CHANGING WEATHER PATTERN IMPACT ON SOIL MICROBIAL EFFICIENCY IN COMMON USED PHARMACEUTICAL CONTAMINANTS BREAKDOWN FROM SOIL ENVIRONMENT

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Abstract. Soil that is a vital life supporting system is degraded mainly due to the pollution with several contaminants resulted usually from anthropogenic activities. At this moment, the pollution due to new emerging pollutants such as pharmaceutical products pose an additional threat to the soil system. Although use of manure as organic amendment has been proved to possess benefit effects, now it could be considered as a source in addition for soil pollution with pharmaceuticals. Soil microbial communities and soil physicochemical parameters are known that influence in most part pollutants behaviour and degradation in soil. Climate change could impact soil in terms of these parameters as well in terms of microbial content, thus pollutants degradation pattern could suffer changes. At this moment there are minor information on how changing climate will affect pharmaceuticals behaviour in the soil system. Therefore, the present work is aimed to assess pharmaceuticals pattern in soil under normal conditions and stress conditions associated with climate change drivers as anomalies of temperature, and wet, as well sudden temperature and wet changes. Soil enzymes catalyse consecutive stages of biodegradation of different contaminant substrates, leading to their decomposition. Climatic and habitat condition are very important determinants of the intensity of these processes. The activity of soil enzymes, which are catalysts of organic matter decomposition are correlated with soil biogeochemical and physical properties, microbial content, vegetation and with occurrence of various anthropogenic factors. Factors that influence soil biology and functioning is complex therefore assessment of soil enzymatic activity constitutes a necessary step towards understanding of pharmaceuticals dynamics and degradation patters in soil, especially under challenge of climate change.

Keywords: weather anomalies, soil functioning, soil extracellular enzymatic activities, pharmaceuticals, transformation products

INTRODUCTION

The fifth Intergovernmental Panel on Climate Change (IPCC) concluded that global warming, followed at regional level by anomalous rainfall patterns, changes of cold-warm and dry-wet season ratio, are the main meteorological drivers of climate change which are felt at far, and are expected to be more pronounced and intense in the future, being associated also with extreme climate events (flood, drought, heat waves, etc.) (IPPC, 2014). This climate change associated meteorological anomalies has exerted inedible impacts on the surrounding environment and biota (Coyle et al., 2017) causing changes in environment properties (Mihailovic et al., 2016; Latocha et al.,

2016), functioning and provided ecosystem services (Wang et al., 2016; Kumari and Maiti, 2016; Tziliyakis et al., 2015).

Soil is one of the major and important environmental compartment which is responsible for sustainability of the ecosystem and society development. It serves as a medium for plant growth (including food and feed) (Henderson-Seller, 1996); settlement for humans and other living organisms; a sink for heat, water, and chemicals (Prado et al., 2016; Wallace at al., 2017); a filter for water (Wallace et al., 2017); and not ultimately as a biological medium for the breakdown of wastes (including pollutants also) and regulation of chemicals in ecosystem (Wallace at al., 2017; Breure, 2004; Rodriguez et al., 2004).

Soil microorganisms communities are a major component of soil and their activity is absolutely crucial for well-functioning of soil. They are involved in most of the key functions that soil provides in term of ecosystem services, by driving many fundamental nutrient cycling processes, soil structural dynamics, degradation of pollutants (Sanz-Cobena et al., 2016; Ying et al., 2017), regulation of plant communities (Rodriguez et al., 2004; Molina-Montenegro et al., 2016); etc. Also, microbial driven soil processes play key roles in mediating global climate change, by acting as carbon sources and sinks and by generation of greenhouse gases such as nitrogen oxides and methane (Prado et al., 2016; Sanz-Cobena et al., 2016).

Enzymes respond to soil changes induced by challenges of climate changes or anthropogenic drivers long before other soil quality indicator changes are detectable (Xue et al., 2017; Duran et al., 2017). Soil microorganism communities control and produce most of the enzymes involved in the soil key functioning processes as breakdown of organic matter, net changes of soil carbon and nutrient cycling, as well pollutants degradation (Boruszko, 2017) through decomposition, mineralization and immobilization processes (Varjani and Upasani, 2017; Li et al., 2017).

Soil pollutants are degraded enzymatically and assaying the activity of extracellular enzymes can provide insight into metabolic requirements of the soil microorganisms communities, and valuable information on nutrient and substrate availability, and, not finally, on pollutant fate in soil (Li et al., 2017; Carr et al., 2011) under different environmental and meteorological conditions.

