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Analysis of pharmaceutical biodegradation of WWTP sludge using composting and identification of certain microorganisms involved in the process

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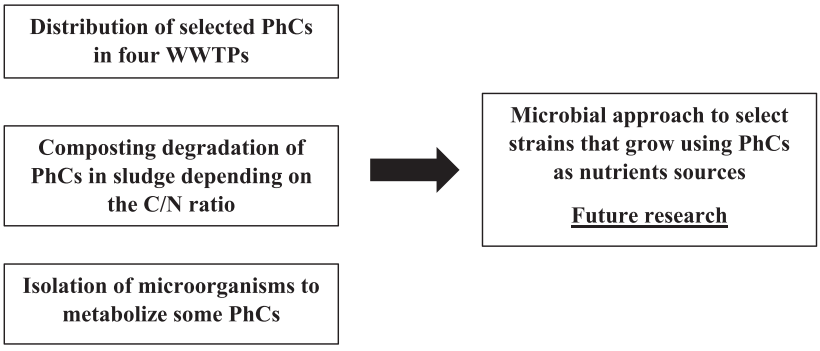
HIGHLIGHTS

- Ten PhCs were analysed during wastewater treatment, and increased in the sewage sludge.
- Composting degraded some pharmaceuticals depending on the C/N ratio.
- Minimal microbiological cultures were applied to isolate degrading microorganisms.
- Strains that metabolize azithromycin, ibuprofen, and irbesartan were isolated.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceuticals (PhCs) are organic contaminants that have been detected in wastewater, surface water, and soils throughout the world. The presence of 10 commonly used PhCs in Spain (azithromycin, benzylpenicillin, citalopram, fluconazole, fluoxetine, ibuprofen, irbesartan, olanzapine, telmisartan, and venlafaxine) was analysed at four wastewater treatment plants, and the changes in their concentrations during treatment were assessed. Although certain some PhCs were degraded in the treated water, their presence in sewage sludge increased in all cases. The sewage sludge was composted using rice straw to degrade the PhCs, and the composting efficiency was modified by changes in the relative C/N ratio of the composting blend. Using a simple microbiological culture process for enrichment, 11 different strains of microorganisms that degraded specific PhCs were identified. Ibuprofen and azithromycin were metabolized by one and four strains, respectively, and both PhCs were used as a carbon source; in addition, six strains used irbesartan as a nitrogen source.

1. Introduction

Since the 1900s, society has increasingly treated diseases with pharmaceuticals (PhCs) to improve human, animal, and plant health. However, the absorption of these chemicals by tissues is incomplete and they are released into waste water. Biologically relevant amounts of

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these substances are present in the environment and are concentrated in residual waters (Ramaswamy et al., 2010). Abnormal accumulations of these chemicals degrade the environment and may negatively impact human health (Verlicchi and Zambello, 2015). Additionally, PhCs are toxic to microorganisms, acting as antimicrobial agents that can reduce the microbial populations in water, soils, and wastewater treatment plants (WWTPs), thereby retarding or blocking the bioremediation of pollutants (Selvam et al., 2012). Moreover, the elevated concentrations of antimicrobial drugs are alarming because of their effects on the development of microbial resistance to chemotherapeutics, including pathogenic microorganism treatments (Gatica and Cytryn, 2013; Rizzo et al., 2013). The global implication of the problems described above are derived from the liberation of PhCs into the environment, as indicated by directives provided by the US Food and Drug Administration (FDA), which stipulates that an environmental risk assessment should be an important part of the approval procedure for new medical substances (Cooper et al., 2008)

Although the wastewater (WW) treatment industry has recently made many changes in response to increasing demands for water conservation and public health concerns, the increase in new contaminants, such as PhCs, in water poses a substantial new challenge to the control of water pollution (Bozkurt et al., 2016). Little information has been published regarding chronic environmental toxicity, their distribution across the land after irrigation, and the effects of pharmaceutical and personal care products alone or in combination on living organisms, including humans (Verlicchi and Zambello, 2015).

Wastewater treatment plants (WWTPs) are used to remove pollutants from wastewater, and the resulting water effluent and sludge are subsequently used in agriculture. PhCs represent an unresolved problem, and different approaches have been proposed to resolve it, including composting (for a review, see Verlicchi and Zambello, 2015). Composting is an aerobic process in which different organic materials (agricultural residues; garden, kitchen, and municipal waste; and sludge, etc.) produce stable organic and inorganic products by converting the compost into a good fertilizer or soil conditioner (O'Callaghan, 2016; Selvam et al., 2012). During composting, pathogenic microorganisms are eliminated, as are different environmental pollutants, including certain PhCs (Kupper et al., 2008; Martín et al., 2015).

Many microorganisms have been identified in the microbial populations involved in degradation during the composting process (Jurado et al., 2014; López-González et al., 2015; MacCready et al., 2013), and the addition of different selected strains improves composting and the elimination of different PhCs (Sánchez et al., 2017; Zhao et al., 2015; Zhang et al., 2016). However, the microorganisms responsible for degradation of PhCs have been not identified in the many composting processes described that reduce the levels of PhCs.

The present study aims to analyse the presence of 10 PhCs used in Spain to determine their elimination efficiency in four WWTPs. Moreover, composting experiments using different blends of sewage sludge and rice straw were performed to determine the effects of composting on the PhC concentrations in the sludge or on high PhC concentrations that were artificially enhanced in the mixed blends. Using a simple selective microbial culture media, microorganisms that can metabolize various PhCs as carbon or nitrogen sources were identified.

