dried biosolids

LOD – limit of detection

LOQ – limit of quantification

K_d – adsorption-desorption distribution coefficient

K_{oc} – organic carbon-water partition coefficient

K_{bw}^{des} – biosolids-water desorption coefficient

$K_{bw,oc}^{des}$ – biosolids-water desorption coefficient normalized to organic carbon

MCZ – Miconazole

MPN – most probable number

OC – organic carbon

OM – organic matter

pK_a – acid dissociation constant

PPCP – pharmaceutical and personal care products

TCC – Triclocarban

TCS – Triclosan

m – slope of the calibration curve

s – standard deviation of replicate samples with concentration 1-5 times the limit of detection

2,4-DCP – 2,4-dichlorophenol

3,4-DCA – 3,4-dichloroaniline

ABSTRACT

Author: McLaughlin, Kyle, N. MS
Institution: Purdue University
Degree Received: August 2019
Title: The Degradation of Resident Biosolids Contaminants within Aerobic Microcosms
Committee Chair: Michael Mashtare

Biosolids-based fertilizers are sold to the public to provide beneficial nutrients and organic matter for plant production. They are commonly applied to community gardens, municipal lands, reclamation projects, and golf courses. These fertilizers, however, may also contain a variety of trace organic contaminants, which can be persistent in the environment. Our work sought to quantify the persistence of biosolids contaminants in community garden soils. The commercial biosolids-based fertilizer, OCEANGRO®, was amended to two community garden soils to determine the first-order half-lives of four model contaminants: carbamazepine, miconazole, triclocarban, and triclosan. The criteria for their selection included biosolids occurrence, ecotoxicity, antimicrobial function, and knowledge gaps. Aerobic biosolids-amended soil microcosms were incubated at 22 ± 1 °C and approximately 80% field capacity. Sacrificial sampling occurred seven times over 180 days through multi-step solvent extractions. Detection and quantification were done on a high-performance liquid chromatograph tandem triple-quadrupole mass spectrometer. Results indicated that biosolids contaminants persist in soils with some having modeled half-lives in the hundreds of days. Additional analyses of solvent-spiked contaminant degradation and porewater desorption were performed to provide greater insight into possible limitations on resident biosolids contaminant degradation and to form a better comparative basis to previous literature. Solvent-spiked contaminants degraded more quickly than those resident within

biosolids, which indicate that data using the former may underestimate persistence in real-world environments. The porewater analysis allowed for the desorption coefficient to be calculated for all four model resident contaminants. Disparities in the trends of these desorption coefficients and solvent-spiked degradation rates showed that desorption from the biosolids matrix may have been a limiting factor to resident degradation for only some of our four model contaminants. Nonetheless, the demonstrated persistence of these contaminants necessitates long-term thinking in relation to biosolids application. More work is needed on the potential hazards associated with biosolids use in public lands regarding ecotoxicity and antimicrobial resistance.

CHAPTER 1. LITERATURE REVIEW

1.1 Biosolids

The management of human wastes has been an ever-present challenge. In ancient Mesopotamia, homes contained deep pits sometimes connected to drainage channels leading to outside streets [1]. At Monticello, the home of Thomas Jefferson, the sewers were cleaned manually by slaves who received monetary compensation for the task [2]. Modern waste management infrastructure was first built in mid-nineteenth century England in response to numerous health crises such as cholera outbreaks [3]. Worcester, MA and Coney Island, NY were home to the first wastewater treatment plants in the United States, which began construction in the late 1880s [4, 5]. Today, municipal wastewater treatment plants are fundamental to life.

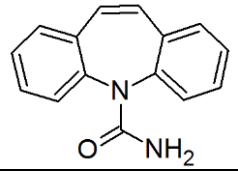
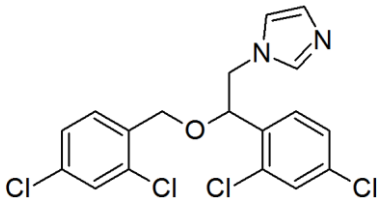
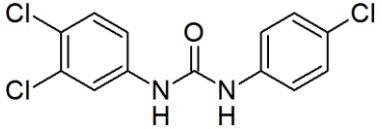
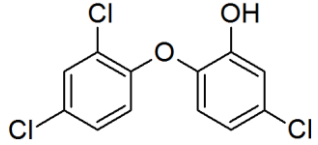
The solid byproduct of wastewater treatment, commonly referred to as "sewage sludge" or "biosolids," can be disposed of by incineration, landfilling, ocean dumping or reuse. Because biosoilds are nutrient-rich and high in organic matter (OM), their reuse via, land application as a fertilizer is widely utilized. As related to their carbon content, they have also found wide scale use to remediate lands disturbed by acid mine drainage or erosion. [6]

In the United States, Environmental Protection Agency (EPA) regulations delineate two classes of biosolids [7]. Class B biosolids must have a fecal coliform population below 2×10^6 most probable number (MPN) per dry gram as fecal bacteria indicate a potential human health risk, which necessitates restrictions on site, quantity, and timing of application. Class A biosolids, which have fecal coliform populations below 1,000 MPN per dry gram, are subject to less stringent regulation. For example, public access to land amended with Class A biosolids is not restricted. An additional distinction, Exceptional Quality (EQ), exists for those Class A biosolids that also meet criteria for reduced heavy metal content and odor. Municipalities commonly sell or give away

EQ Class A biosolids derived from their wastewater treatment operations directly to consumers under a number of commercial trade names including Miloganite, Oceangro, or Dillo Dirt. [6, 8]

While pathogen and heavy metal concentrations are well characterized and regulated [6, 8], recent scientific interest has been focused on the anthropogenic organic contaminants present within biosolids, including most EQ Class A commercial biosolids-based fertilizers. These contaminants include pharmaceutical and personal care products (PPCPs) from a diverse array of compound classes. [9, 10] Our interest is in four such pharmaceuticals that occur regularly in biosolids and have received attention for their potential environmental persistence and ecotoxicological effects. These pharmaceuticals are: carbamazepine (CBZ), miconazole (MCZ), triclocarban (TCC), and triclosan (TCS). These represent a major cross-section of the materials commonly encountered as they include: two ionizable (TCS, MCZ) a heavily halogenated material (MCZ) and a non-halogenated form (CBZ). Their structure, formula, CAS number as well as physiochemical properties including molecular mass, pK_a, Log K_{ow}, and Log K_{oc} are shown in Table 1.1.

Table 1: Model Contaminant Properties

	Structure	Formula CAS Number	Molecular Mass (u)	pK_a	Log K_{ow}	Log K_{oc}
CBZ		$C_{15}H_{12}N_2O$ 298-46-4	236.274	13.9[a]	2.25[a]	2.02[b] - 3.10[c]
MCZ		$C_{18}H_{14}Cl_4N_2O$ 22916-47-8	416.123	6.65[d]	6.25[e]	5.74[f]
TCC		$C_{13}H_9Cl_3N_2O$ 101-20-2	315.578	12.77[g]	4.9[e]	3.65 - 3.96[h]
TCS		$C_{12}H_7Cl_3O_2$ 3380-34-5	289.536	8.14[i]	4.76[e]	3.81[c] - 4.38[i]

[a] Jones et al. (2002) [11] [b] Williams et al. (2006) [12] [c] Yu et al. (2013) [13] [d] Bossche et al. (1987) [14] [e] EPI Suite Model, Chen et al. (2012) [15] [f] EPI Suite Model, Chen et al. (2015) [16] [g] Solaris V4.67, Sapkota et al. (2007) [17] [h] Agyin-Birikorang et al. (2010) [18] [i] Reiss et al. (2002) [19]

1.2 Model Biosolids Contaminants

1.2.1 Carbamazepine

Carbamazepine (5*H*-dibenzo[*b,f*]azepine-5-carboxamide) is a potentially teratogenic [20] pharmaceutical used to treat epilepsy [21], nerve pain [22], and depressive disorders [23]. As much as 5.2 x 10^4 kg were produced annually in the countries of France, Spain, and the United Kingdom during the early 2000s [24]. Carbamazepine is listed as an "essential medicine" by the World Health Organization [25]. Detected concentrations found in biosolids surveys within the United States have ranged from 8 µg/kg [26] to 6,030 µg/kg [10]. Details on the occurrence (%), average (µg/kg), and range (µg/kg) data found by such biosolids surveys is shown in Table 1.2.

Table 2: Summary of Biosolids Contaminant Occurrence Surveys

		Kinney et al. (2006) [26]	**US Environmental Protection Agency (2009) [10]**	**McClellan and Halden (2010) [27]**
CBZ	**Occurrence (%)**	100	95	100
	Average (µg/kg)	-	-	163
	Range (µg/kg)	8 - 390	8.74 - 6,030	[NA] - 238
MCZ	**Occurrence (%)**	89	95	100
	Average (µg/kg)	-	-	777
	Range (µg/kg)	14 - 460	14.2 - 9,210	[NA] - 1,100
TCC	**Occurrence (%)**	-	100	100
	Average (µg/kg)	-	-	36,060
	Range (µg/kg)	-	187 - 441,000	[NA] - 48,100
TCS	**Occurrence (%)**	100	94	100
	Average (µg/kg)	-	-	12,640
	Range (µg/kg)	443 - 10,500	430 - 133,000	[NA] – 19,700

[NA]: Not available

Despite the potential benefits to human health, CBZ has exhibited the potential for bioaccumulation and ecotoxicity in plant and animal species. Uptake of CBZ has been observed in many plant species with the highest edible tissue concentrations in the literature occurring in collard greens (*Brassica oleracea*, Vates variety) [28, 29]. It has been shown to bioaccumulate

within rainbow trout (*Oncorhynchus mykiss*) [30] and have growth retarding and organ deforming effects on zebrafish embryos (*Danio rerio*) [31].

1.2.2 Miconazole

Miconazole ((*RS*)-1-(2-(2,4-dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-1*H*-imidazole), is a broad spectrum antifungal and antibacterial compound [32]. It is listed as an "essential medicine" by the World Health Organization [25]. The Water Environment Research Foundation, a not-for-profit fund for water quality research, labeled MCZ a "High Priority" trace organic contaminant. This classification was applied to those contaminants that either occurred at concentrations greater than 1,000 µg/kg in national biosolids surveys or had potential for bioaccumulation and ecotoxicity in aquatic environments [9]. Detected concentrations found in biosolids surveys within the United States have ranged from 14 µg/kg [26] to 1,100 µg/kg [27]. Details on the occurrence (%), average (µg/kg), and range (µg/kg) data found by such biosolids surveys is shown in Table 1.2.

Miconazole is labeled by the Food and Drug Administration as a Pregnancy Category C drug, which means it has been shown to have adverse effects on animal fetuses [9].Azole antifungals, such as MCZ, primarily function via disruption of ergosterol biosynthesis through the inhibition of the cytochrome P450 enzyme sterol 14α-demethylase. This can also interfere with sex hormone synthesis through aromatase inhibition in rats, turtles, lizards, and non-human primates. [33]

1.2.3 Triclocarban and Triclosan

Triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol) and TCC (3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea) are closely related broad spectrum antimicrobial agents utilized in a wide array of personal care products such as hand soaps and toothpaste [34]. Triclosan and TCC

are regarded as “High Priority” trace organic contaminants by the Water Environment Research Foundation [9]. After the FDA determined that TCC and TCS in over-the-counter products could not be considered safe and effective [35], manufacturers have begun to phase them out. This was reflected in a study of Chicago wastewater treatment plants, which saw the concentrations of TCC and TCS in biosolids from 2012 to 2017 decline by 70 and 80%, respectively [36]. Unless similar regulations are put into place in other countries, waterbodies with concentrations exceeding a point no-effect concentration of 26.2 ng/L have been projected to nearly double by the year 2050 [37]. However, some countries, Switzerland, for example, do not land apply biosolids [38], while other countries differ greatly in the proportion they divert to land application [39].

Concern for TCC and TCS stems from their potential ecotoxicity in humans and non-target species as well selection for antimicrobial resistance. Their mode of action is linked to their effect on the biosynthesis of fatty acids in microbes [40]. Triclosan has been shown to interfere with the enzymatic nitrification activity of soil microbes [41]. Both contaminants have been shown to inhibit algal growth [42]. Bioaccumulation of TCS in earthworms (*Eisenia foetida*) [43] and snails (*Helisoma trivolvis* and *Cladophora* spp.) [44] has been observed. In one study, TCC at sub µM concentrations caused an increase in water flea (*Daphnia similis*) mortality [45]. Triclosan and TCC have the potential to translocate into plant roots, stems, leaves, and edible tissue [46]. For these two contaminants, hazard quotients for humans are generally low, but TCS uptake by biosolids-amended crops could have adverse biological effects if consumed by toddlers [28].

1.2.4 Ionizability

The charge of a contaminant can be a major characteristic governing its environmental fate. Two of the model contaminants included here can exist as ions at environmentally relevant pH, which is approximately 4-8. Miconazole can exist as a cation due to its imidazole group. The

alcohol group of TCS can become anionic. At a neutral pH of 7, approximately that of our microcosms, 31% of MCZ is cationic and 7% of TCS is anionic. The anionic fraction of TCS proportional and the cationic fraction of MCZ are inversely proportional to pH. The cationic speciation of MCZ can be calculated using Equations 1.1, while the anionic speciation of TCS can be calculated using Equation 1.2. The pK_a values of all four model contaminants can be found in Table 1.1.

Equation 1.1: Speciation of MCZ

$$f_{BH^+} = \frac{10^{-pH}}{10^{-pH} + 10^{-pK_a}}$$

Equation 1.2: Speciation of TCS

$$f_{A^-} = \frac{10^{-pK_a}}{10^{-pH} + 10^{-pK_a}}$$

1.2.5 Sorption

The ionizability of TCS has been shown to affect its sorption to OM. As the anionic fraction of TCS increases, its sorption to OM decreases [47, 48]. An experimental K_d has not been reported for MCZ in soils or biosolids, but when positively charged it would be able to sorb to cation exchange sites in soils. One study could not obtain a sorption isotherm for sewage sludge due to speculated high sorption to glassware [49]. Data has been gathered in clinical setting with one studying determining negligible sorption to the plastics of intravenous infusion sets [49, 50]. Carbamazepine, TCC, and TCS sorb strongly in biosolids-amended soils and exhibit hysteresis [12, 48, 51]. The Log K_{oc} for these contaminants in biosolids matrices follows the general trend of TCS > TCC > CBZ [12, 13, 18, 19]. Values for Log K_{oc} for the contaminants can be found in Table 1.1.

1.2.6 Antimicrobial Resistance

Three of our model contaminants (MCZ, TCC, and TCS) are used as antimicrobial products. Their release into the environment has the potential to select for the expression of antimicrobial resistance genes in microbes. This phenomenon of antimicrobial resistance is emerging as a major concern in global health. The most concerning hazard of which is the transfer of antimicrobial resistance genes to human pathogens. This could lead to conventional antimicrobial medications becoming ineffective against normally susceptible pathogenic illnesses. [52, 53] If these contaminants can contribute to such a phenomenon, understanding their release and fate in the environment is increasingly important.

1.3 Experimental Methodologies

1.3.1 Scale

Studies examining the decline of contaminant concentrations take place at one of three scales: macro-, meso-, and micro-. Macroscale studies are those that occur in soil exposed to the uncontrolled elements of nature, while mesoscale typically utilize greenhouses or soil columns. These distinctions are important when considering the values offered in previous degradation studies. For macro- and some mesoscale studies, authors usually refer to the observed phenomena in these studies as “dissipation” as opposed to “degradation” because all mechanisms of contaminant loss take place.

Our study was performed at the microscale, which precluded leaching and sought to isolate microbial activity as the primary driver of contaminant loss. Soil microcosms were incubated at 22 ± 1 °C and approximately 80% field capacity.

1.3.2 Solvent Carriers

The most common method of amending soils in microscale degradation studies is with solvent-carriers such as methanol [54], acetone [48], or acetonitrile [55]. Solvents such as these can decrease microbial populations and inhibit nitrification [56, 57]. They may also increase available organic carbon (OC) and when the solvent fraction is above 0.1%, it can potentially facilitate deeper penetration into pores depending on the soil and type of contaminant [58, 59]. Talc has been used as a carrier to avoid altering microbial behavior and increase homogeneity [60]. Our study primarily examined the degradation of resident contaminants (those found within the influent waste stream and eventually present in the biosolids that are subsequently formed), but also used an abbreviated solvent-spiked degradation study to deepen our data interpretation and serve as a point of comparison to the literature.

1.4 Degradation of Model Contaminants

1.4.1 Carbamazepine

In biosolids-free soil microcosms amended with solvent, observed CBZ half-lives range from 28.0 days [13] to 46.2 days [61]. In biosolids-amended soil microcosms, where CBZ is introduced through a solvent-spike, significant degradation has not been observed for experimental periods of up to 120 days [61-63]. There are, however, exceptions to these general trends. In Li et al. (2013) [61], one biosolids-free silty clay did not see any CBZ degradation over 120 days, but the half-life of CBZ increased from 46.2 to 108.3 days in a sandy clay loam after amendment with biosolids. Other studies have been able to document slight CBZ degradation in biosolids-amended soils even though they do not reach a half-life [61, 62]. Biosolids increasing persistence may be explained by evidence showing that they increase sorption of CBZ in soils [12], which would limit CBZ availability to microbial degradation.

Carbamazepine can also persist at larger scales of experimentation, but dissipates more quickly when it can be removed through leaching. In column and field studies, CBZ had 50% dissipation times (DT_{50}) of 97.6 days [64] and 46 days [65], respectively. In a greenhouse study with solvent-spiked CBZ in biosolids-free pots of soil, no significant degradation took place over 40 days [66]. In a 994 day greenhouse study, in which leachate was allowed to drain freely, resident CBZ had a half-life of 495 ± 36 days [67]. The rate of biosolids application in this study was relatively high at 2:1 and no effort was made to analyze the leachate. In Gottschall et al. (2012) [65], CBZ was the only compound to be detected multiple times in groundwater discharge. With a relatively low Log K_{oc} of 2.25, CBZ, while resistant to microbial degradation, may readily leach.

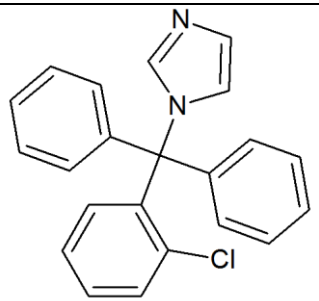
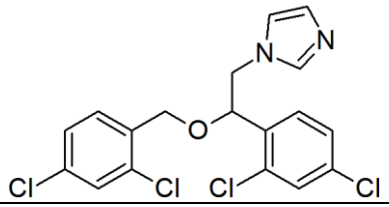
1.4.2 Miconazole

To date, research on MCZ persistence have been limited to three macroscale studies. Half-lives range from an observed 347 days [65] to an extrapolated 1,386 days [67]. Once again, biosolids amendment is correlated with increased contaminant persistence. In Chen et al. (2013) [68], no significant degradation took place in a plot that had received 3 annual biosolids applications. The persistence of MCZ may be enhanced by cation exchange capacity (CEC), as it can exist as a cation in the environmental pH range and thereby sorb to cation exchange sites.

Another azole fungicide, clotrimazole, has been the subject of microscale degradation studies by Sabourin et al. (2011) [69] and García-Valcárcel and Tadeo (2012) [70]. They reported DT_{50} values of 31 and 36.2-68.0 days, respectively. In the previously mentioned Chen et al. (2013) [68] study, clotrimazole had a DT_{50} of 1,019 days after a single biosolids application treatment. An important distinction between the two compounds is that clotrimazole is estimated to have a higher Log K_{oc} than MCZ, which would make more strongly sorbed in soil and, therefore, likely more persistent. Also, their difference in pK_a means that clotrimazole would have a smaller

cationic fraction than MCZ at a given environmental pH. The structure, molecular mass, pK_a, Log K_{ow}, and Log K_{oc} of clotrimazole are shown in Table 1.3 with a comparison to MCZ. These results suggest that a microscale study of solvent-spiked MCZ, a target contaminant of this study, may underestimate compound persistence in comparison to real-world environments due to ideal conditions that exist for microbial degradation in controlled incubations.