A vast number of chemical substances is used routinely in society; pharmaceuticals are an important class of those, and which in the past decades started to receive attention and began to be considered as a new class of possible emerging pollutants (Hursthouse and Kowalczyk, 2009). Although there is an extensive and continuous growing interest on pharmaceutical products occurrence, fate, removal and possible toxicological effects on environment and biota, and unfortunately, at this moment there are no statutory regulations at worldwide level, defining a maximum safe contamination levels of pharmaceutical products in water (drinking water, sewage effluent, etc.) or soil environment (Mansour et al., 2016; Straub and Hutchinson, 2012). Until now, in 2013 only European Union through Directive 2013/39/EU amended an earlier directives on priority substances in the field of water policy (EC, 2013), throughout EU calls for the inclusion of 17-α-ethinylestradiol, 17-β-estradiol and diclofenac pharmaceutical products onto the first watch list of chemical substances (Mansour et al., 2016).

Pharmaceutical products are extensively used both for human health and livestock health assurance. They are a large group of possible emerging contaminants from different chemical classes with different physico-chemical properties (polar, semi polar, non-polar; acidic, basic, neutral; strongly, moderately and weakly sorbed; etc.) (Ho et al., 2014). After treatment, most pharmaceutical products are excreted from the treated body, either unaltered (parent compound) or as metabolites, some of which are still bioactive and whose often could be more intense in effects that parent compound, these making them potentially hazardous to non-target microorganism communities and other non-target organisms from the environment (Lukaszewicz et al., 2016; Martinez-Hernandez et al., 2017). The dominant pathway for antibiotic release in the terrestrial environment is via the application of animal manure and biosolids containing excreted pharmaceutical products to agricultural land as fertilizer. They also can enter in agricultural soil through irrigation with reclaimed wastewater, since they have been frequently detected in the raw and treated sewage wastewaters (Gatica and Cytryn, 2013).

Pharmaceutical products due to their medical properties have an inherent biological effect; furthermore, they behave as persistent pollutants because of their continual infusion into soil ecosystem (Hoyett et al., 2016).

Although soil properties and characteristics largely impact the fate of pharmaceuticals in soil, anomalies and changing meteorological pattern are expected to influence more their breakdown and fate in soil environment (Carr et al., 2011, b; Teng et al., 2012). Thus, assessing the impact climate warming and anomalous rainfall events on the pharmaceutical fate and pathway within soil environmental, especially agricultural soils, is important for understanding their cycling processes and formulating relevant protection and mitigation strategies. Therefore, the primary objective of this study was to lay foundations for a better understanding of the behaviours of pharmaceutical products with respect to climate and land use challenges.

MATERIAL AND METHODS

Table 1

Soil sample main properties

Properties parameters	Soil sample
pH	7.25
EC ($dS \cdot m^{-1}$)	0.62
Organic carbon (according with Walkley-Black, 1934)	0.71
Sand (%)	76.25
Silt (%)	12.8
Clay (%)	10.95

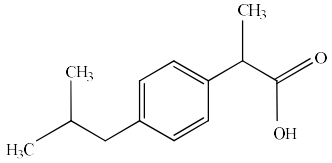
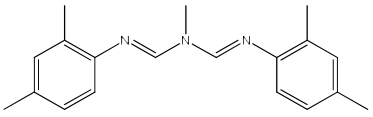
Soil sampling: In order to avoid any result biasing considering pharmaceutical contamination sources, soil samples (first 50 cm depth) were collected from an agricultural land free of wastewater irrigation processes and any organic farming management practices (manure, biosolids, etc.) from Cluj County, Romania. Soil samples were collected by digging, from three different plots in duplicate for

laboratory artificial exposure experiments with pharmaceuticals (exposure polluted water infiltration, and direct contamination of soil). Samples for blank assay were also collected and performed for each experiment cases. Soils were collected on layers with steps of 5 cm after that the column packing process was started respecting layers succession. Soil sample main characteristics could be summarized as presented in Table 1.

***Experimental setup and concept*:** Two frequently used pharmaceutical products as ibuprofen (non-steroidal anti-inflamatory drug for human consumption) and amitraz (veterinary used anti-parasitic agent acting against ticks, lice, mites) were selected for this study. Pharmaceuticals characteristics and properties are included in Table 2.