2. Materials and methods

2.1. Samples and sampling

Samples were collected from four municipal WWTPs with different flow ratios (FR, m^3d^{-1}) around the Valencia City (Spain) area as follows: Alcoi (FR 14.500), Paterna (FR 10.000), Pinedo (FR 96.000), and Quart (FR 30.000). These plants treated urban residual waters using essentially the same treatment method. In brief, the waste water treatment process consisted of pretreatment, primary treatment, and the biological treatment of activated sludge. Many Spanish WWTPs only perform primary and secondary treatments, as in the Alcoi WWTP (Gros et al., 2010), whereas the Quart, Paterna, and Pinedo WWTPs performed a tertiary treatment by coagulation/flocculation and filtration followed by UV treatment.

For the composting studies, sludge was obtained from the Paterna WWTP, and it had the following characteristics: moisture 60 °C (75.5%), density (1 g cm^{-3}), pH (7.02), total organic matter (58.6%), oxidizable organic carbon (26.0%), C/N (7.4), and other parameters that were similar to those described before (Iranzo et al., 2004), given that we normally we used the sludge from this WWTP. A total of 20 rice straw samples were collected from Albufera Natural Park, over-dried at 60 °C, pulverized, and stored until the compost was produced. The rice straw had similar characteristics to that described before (Iranzo et al., 2004), namely, the following: moisture 60 °C (12%), density (0.06 g cm^{-3}), total organic matter (81.4%), oxidizable organic carbon (34.5%), and C/N (41.8). Quantitative data was obtained for three samples with SD < 5%. The mixture content (over-dried at 105 °C for 24 h) of total organic matter (weight loss upon ignition at 550 °C for 72 h), oxidizable organic carbon (Walkley-Black method), and total nitrogen (Kjeldahl method) were determined (Page et al., 1982).

For the sludge three samples, 3 kg or 2 L of water was collected in the summer and winter and stored at 4 °C until analysis.

2.2. Standards and chemicals

The standards (>98% pure) for the analysed compounds were a gift from CINFA Pharmaceutical Laboratories (Pamplona, Spain). HPLC-grade methanol and HPLC-grade acetonitrile (ACN) were purchased from Scharlab (Barcelona, Spain). HPLC-grade water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Formic acid (>98%, analysis grade), ammonium formate (>99%, reagent grade) and other commonly used reactive compounds were supplied by Sigma-Aldrich.

The following 10 PhCs were analysed in this study: azithromycin, benzylpenicillin, citalopram, fluconazole, fluoxetine, ibuprofen, irbesartan, olanzapine, telmisartan, and venlafaxine. These PhCs were the most commonly used compounds in Spain over the last 10 years, according to the Spanish Agency of Medicines and Medical Devices (AEMPS, 2015).

2.3. Sample treatment, liquid chromatography and mass spectrometry

Three samples for each point were treated and analysed according to the UNE standards and the official analysis methods of the Spanish Ministry of Agriculture, Fishing and Food. The sludge, compost, and waste samples were dried at 40 °C, ground, and sieved through a 2 mm sieve, and one portion was crushed.

Liquid chromatography and mass spectrometry were performed as described by Peysson and Vulliet (2013). For solid samples, approximately 5 g of each homogenized sample was placed into a 50 mL centrifuge tube. To perform the extraction process, 20 mL of 75% (v/v) ACN aqueous solution with 1% (v/v) formic acid was added, and the sample was homogenized over 60 min and then centrifuged at 4000 rpm for 5 min. The extract was filtered, and liquid chromatography was performed with an HP1200 HPLC system (Agilent Technologies Spain S.L., Madrid) equipped with a degasser, a binary pump, an auto-sampler, and a column oven. The chromatographic separation was performed with an Atlantis T3-C18 column (Waters, Milford, MA, USA) that was maintained at 5 °C and the samples were added at 40 °C.

For mass spectrometry analysis, a triple-stage quadruple mass spectrometer (3200 Qtrap, AB SCIEX, Concord, ON) with an electrospray ion (ESI) source was used with nitrogen as the nebulizer gas. The analytes were identified by their retention times and by having 2 or more selected reaction monitoring (SRM) transitions.

The MS/MS conditions of the analytes were obtained via direct injection of 1 mg/L water/acetonitrile (98:2 v/v) of single standard solutions at a flow rate of 10 μL/min. The addition of formic acid to the infusion vial favoured $[M + H^+]$ formation, and the samples were ionized in the positive ionization mode. With the exception of ibuprofen and benzylpenicillin, which were ionized in the negative mode, the positive ionization mode was selected because of its superior sensitivity. Two or three SRM transitions were selected for each compound to ensure reliable confirmation of the detected compounds. The most abundant product ion was used for quantification, and secondary sensitive transitions were used for confirmation. To control for possible matrix effects that would affect the accuracy, matrix-matched calibration curves were prepared for control and quantification purposes. One blank sample was fortified by spiking it with different aliquots of a standard solution mixture ranging from 1 to 500 μg/L.

2.4. Validation study

The linearity of the method was studied by analysing the standard solutions at five concentrations ranging from 1 to 500 μg/L. Satisfactory linearity using a least squares regression was assumed when the correlation coefficient (r^2) was higher than 0.99 and when the residuals, using analyte peaks, were lower than 30% without a significant trend. The accuracy (expressed as percentage recovery) and precision (expressed in terms of the relative standard deviation, RSD) were studied using recovery experiments, which were performed in five replicates spiked at the following two levels: surface water and WW at 0.8 μg/L and 8.0 μg/L, compost, and 0.8 μg/kg and 20 μg/kg for PhCs. The limit of quantification (LOQ) and the limit of detection (LOD) were estimated for signal-to-noise ratios of 10 and 3, respectively, from the sample chromatograms at the lowest validation level tested. The quantification transition and the ion transition ratio were used for confirmation.