Table 3: Biosolids Contaminant Properties

	Structure	Formula CAS number	Molecular Mass (u)	pK_a	Log K_{ow}	Log K_{oc}
Clotrimazole		$C_{22}H_{17}ClN_2$ 23593-75-1	344.842	6.12[a]	4.1[a]	6.43[b]
MCZ		$C_{18}H_{14}Cl_4N_2O$ 22916-47-8	416.123	6.65[c]	6.25[d]	5.74[b]

Miconazole shown again for comparison

[a] García-Valcárcel and Tadeo (2012) [70] [b] EPI Suite Model, Chen et al. (2015) [16] [c] Bossche et al. (1987) [14] [d] EPI Suite Model, Chen et al. (2012) [15]

1.4.3 Triclocarban

Previously reported half-lives for solvent-spiked TCC at the microscale range from 74-231 days in biosolids-free soils [48, 54, 55, 71, 72]. A meta-analysis performed by Fu et al. (2016) [54] strongly supported biosolids' effect of increasing TCC persistence. Along with their review, they provided original data showing an increase in TCC half-life from 74 to 133 days in a soil after biosolids amendment at a 10% rate.

The persistence of TCC has also been documented at the meso- and macroscale. A column study by Al-Rajab et al. (2015) [64] found a half-life of 157.5 days for solvent-spiked TCC in a biosolids-amended soil. A longer half-life was determined for residual concentrations of TCC in farmland that had received biosolids application with an estimated half-life of 287.5 days [73].. These results are in contrast to the greenhouse Walters et al. (2010) study [67], where resident TCC concentrations showed no decrease over a 994-day experimental period.

1.4.4 Triclosan

Triclosan has received the most literature attention of the model contaminants included here. Half-lives for solvent-spiked TCS in biosolids-free soils at the microscale range from 2.0-83 days [13, 48, 54, 55, 71, 72, 74]. Once again, the meta-analysis by Fu et al. (2016) [54] found a positive correlation between TCS persistence and biosolids amendment. Their own data showed an increase in half-life from 10 to 63 days in a soil before and after amending with biosolids at a 10% rate. Due to their similarities in function and concomitance, TCS and TCC are often studied together, the latter consistently being found more persistent [48, 54, 55, 72].

Study of TCS at larger scales of experimentation seem to show increased persistence compared to the microscale. Solvent-spiked TCS in a soil column study underwent a DT_{50} of 72.9 days [64]. Walters et al. (2010) [67] and Gottschall et al. (2012) [65] reported similar values for

resident TCS of 187 and 182 days, respectively. However, at the macroscale, Langdon et al. (2012) [75] detected no resident TCS concentration decreases over 336 days in a biosolids-amended soil. The monitoring by Lozano et al. (2010) [76] of TCS concentrations in agricultural field soils previously amended with biosolids in years prior estimated a half-life of 107.4 days. In a follow-up to that study under more controlled conditions, TCS had a half-life of 104 days in an experimental agricultural plot [77].

1.5 Community Gardening

The phrase "community garden", along with its variants, was nearly absent from the English lexicon until World War I when the federal government and private organizations began promoting "Liberty Gardens" to ease wartime resource burdens and ensure agricultural resiliency [78]. These projects, later dubbed "Victory Gardens" [79], continued on through the Great Depression [80] and once again played an important role in American life during World War II [81]. After the war, use of the term declined to interwar levels until a resurgence in the 1970s, presumably due to fuel crises raising food prices. Community and urban gardening is commonly perceived as creating more beautiful, relaxing, and safe environments [82]. The occurrence of "community gardens" and similar phrases in the English lexicon can be found in Figure 1.1.

However, exposure to paints, fossil fuels, and pesticides has made lead (Pb) the most common hazardous contaminant in urban environments [83]. Legacy organic pollutants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls have also been detected in community gardens worldwide [84]. Class A biosolids are applied to community gardens, golf courses, and other public lands [6], but the environmental fate of the incident PPCPs following such uses has not been thoroughly researched.

1.6 Objective

The availability of numerous EQ Class A biosolids fertilizers that potentially harbor persistent, ecotoxicological contaminants necessitates the investigation of their environmental fate following application. The objective of this study was to answer the following: how will four model biosolids contaminants persist in community garden soils? Our hypothesis was that TCS would be the least persistent contaminant of the four and all concentrations would decrease to recalcitrant fractions. Results from previous studies had shown shorter half-lives of TCS relative to the other compounds as well as a tendency for contaminant concentrations to level-off, which likely signifies the existence of a non-bioavailable recalcitrant fraction.

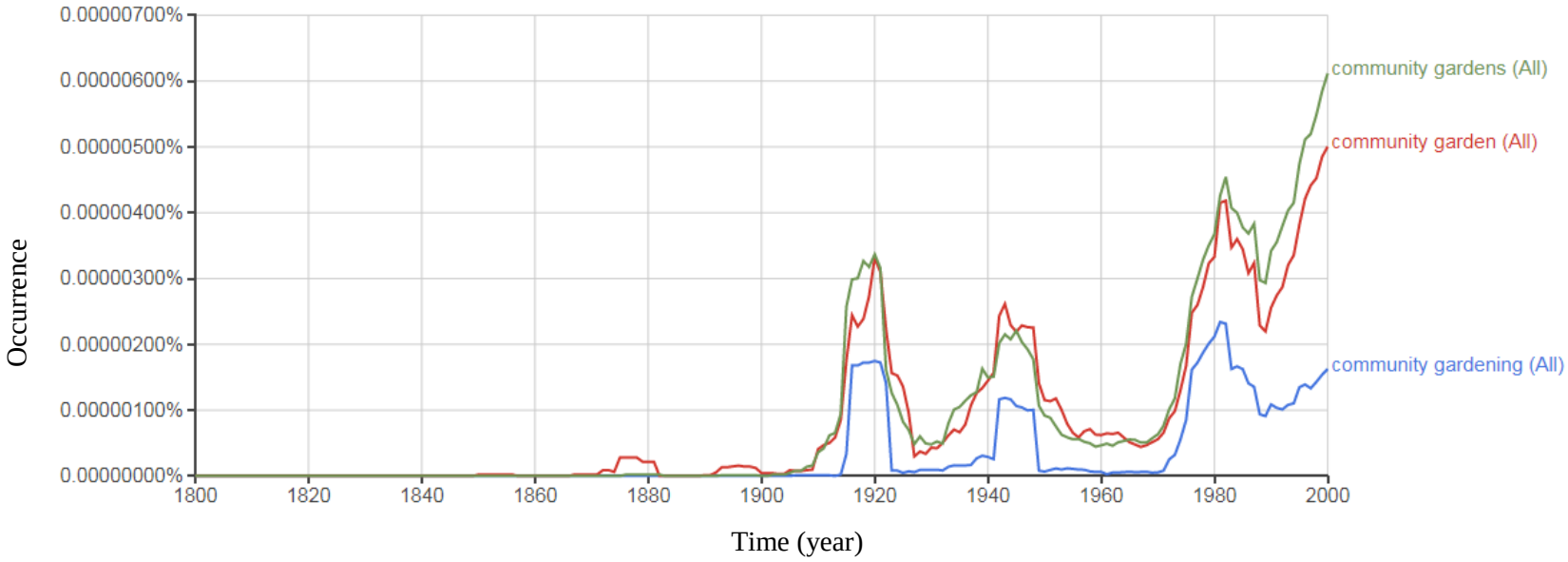


Figure 1: Occurrence (%) of “Community Garden” Phrasings in the English Lexicon over Time (year) [85]

CHAPTER 2. THE DEGRADATION OF RESIDENT BIOSOLIDS CONTAMINANTS WITHIN AEROBIC MICROCOSMS

2.1 Introduction

Exceptional Quality Class A biosolids can harbor numerous PPCPs in excess of 1,000 μg/kg [9], even though these are commonly applied to lands such as golf courses and community gardens [6]. The persistence of these contaminants has been an increasing focus of scientific inquiry, but the majority of research thus far seems to have been performed using municipal biosolids and agricultural soils. Understanding the environmental fate of these contaminants is vital to managing the hazards they may pose to humans, macrofauna, and soil microbes.

To better understand the persistence of these contaminants,four model compounds were selected: CBZ, MCZ, TCC, and TCS. Physiochemical properties including chemicalstructures, molecular mass, pK_a, Log K_{ow}, and Log K_{oc} are provided in Table 1.1. Miconazole and TCS are both ionizable, which adds an additional layer of complexity to their environmental behavior. Triclocarban and TCS have received more attention than CBZ and MCZ, but provide value to this study as a basis of comparison to previous literature. All four have been shown to be environmentally persistent with potentially ecotoxicological effects [28, 30, 31, 33, 41, 45]. Three have antimicrobial properties, which makes their release into the environment a potential driver in the propagation of antimicrobial resistance genes. Additional uncertainties, such as the limiting effects imposed by the biosolids matrices on degradation and how closely experimental methodologies reflect real-world systems are also considered [86].

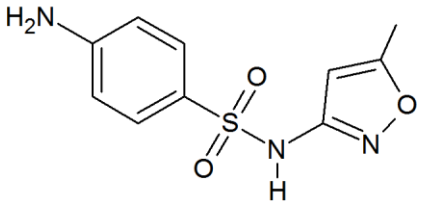
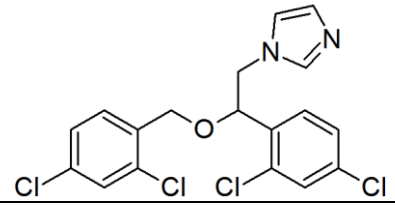
Commercially available biosolids fertilizers are available in pelletized, composted, cake, or semi-liquid forms [87] and there may be differences in contaminant fate between them. Langdon et al. (2011, 2013) [86, 88] found significant differences in resident TCS degradation between

centrifuge (CDB) and lagoon-dried biosolids (LDB), which the authors attributed to differences in percent and nature of OC. Such findings may mean there are differences in contaminant behavior between municipal biosolids and those EQ Class A biosolids sold as commercial products. The former being more heavily favored for use in previous degradation studies.

Langdon et al. (2013) [86] also observed significant differences in the degradation of resident and solvent-spiked contaminants. Solvent-spiked TCS degraded 27 times slower than resident for CDB and 1.6 times faster than that of LDB, but the solvent-spiked form still degraded at a similar rate between the two biosolids. The results for CDB run counter to the general observation of biosolids increasing persistence of solvent-spiked compounds [54, 61], but the recalcitrant fraction of resident TCS in CDB was higher than that of the spiked. Resident TCS has also been found to be more bioavailable to plants than when solvent-spiked [89]. These findings suggest half-lives derived from solvent-spiked degradation methods may not adequately reflect degradation of those contaminants resident in biosolids.

The observed behavior of our target contaminants in previous studies indicates that diffusion from within the biosolids matrix may be a limiting factor to degradation. The sorption of CBZ, TCC, and TCS increases with biosolids addition [12, 18, 48] and all three exhibit strong hysteresis [12, 48, 51]. No batch equilibration sorption studies exist for MCZ, but it has been shown to partition predominantly to sludge in wastewater treatment [90] and similar azoles have a high K_d in a variety of soils [91]. Biosolids aggregate interiors also have reduced oxygen concentrations [92], which have been shown to inhibit the degradation of CBZ [93], TCC, and TCS [72]. No relevant data exists for MCZ, but the similar azole sulfamethoxazole does dissipate more slowly under anoxic conditions [94]. The structure, molecular mass, pK_a, Log K_{ow}, and Log K_{oc} of sulfamethoxazole are shown in Table 2.1 with a comparison to MCZ.

Table 4: Biosolids Contaminants Properties

	Structure	Formula CAS number	Molecular Mass (u)	pK_a	Log K_{ow}	Log K_{oc}
Sulfamethoxazole		$C_{10}H_{11}N_3O_3S$ 723-46-6	253.276	1.69, 5.57[a]	0.89[b]	1.91 at pH 6.9[c]
MCZ		$C_{18}H_{14}Cl_4N_2O$ 22916-47-8	416.123	6.65[d]	6.25[e]	5.74[f]

Miconazole shown again for comparison

[a] Lucida et al. (2000) [95] [b] Demoling et al. (2009) [96] [c] Liu et al. (2010) [94] [d] Bossche et al. (1987) [14] [e] EPI Suite Model, Chen et al. (2012) [15] [f] EPI Suite Model, Chen et al. (2015) [16]

Broadly, our study aims to fill knowledge gaps on microscale degradation of environmentally persistent contaminants resident in commercially available EQ Class A biosolids when applied to community gardens. Results from previous studies have shown that TCS has shorter half-lives relative to our other model contaminants and a leveling of contaminant concentrations over time signifying a non-bioavailable recalcitrant fraction. This led us to hypothesize that TCS would be the least persistent and that concentrations of all four resident contaminants would reach a recalcitrant fraction. That is, a portion of contaminant that degrades at a much slower rate and is less bioavailable. Strong sorption to clay or OM can be one cause of contaminant recalcitrance. To test our hypothesis, biosolids-amended soil microcosms were incubated at 22 ± 1 °C and approximately 80% field capacity. They underwent sacrificial solvent extractions periodically over six months. Solvent-spiked contaminant degradation over 30 days and porewater desorption of resident contaminants were also performed. These additional analyses provided insight into our understanding as to what may limit resident contaminant degradation These quantified. Our study may be the first to quantify resident TCC, CBZ, and MCZ and solvent-spiked MCZ degradation at the microscale.

2.2 Materials and Methods

2.2.1 Soils and Biosolids

Soil samples were taken from the top 3 inches of two community gardens in Lafayette, Indiana. One soil, PCG-53, was taken from a community garden whose irrigated plots are rented to interested community members. The plots operated during the growing season from April to October and tilled before once they are rented. The second soil, BSC-54, was taken from an organically managed raised bed site. The soils were selected for their differences in OM. Soil properties including OM%, pH, CEC, texture, field capacity, and native moisture content can be

found in Table 2.2. The properties of pH, OM%, and CEC were determined by A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN). Soils were passed through a 2-mm sieve, homogenized, and stored at 4 °C until use. Their native moisture contents were approximately 80% field capacity.

Table 5: Soil Properties

	pH[a]	**OM[b] (%)**	**CEC[c]** $\left(\frac{meq}{100\ g}\right)$	**Sand[d] %**	**Silt[d] %**	**Clay[d] %**	**Field Capacity[e] (Θ_g, %)**	**Native Moisture (Θ_g, %)**
PCG-53	6.9	3.8	16.7	41 ± 1	27 ± 1	32 ± 0	21.46 ± 1.35	16.87 ± 0.48
BSC-54	7.3	16.4	38.9	51 ± 1	17 ± 1	32 ± 0	44.30 ± 0.67	35.40 ± 0.63

[a] 1:1 soil (g) : water (mL) slurry [b] loss on ignition [c] Mehlich-3 [d] hydrometer method; [97] [e] Pressure Plate Method; [98]

The biosolids used were the EQ Class A commercial product OCEANGRO®, which is generated by a Direct Drying System where the biosolid is dried by air at 850 °F and then pressed to to form dry pellets 1.5-2.5 mm in diameter [99]. It has a fertilizer N-P-K analysis of 5-5-0 [100]. Organic carbon content, analyzed with a FlashEA 1112 Nitrogen and Carbon analyzer (Thermo Fisher Scientific, Waltham, MA) was 36.1 ± 0.5%. Upon receipt, the biosolids were kept in an amber jar at room temperature.

2.2.2 Chemicals

High-performance liquid chromatography grade methanol (MeOH) and ethylacetate (EtOAc) were purchased from Thermo Fisher Scientific (Waltham, MA). Sulfuric acid (H_2SO_4, 98+% pure) was purchased from AMRESCO, Inc. (Cleveland, OH). Formic acid (98+% pure) was purchased from ACROS Organics. Ultrapure water was generated by a Barnstead™ Nanopure™ (D11901) water purification system with a Barnstead™ Final Filter (D3750).(±)-Miconazole nitrate salt (98+% pure)was supplied by Sigma-Aldrich (Darmstadt, Germany). Triclocarban (98+% pure) was supplied by Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Carbamazepine (98% pure) and TCS (99% pure) were supplied by Alfa Aesar (Tewksbury, MA).Deuterated molecules

were used as surrogate standards. (±)-Miconazole-D5 nitrate salt (98% pure), TCS-D3 (97% pure), and TCC-D4 (99% pure) were supplied by C/D/N Isotopes Inc. (Quebec, Canada). Carbamazepine-D10 (98+%) was supplied by the Cerilliant Corporation (Round Rock, TX).

2.2.3 Microcosm Preparation

The microcosms were prepared by adding 5.0 ± 0.1 g of soil (oven dry mass basis) to 35-mL glass round-bottom centrifuge tubes. The soil microcosms, at the native moisture content, which were approximately 80% of field capacity, were incubated in a dark Heraeus B12 incubator (Hanau, Germany) at 22 ± 1 °C for 7 days. Microcosms were prepared in triplicate replications. Experimentation with both soils was performed simultaneously.

2.2.4 Resident Compound Microcosm Preparation

After the initial 7-day incubation period, 300 ± 10 mg of biosolids were added to each microcosm and incorporated with light shaking. The biosolids to soil ratio was based on the recommended application rate. To maintain the ambient moisture levels in the soils after biosolids addition, 100 μL of ultrapure water was added to the microcosms. Samples for both soils were prepared in triplicate for each of the seven sampling times and stored in the incubator until time of sacrifice. To maintain an aerobic environment, microcosms were removed from the chamber and uncapped to aerate the headspace weekly. Moisture levels were kept consistent and adjusted as needed on a gravimetric basis.

2.2.5 Solvent-spiked Microcosm Preparation

After an initial 7-day incubation period, 300 ± 10 mg of biosolids were incorporated into each microcosm, which were similarly prepared in triplicate, amended with 100 μL of ultrapure water, and allowed to equilibrate for 3 hours. A 100 μL aliquot of MeOH containing a mix of

unlabeled CBZ, MCZ, TCC, and TCS was then added to each microcosm. Methanol had been used as a carrier solvent in previous degradation studies [54, 86]. Spiked concentrations of each chemical were at least twice that found native in the biosolids so as to allow differences in persistence to be more easily detectable. Ratios of solvent volume and contaminant concentration to oven-dry soil mass were similar to those reported previously [48]. The concentrations of solvent-spiked compounds within the microcosms can be found in Table 2.3. After solvent addition, the samples were left uncapped in a fume hood for 1 hour to allow the solvent to evaporate. After this, the samples were thoroughly mixed with a narrow steel spatula. The conditions of incubation were identical to resident degradation microcosms.

Table 6: Concentrations of Solvent-Spiked[a] Contaminants (µg/kg oven-dry mass)

	µg/kg oven-dry mass
CBZ	0.1
MCZ	0.5
TCC	3.0
TCS	1.0

[a] aliquot spike was 100 µL of a standard mix with the following concentrations (mg/L): 5.3 CBZ, 26.5 MCZ, 159.0 TCC, and TCS 53.0

2.2.6 Extraction

Resident contaminant microcosms were sacrificed 7 times at 0, 3, 30, 60, 105, 140, and 180 days. Solvent-spiked contaminant microcosms were sacrificed 4 times at 0, 3, 15, and 30 days. The extraction and cleanup methods were adapted in part from Chen et al. (2012) [15]. The following three sequential solvent equilibrations were performed: 48 hours in 20 mL MeOH, 24 hours in 10 mL MeOH, and 24 hours in 20 mL 10:10 v/v MeOH / 0.1% formic acid by volume / in ultrapure water. Surrogate standards in 1 mL MeOH were added along with solvent at the first extraction. After the addition of solvent at each step, tubes were vortexed for 1 min, sonicated for 30 min in a Branson 5800 ultrasonic bath (Branson Ultrasonics Corporation; Danbury, CT), and

equilibrated on an end-over-end rotator (Glas-Col; Terre Haute, IN) for either 24 or 48 hours. Tubes were covered in foil during rotation to limit potential photolysis. Solvent extracts were combined in 50-mL polypropylene centrifuge tubes and stored at 4 °C.