Table 2

Physicochemical properties and information about the therapeutic use of the studied pharmaceuticals

Pharmaceuticals name	**Ibuprofen**(Pubchem, 2017, a)	**Amitraz**(Pubchem, 2017, b)
IUPAC name	2-[4-(2-methylpropyl)phenyl]-propanoic acid	N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)iminomethyl]-N-methylmethanimidamide
Molecular formula	$C_{13}H_{18}O_2$	$C_{19}H_{23}N_3$
CAS	15687-27-1	33089-61-1
Molecular weight	206.285 g·mol^{-1}	293.414 g·mol^{-1}
Drug class	Non-steroidal anti-inflammatory drugs	Monoamine oxidase inhibitor
Therapeutic use	Anti-inflammatory	Anti-parasitic agent
pKa	4.91	4.20
Water solubility	21 mg·L^{-1} (at 25°C)	1 mg·L^{-1} (at 25°C)
Log K_{ow}	3.97	5.50
Molecular structure		

Impact of weather changes, as temperature and rainfall pattern, was studied on soil columns in a controlled climate chamber. The column experiments had the following exposure setups for each pharmaceutical contaminants: (1.) column experiment (CE-1) exposed at 20 °C for 30 days where in every 4 days soil watering was performed, maintaining thus an optimal soil humidity; (2.) column experiment (CE-2) exposed at 18 °C for 30 days where watering was performed constantly for 7 days followed by a pause for 10 days after that similar watering process (as before

mentioned) was resumed, thus simulating an excess rainfall events; and (3.) column experiment (CE-3) exposed at 30 °C for 30 days where soil watering was performed twice (15 days) during the 30 days experiment, thus simulating drought condition.
Schematic diagram of soil watering regime is presented in Table 3. Control experiment without contaminant addition were performed for each experiment case setup. Contamination experiments were performed for the following concentrations: 5 mg·kg^{-1} and 50 mg·kg^{-1} for ibuprofen (IBU), and at 10 mg·kg^{-1} and 50 mg·kg^{-1} for amitraz (AMT), respectively.

Table 3

Schematic diagram of soil watering regime under the 30th days of applied on CE-1, CE-2 and CE-3 column experiments

Days	Experiments set-up		
	CE-1	CE-3	CE-2
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
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30			

***Pharmaceuticals analysis*:** Ibuprofen analysis from soil samples was performed according with method described by Aznar et al. (2014) with minor

modification. Briefly, 5 g of soil samples were placed in laboratory prepared SPE cartridge with anhydrous sodium sulphate (5 g) as stationary phase. 10 mL of a basic solution (acetonitrile solution with 2 % NH_4OH) were added at SPE cartridge and left for equilibration for 10 minutes after the cartridges were placed in an ultrasonic water bath for 30 min at ambient temperature in order to allow target pharmaceutical products extraction. After extraction, the SPE cartridge was placed on the multiport vacuum manifold where the solvent was collected in graduated tubes. The sample was washed with 5 mL of additional basic solution and the obtained extract was evaporated to dryness. The soil samples were extracted a second time with 10 mL acidic solvent (acetonitrile solution with 2 % CH_2O_2) through 30 min at ambient temperature in an ultrasonic water bath. After the extraction, the SPE cartridge was placed on the multiport vacuum manifold where the solvent was collected in the graduated tube used in the first extraction step. The sample was washed with 5 mL of additional basic solution and the obtained extract was evaporated to dryness. Trimethylsylil-oxime derivatization was performed in two steps, at first time the extract residues was treated with 125 µL hydroxylamine hydrochloride (2.5 g/100 mL) containing pyridine (1:5) and heated in oven at 70 °C for 30 minutes. Thereafter, silylation was continued with 225 µL HMDS and 25 µL TFA and heated also at 70 °C for 90 minutes. Sample was taken for the analysis after dilution with 500 µL HMDS and 1 µL of the diluted solution was injected with an automated sample injector CTC PAL G 6509-B (Agilent Technologies) into the GC-MS system (GC System 7890A coupled with a 5975 Mass Spectrometer, Agilent Technologies) operating at 70 eV. The column used was a capillary HP5-MS column (5 % diphenyl 95 % dimethylpolysiloxane) with 30 m x 0.25 mm I.D. x 0.25 µm film thickness. Helium (purity: 6.0, which means He of 99.9999 %) was used as a carrier gas, with the column flow rate fixed at 1 $mL \cdot min^{-1}$. The temperature of the ion source and GC-MS transfer line was 230 and 280 °C, respectively. MS detector was used in full scan mode. GC injector temperature was set at 250 °C. The oven temperature was set at 40 °C and hold for 3 min after that the temperature was increased with 7 $°C \cdot min^{-1}$ until 160 °C and kept at this temperature for 6 minutes followed by a new increases with 12 $°C \cdot min^{-1}$ at 270 °C and maintained at this temperature for 6 min.

Amitraz extraction from soil samples and instrumental analysis were performed as described by Jimenez et al., (2004) without modifications.