2.5. Composting procedure and sampling

Composting processes were performed with rice straw mixed with sewage sludge from Paterna WWTP using different C/N ratios (17, 20, 24, 29, and 37) to evaluate the possibility of different concentration effects on pharmaceutical degradation. The compost mixtures were prepared in 8 L Dewar vessels (KGW-Isotherm Karlsruhe, Germany). The characteristics of the rice straw and sewage were determined as previously described (Iranzo et al., 2004), and the composting process was followed by the determination of different commonly used parameters, including the temperature, C/N ratio, organic matter, etc. (Roca-Pérez et al., 2009).

Three samples were collected at different composting time (every 2 days until day 32) from different points on the Dewar to ensure homogeneity and representativeness. For the microbial studies, an additional sample was collected before the temperature reached 40 °C. The samples were fractionated and treated according to specific protocols or frozen at −40 °C for future analysis.

2.6. Microbial analyses

The compost samples (1 g) for the microbial analysis were suspended in 20 mL of sterile saline solution, and the resuspensions were shaken (150 rpm) at 28 °C for 1 h. Aliquots of 100 μL of each suspension were grown in liquid medium YPD and complete minimal medium (glucose 5 g, monobasic ammonium phosphate 1 g, sodium chloride 5 g, magnesium sulphate 0.2 g, and potassium phosphate 1 g per L). We used minimal medium as the complete medium without a carbon source (glucose) and without a nitrogen source (ammonium phosphate), which was supplemented with the different pure PhCs (1 g/L) as carbon and/or nitrogen sources. The incubations were performed over 48 h with agitation at 28 °C. From minimal liquid media with visible growth, a three-step enrichment process in the same medium was performed. The samples were then plated in the same minimal medium with 2% agar and incubated at 28 °C until colony growth was observed. The different colonies were picked, grown twice as pure cultures in the same minimal solid medium, and finally passed to YPD medium for identification at the laboratory of the Spanish Type Culture Collection (CECT). All the processes described here were complemented by growing and plating the microbes in YPD and complete solid medium to obtain pure cultures to maintain the different microorganisms for future studies.

2.7. Molecular identification of isolates

The identification of the strains that were able to degrade certain pharmaceutical products was performed by direct amplification of the 16S RNA gene by PCR, the partial sequencing of the gene in both directions, and sequence analysis (Arahal et al., 2008) at the CECT. The assembled sequences obtained by alignment of the forward and reverse amplification products were compared for similar nucleotide sequences with EzTaxon (Kim et al., 2012) and BLAST (Altschul et al., 1997).

3. Results and discussion

3.1. Recovery and validation studies

Because the quality of data obtained from the determination of pharmaceuticals in different matrices is an important issue, an initial validation study was performed. The efficiency of the extraction and quantification of PhCs was determined with three matrices (compost, sewage sludge, and water). The PhCs were spiked with matrices (to 1 mg/L/mg kg^{-1}), extracted with ACN, and analysed by HPLC-MS.

Table 1 shows the results obtained with compost, the matrix in which the lowest recovery was found. Of the 10 PhCs tested here, only one (olanzapine) showed <50% recovery; for the others, the recovery ranged from 80% to 100%. Over a concentration range from 1 μg/L to 100 μg/L, all the target compounds exhibited good linearity; and the R^2 values ranged from 0.9900 to 1.0000. The limit of quantification for the detected compounds showed lower variation, from 0.8 μg/kg to 4.0 μg/kg; and all the PhCs, with the exception of ibuprofen (2.0 μg/kg) and olanzapine (4.0 μg/kg), showed an LOQ of 0.8 μg/kg.

The relative standard derivation of the spiked samples was lower than 10% with the exception of olanzapine (20%). In conclusion, compared with the other determinations described for the quantitative analysis of PhCs (Peysson and Vulliet, 2013), our data was comparable in terms of specificity and precision.

3.2. Incidence of pharmaceutical sewage in WWTPs

In previous studies on PhC degradation in WWTPs, wastewaters (influent and effluent) and sewage sludge were often analysed to determine the contaminant removal efficiency (Bozkurt et al., 2016; Jelic et

Table 1
Analytical method validation and performance criteria.

Pharmaceuticals	Limit of quantification (μg/kg)	Expanded uncertainty (%)	RSD[a]
Azithromycin	0.8	86	4
Benzilpenicilin	0.8	85	8
Citalopram	0.8	96	3
Fluconazole	2.0	109	6
Fluoxetine	0.8	78	6
Ibuprofen	2.0	79	8
Irbesartan	0.8	87	4
Olanzapine	4.0	24	20
Telmisartan	0.8	90	2
Venlafaxine	0.8	88	2

[a] Relative standard derivation.

Table 2
Quantification of pharmaceutical levels detected in wastewater influent (WWI), WW effluent (WWE), the solid fraction (SF), and sewage sludge (SS) from the wastewater treatment plants (WWTPs). The concentrations of pharmaceuticals in the liquid fraction are presented as μg/L and in the solid fraction as μg/kg dry weight.