Sample clean-up began with the combined solvent extracts being centrifuged (Jouan, Inc.; Winchester, VA) at 3000 rpm for 15 min to separate remaining solids. The liquid contents were then transferred to a 0.5-L amber bottle and diluted to with ultrapure water so the final solution was ~8% MeOH by volume. The pH of diluted solvent extracts was lowered to pH 3 using H_2SO_4. Solid-phase extraction was performed using Oasis HLB 6 cc 200 mg cartridges (Waters Corp.; Milford, MA) on a Visiprep^{TM} 24 apparatus (Supelco; St. Louis, MO). The cartridges were preconditioned with 10 mL of MeOH followed by 10 mL of ultrapure water. The diluted samples were passed by vacuum via Teflon tubing through the cartridges at a dropwise rate of approximately 3 mL min^{-1}. When the initial volume was depleted, 100 mL of a 95:5 v/v of ultrapure water / MeOH was added to obtain any residuals in the bottle and tubing. Once transfer was complete, the cartridges were dried under vacuum for at least 15 min. Elution of the cartridges was performed three times with 4 mL of EtOAc. The resulting extract was brought to near dryness under a gentle stream of nitrogen gas in a RapidVap vacuum device (Labconco; Kansas City, MO). Samples were reconstituted in MeOH.

Relative recovery (Table 2.4) and matrix effects (Table 2.5) were determined using methods adapted from Huang et al. (2010) [101]. To determine extraction yield from the biosolids, duplicate samples were created that underwent a 4th solvent extraction (10 mL of MeOH equilibrated for 24 hours) following the usual 3-step solvent extraction method. The established contaminant concentration (ug / kg biosolid) obtained from the 3-step extractions of previous samples was divided by the sum of the same 3-step contaminant concentration and that determined

in the 4th step extractions of the duplicate samples. Using Equation 2.1, we found that extraction yield exceeded 90% for all contaminants. Extraction yield values shown in Table A5.

Equation 2.1: Extraction Yield (%)

$$\frac{3\text{-}step\ extract\ conc.\left(\frac{\mu g}{kg\ biosolids}\right)}{3\text{-}step\ extract\ conc.\left(\frac{\mu g}{kg\ biosolids}\right) + 4^{th} step\ extract\ conc.\left(\frac{\mu g}{kg\ biosolids}\right)} \times 100 = Extraction\ Yield\ (\%)$$

Table 7: Relative Recovery (%) for the Two Biosolids-Amended Soil Microcosms

	PCG-53	**BSC-54**
CBZ	142.7 ± 5.7	147.2 ± 6.2
MCZ	98.5 ± 3.2	94.0 ± 2.2
TCC	96.4 ± 4.1	102.4 ± 3.3
TCS	98.0 ± 4.9	102.6 ± 5.5

Table 8: Matrix Effect (%) for the Two Biosolids-Amended Soil Microcosms

	PCG-53	**BSC-54**
CBZ	139.7 ± 7.8	144.2 ± 6.6
MCZ	91.7 ± 2.8	92.2 ± 1.2
TCC	79.6 ± 10.0	76.7 ± 8.0
TCS	90.1 ± 4.9	78.4 ± 4.2

2.2.7 Porewater

This method was adapted from Choi et al. (2019) [102]. Glass syringes were rinsed with acetone and air-dried. Their outlets were capped and glass wool loaded within before adding 2 g biosolids, and 6 mL of an electrolyte solution of 0.5 mM calcium chloride and 3.08 mM sodium azide. The calcium chloride mimicked ionic conditions in soil and sodium azide inhibited microbial degradation of the contaminants. The syringes were incubated in darkness for 48 hours with plungers partially inserted to maintain constant headspace. After incubation, the aqueous solution was collected in a glass test tube. A pH measurement was taken for an aqueous solution. The spent biosolids were retained in the syringe and freeze-dried. Surrogate standard was added to the aqueous solution before cleanup with SPE in the same method as previously described. A

0.5 g subset of the freeze-dried biosolids underwent the same extraction and cleanup procedure as previously described. These data allowed us to calculate a single-point biosolids-water desorption coefficient (K_{bw}^{des}). Equation 2.2 shows the calculation for K_{bw}^{des}, while Equation 2.3 shows the calculation for K_{bw}^{des} normalized to OC ($K_{bw,oc}^{des}$).

Equation 2.2: Biosolids-water Desorption Coefficient

$$K_{bw}^{des} = \frac{C_s \left(mol/_{kg}\right)}{C_w \left(mol/_{L}\right)}$$

Equation 2.3: Biosolids-water Desorption Coefficient Normalized to Organic Carbon

$$K_{bw,oc}^{des} = \frac{K_{bw}^{des}}{f_{oc}}$$

2.2.8 Detection and Quantification

Sample analysis was performed on a Shimadzu 8040 LC-MS/MS equipped with electrospray ionization and accompanying LabSolutions Version 5.89 software (Shimadzu North America; Columbia, MD). The column used was a Kinetex 5 µm EVO C18 100 Å (Torrance, CA). Samples were run in 1:1 (v/v) MeOH / ultrapure water mixtures.

The method for CBZ and MCZ used 0.1% formic acid by volume in ultrapure water and ACN as mobile phases at a 0.4 mL min^{-1} flow rate in positive ion mode. The method for TCC and TCS used 0.15% acetic acid by volume in ultrapure water and MeOH for mobile phases at a 0.3 mL min^{-1} flow rate in negative ion mode. Both methods were run on a binary gradient. Product ions can be found in Table 2.6. Limits of detection (LOD) and quantification (LOQ) (Table 2.7) were determined at a 25 µL injection volume for all compounds according to the method found in Harris (2010) [103]. The definitions for the LOD and LOQ for this method are shown in Equations 2.4 and 2.5.

Equation 2.4: LOD Definition

$$\text{LOD} = \frac{3s}{m}$$

Equation 2.5: LOQ Definition

$$\text{LOQ} = \frac{10s}{m}$$

Table 9: Product Ions (m/z) and Retention Time (min)

	Product Ion (m/z)	**Retention Time (min)**
CBZ	236.80 > 194.10	2.7
CBZ-D10	246.90 > 204.25	2.7
MCZ	416.75 > 159.10	3.1
MCZ-D5	421.75 > 164.00	3.1
TCC	313.10 > 160.10	6.6
TCC-D4	319.05 > 162.05	6.6
TCS	287.10 > 35.00	6.7
TCS-D3	290.05 > 34.90	6.6
2,4-DCP	160.95	4.6
3,4-DCA	161.90 > 127.00	4.2

Table 10: Limits of Detections and Quantification for Target Contaminants (ng/L)

	LOD	**LOQ**
CBZ	6	19
MCZ	31	104
TCC	27	90
TCS	119	397
2,4-DCP	298	994
3,4-DCA	1314	4381

2.2.9 Modeling

First-order half-lives (β_1) were calculated using linearized semi-ln plots of the concentration in µg of contaminant per kg of biosolids vs time in days (Equation 2.6). Solvent-spiked concentrations were also presented in this manner in order to have a basis of comparison with resident degradation data. Furthermore, resident contaminant concentrations were subtracted from solvent-spiked data to better isolate the degradation of the solvent-spiked contaminants. When analyzing data for a single resident or solvent-spiked contaminants, the t0 data from both

soils was aggregated. The justification being that no degradation had yet occurred, so there had been no soil effect.

Equation 2.6: First-Order Degradation Model

$$ln\ concentration\left(\frac{\mu g\ contaminant}{kg\ biosolids}\right) = \beta_1 \times time\ (days) + \beta_0$$

2.2.10 Statistical Analysis

All statistical analyses were performed at the 95% confidence level. The software used was SAS 9.4 (SAS Institute, Inc.; Cary, NC). Two analyses were performed: a determination of whether the mean degradation rates were significantly different from zero (H_0: $\beta_1 = 0$; H_a: $\beta_1 \neq 0$) and a determination of differences between mean degradation rates (H_0: $\beta_1 = \beta_1'$; H_a: $\beta_1 \neq \beta_1'$). For both, an F-test was performed for the model and a t-test was performed for the regression coefficients, i.e., degradation rates. The codes and example SAS 9.4 outputs for both tests are provided in Figures A4 and A5. Comparisons of degradation rates were done between either the two soils or the two carrier matrices. A small number of outlier points in resident degradation were identified by the method described in Timme and Frehse (1980) [104] and therefore not included in data analysis. Standard error of the half-life was calculated according to method found in Bryan et al. (1990) [105].

2.3 Results and Discussion

Resident TCS, in both soils, was the only contaminant for which a half-life was observed. Concentrations for resident CBZ, MCZ, and TCC did not decrease below 50% in the 180-day time period. Normalized resident concentration trends are shown in Figure 2.1. No solvent-spiked contaminant concentrations decreased below 50%. Normalized solvent-spiked concentrations trends are shown in Figure 2.2. For those resident or solvent-spiked contaminants with statistically

significant mean concentration decreases over time (p-values for this determination found in Table 2.8), the half-life provided is one that is modeled outside the experimental timeframe. Degradation rates are listed in Table 2.9, while half-lives are listed in Table 2.10. Soil type was found to have nearly no impact on contaminant degradation (p-values for these comparisons are in Table 2.11). The complete datasets showing concentration over time for the resident and solvent-spiked degradation studies is shown in Tables A1, A2, and A3 and Figures A1 and A2.

Table 11: p-Values for the F-test of Degradation Rate (H_0: $\beta_1=0$; Ha: $\beta_1\neq0$)

	PCG-53	**BSC-54**
Resident CBZ	0.0003	0.0228
Solvent-spiked CBZ	0.0041	0.0001
Resident MCZ	<0.0001	<0.0001
Solvent-spiked MCZ	0.0008	0.0005
Resident TCC	0.2334	0.4424
Solvent-spiked TCC	0.0132	0.1036
Resident TCS	<0.0001	<0.0001
Solvent-spiked TCS	0.0422	0.0266

Table 12: First-Order Contaminant Degradation Rates ($days^{-1}$)

	PCG-53	**BSC-54**
Resident CBZ	1.93×10^{-3} (4.59×10^{-4})	1.53×10^{-3} (6.32×10^{-4})
Solvent-spiked CBZ	3.42×10^{-3} (9.82×10^{-4})	5.55×10^{-3} (1.01×10^{-3})
Resident MCZ	1.34×10^{-3} (2.53×10^{-4})	1.23×10^{-3} (1.98×10^{-4})
Solvent-spiked MCZ	6.56×10^{-3} (1.52×10^{-3})	6.33×10^{-3} (1.38×10^{-3})
Resident TCC	1.24×10^{-4} (1.02×10^{-4})	1.53×10^{-4} (1.97×10^{-4})
Solvent-spiked TCC	3.53×10^{-3} (1.23×10^{-3})	3.32×10^{-3} (1.89×10^{-3})
Resident TCS	4.95×10^{-3} (2.85×10^{-4})	6.58×10^{-3} (6.06×10^{-4})
Solvent-spiked TCS	4.88×10^{-3} (2.12×10^{-3})	5.14×10^{-3} (2.06×10^{-3})

Resident rates over 180 days, solvent-spiked rates over 30 days
Standard error shown in parentheses

Table 13: First-Order Half-Lives (days) in the Two Biosolid-Amended Soils PCG-53 and BSC-54

	PCG-53	**BSC-54**
Resident CBZ	*359 (85)*	*453 (187)*
Solvent-spiked CBZ	*203 (58)*	*125 (23)*
Resident MCZ	*516 (97)*	*562 (90)*
Solvent-spiked MCZ	*106 (24)*	*110 (24)*
Resident TCC	*5582 (4580)*[a]	*4516 (5792)*[a]
Solvent-spiked TCC	*196 (68)*	*209 (119)*[a]
Resident TCS	140 (8)	105 (10)
Solvent-spiked TCS	*142 (62)*	*135 (54)*

Modeled values outside experimental timeframe shown in italics
Standard error shown in parentheses
[a] Degradation rate not significantly different from zero

Table 14: p-Values for t-test Comparison of Degradation Rate between Biosolid-Amended Soils PCG-53 and BSC-54 (H_0: $\beta_1 = \beta_1'$; H_a: $\beta_1 \neq \beta_1'$)

Resident CBZ	0.6072
Solvent-spiked CBZ	0.1414
Resident MCZ	0.7342
Solvent-spiked MCZ	0.9117
Resident TCC	0.8953
Solvent-spiked TCC	0.9241
Resident TCS	0.0193
Solvent-spiked TCS	0.6072

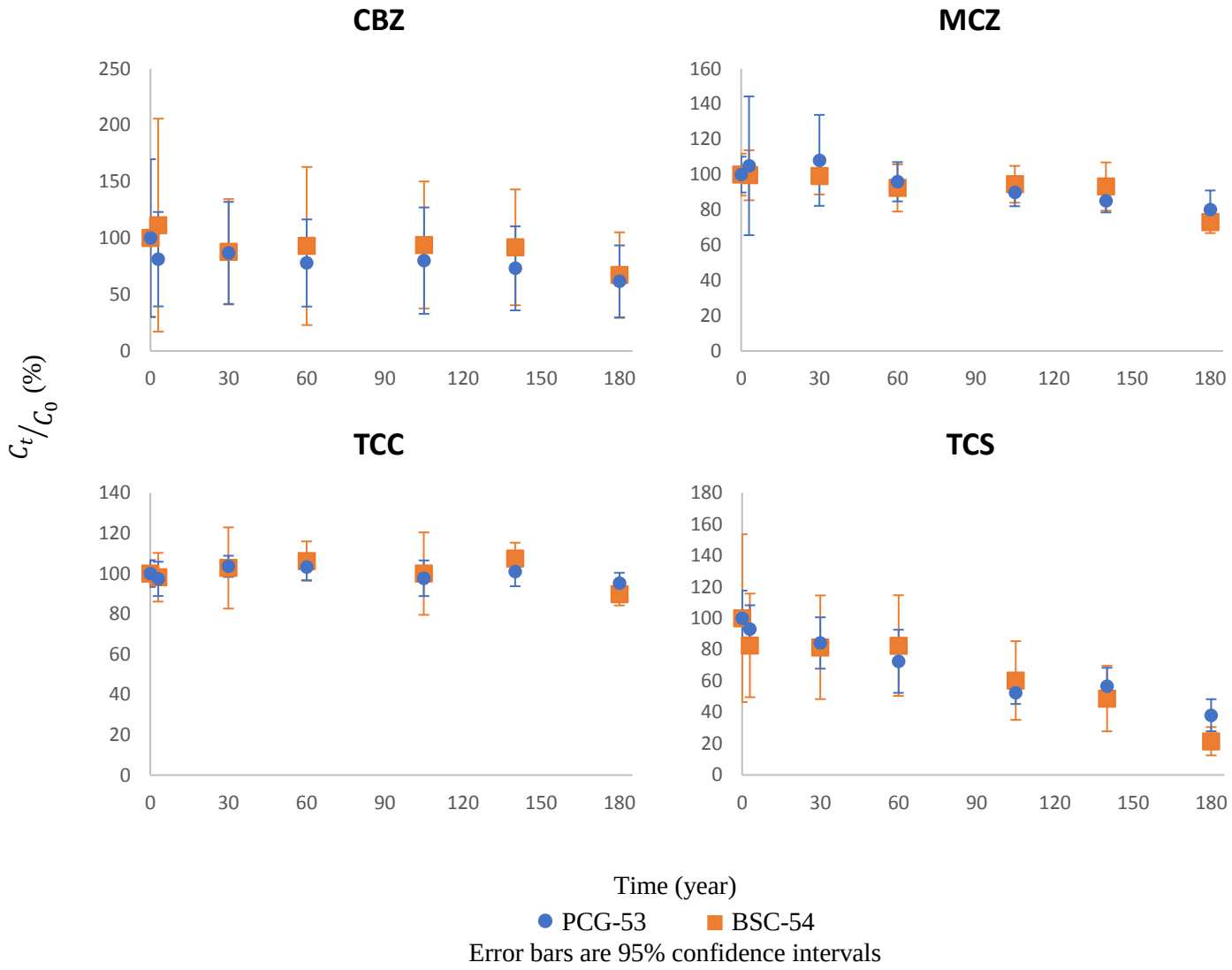


Figure 2: Degradation of Resident Biosolids Contaminants Normalized by t=0 Concentration

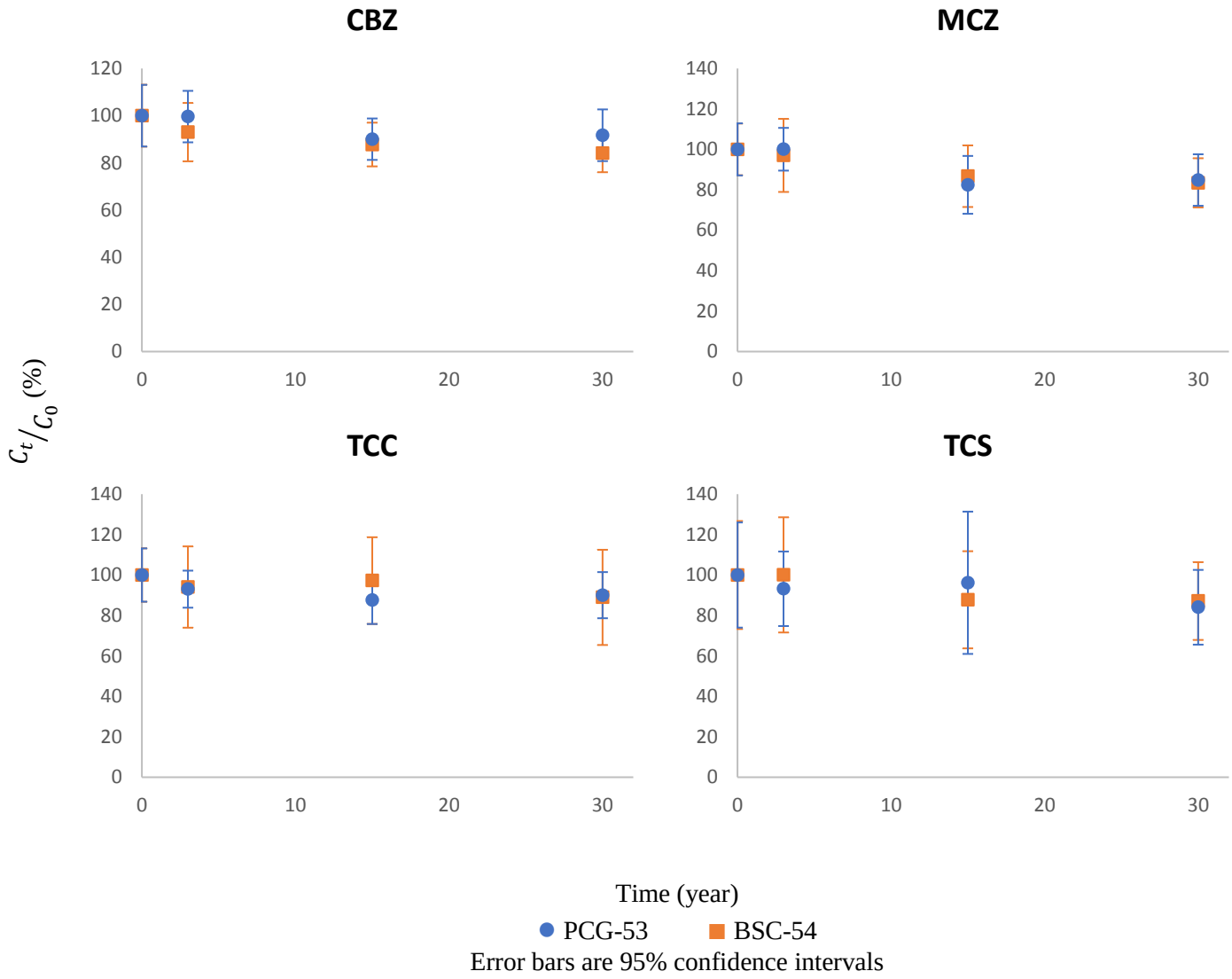


Figure 3: Degradation of Solvent-spiked Biosolids Contaminants Normalized by t=0 Concentration

The supporting solvent-spiked degradation and porewater desorption experiments sought to provide some insight into the role of the biosolids matrix on resident contaminant degradation. The conclusions drawn from the available literature led us to believe that desorption from the biosolids matrix may have been a limiting step in degradation.

Some statistically significant differences were found between the mean degradation rates of resident and solvent-spiked contaminants. Solvent-spiked MCZ in both soils and solvent-spiked TCC in PCG-53 had significantly mean higher degradation rates than their resident counterparts (p-values for these comparisons are in Table 2.12).