Pharmaceuticals	WWTP	WWI	WWE	SF	SS
Azithromycin	Alcoi	7.52	0.56	9203.4	922.9
	Paterna	2.43	0.16	17,182.5	394.5
	Pinedo	1.58	0.62	573.7	588.0
	Quart	0.40	0.12	551.4	582.1
Citalopram	Alcoi	0.10	0.11	130.5	101.1
	Paterna	0.34	0.15	105.1	53.2
	Pinedo	0.14	0.10	123.7	106.2
	Quart	0.13	0.10	8.2	91.6
Fluoxetine	Alcoi	2.84	nd	123.8	12.9
	Paterna	2.20	nd	1485.1	14.8
	Pinedo	<LOQ	<LOQ	120.1	39.3
	Quart	0.27	<LOQ	317.0	33.0
Irbesartan	Alcoi	0.46	0.83	617.4	23.3
	Paterna	0.63	1.14	453.5	20.3
	Pinedo	0.45	0.43	546.2	107.6
	Quart	<LOQ	0.57	42.8	14.2
Telmisartan	Alcoi	0.85	2.27	2572.8	529.2
	Paterna	2.49	2.70	2354.8	676.3
	Pinedo	1.31	1.93	2527.5	2733.5
	Quart	1.76	2.74	231.7	1052.3
Venlafaxine	Alcoi	0.25	0.30	23.7	24.5
	Paterna	0.99	0.31	10.7	18.9
	Pinedo	<LOQ	<LOQ	120.1	39.3
	Quart	0.51	0.27	<LOQ	39.1

LOQ, limit of quantification (0.10 μg/L); nd, not detected.

al., 2011). In the present study, PhCs linked to the solid fraction of the wastewater effluent were determined to analyse a more accurate partition of the PhCs during WW treatment. Table 2 shows the quantification of the pharmaceutical levels in water influent, water effluent, solid particles, and sewage sludge from the four WWTPs. To prevent the sampling or weather changes from having any influence on the results, three samples were taken for each condition in the summer and winter, and no notable differences were detected (data not shown).

Six of the 10 analysed PhCs were found in the wastewater influent (WWI), although high concentration variation was detected among the WWTPs studied here. Azithromycin was detected in WWI at elevated concentrations in all WWTPs with high variability, from 7.52 μg/L in Alcoi to 0.40 μg/L in Quart. Citalopram (0.10–0.34 μg/L) and irbesartan (0.46–0.63 μg/L) were detected in WWI at similar amounts among the four WWTPs, although irbesartan was found below the limit of quantification (LOQ) in Quart. Fluoxetine ranged from 2.84 μg/L in Alcoi to 0.27 μg/L in Quart. For reasons that remain unclear, telmisartan and venlafaxine were found at surprisingly high levels in the Paterna WWTP. The occurrence of PhCs in untreated WW from different WWTPs and comparisons with the literature from different countries showed important differences in their presence and/or concentration (Muter et al., 2017). These results clearly indicate that human PhCs reach the water in consistent concentrations, representing a critical problem (Zhao et al., 2015).

During wastewater treatment, the reduction in the concentrations of several PhCs in the wastewater effluents (WWE) showed differences depending on the WWTP; these differences arose for different reasons than those described by previous authors (Jelic et al., 2011; Muter et al., 2017; Sun et al., 2016). However, in the case of irbesartan and telmisartan, both levels increased in the WWE. The reduction of PhCs or different chemicals in general were shown previously during WW treatment (Jelic et al., 2011) and could not represent a degradation process because the determination of the pharmaceutical concentration present in sludge increased drastically in all cases.

However, the presence of azithromycin or other antibiotics in the water influent might represent an additional problem because of their antimicrobial effects within the WWTP microbial communities (Barra Caracciolo et al., 2015). The PhC compounds used here have different structural and physicochemical properties, but almost 100% of them were associated with the solid fraction present in WWTP influents. This is the primary reason why increased levels of medical compounds (in contrast with the influent levels) were found in the sewage sludge after depuration, as previously described for other PhCs (Yang et al., 2016).

The WWTPs analysed here removed different proportions of several PhCs (Table 2), but in all cases, important concentrations of contaminants were found in the water effluents or in the sewage sludge. In the case of azithromycin, irbesartan, telmisartan, and venlafaxine, the PhC concentration increased during the process, clearly indicating an accumulation process in the WWTPs, as in the case of other PhCs (Gros et al., 2010).

The fact that water effluents and sludge are commonly used for farmlands may result in the accumulation of persistent PhCs in soil, which represents an important ecological problem with many negative

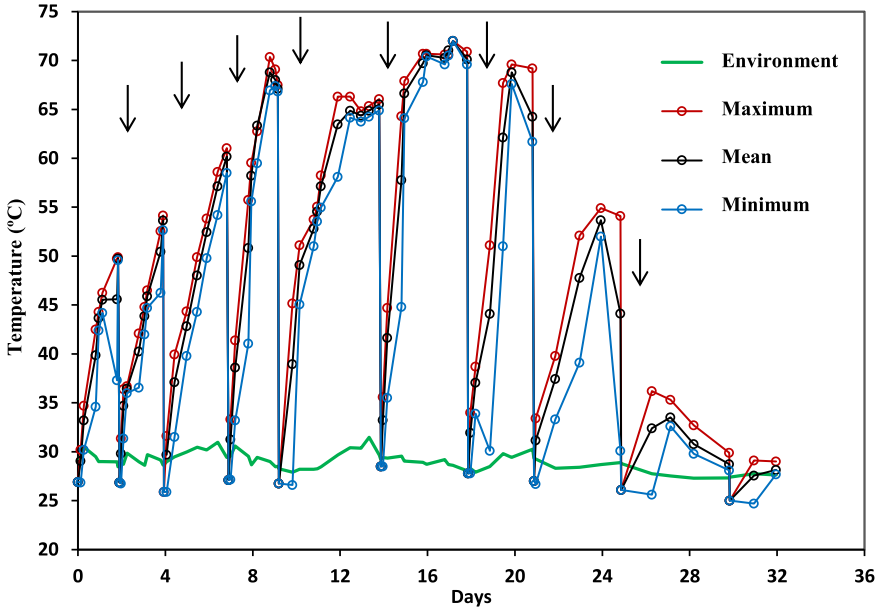

Fig. 1. Temperature profiles during the first mesophilic and thermophilic phases during the composting process with rice straw and sewage sludge. Arrows indicate turning.

implications (Cucina et al., 2017a, 2017b; Verlicchi and Zambello, 2015). One such risk for human health is environmental antibiotic resistance (AR), which contributes to the global propagation of clinical AR (Gatica and Cytryn, 2013).

3.3. Effects of composting on pharmaceuticals in the sewage sludge

We previously described the challenges of using rice straw in the Valencia area (Spain) and the possibility of composting with sewage sludge to minimize the environmental impacts (Iranzo et al., 2004). After several years, the problem persists, and here we analyse the effects of sewage sludge composting with rice straw on the PhC concentrations in the sludge.

Composting processes were performed using rice straw mixed with contaminated sewage sludge at a C/N ratio of approximately 20 (Fig. 1). In general, the parameters monitored during composting were similar to those obtained in previous work (Iranzo et al., 2004; Roca-Pérez et al., 2009), producing a mature compost at approximately 80–100 days (data not shown).

Fig. 2 shows the changes over time in the concentration of the PhCs during the composting process. Initially, azithromycin, citalopram, irbesartan, fluoxetine, telmisartan, and venlafaxine were measured at different concentrations in the sludge blend mixed from different WWTPs. Using these composting conditions (C/N ≈ 20), the biodegradation of azithromycin, irbesartan, fluoxetine, and citalopram was identified, whereas the concentrations of telmisartan and venlafaxine were not affected.

The azithromycin levels were reduced by up to 50%, whereas that of citalopram was reduced by 10%, and fluoxetine was completely biodegraded over 15 days. These results confirm that

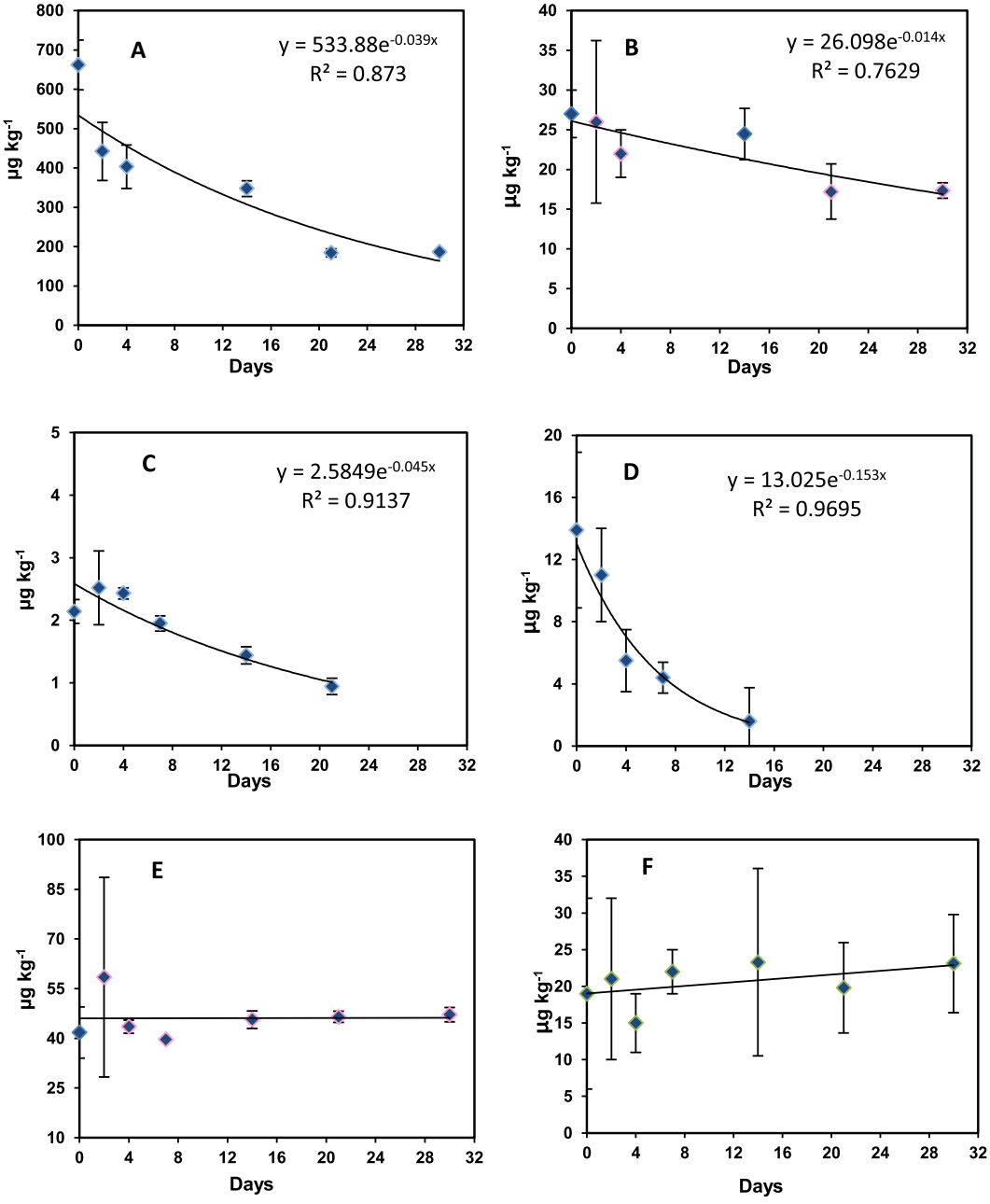

Fig. 2. Changes in the pharmaceutical concentrations during composting with rice straw and sewage sludge described in Fig. 1: azithromycin (A), citalopram (B), fluoxetine (C), irbesartan (D), telmisartan (E), and venlafaxine (F). The error bars represent the standard deviation from the means (n = 3).