Table 15: p-Values for t-test Comparison of Degradation Rates between Resident and Solvent-Spiked Contaminants in the Two Biosolid-Amended Soils PCG-53 and BSC-54 (H_0: $\beta_1 = \beta_1'$; H_a: $\beta_1 \neq \beta_1'$)

	PCG-53	**BSC-54**
CBZ	0.8613	0.8120
MCZ	0.0040	0.0012
TCC	0.0011	0.0551
TCS	0.8751	0.9780

The trend of these desorption coefficients was TCS > TCC > CBZ (K_{bw}^{des} and $K_{bw,oc}^{des}$ values found in Table 2.13). For MCZ, the aqueous phase concentration was below LOD, so a Log K_{bw}^{des} and $K_{bw,oc}^{des}$ could only be estimated as > 4.80 and > 5.24, respectively. The Log K_{oc} of MCZ has been modeled as 5.74 [16], which would make it strongly sorbed to biosolids OM. This strong sorption may be partly explained by its cationic quality and sorption to cation exchange sites. While no comparable sorption data for MCZ are available, our $K_{bw,oc}^{des}$ values for CBZ, TCC, and TCS were similar to K_{oc} values previously reported [13, 18]. The datasets showing C_s and C_w for the porewater desorption study in shown in Table A4.

Table 16: Partition Concentrations and Coefficients (L/kg)

	C_s (mol/kg)	C_w (mol/L)	K_{bw}^{des}	Log K_{bw}^{des}	$K_{bw,oc}^{des}$	Log $K_{bw,oc}^{des}$	Literature Log K_{oc} values
CBZ	$4.18 \pm 0.18 \times 10^{-07}$	$7.82 \pm 0.34 \times 10^{-09}$	53.6	1.73	1.48	2.14	2.02[b]-3.10[c]
MCZ	$5.74 \pm 0.32 \times 10^{-06}$	< LOD	> 6323[a]	> 4.80[a]	> 175,140[a]	> 5.24	5.74[f]
TCC	$7.77 \pm 0.20 \times 10^{-05}$	$3.18 \pm 0.71 \times 10^{-08}$	2530	3.40	70.0	3.91	3.65-3.96[h]
TCS	$2.18 \pm 0.12 \times 10^{-05}$	$2.50 \pm 0.52 \times 10^{-09}$	8940	3.95	247	4.41	3.81[c]-4.38[i]

[a] parameters estimated using LOQ

When investigating sorption, ionizability must be considered. The pH of OCEANGRO® is 7 [100]. The pH of the aqueous solution collected in the porewater analysis was 6.74. The biosolids-amended PCG-53 microcosm had a pH of 6.83 ± 0.20, while that of BSC-54 was 7.12 ± 0.01. As influenced by their cationic and anionic speciation (Equations 1 and 2), both MCZ and TCS may have been more strongly sorbed in the porewater analysis than in the biosolids-amended soil microcosms. MCZ would have had a higher cationic fraction in the porewater solution, while TCS would have had a higher anionic fraction within the biosolids-amended soil microcosms.

Taken together, the data from these two studies provides evidence that degradation of some contaminants is limited by the biosolids matrix. Miconazole was likely the most strongly sorbed contaminant and likewise saw the greatest increase in degradation rate for the solvent-spiked form. Although CBZ had the lowest K_{bw}^{des} of all model contaminants, its persistence might be explained by limitations in the microbial communities ability to degrade the contaminant given its triple-ring aromatic structure Carbamazepine has been detected in numerous waterbodies and is considered one of the most environmentally persistent pharmaceutical contaminants [12]. Therefore, desorption may have been but one possible limitation in contaminant degradation. Other possible limitations being microbial enzymatic effectiveness.

The chemical structures of all four model contaminants have elements that make degradation by microbes more difficult. The halogen groups of MCZ, TCC, and TCS and the aromaticity of all four provides electronegative stability that imposes an energetic barrier to the chemical reactions underlying microbial attack. Their high molecular masses also deprioritize them as potential microbial energy sources. In addition, the former three contaminants are antimicrobials whose function is to inhibit microbial activity. [106]

Triclosan having an anionic fraction, due to its hydroxyl functional group, may partly explain its rapid degradation relative to the other contaminants. Anionic TCS sorbs less strongly to OM than its neutral form [47, 48]. Decreases in sorption allow for greater access by soil microbes due to increased solubility. Anionic TCS may have been able to diffuse over the experimental timeframe in a way other contaminants could not.

Contaminant desorption from biosolids has been described in previous macroscale studies. Butler et al. (2012) [107] demonstrated that TCS does diffuse from heat-dried biosolids when applied to fields. Although the majority of recovered contaminant (either as TCS or methyl-TCS) was found in the top 10 cm of soil over a 12-month period, which means only a minor fraction may actually enter waterbodies through such a pathway. Gottschall et al. (2012) [65] detected CBZ, TCC, TCS, but not MCZ in groundwater following a single application of dewatered municipal biosolids. This mirrors our own results in which MCZ was the only contaminant not detected in the aqueous phase of our porewater desorption analysis. While these results provide support for the possibility that our target contaminants (with the possible exception of MCZ) can diffuse from a biosolids matrix, unlike in our microcosms, conditions likely included saturation. Therefore, such diffusion would have been diminished in our relatively stagnant systems.

Our data shows that, at least for some contaminants whose degradation may be desorption limited, solvent-spike methodologies may underestimate resident contaminant persistence. Any solvent-spiked contaminant may have sorbed more weakly to soil OM than its resident counterpart was sorbed to biosolids due to a lack of age effects [108, 109] or not sorbed at all before being degraded by microbes.

The solvent-spike data also allowed for comparisons to previous literature that also employed such a method with our target compounds. Comparison of our data to a meta-analysis

of half-life correlated to soil OC as reviewed by Fu et al. (2016) [54] revealed that our modeled half-lives of solvent-spiked TCC and TCS are within calculated prediction intervals (shown in Figure A3). These data also reveal that the soil OC of our microcosms were in fact higher than in nearly all previous studies, which shows our community garden soils were somewhat atypical from those commonly included in previous studies.

Solvent-spiked CBZ in our study had a comparable modeled half-life to that reported by Li et al. (2013) [61] with 108.3 days observed in biosolids-amended soils. Yu et al. (2013) [13] found shorter half-lives of approximately 30 days while other studies reported no degradation of CBZ [61-63, 66]. Miconazole has not been the subject of any previous microscale studies, but the resident persistence conforms to the trend established by high OC% field studies [65, 67, 68].

Langdon et al. (2011, 2013) [86, 88], the only previous studies to examine the degradation of the resident organics in biosolids for any of our target compounds provide a valuable point of comparison. In those studies, TCS degraded to recalcitrant fractions between 1 and 50 days. However, the dissimilarities between our results supports a postulation of these two studies in that differences in biosolids matrices can lead to variable degradation behaviors. Our microcosms had OC of approximately 4.1 and 11.0%, while theirs was approximately 2%. Although not explicitly described by the authors, their biosolids may have had a cake-like consistency, which would have allowed for more thorough incorporation and made the contaminants more accessible to soil microbes.

To confirm the degradation trend beyond statistical means, an attempt was made to quantify the presence of known TCC and TCS metabolites, which were the two most abundant target contaminants. However, neither 3,4-dichloroaniline nor 2,4-dichlorophenol concentrations were above the LOD in any samples. Limits of detection listed in Table 2.7. Kwon and Xia (2012)

[110] used biosolids with similar TCC concentrations as our own and were also not able to detect 3,4-DCA nor three other metabolites. 2,4-dichlorophenol has been shown to be a TCS metabolite [111, 112], but has not yet been used to confirm its degradation in a study such as this. The degradation pathways of our contaminants may have progressed in a manner wherein these metabolites were not present in detectable concentrations for appreciable amounts of time.

2.4 Conclusion

Our results indicate that the resident biosolids contaminants CBZ, MCZ, TCC, and TCS have the potential for long-term persistence within soil. The persistence of all four of our model contaminants would exceed the 90-day criterion to be considered “persistent” by the EPA [113]. Our solvent-spiked degradation and porewater data suggested that desorption might not be the limiting factor to degradation for all resident biosolids contaminants and that solvent-spiked half-lives may underestimate real-world persistence. This would imply that even if a contaminant can readily enter the aqueous phase, there might still be limitations in terms of microbial degradation capability. Such contaminants that are both persistent and mobile would likely be the most hazardous.

CHAPTER 3. IMPLICATIONS AND FUTURE WORK

This study sought to quantify the persistence of four model contaminants resident in the commercially available EQ Class A biosolids product OCEANGRO®. This was performed using community garden soils, which are typically amended with such products. This analysis demonstrated that resident contaminants can be persistent in biosolids-amended soils.

Our solvent-spiked degradation and porewater studies showed that desorption from the biosolids matrix may present a limiting factor to degradation, but not for all of our model contaminants. These results also provided insight into the utility of common literature methodologies by suggesting that the solvent-spiked half-lives underestimates persistence of resident contaminants. These potential differences between the real world and research at the lab scale must be taken into consideration by future researchers during literature review and data interpretation. Nonetheless, half-lives of solvent-spike contaminants still hold value as representations of degradation after desorption has taken place or in mimicking the behavior of compounds present in solvent contamination.

These data on contaminant fate are important as to further our understanding of the risk such contaminants may pose to non-target species. What is vital to consider is that those contaminants that are more persistent may not necessarily be more hazardous, if that persistence is facilitated by strong sorption, meaning they would not be bioavailable [114, 115]. This is likely the case for MCZ, which had a modeled half-life in the hundreds of days and was likely the contaminant most strongly bound to the biosolids matrix. However, when the persistence is not a result of strong sorption, the contaminant may still be mobile. In our study, CBZ was the least strongly bound while also being comparably persistent. Carbamazepine is, in fact, ubiquitous in

waterbodies and considered one of the most persistent pharmaceutical contaminants [12]. Its persistence may be the result of microbial difficulty in degrading its three-ring aromatic structure.

The literature consensus seems to be that PPCP contaminants in biosolids present a low risk for toxic effects in humans, although data gaps remain as to possible synergistic effects [28, 116, 117]. The porewater concentrations we measured for TCC (4.00 ± 0.10 Log ng/L) and TCS (2.86 ± 0.09 Log ng/L) exceed toxicity thresholds for crustacea and algae, respectively [118].

Ecotoxicity may be of lower priority than the emerging concern of antimicrobial resistance. This is the phenomenon whereby the release of antimicrobial compounds selects for resistance genes in the environment. This selection pressure can potentially lead to conventional antimicrobial medications becoming ineffective against human illnesses. [52, 53] The Center for Disease Control estimates there are at least 23,000 deaths per year attributable to antimicrobial-resistant illnesses [119].

The model contaminants used in this study have a direct relationship to this environmental antimicrobial resistance. Miconazole, TCC, and TCS are designed as antimicrobial compounds and can impose this selection pressure. Miconazole-resistant *Candida* has been isolated in clinical environments [120] and a relationship between agricultural fungicide use and the occurrence of antifungal-resistant infections has been proposed [121]. Exposure to TCC and TCS does increase resistance to those compounds in environmental bacteria [122, 123]. Carbamazepine is not intended to function as an antimicrobial, but has been shown to affect abundances of microbial taxa in soil [124]. It is important to consider that exposure to even a single antimicrobial can induce broad-spectrum resistance to a suite of different compound classes [125].

The interplay between organic amendments and soil in propagating antimicrobial resistance genes is a topic still being pursued by researchers. Most studies thus far have focused

on agricultural lands, in part due to the pervasive administration of antibiotics to livestock. Links between animal manure and antimicrobial-resistant bacteria have been made in numerous studies [126-128]. Although at lower concentrations than in manure, antimicrobial resistance genes have also been detected in biosolids [129]. As in all soils, diverse sequences of antimicrobial resistance genes are present in urban community gardens [130]. The links between commercial EQ Class A biosolids and antimicrobial-resistant microbes have not been thoroughly explored. Such research is challenging, but given the pervasiveness of our model contaminants and other antimicrobials in the environment, global involvement in the surveillance of these genes will be crucial to safeguarding the public health [52].

Our results, demonstrating the persistence of ecotoxic and antimicrobial organic contaminants, have important implications for the contaminant profiles of community garden soils. Some contaminants may be strongly bound to OM, therefore not mobile nor bioavailable, but soil concentrations will accumulate if inputs continually exceed outputs. Such accumulation may increase risks of unloading events should there be marked changes in soil properties. The behaviors of MCZ and TCS would be particularly susceptible to pH changes due to their ionizability. Lime application would raise pH and potentially decrease sorption for both compounds. Community gardens may also receive lower pH with sulfur applications for pest or fungi control as well as maximize growth for specific crops being grown. Changes in moisture content or OM would also affect contaminant partitioning and environmental fate. Rain events also increase risks of erosion, which can transport contaminated soils to waterways. The impact of management practices presents a special challenge in the context of community gardens as those operating them may not be as well informed on best management practices. Over-application of biosolids fertilizer is one example of an improper practice that could hasten contaminant accumulation.

Given that those community gardens and other public lands amended with biosolids are likely to harbor persistent organic contaminants, their continued monitoring is warranted. Several studies have characterized contaminant occurrence in agricultural fields years after biosolids application [73, 76]. To date, studies on community gardens have focused on well-known legacy pollutants such as PAHs and PCBs [84]. One potential barrier to a comprehensive understanding of the link between present PPCP contaminant concentrations and previous biosolids use in community gardens may be a lack of recordkeeping that is typical of agricultural operations.

Biosolids application practices are generally accepted by the public, but some reservations concerning quality of life and public health remain [131, 132]. Community engagement must remain central to holistic strategies that continue to monitor and address the possible externalities of biosolids use. The results of this degradation study and others like it should be incorporated into the groundwork of such assessments.

For most people, when something goes down the drain, it is no longer their concern. In a world of complex environmental matrices, this is only the beginning. Moving forward, a culture of education, rooted in mindfulness and vigilance, will be vital if the harms of anthropogenic compounds are to be minimized or avoided entirely.

APPENDIX

Table A1: Concentration of Resident Contaminants (µg/kg biosolids)

	CBZ		MCZ		TCC		TCS	
	PCG-53	BSC-54	PCG-53	BSC-54	PCG-53	BSC-54	PCG-53	BSC-54
180-1	53	53	2057	1765	20253	18606	2228	1084
180-2	51	63	1956	1770	19991	19305	1997	1308
180-3	46	48	1826	1758	19688	18602	1746	1199
140-1	62	71	2087	2098	20923	22976	3248	2823
140-2	61	67	2086	2308	20749	22857	2821	2926
140-3	55	86	2035	2358	21843	21803	2810	2396
105-1	57	85	2165	2356	21425	19597	2703	3282
105-2	77	82	2248	2193	19977	19881	2683	3099
105-3	60	61	2144	2307	20060	23424	2824	3682
60-1	64	63	2446	2360	21440	22234	3823	4397
60-2	62	62	2321	2093	22179	21396	4249	4822
60-3	62	100	2230	2252	21310	23198	3292	4565
30-1	65	66	2382	2347	21656	23689	4772	4712
30-2	70	79	2531	2490	21982	21481	4121	4141
30-3	77	68	2969	2361	21544	19502	4302	4750
3-1	61	*245*	2331	2642	20114	20299	4925	4594
3-2	63	80	2397	2330	19897	22312	4990	4674
3-3	64	68	3401	2292	20642	20987	4998	4795
3-1rep	70	137	2393	2343	21668	19019	5009	4086
3-2rep	72	75	2239	2439	19786	20341	4402	4854
0-1	63	76	2465	*1372*	21370	21370	5568	5080
0-2	73	72	2415	2415	21807	21807	5801	5568
0-3	64	92	2603	2603	20765	20765	*8315*	5801
0-1rep	64	121	2300	2409	20171	20760	5119	4891
0-2rep	104	-	2435	-	20690	-	4875	-

Sample identifiers follow the format “day-replicate number”, those with “rep” are replicate samples that were created at a later time than the originals, outliers in red italics

Table A2: Concentration of Solvent-spiked Contaminants (µg / kg biosolids)

	CBZ		**MCZ**		**TCC**		**TCS**	
	PCG-53	BSC-54	PCG-53	BSC-54	PCG-53	BSC-54	PCG-53	BSC-54
30-1	1,776	1,640	11,725	11,313	74,226	79,844	18,938	17,441
30-2	1,689	1,626	11,692	12,421	78,148	80,903	17,329	18,316
30-3	1,817	1,601	12,724	11,933	78,748	68,307	17,734	19,044
15-1	1,760	1,691	11,296	13,057	72,755	79,319	22,967	17,259
15-2	1,708	1,651	11,553	11,536	78,143	76,836	19,959	19,937
15-3	1,729	1,734	12,626	12,331	75,689	88,527	17,977	19,311
3-1	1,966	1,848	14,135	14,008	78,848	75,573	20,422	19,210
3-2	1,909	1,826	13,526	14,092	79,957	77,113	19,463	22,264
3-3	1,856	1,695	13,933	12,457	78,083	86,184	20,444	22,095
0-1	1,984	1,989	14,769	14,173	85,879	83,066	21,949	20,464
0-2	2,010	1,847	13,677	13,792	77,873	84,005	23,411	22,141
0-3	1,874	1,805	13,385	13,410	83,132	85,980	20,642	19,102

Sample identifiers follow the format “day-replicate number”

Table A3: Concentration of Solvent-spiked Contaminants after Resident Concentrations Subtraction (µg / kg biosolids)

	CBZ		**MCZ**		**TCC**		**TCS**	
	PCG-53	BSC-54	PCG-53	BSC-54	PCG-53	BSC-54	PCG-53	BSC-54
30-1	1,706	1,563	9,314	8,957	53,283	58,769	14,549	12,923
30-2	1,619	1,548	9,281	10,065	57,205	59,827	12,940	13,797
30-3	1,747	1,524	10,313	9,577	57,805	47,231	13,345	14,525
15-1	1,688	1,612	8,836	10,657	51,773	58,195	18,239	12,271
15-2	1,636	1,571	9,093	9,136	57,161	55,712	15,232	14,949
15-3	1,657	1,654	10,166	9,931	54,707	67,403	13,249	14,323
3-1	1,893	1,768	11,636	11,573	57,835	54,410	15,405	13,812
3-2	1,835	1,745	11,027	11,657	58,944	55,950	14,446	16,866
3-3	1,782	1,614	11,433	10,022	57,070	65,021	15,428	16,698
0-1	1,910	1908	12259	11729	64,858	61,894	16858	14,959
0-2	1,936	1766	11167	11348	56,852	62,832	18320	16,636
0-3	1,800	1723	10875	10966	62,111	64,808	15550	13,597

Sample identifiers follow the format “day-replicate number”

Table A4: Porewater Desorption Concentrations

	C_s (mol/kg)	C_w (mol/L)
CBZ	4.14×10^{-7}	8.21×10^{-9}
	4.03×10^{-7}	7.69×10^{-9}
	4.38×10^{-7}	7.57×10^{-9}
MCZ	6.08×10^{-6}	< LOD
	5.71×10^{-6}	< LOD
	5.44×10^{-6}	< LOD
TCC	7.91×10^{-5}	2.72×10^{-8}
	7.54×10^{-5}	4.00×10^{-8}
	7.86×10^{-5}	2.82×10^{-8}
TCS	2.04×10^{-5}	2.21×10^{-9}
	2.27×10^{-5}	2.19×10^{-9}
	2.23×10^{-5}	3.10×10^{-9}

Table A5: Extraction Yield from Biosolids (%)