composting is an efficient process to remove PhCs, as has previously been described by different researchers (i.e. Ho et al., 2013; Ramaswamy et al., 2010; Verlicchi and Zambello, 2015). In this context, an interesting paper was recently published by Cucina et al. (2017a, 2017b) on the comparison of two composting treatments of a pharmaceutical organic sludge to remove its phytotoxicity and thus obtain a high-quality organic fertilizer. The results show the possibility of using highly PhC-contaminated sludge, such as that described in our work, with agronomic value after improving it through composting.

3.4. Effects of the C/N ratio of composting blends on pharmaceutical degradation

To optimize the C/N ratio in an initial blend to remove the PhCs during biosolid composting, five different C/N ratios (17, 20, 24, 29, and 37) were used to study several PhCs that were previously explored during the composting process in addition to benzylpenicillin, ibuprofen, and olanzapine, the most commonly used PhCs not present in the previous experiment. Pure PhCs (1.5 g/kg) were added to each blend, and the differences in

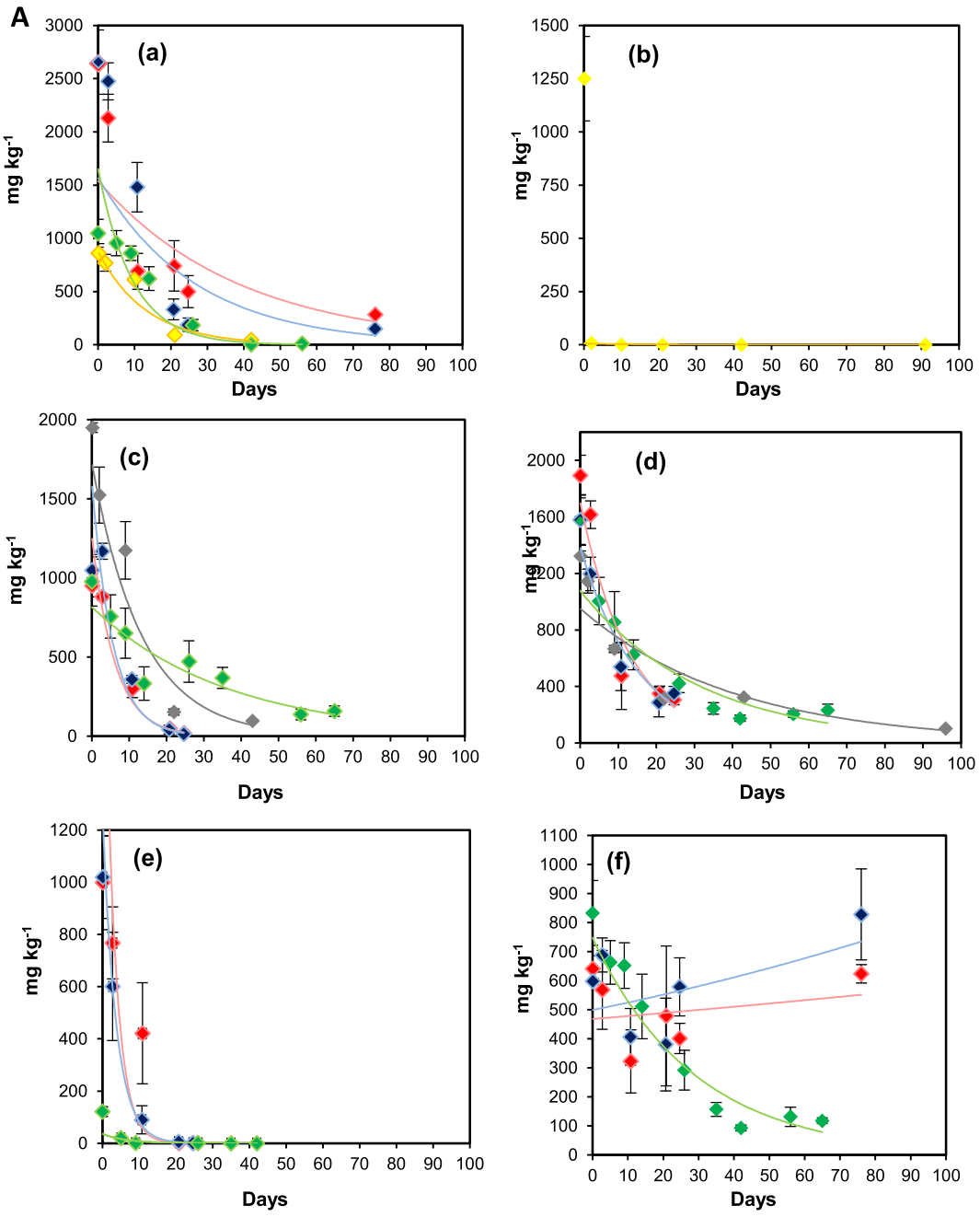

Fig. 3. a. Determination of the drug concentrations during composting of M1 (C/N 17) (◊), M2 (C/N 20) (◊), M3 (C/N 24) (◊), M4 (C/N 29) (◊), and M5 (C/N 37) (◊): azithromycin (a); benzylpenicillin (b); ibuprofen (c); irbesartan (d); olanzapine (e); and telmisartan (f). The error bars represent the standard deviation from the means (n = 3). b. Determination of the drug concentrations during composting of M1 (C/N 17) (◊), M2 (C/N 20) (◊), M3 (C/N 24) (◊), and M5 (C/N 37) (◊): citalopram (a); fluconazole (b); fluoxetine (c); and venlafaxine (d). The error bars represent the standard deviation from the means (n = 3).