CBZ	> 99.4[a]
MCZ	94.2 ± 4.8
TCC	96.9 ± 3.2
TCS	98.0 ± 8.7

[a] estimated using LOQ

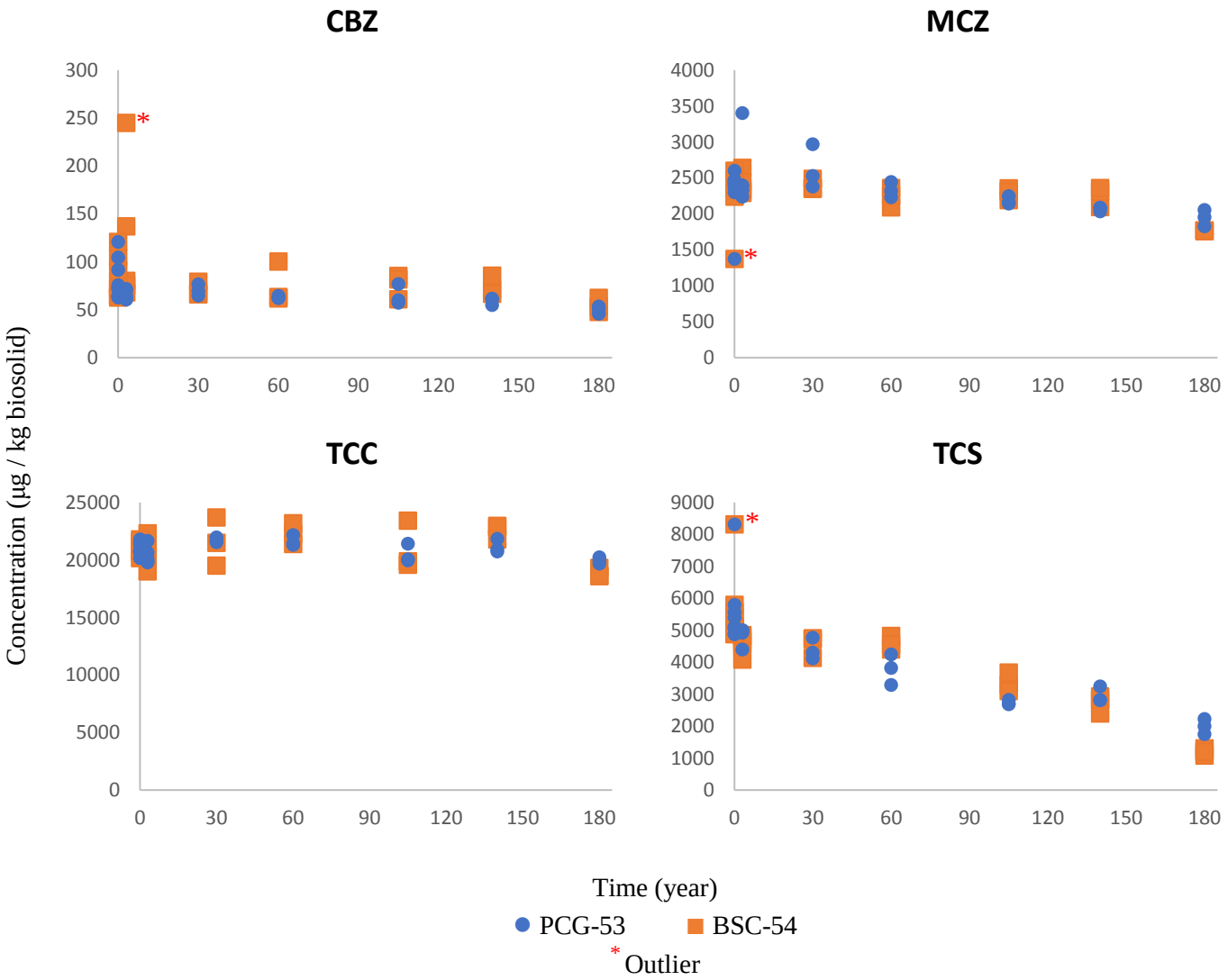


Figure A1: Degradation of Resident Contaminants in Two Soils

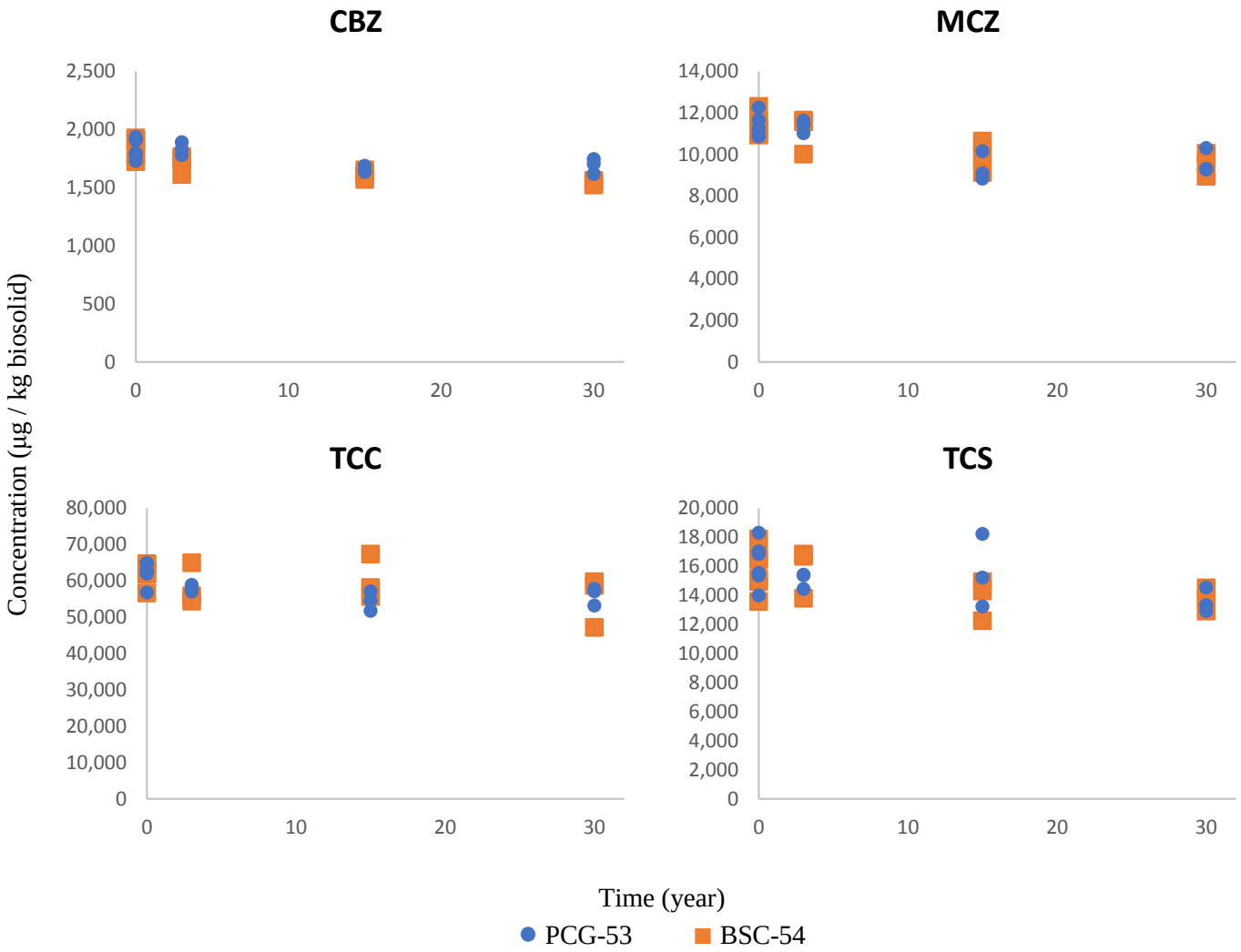


Figure A2: Degradation of Solvent-spiked Contaminants in Two Soils

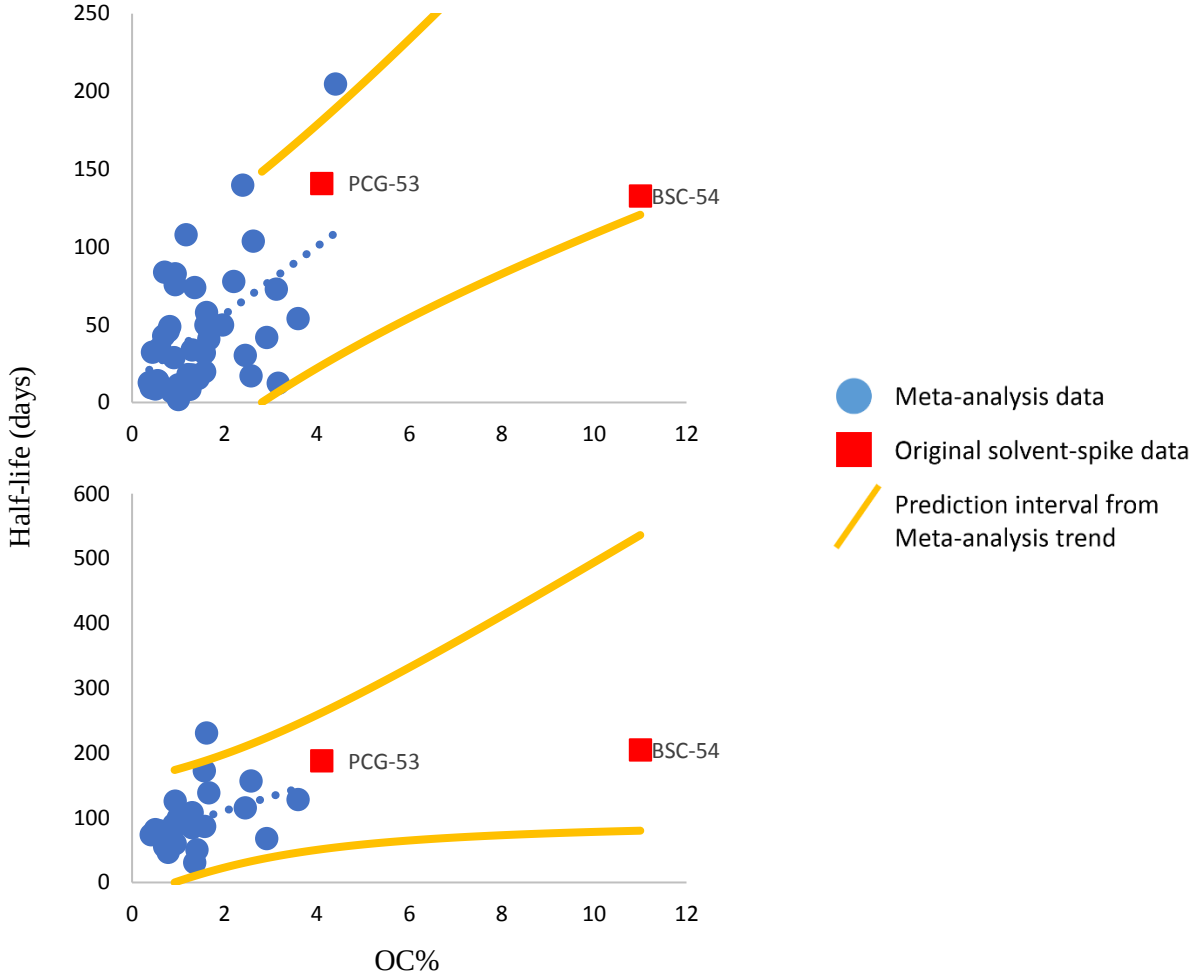


Figure A3: Comparison of Solvent-Spiked Data with the Fu et al. (2016) [54] Meta-Analysis

The SAS System

The REG Procedure
Model: MODEL1
Dependent Variable: conc

Number of Observations Read	28
Number of Observations Used	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.83375	2.83375	301.95	<.0001
Error	26	0.24400	0.00938		
Corrected Total	27	3.07775			

Root MSE	0.09687	R-Square	0.9207
Dependent Mean	8.25968	Adj R-Sq	0.9177
Coeff Var	1.17286		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	8.53540	0.02423	352.31	<.0001
day	1	-0.00495	0.00028480	-17.38	<.0001

Figure A4: SAS 9.4 Code and Example Output (Resident TCS in PCG-53) for of Degradation Rate (H_0: $\beta_1=0$; Ha: $\beta_1\neq0$)

Figure A4 continued

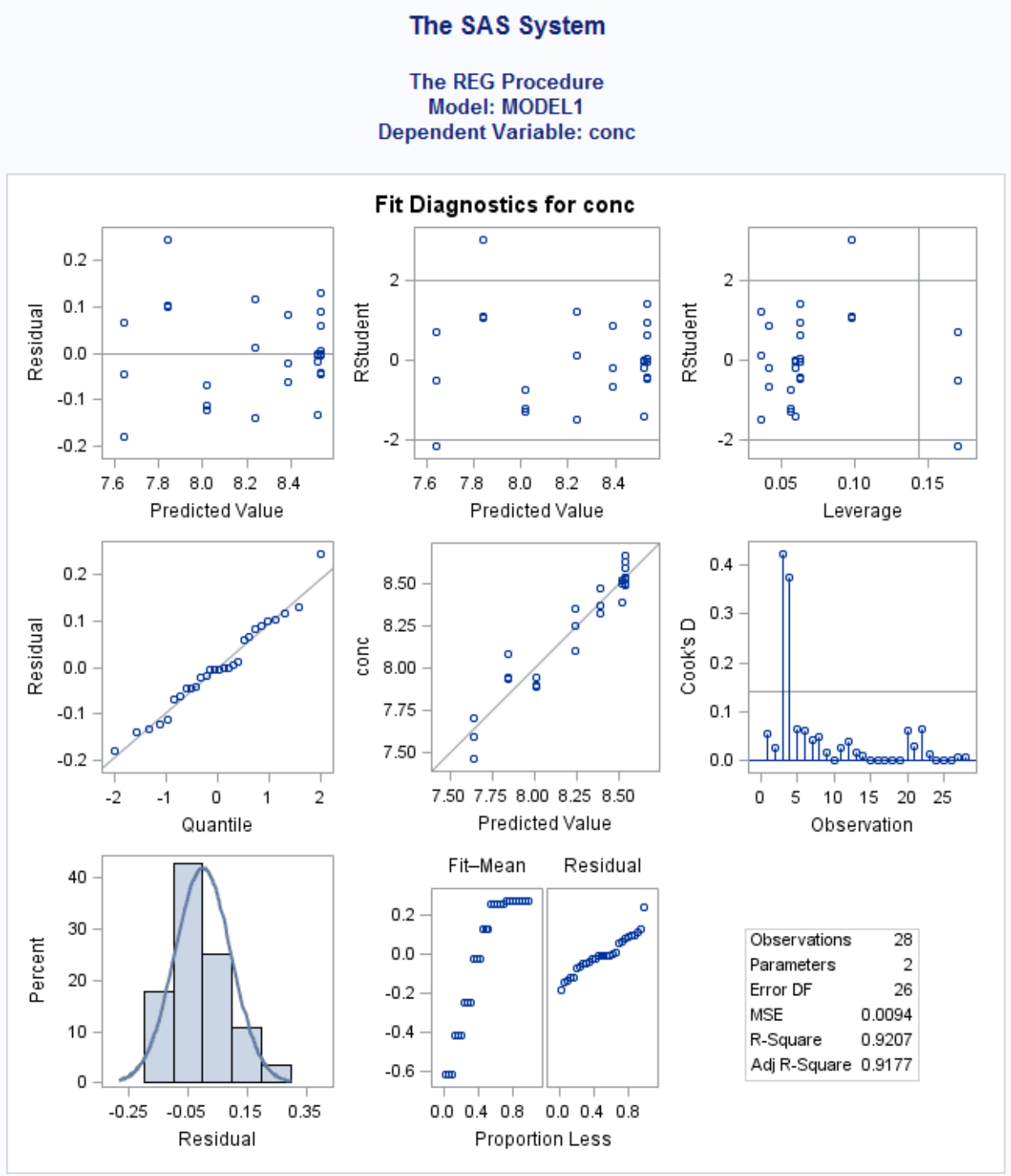


Figure A4 continued

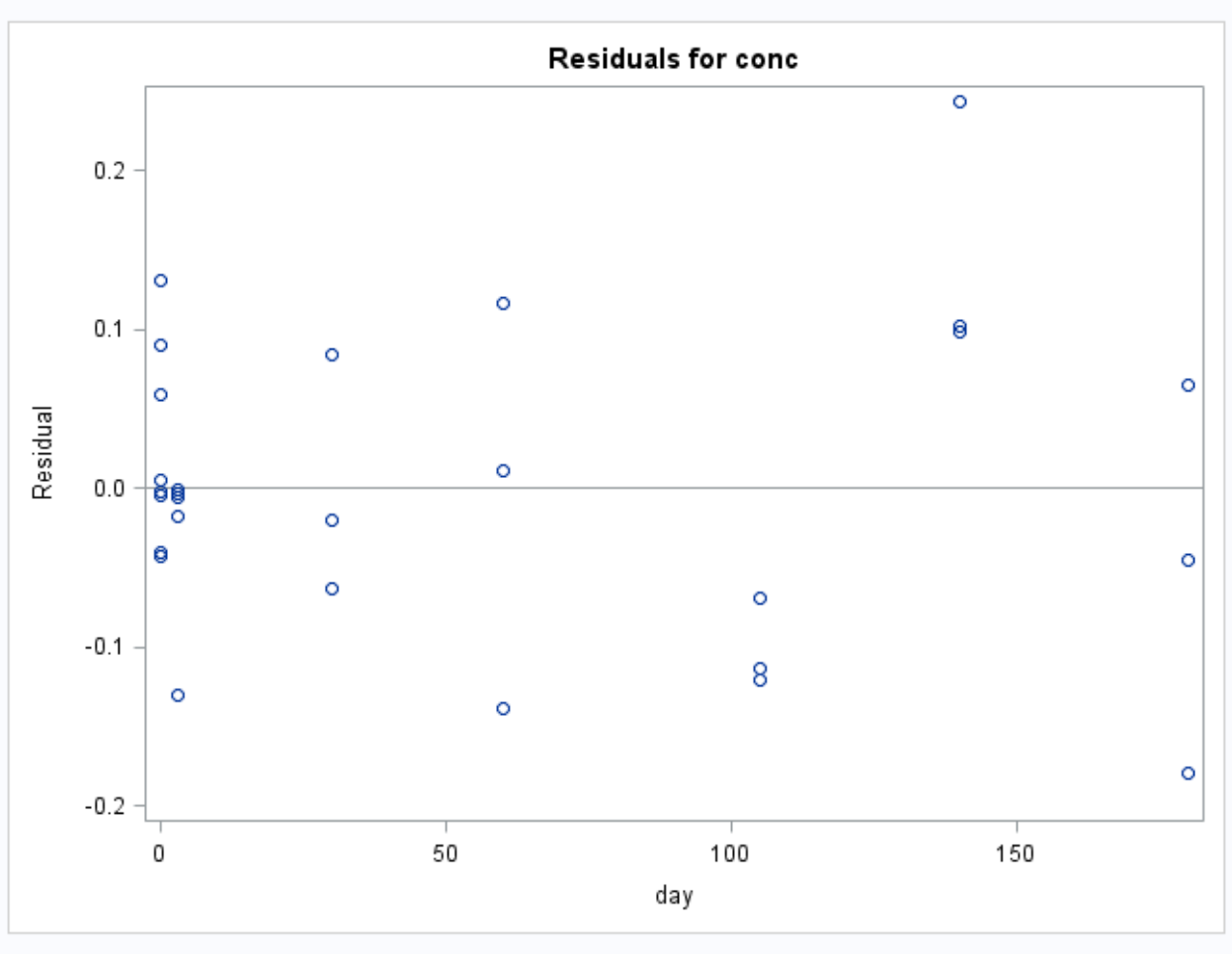
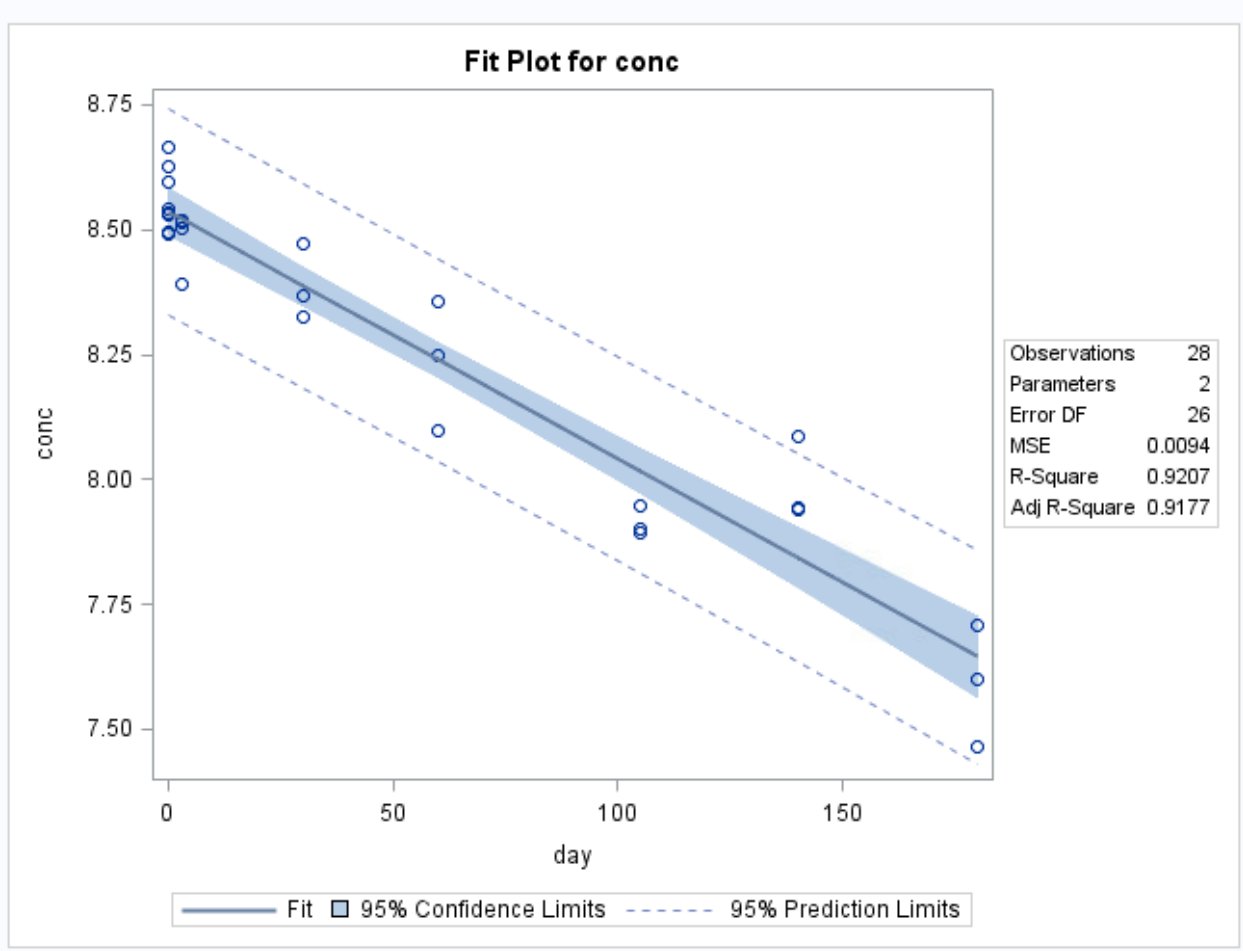


Figure A4 continued




```
data che;/*the name of dataset is che*/
input conc day@@;/*indicate variables*/
datalines;
[ln concentration] [time (day)]
;
/*proc print data=che;run;*/ /*check SAS reads the data*/
proc reg;/*liear regression*/
model conc=day;
run;
```

The SAS System

The REG Procedure
Model: MODEL1
Dependent Variable: resp

dif=pi

Number of Observations Read	28
Number of Observations Used	28

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.83375	2.83375	301.95	<.0001
Error	26	0.24400	0.00938		
Corrected Total	27	3.07775			

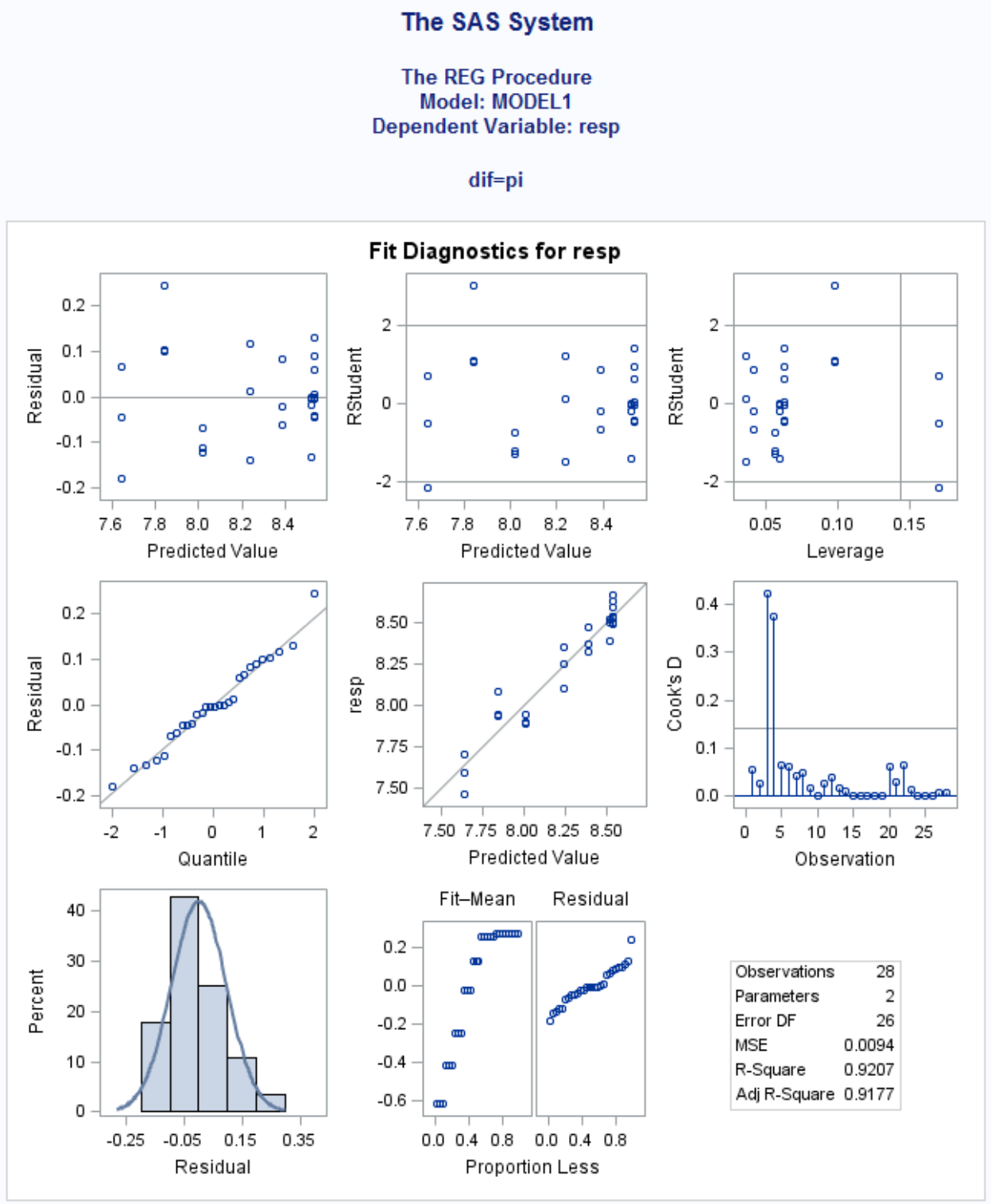
Root MSE	0.09687	R-Square	0.9207
Dependent Mean	8.25968	Adj R-Sq	0.9177
Coeff Var	1.17286		

Parameter Estimates

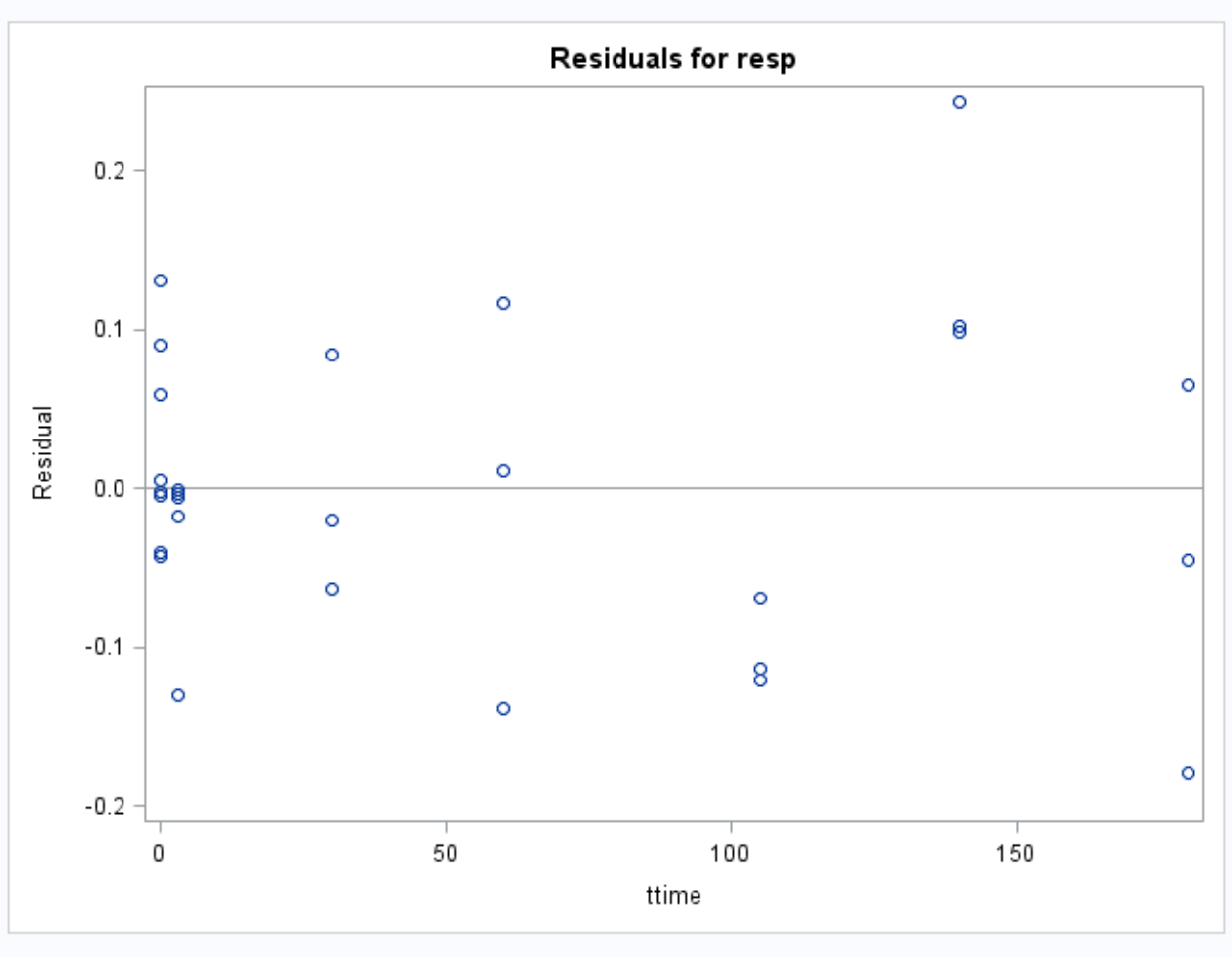
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	8.53540	0.02423	352.31	<.0001
ttime	1	-0.00495	0.00028480	-17.38	<.0001

Figure A5: SAS 9.4 Code Example Output (Resident TCS in PCG-53 and BSC-54) for Comparison of Degradation Rates (H_0: $\beta_1 = \beta_1'$; H_a: $\beta_1 \neq \beta_1'$)

Figure A5 continued



Figured A5 continued



Figured A5 continued

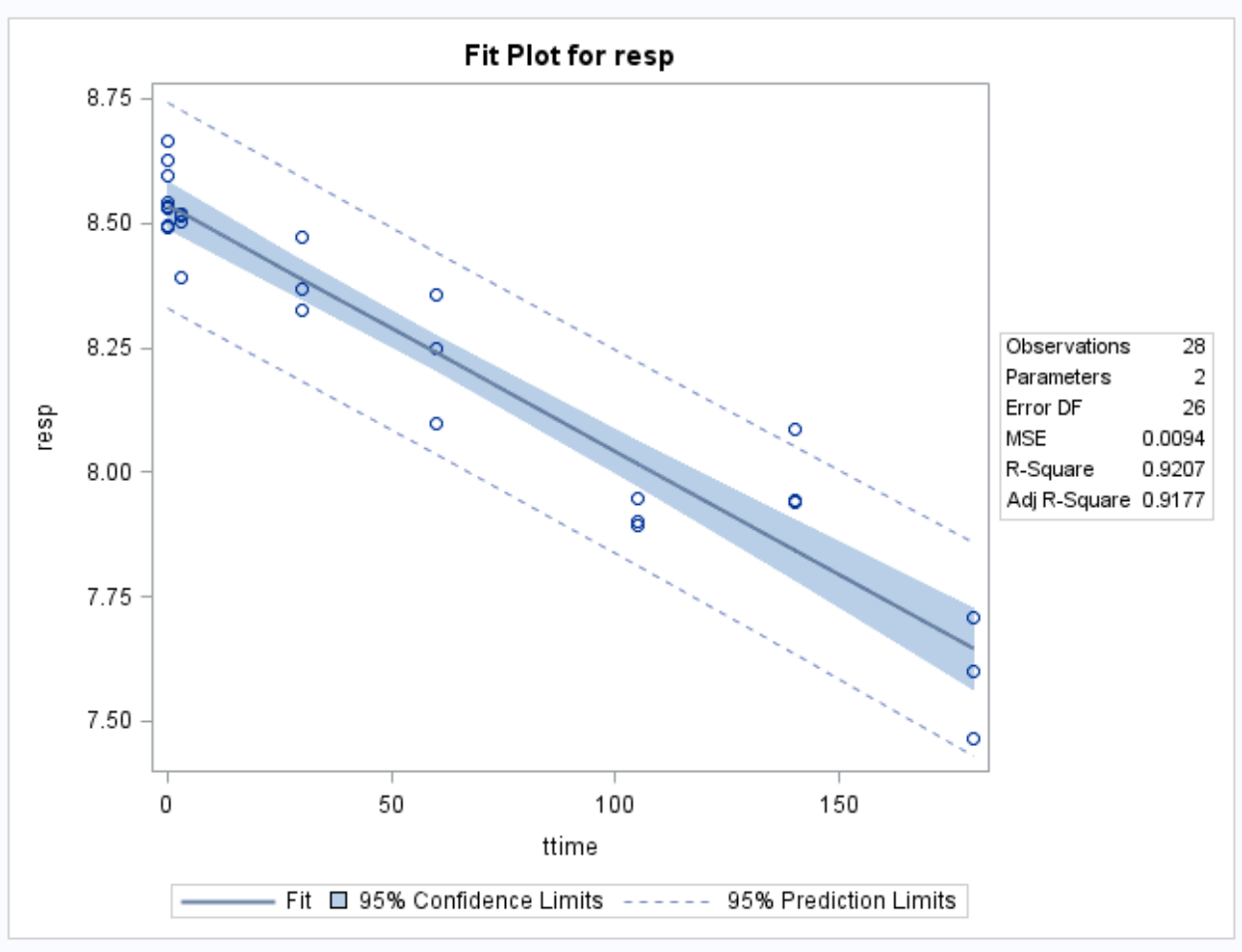


Figure A5 continued

The SAS System

The REG Procedure
Model: MODEL1
Dependent Variable: resp

dif=pii

Number of Observations Read	29
Number of Observations Used	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5.14368	5.14368	117.81	<.0001
Error	27	1.17885	0.04366		
Corrected Total	28	6.32254			

Root MSE	0.20895	R-Square	0.8135
Dependent Mean	8.25935	Adj R-Sq	0.8066
Coeff Var	2.52989		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	8.61346	0.05069	169.91	<.0001
ttime	1	-0.00658	0.00060648	-10.85	<.0001