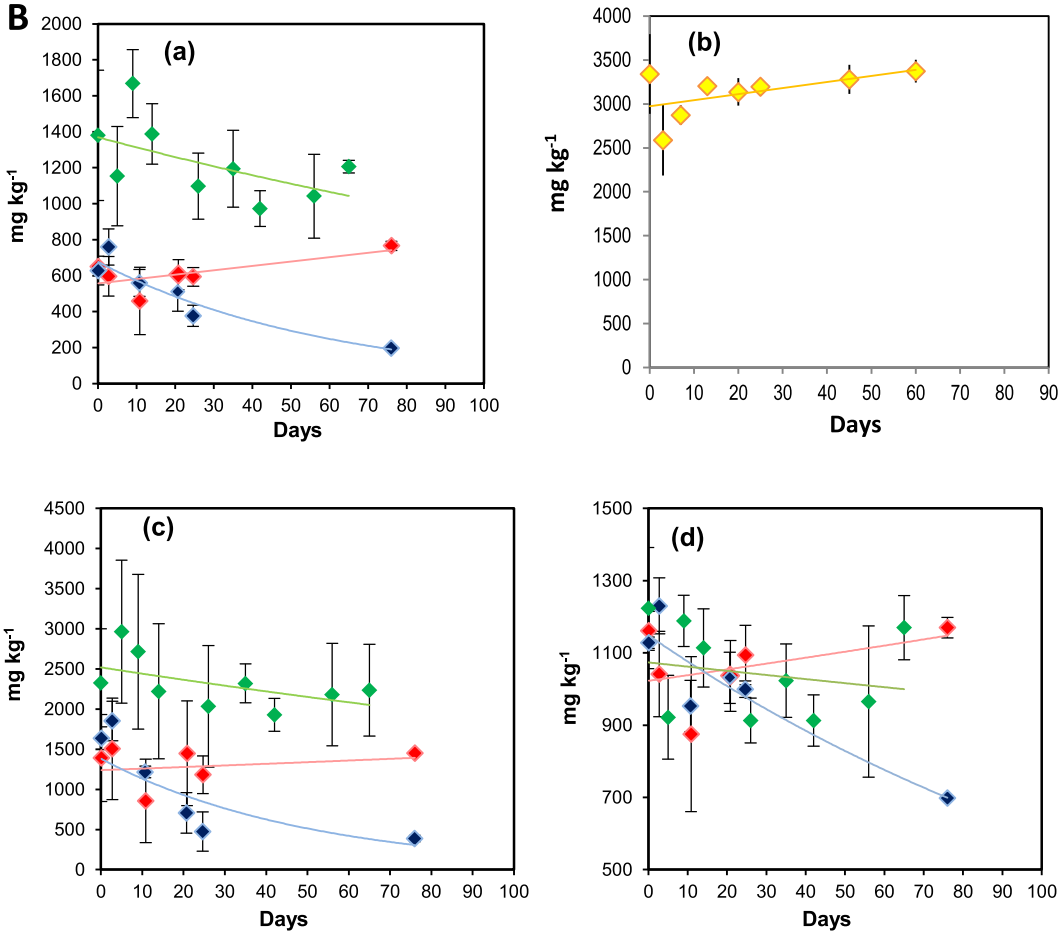

Fig. 3 (*continued*).

the initial measurements (Fig. 3) must have been caused by the different solubility levels of the molecules.

As shown in Fig. 3, azithromycin, ibuprofen, irbesartan, olanzapine, and benzylpenicillin were removed during all the composting processes at different rates of degradation. The rapid removal of the antimicrobial benzylpenicillin during the five processes indicates the presence of notable populations of β-lactamase-producing microorganisms (Dantas et al., 2008; Gatica and Cytryn, 2013). A similar result was obtained with olanzapine, which was almost completely removed during all the processes before 10 days had passed. The concentration of telmisartan was reduced only in M5, which had a high C/N ratio (37), whereas fluoxetine, venlafaxine, and citalopram were reduced in M2, which had the most common C/N (20) ratio used in composting. In the case of fluconazole, no reduction was detected in M3 (the only compost studied); this finding will be further investigated in a future study.

The determination of the semi-reaction periods (SRPs) of the biodegraded PhCs (Table 3), revealed important differences. For azithromycin, the SRPs ranged from 6.5 to 26.7 days (M1), whereas in the case of olanzapine, the SRPs were very close (2 to 4.5 days). In contrast to azithromycin, ibuprofen had an SRP of 39 days in the M2 blend versus 25.5 days in the M5 blend. Similarly, the irbesartan SRPs increased from approximately 10 days (M1 and M2) to 25.7 days (M5). These results clearly indicate that different composting blends have different effects on the degradation of different PhCs, suggesting that for the ideal degradation of PhCs through composting, a study of the composition of the blends in use is required. Furthermore, the addition of compost to the soil produces important improvements in soil characteristics that could be fundamental to reducing degradation in certain semi-desert areas, such as the southern Valencia area. We intend to study this approach in the near future.

Table 3
Variables corresponding to the first order equation kinetics of drug degradation during the composting of blends M1, M2, M3, M4, and M5.

Compound	Blend	Variables			
		C_0	k	R^2	$t_{1/2}$ (days)
Azithromycin	M1	1540.6	−0.026	0.7063	26.7
	M2	1571.2	−0.038	0.6509	18.2
	M3	875.3	−0.076	0.9149	9.1
	M5	1651.1	−0.106	0.8412	6.5
Citalopram	M2	677.0	−0.017	0.9422	40.8
Fluoxetine	M2	1381.5	−0.020	0.7137	34.7
Ibuprofen	M1	1264.5	−0.162	0.9799	4.3
	M2	1600.4	−0.178	0.9708	3.9
	M4	1727.2	−0.075	0.8955	9.2
	M5	815.2	−0.027	0.8758	25.7
Irbesartan	M1	1707.4	−0.077	0.9117	9.0
	M2	1388.4	−0.066	0.9182	10.5
	M4	951.2	−0.025	0.8868	27.7
	M5	1091.0	−0.029	0.8663	23.9
Olanzapine	M1	2295.5	−0.339	0.9044	2.0
	M2	1266.4	−0.273	0.9928	2.5
	M5	35.8	−0.153	0.8616	4.5
Telmisartan	M5	748.5	−0.035	0.8667	19.8
Venlafaxine	M2	1148.8	−0.007	0.8807	99.0

Table 4
Identification of microorganisms able to degrade some pharmaceuticals.

Pharmaceutical	Species	Accession number	Sequence bp	Similarity (%)
Azithromycin	*Alcaligenes faecalis* subsp. *faecalis*	AB680368	901	99,8
	Bacillus cereus	AE016877	990	99,9
	Brevundimonas naejangsanensis	FJ544245	927	99,5
	Klebsiella oxytoca	AB004754	945	100
Ibuprofen	*Alternaria alternata*	KP701250	570	100
Irbesartan	*Aspergillus terreus*	NR131276	608	100
	Klebsiella michiganensis	JQ070300	941	100
	Klebsiella pneumoniae subsp. *ozaneae*	Y17654	957	100
	Klebsiella pneumoniae subsp. *pneumoniae*	AJJI01000018	944	100
	Klebsiella quasipneumoniae subsp. *similipneumoniae*	CBZR010000040	944	100
	Papiliotrema terrestris	NR073350	508	99

3.5. Isolation and characterization of microorganisms able to degrade pharmaceuticals

Azithromycin, citalopram, fluoxetine, irbesartan, ibuprofen, olanzapine, and penicillin G were degraded by composting, and we therefore attempted to isolate the microorganisms that can metabolize these PhCs from the samples taken during the corresponding composting processes according the protocol described in the Materials and Methods.

For the isolation of the degrading strains, the samples used here were obtained during the first mesophilic phase of the composting process, before the temperatures reached 40 °C, because the PhC degradations began at the initiation of the composting process. During the initial testing, the possibility that the different PhCs could be used as carbon and/or nitrogen sources was analysed; positive growth with all PhCs except for fluoxetine and benzylpenicillin was detected in liquid media. Moreover, no PhCs were used for both sources together (carbon and nitrogen). The fact that a PhC was degraded does not indicate that it can be metabolized for growth, and it was clear that benzylpenicillin was degraded by β-lactamases (Brown, 2015), but the antibiotic could not be used as a carbon or nitrogen source by the β-lactamase-producing microorganisms.

After growth in minimal media, similar solid media were spread to obtain isolated colonies after incubation until the appearance of different colonies; the plates containing citalopram and olanzapine showed no growth, suggesting a type of consortium that was able to degrade the PhCs, as previously described (Kästner and Miltner, 2016). However, no studies have investigated this issue, and the colonies obtained on YPD were saved for future analysis.

After the enrichment and screening process described in Materials and Methods, 11 strains (Table 4) that were able to degrade, and more importantly metabolize, high PhC concentrations were identified by BLAST analysis.

Azithromycin and ibuprofen were used as carbon sources by all the strains identified here, whereas irbesartan was used as a nitrogen source (Table 4). To the best of our knowledge, there is no prior description of the ability to degrade these PhCs by these microorganisms. *Alcaligenes faecalis* degrades sulfamethoxazole (Zhang et al., 2016) and sulfamethazine (Islas-Espinoza et al., 2012), and *Bacillus cereus* is involved in the degradation of oxytetracycline (Wang et al., 2015). Using this culture process with these minimal media or others with different compositions should be an interesting approach for isolating microbial strains that are able to degrade pollutants through composting or other decontamination processes.

Although this subject was not the focus of this work, the fact that azithromycin and benzylpenicillin, members of two important chemotherapeutic groups, were detected in this study represents an example of the AR problem (Dantas et al., 2008). Concern about antibiotic resistance is growing, and changes in the usual clinical infection treatment protocols might be necessary (Brown, 2015).

4. Conclusions

Composting sludge with rice straw is a feasible method for PhC degradation and its efficiency can be increased by changing the C/N ratio. To select the appropriate composting blend, small-scale studies might be necessary prior to full-scale treatments, as we describe in this manuscript. The identification of specific microbial strains that are able to metabolize PhCs was possible using simple microbiological methods for enrichment and selection. Further research is needed to identify more microorganisms that are involved in each biodegradation and these isolated microorganisms could be used in composting or other detoxification processes in situ or ex situ.

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