Figure A5 continued

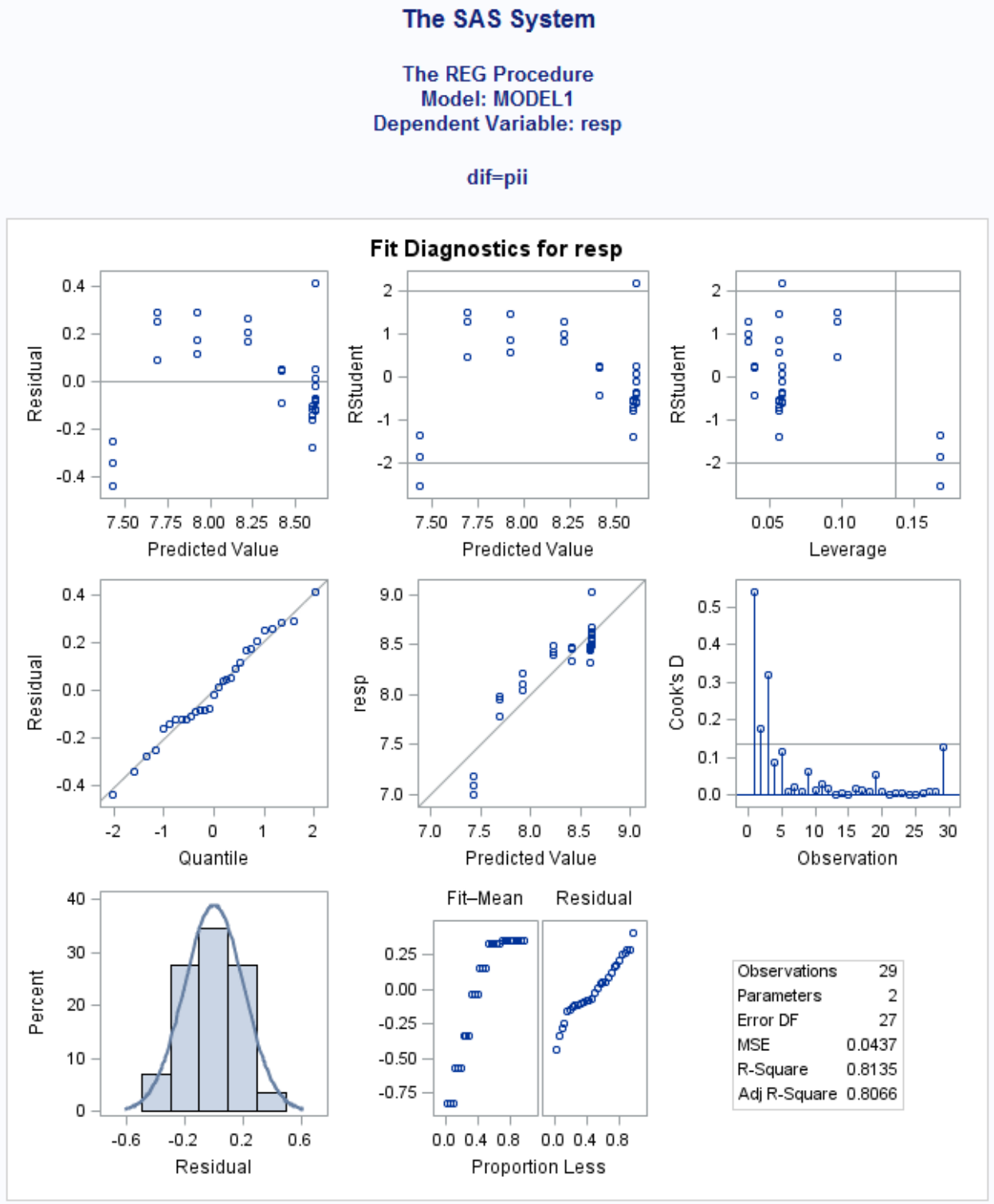


Figure A5 continued

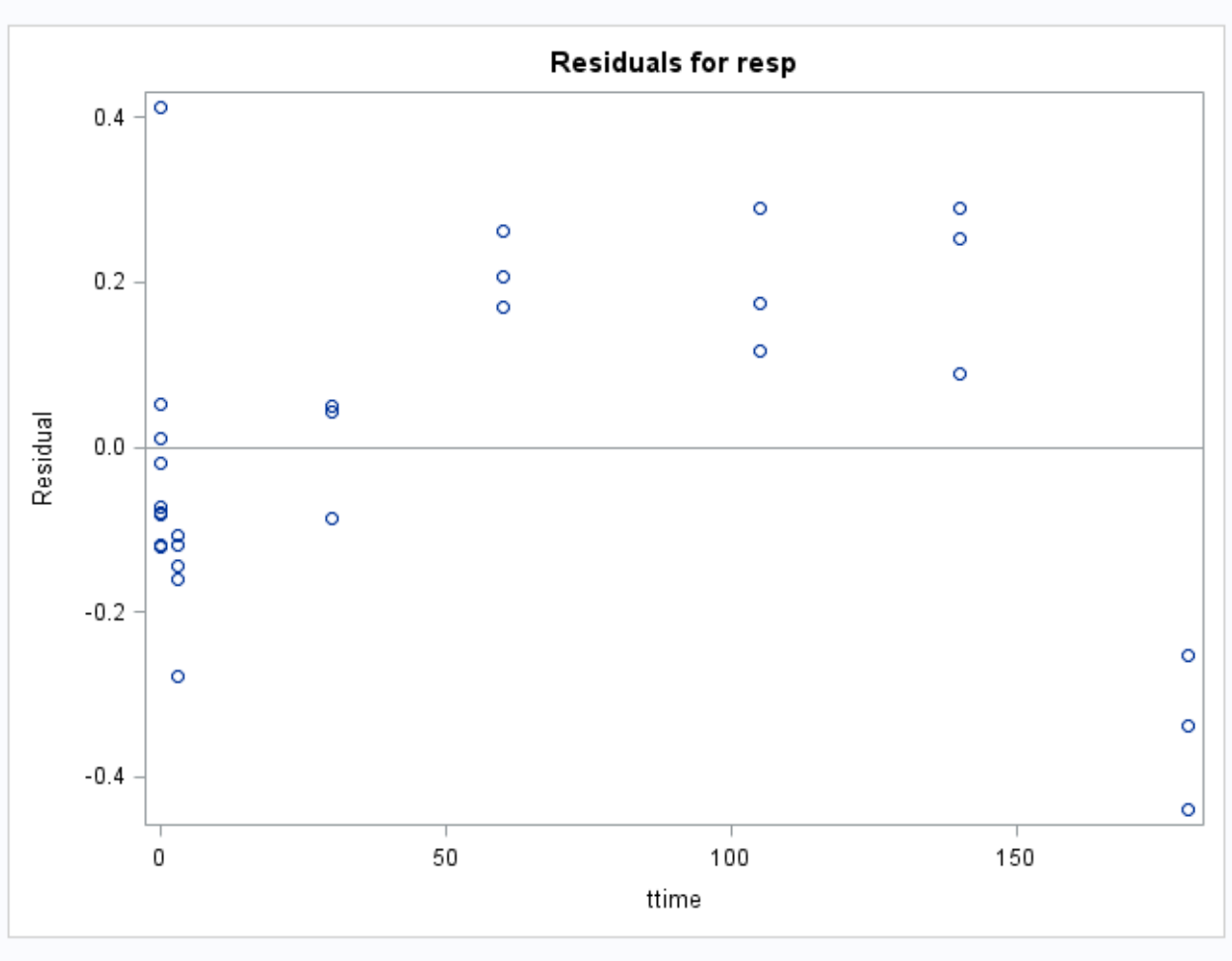


Figure A5 continued

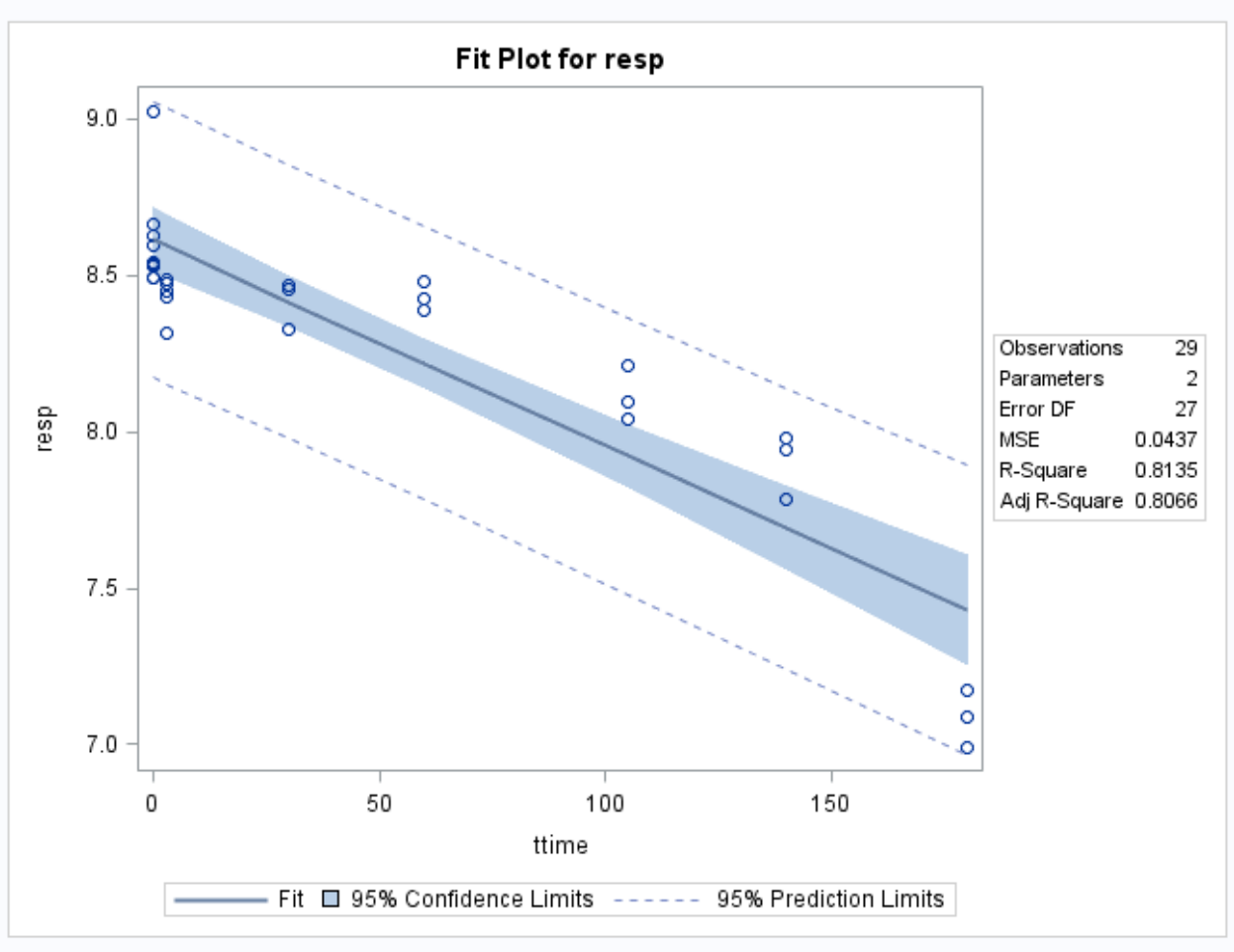


Figure A5 continued

The SAS System

The REG Procedure
Model: MODEL1
Dependent Variable: resp

Number of Observations Read	57
Number of Observations Used	57

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7.97743	2.65914	99.05	<.0001
Error	53	1.42286	0.02685		
Corrected Total	56	9.40029			

Root MSE	0.16385	R-Square	0.8486
Dependent Mean	8.25951	Adj R-Sq	0.8401
Coeff Var	1.98376		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	8.61346	0.03975	216.68	<.0001
pi	1	-0.07806	0.05709	-1.37	0.1773
ttime	1	-0.00658	0.00047557	-13.84	<.0001
pittime	1	0.00163	0.00067690	2.41	0.0193

Figure A5 continued

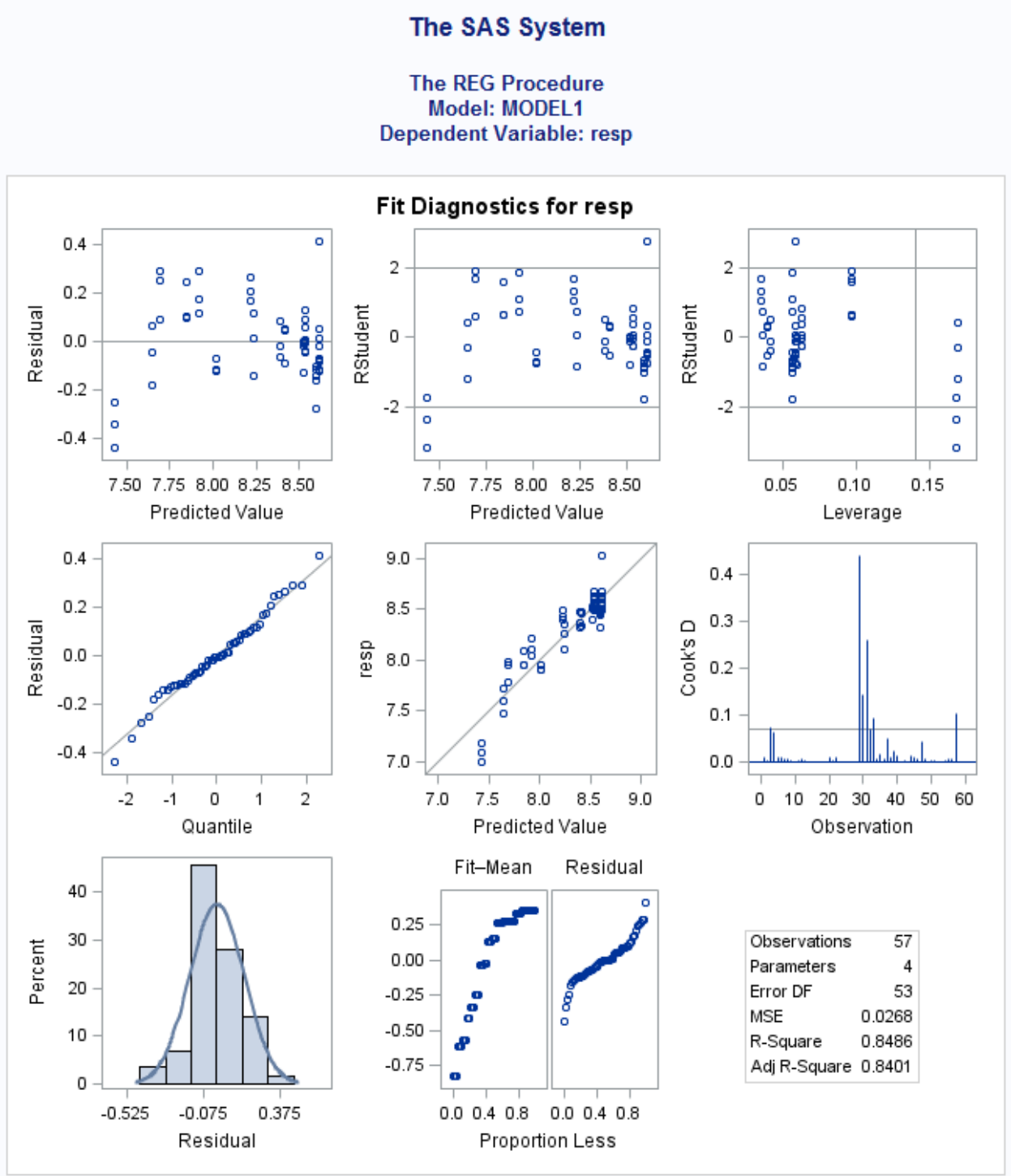


Figure A5 continued

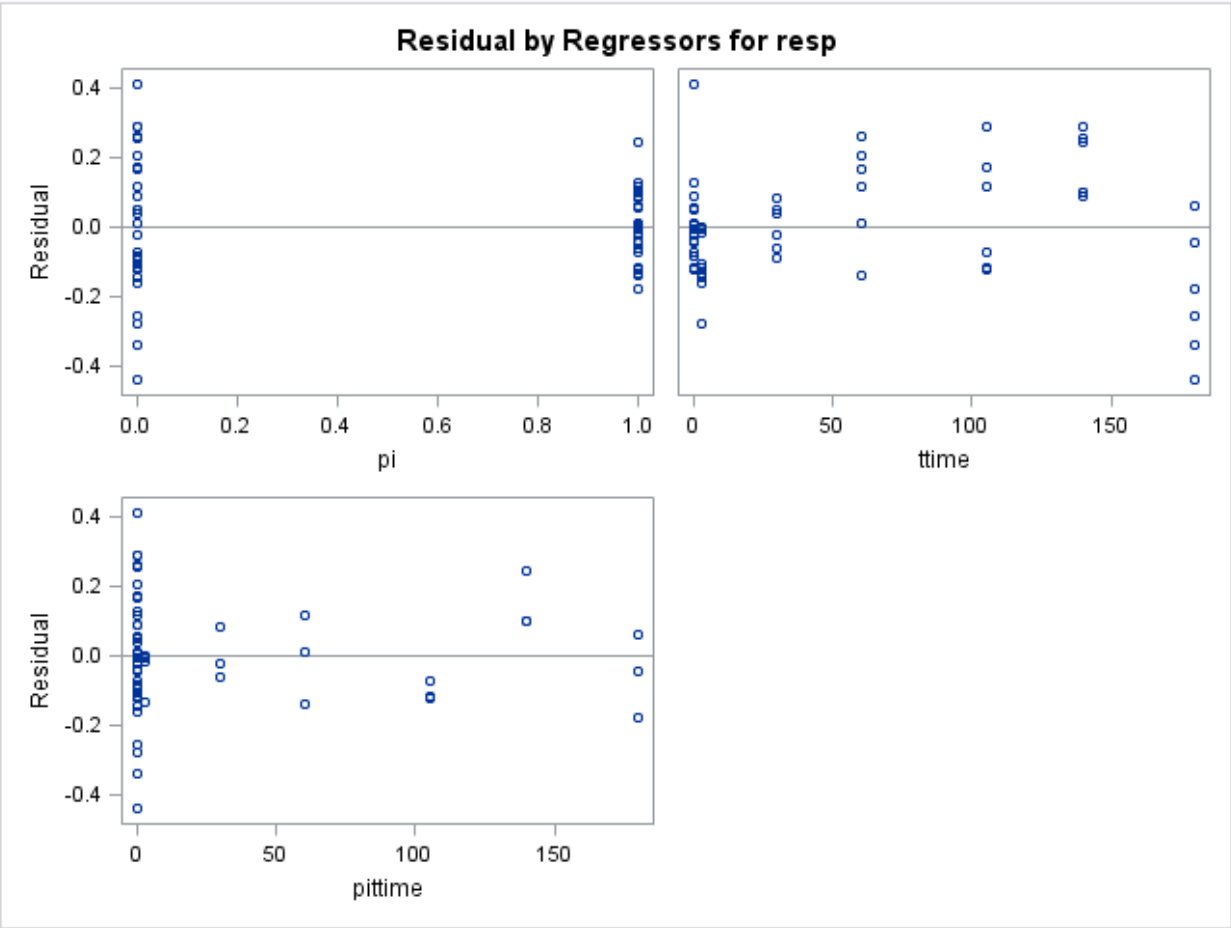


Figure A5 continued

```
data first;
input resp ttime dif$ @@;
datalines;
[ln concentration] [time (day)] [category]
;
run;
proc print data=first;run;
/*PROC GLM DATA=first ;
  CLASS dif ;
  MODEL resp = dif ttime dif*ttime / SOLUTION ;
RUN; */
PROC REG DATA=first;
   BY dif;
   MODEL resp = ttime ;
RUN;
data first2;
  set first;

  pi = . ;
  IF dif = "pi" then pi = 1;
  IF dif = "pii" then pi = 0;

  pittime = pi*ttime ;

RUN;

PROC REG DATA=first2 ;
   MODEL resp = pi ttime pittime ;
RUN;
```

REFERENCES

1. McMahon, A., *Waste management in early urban southern Mesopotamia*, in *Sanitation, Latrines and Intestinal Parasites in Past Populations*. 2016, Routledge. p. 31-52.
2. Beiswanger, W. *Privies*. 2003 09/06/2018]; Available from: https://www.monticello.org/site/house-and-gardens/privies.
3. Angelakis, A. and S. Snyder, *Wastewater Treatment and Reuse: Past, Present, and Future.* Water, 2015. **7**(9): p. 4887.
4. Metcalf, L. and H.P. Eddy, *American Sewerage Practice*. Vol. 1. 1914: McGraw-Hill.
5. Stoddard, A., *Municipal wastewater treatment : evaluating improvements in national water quality*. 2002, New York: New York : J. Wiley.
6. Lu, Q., Z.L. He, and P.J. Stoffella, *Land Application of Biosolids in the USA: A Review.* Applied and Environmental Soil Science, 2012. **2012**: p. 11.
7. Walker, J., L. Knight, and L. Stein, *Plain english guide to the EPA part 503 biosolids rule*, in *Plain english guide to the EPA part 503 biosolids rule*. 1994, EPA.
8. Iranpour, R., et al., *Regulations for biosolids land application in US and European Union.* Journal of Residuals Science & Technology, 2004. **1**(4): p. 209-22.
9. Higgins, C., et al., *Trace organic chemicals in biosolids-amended soils*, in *Water Environment Researh Foundation, Alexandria, VA, USA*. 2010.
10. US Environmental Protection Agency, *Targeted National Sewage Sludge Survey Sampling and Analysis Technical Report*. 2009: Washington, DC.
11. Jones, O.A.H., N. Voulvoulis, and J.N. Lester, *Aquatic environmental assessment of the top 25 English prescription pharmaceuticals.* Water Research, 2002. **36**(20): p. 5013-5022.
12. Williams, C.F., C.F. Williams, and F.J. Adamsen, *Sorption–Desorption of Carbamazepine from Irrigated Soils.* Journal of Environmental Quality, 2006. **35**(5): p. 1779-1783.
13. Yu, Y., Y. Liu, and L. Wu, *Sorption and degradation of pharmaceuticals and personal care products (PPCPs) in soils.* Environmental Science and Pollution Research, 2013. **20**(6): p. 4261-4267.
14. Bossche, H.V., G. Willemsens, and P. Marichal, *Anti-Candida drugs—the biochemical basis for their activity.* CRC Critical Reviews in Microbiology, 1987. **15**(1): p. 57-72.
15. Chen, Z.-F., et al., *Determination of biocides in different environmental matrices by use of ultra-high-performance liquid chromatography–tandem mass spectrometry.* Analytical and bioanalytical chemistry, 2012. **404**(10): p. 3175-3188.
16. Chen, Z.F. and G.G. Ying, *Occurrence, fate and ecological risk of five typical azole fungicides as therapeutic and personal care products in the environment: A review.* Environ Int, 2015. **84**: p. 142-53.
17. Sapkota, A., J. Heidler, and R.U. Halden, *Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry.* Environmental Research, 2007. **103**(1): p. 21-29.
18. Agyin-Birikorang, S., M. Miller, and G.A. O'Connor, *Retention-release characteristics of triclocarban and triclosan in biosolids, soils, and biosolids-amended soils.* Environ Toxicol Chem, 2010. **29**(9): p. 1925-33.
19. Reiss, R., et al., *An ecological risk assessment for triclosan in lotic systems following discharge from wastewater treatment plants in the United States.* Environmental Toxicology and Chemistry, 2002. **21**(11): p. 2483-2492.
20. Kaneko, S., et al., *Malformation in infants of mothers with epilepsy receiving antiepileptic drugs.* Neurology, 1992. **42**(4 Suppl 5): p. 68-74.

21. Livingston, S., L.L. Pauli, and W. Berman, *Carbamazepine (Tegretol) in epilepsy. Nine year follow up study with special emphasis on untoward reactions*, in *Disease of the Nervous System*. 1974.
22. Taylor, J., S. Brauer, and M. Espir, *Long-term treatment of trigeminal neuralgia with carbamazepine.* Postgraduate Medical Journal, 1981. **57**(663): p. 16-18.
23. Post, R.M., et al., *Antidepressant effects of carbamazepine.* The American Journal of Psychiatry, 1986. **143**(1): p. 29-34.
24. Monteiro, S.C. and A.B.A. Boxall, *Occurrence and Fate of Human Pharmaceuticals in the Environment*, in *Reviews of Environmental Contamination and Toxicology*. 2010, Springer New York: New York, NY. p. 53-154.
25. WHO, *WHO Model List of Essential Medicines*. 2015.
26. Kinney, C.A., et al., *Survey of Organic Wastewater Contaminants in Biosolids Destined for Land Application.* Environmental Science & Technology, 2006. **40**(23): p. 7207-7215.
27. McClellan, K. and R.U. Halden, *Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey.* Water Research, 2010. **44**(2): p. 658-668.
28. Prosser, R.S. and P.K. Sibley, *Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation.* Environment International, 2015. **75**: p. 223-233.
29. Wu, C., et al., *Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil.* Ecotoxicology and Environmental Safety, 2012. **85**: p. 104-109.
30. Li, Z.-H., et al., *Acute toxicity of carbamazepine to juvenile rainbow trout (Oncorhynchus mykiss): Effects on antioxidant responses, hematological parameters and hepatic EROD.* Ecotoxicology and Environmental Safety, 2011. **74**(3): p. 319-327.
31. van den Brandhof, E.-J. and M. Montforts, *Fish embryo toxicity of carbamazepine, diclofenac and metoprolol.* Ecotoxicology and Environmental Safety, 2010. **73**(8): p. 1862-1866.
32. Van Cutsem, J.M. and D. Thienpont, *Miconazole, a Broad-Spectrum Antimycotic Agent with Antibacterial Activity.* Chemotherapy, 1972. **17**(6): p. 392-404.
33. Zarn, J.A., B.J. Brüschweiler, and J.R. Schlatter, *Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14 alpha-demethylase and aromatase.* Environmental Health Perspectives, 2003. **111**(3): p. 255-261.
34. Dodson, R.E., et al., *Endocrine disruptors and asthma-associated chemicals in consumer products.* Environmental health perspectives, 2012. **120**(7): p. 935.
35. FDA, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use*, H.a.H. Services, Editor. 2017: Rockville, MD. p. 60474-60503.
36. Brose, D.A., et al., *A reduction in triclosan and triclocarban in water resource recovery facilities' influent, effluent, and biosolids following the U.S. Food and Drug Administration's 2013 proposed rulemaking on antibacterial products.* Water Environment Research, 2019.
37. van Wijnen, J., A.M.J. Ragas, and C. Kroeze, *River export of triclosan from land to sea: A global modelling approach.* Science of The Total Environment, 2018. **621**: p. 1280-1288.
38. Evans, T., *Biosolids in Europe.* Proceedings of the Water Environment Federation, 2012. **2012**(2): p. 108-117.
39. Christodoulou, A. and K. Stamatelatou, *Overview of legislation on sewage sludge management in developed countries worldwide.* Water Science and Technology, 2015. **73**(3): p. 453-462.
40. Schweizer, H.P., *Triclosan: a widely used biocide and its link to antibiotics.* FEMS Microbiology Letters, 2001. **202**(1): p. 1-7.
41. Waller, N.J. and R.S. Kookana, *Effect of triclosan on microbial activity in australian soils.* Environmental Toxicology and Chemistry, 2009. **28**(1): p. 65-70.

42. Yang, L.-H., et al., *Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata.* Environmental Toxicology and Chemistry, 2008. **27**(5): p. 1201-1208.
43. Higgins, C.P., et al., *Persistence of triclocarban and triclosan in soils after land application of biosolids and bioaccumulation in Eisenia foetida.* Environ Toxicol Chem, 2011. **30**(3): p. 556-63.
44. Coogan, M.A. and T.W.L. Point, *Snail bioaccumulation of triclocarban, triclosan, and methyltriclosan in a north texas, usa, stream affected by wastewater treatment plant runoff.* Environmental Toxicology and Chemistry, 2008. **27**(8): p. 1788-1793.
45. Satyro, S., et al., *Triclocarban: UV photolysis, wastewater disinfection, and ecotoxicity assessment using molecular biomarkers.* Environmental Science and Pollution Research, 2017. **24**(19): p. 16077-16085.
46. Wu, X., et al., *Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review.* Sci Total Environ, 2015. **536**: p. 655-66.
47. Behera, S.K., S.-Y. Oh, and H.-S. Park, *Sorption of triclosan onto activated carbon, kaolinite and montmorillonite: Effects of pH, ionic strength, and humic acid.* Journal of Hazardous Materials, 2010. **179**(1): p. 684-691.
48. Wu, C., A.L. Spongberg, and J.D. Witter, *Adsorption and degradation of triclosan and triclocarban in soils and biosolids-amended soils.* J Agric Food Chem, 2009. **57**(11): p. 4900-5.
49. Horsing, M., et al., *Determination of sorption of seventy-five pharmaceuticals in sewage sludge.* Water Res, 2011. **45**(15): p. 4470-82.
50. McGookin, A.G., J.S. Millership, and E.M. Scott, *MICONAZOLE SORPTION TO INTRAVENOUS INFUSION SETS.* Journal of Clinical Pharmacy and Therapeutics, 1987. **12**(6): p. 433-437.
51. Karnjanapiboonwong, A., et al., *Sorption of estrogens, triclosan, and caffeine in a sandy loam and a silt loam soil.* Journal of Soils and Sediments, 2010. **10**(7): p. 1300-1307.
52. Berendonk, T.U., et al., *Tackling antibiotic resistance: the environmental framework.* Nature Reviews Microbiology, 2015. **13**(5): p. 310-317.
53. Sanderson, H., et al., *Antibiotic resistance genes as an emerging environmental contaminant.* Environmental reviews, 2016. **24**(2): p. 205-218.
54. Fu, Q., et al., *Meta-analysis of biosolid effects on persistence of triclosan and triclocarban in soil.* Environ Pollut, 2016. **210**: p. 137-44.
55. Cha, J. and A.M. Cupples, *Triclocarban and triclosan biodegradation at field concentrations and the resulting leaching potentials in three agricultural soils.* Chemosphere, 2010. **81**(4): p. 494-9.
56. Miller, J.L., et al., *Effect of application solvents on heterotrophic and nitrifying populations in soil microcosms.* Environmental Toxicology and Chemistry, 1997. **16**(3): p. 447-451.
57. Reid, B.J., et al., *Evaluation of Spiking Procedures for the Introduction of Poorly Water Soluble Contaminants into Soil.* Environmental Science & Technology, 1998. **32**(20): p. 3224-3227.
58. Northcott, G.L. and K.C. Jones, *Spiking hydrophobic organic compounds into soil and sediment: A review and critique of adopted procedures.* Environmental Toxicology and Chemistry, 2000. **19**(10): p. 2418-2430.
59. Nkedi-Kizza, P., P.S.C. Rao, and A.G. Hornsby, *Influence of organic cosolvents on sorption of hydrophobic organic chemicals by soils.* Environmental Science & Technology, 1985. **19**(10): p. 975-979.
60. Khan, B. and L.S. Lee, *Soil temperature and moisture effects on the persistence of synthetic androgen 17α-trenbolone, 17β-trenbolone and trendione.* Chemosphere, 2010. **79**(8): p. 873-879.
61. Li, J., et al., *Degradation kinetics and metabolites of carbamazepine in soil.* Environ Sci Technol, 2013. **47**(8): p. 3678-84.
62. Monteiro, S.C. and A. Boxall, *Factors affecting the degradation of pharmaceuticals in agricultural soils.* Environmental Toxicology and Chemistry, 2009. **28**(12): p. 2546-2554.

63. Wu, C., et al., *Dissipation and Leaching Potential of Selected Pharmaceutically Active Compounds in Soils Amended with Biosolids.* Archives of Environmental Contamination and Toxicology, 2010. **59**(3): p. 343-351.
64. Al-Rajab, A.J., et al., *Dissipation of triclosan, triclocarban, carbamazepine and naproxen in agricultural soil following surface or sub-surface application of dewatered municipal biosolids.* Sci Total Environ, 2015. **512-513**: p. 480-8.
65. Gottschall, N., et al., *Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field.* Chemosphere, 2012. **87**(2): p. 194-203.
66. Carter, L.J., et al., *Fate and Uptake of Pharmaceuticals in Soil–Plant Systems.* Journal of Agricultural and Food Chemistry, 2014. **62**(4): p. 816-825.
67. Walters, E., K. McClellan, and R.U. Halden, *Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms.* Water Res, 2010. **44**(20): p. 6011-20.
68. Chen, Z.F., et al., *Occurrence and dissipation of three azole biocides climbazole, clotrimazole and miconazole in biosolid-amended soils.* Sci Total Environ, 2013. **452-453**: p. 377-83.
69. Sabourin, L., et al., *Fate of the antifungal drug clotrimazole in agricultural soil.* Environ Toxicol Chem, 2011. **30**(3): p. 582-7.
70. García-Valcárcel, A.I. and J.L. Tadeo, *Influence of moisture on the availability and persistence of clotrimazole and fluconazole in sludge-amended soil.* Environmental Toxicology and Chemistry, 2012. **31**(3): p. 501-507.
71. Kwon, J.-W., K.L. Armbrust, and K. Xia, *Transformation of Triclosan and Triclocarban in Soils and Biosolids-applied Soils.* Journal of Environment Quality, 2010. **39**(4): p. 1139.
72. Ying, G.G., X.Y. Yu, and R.S. Kookana, *Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling.* Environ Pollut, 2007. **150**(3): p. 300-5.
73. Lozano, N., et al., *Fate of triclocarban in agricultural soils after biosolid applications.* Environmental Science and Pollution Research, 2018. **25**(1): p. 222-232.
74. Xu, J., L. Wu, and A.C. Chang, *Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils.* Chemosphere, 2009. **77**(10): p. 1299-305.
75. Langdon, K.A., et al., *Field dissipation of 4-nonylphenol, 4-t-octylphenol, triclosan and bisphenol A following land application of biosolids.* Chemosphere, 2012. **86**(10): p. 1050-1058.
76. Lozano, N., et al., *Fate of triclosan in agricultural soils after biosolid applications.* Chemosphere, 2010. **78**(6): p. 760-6.
77. Lozano, N., et al., *Fate of Triclosan and Methyltriclosan in soil from biosolids application.* Environmental Pollution, 2012. **160**: p. 103-108.
78. Hayden-Smith, R., *Sowing the Seeds of Victory: American Gardening Programs of World War I.* 2014: McFarland.
79. Thone, F., *Victory Gardens.* The Science News-Letter, 1943. **43**(12): p. 186-188.
80. Lawson, L.J., *City Bountiful: A Century of Community Gardening in America*. 2005, Berkeley: Berkeley : University of California Press.
81. Miller, C., *In the sweat of our brow: citizenship in American domestic practice during WWII-victory gardens.* The Journal of American Culture, 2003. **26**(3): p. 395.
82. Dunnett, N. and M. Qasim, *Perceived benefits to human well-being of urban gardens.* HortTechnology, 2000. **10**(1): p. 40-45.
83. Attanayake, C.P., et al., *Field Evaluations on Soil Plant Transfer of Lead from an Urban Garden Soil.* Journal of Environmental Quality, 2014. **43**(2): p. 475-487.
84. Li, G., et al., *Urban soil and human health: a review.* European Journal of Soil Science, 2018. **69**(1): p. 196-215.
85. Google Books Ngram Viewer. [cited 2019 July 24]; Available from: https://books.google.com/ngrams/graph?content=community+garden%2C+community+gardens

%2C+community+gardening&year_start=1800&year_end=2000&corpus=15&smoothing=3&share=&direct_url=t1%3B%2Ccommunity%20garden%3B%2Cc0%3B.t1%3B%2Ccommunity%20gardens%3B%2Cc0%3B.t1%3B%2Ccommunity%20gardening%3B%2Cc0#t1%3B%2Ccommunity%20garden%3B%2Cc0%3B.t1%3B%2Ccommunity%20gardens%3B%2Cc0%3B.t1%3B%2Ccommunity%20gardening%3B%2Cc0.

86. Langdon, K.A., et al., *Comparison of degradation between indigenous and spiked bisphenol A and triclosan in a biosolids amended soil.* Sci Total Environ, 2013. **447**: p. 56-63.
87. Jones-Lepp, T.L. and R. Stevens, *Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation.* Analytical and Bioanalytical Chemistry, 2007. **387**(4): p. 1173-1183.
88. Langdon, K.A., et al., *Degradation of 4-nonylphenol, 4-t-octylphenol, bisphenol A and triclosan following biosolids addition to soil under laboratory conditions.* Chemosphere, 2011. **84**(11): p. 1556-62.
89. Jachero, L., et al., *Decreases in the bioconcentration of triclosan in wheat plants according to increasing amounts of biosolids added to soil.* Geoderma, 2016. **276**: p. 19-25.
90. Peng, X., et al., *Distribution, behavior and fate of azole antifungals during mechanical, biological, and chemical treatments in sewage treatment plants in China.* Science of The Total Environment, 2012. **426**: p. 311-317.
91. Rütters, H., et al., *Sorption Behavior of Prochloraz in Different Soils.* Journal of Agricultural and Food Chemistry, 1999. **47**(3): p. 1242-1246.
92. Hesselsøe, M., et al., *Degradation of 4-Nonylphenol in Homogeneous and Nonhomogeneous Mixtures of Soil and Sewage Sludge.* Environmental Science & Technology, 2001. **35**(18): p. 3695-3700.
93. Conkle, J.L., J. Gan, and M.A. Anderson, *Degradation and sorption of commonly detected PPCPs in wetland sediments under aerobic and anaerobic conditions.* Journal of Soils and Sediments, 2012. **12**(7): p. 1164-1173.
94. Liu, F., et al., *Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions.* Environmental Chemistry, 2010. **7**(4): p. 370-376.
95. Lucida, H., J.E. Parkin, and V.B. Sunderland, *Kinetic study of the reaction of sulfamethoxazole and glucose under acidic conditions: I. Effect of pH and temperature.* International Journal of Pharmaceutics, 2000. **202**(1-2): p. 47-62.
96. Demoling, L.A., et al., *Effects of sulfamethoxazole on soil microbial communities after adding substrate.* Soil Biology and Biochemistry, 2009. **41**(4): p. 840-848.
97. Bouyoucos, G.J., *Hydrometer Method Improved for Making Particle Size Analyses of Soils.* Agronomy Journal, 1962. **54**(5): p. 464-465.
98. Dane, J.H. and J.W. Hopmans, *Pressure Plate Extractor*, in *Methods of soil analysis. Part 4—Physical Methods*, J.H. Dane and G.C. Topp, Editors. 2002, SSSA: Madison, WI. p. 688-690.
99. The Ocean County Utilities Authority, *OCEANGRO® FERTILIZER DRYING SYSTEM PROCESS AND PRODUCT USE DESCRIPTION*. 2016: Bayville, NJ.
100. The Ocean County Utilities Authority. *FAQ*. 2017 [cited 2018 July 19]; Available from: http://www.ocua.com/OceanGro/SitePages/FAQ.aspx.
101. Huang, Q., et al., *Determination of commonly used azole antifungals in various waters and sewage sludge using ultra-high performance liquid chromatography-tandem mass spectrometry.* J Chromatogr A, 2010. **1217**(21): p. 3481-8.
102. Choi, Y.J., et al., *Perfluoroalkyl Acid Characterization in U.S. Municipal Organic Solid Waste Composts.* Environmental Science & Technology Letters, 2019. **6**(6): p. 372-377.
103. Harris, D.C., *Quantitative Chemical Analysis*. 8th ed. 2010, New York: W.H. Freeman and Co.
104. Timme, G. and H. Frehse, *Statistical interpretation and graphic representation of the degradation behaviour of pesticide residues. 1.* Pflanzenschutz-Nachrichten Bayer, 1980. **33**(1): p. 47-60.

105. Bryan, M., J. Zimmerman, and W. Berry, *The use of half-lives and associated confidence intervals in biological research.* Veterinary research communications, 1990. **14**(3): p. 235-240.
106. Maier, R.M., I.L. Pepper, and C.P. Gerba, *Chapter 20 - Microorganisms and Organic Pollutants*, in *Environmental Microbiology*. 2009, Academic Press: San Diego. p. 387-420.
107. Butler, E., et al., *Fate of triclosan in field soils receiving sewage sludge.* Environ Pollut, 2012. **167**: p. 101-9.
108. Pignatello, J.J. and B. Xing, *Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles.* Environmental Science & Technology, 1996. **30**(1): p. 1-11.
109. Snyder, E.H., G.A. O'Connor, and D.C. McAvoy, *Fate of 14C–triclocarban in biosolids-amended soils.* Science of The Total Environment, 2010. **408**(13): p. 2726-2732.
110. Kwon, J.W. and K. Xia, *Fate of triclosan and triclocarban in soil columns with and without biosolids surface application.* Environ Toxicol Chem, 2012. **31**(2): p. 262-9.
111. Kim, Y.-M., et al., *Triclosan susceptibility and co-metabolism – A comparison for three aerobic pollutant-degrading bacteria.* Bioresource Technology, 2011. **102**(3): p. 2206-2212.
112. Lee, D.G., et al., *Biodegradation of triclosan by a wastewater microorganism.* Water Research, 2012. **46**(13): p. 4226-4234.
113. US Environmental Protection Agency, *Category for persistent, bioaccumulative and toxic new chemical substances*, U.E.P. Agency, Editor. 1999: Federal Register. p. 60194-60204.
114. Ogram, A.V., et al., *Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy) acetic acid in soils.* Applied and Environmental Microbiology, 1985. **49**(3): p. 582-587.
115. Weissenfels, W.D., H.-J. Klewer, and J. Langhoff, *Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity.* Applied Microbiology and Biotechnology, 1992. **36**(5): p. 689-696.
116. Roccaro, P. and F. Vagliasindi, *Risk assessment of the use of biosolids containing emerging organic contaminants in agriculture.* CHEMICAL ENGINEERING, 2014. **37**.
117. Snyder, E.H. and G.A. O'Connor, *Risk assessment of land-applied biosolids-borne triclocarban (TCC).* Science of The Total Environment, 2013. **442**: p. 437-444.
118. Chalew, T.E.A. and R.U. Halden, *Environmental Exposure of Aquatic and Terrestrial Biota to Triclosan and Triclocarban1*. JAWRA Journal of the American Water Resources Association, 2009. **45**(1): p. 4-13.
119. CDC, *Antibiotic resistance threats in the United States, 2013*. 2013, Atlanta, GA: US Department of Health and Human Services.
120. Holt, R.J. and A. Azmi, *MICONAZOLE-RESISTANT CANDIDA.* The Lancet, 1978. **311**(8054): p. 50-51.
121. Verweij, P.E., et al., *Azole resistance in Aspergillus fumigatus: a side-effect of environmental fungicide use?* The Lancet Infectious Diseases, 2009. **9**(12): p. 789-795.
122. Carey, D.E., et al., *Triclocarban Influences Antibiotic Resistance and Alters Anaerobic Digester Microbial Community Structure.* Environmental Science & Technology, 2016. **50**(1): p. 126-134.
123. Drury, B., et al., *Triclosan Exposure Increases Triclosan Resistance and Influences Taxonomic Composition of Benthic Bacterial Communities.* Environmental Science & Technology, 2013. **47**(15): p. 8923-8930.
124. Thelusmond, J.-R., T.J. Strathmann, and A.M. Cupples, *The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities.* Science of The Total Environment, 2016. **571**: p. 1241-1252.
125. Venter, H., Michael L. Henningsen, and Stephanie L. Begg, *Antimicrobial resistance in healthcare, agriculture and the environment: the biochemistry behind the headlines.* Essays In Biochemistry, 2017. **61**(1): p. 1.
126. Binh, C.T.T., et al., *Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids.* FEMS Microbiology Ecology, 2008. **66**(1): p. 25-37.
127. Kumar, K., et al., *Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure.* Journal of Environmental Quality, 2005. **34**(6): p. 2082-2085.

128. Marti, R., et al., *Impact of Manure Fertilization on the Abundance of Antibiotic-Resistant Bacteria and Frequency of Detection of Antibiotic Resistance Genes in Soil and on Vegetables at Harvest.* Applied and Environmental Microbiology, 2013. **79**(18): p. 5701.
129. Munir, M. and I. Xagoraraki, *Levels of Antibiotic Resistance Genes in Manure, Biosolids, and Fertilized Soil Supplemental data file available online for this article.* Journal of Environmental Quality, 2011. **40**(1): p. 248-255.
130. Mafiz, A.I., et al., *Case study on the soil antibiotic resistome in an urban community garden.* International Journal of Antimicrobial Agents, 2018. **52**(2): p. 241-250.
131. Khuder, S., et al., *Health Survey of Residents Living Near Farm Fields Permitted to Receive Biosolids.* Archives of Environmental & Occupational Health, 2007. **62**(1): p. 5-11.
132. Robinson, K.G., et al., *Public attitudes and risk perception toward land application of biosolids within the south-eastern United States.* Journal of Environmental Management, 2012. **98**: p. 29-36.