***Extracellular enzymatic activity*:** *Soil dehydrogenase activity* was determined using method described by Tabatabai (1982) where dehydrogenase converts 2.3.5-triphenyl tetrazolium chloride to formazan that could be read spectrophotometrically at 485 nm. Minor modifications were done on method, shortly 5 g soil samples were placed in test tube and mixed with 1 mL 2.3.5-triphenyl tetrazolium chloride aqueous solution (3 %, w/v) and stirred mechanically for 10 minutes after that it was placed at incubation for 24 h period at 37 °C. After incubation 10 mL of ethanol were added at test tubes and the suspension was shaken for 1 min manually, then the tube was allowed for equilibration in order that suspended soil to settle. Obtained supernatant was removed carefully and read spectrophotometrically. The amount of formazan was determined using extinction coefficient of 15433 $mol \cdot cm^{-1}$ (Achuba and Peretiemo-Clark, 2008).

Soil catalase activity was expressed according with method presented by Cohen et al. (1970) and Achuba et al., (2008) where decomposed hydrogen peroxide is measured by reacting it with excess of potassium tetraoxomanganate (VII), $KMnO_4$ and residual $KMnO_4$ is measured spectrophotometrically at 480 nm.

***Auxiliar soil analysis*:** *Soil respiration* was assayed and used as additional indicator of soil microbial activity. The effect on soil microbial respiration was assayed according with method described by Liu et al., (2009) and Haney et al., (2008). Briefly, 50 g of test soil were placed in an air-tight plastic jar bottle, and spiked separately with mentioned pharmaceuticals at presented concentrations, and with 1 mL glucose solution (0.1 M), 10 mL Millipore's ultrapure water in order to assure soil moisture level at 25 % MWHC, and 10 mL of NaOH solution (0.15 N). Blank samples were prepared in similar conditions but without addition of soil. CO_2 was determined by titration of NaOH solution at different intervals for each experiment set-up (CE-1: once at every second day; CE-2: days 1, 5, 10, 15, 16, 20, 25, 28, 30; and CE-3: days 1, 3, 6, 8, 13, 17, 18, 22, 24, 25, 28, 30) with HCl (0.1 N) to a phenolphthalein endpoint which is relative to the amount of CO_2 released by soil microorganisms.

Fatty acid methyl esters (FAMEs) profile was used to characterize soil microbial communities, because it could be considered as the earliest predictors of soil quality changes. FAMEs from soil may be derived from live cells, dead cells, humic materials, as well as plant and root exudates. FAMEs extraction from soil samples was performed according with method described by Banowetz et al., (2006). Analyses were performed by gas chromatography with flame ionization detector (GC-FID, Agilent 7890A). DB-Wax capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness) was used to separate fatty acid methyl esters (37 component FAME mix, Supelco). Oven temperature set for column was set at initial 50 °C (hold for 3 min) followed by an increase with 10 °C·min^{-1} until 170 °C and maintained at this temperature for 1 min and increased again with 5 °C·min^{-1} at 310 °C and maintained for 10 min at this temperature in order to allow column cleaning. Hydrogen was used as a carrier gas, with the column flow rate fixed at 1 mL·min^{-1}. Detector and inlet temperature were set at 250 °C. FAMEs were named in accordance with standard nomenclature as presented by Cardinalli et al. (2015) and Ibekwe and Kennedy (1999): the total number of carbon atoms, followed by a colon and the number of double bonds. The position of the first double bound is indicated by ω followed by the number of carbon atoms from the aliphatic end. The suffixes *c* and *t* refer to the *cis* and *trans* isomers, respectively. Methyl branching at the *iso* and *anteiso* position and are designated by the prefixes *i* and *a*, respectively. The *cy* prefix denotes cyclopentane fatty acids.

Statistical analysis: The experimental results were expressed as: (a) mean value ± standard error when soil respiration were expressed for soil samples; (b) mean value ± relative standard deviation when soil FAMEs content were expressed; and as (c) mean ± standard error of the mean when soil enzymatic activities were expressed. All results were compared with those obtained from control experiments and comparison between pot experiments with those from control experiments were made through analysis of variance (ANOVA) and differences at $p < 0.05$ were considered as significant.

RESULTS RESULTS AND DISCUSSION

Incubation experiment – meteorological anomalies impact on soil microbial communities: The mean percent distribution of the fatty acids from soil samples after finishing the experiment is shown in Table 4.

Table 4

Fatty acid methyl esters composition of soil after the end of experiments.

Measurement unit	Area percent (mean ±SD)					
Experiment pot	CE-1		CE-2		CE-3	
Pharmaceutical product	IBU*	AMT**	IBU*	AMT**	IBU*	AMT**
Hydroxyl FA						
10:0 2OH	0.57±0.09	0.41±0.09	0.39±0.04	0.41±0.05	0.32±0.07	0.12±0.06
10:0 3OH	0.43±0.11	0.38±0.11	0.24±0.08	0.38±0.11	0.17±0.15	0
12:0 2OH	0.49±0.18	0.27±0.15	0.18±0.03	0.10±0.05	0	0
12:0 3OH	0.51±0.15	0.22±0.18	0.23±0.02	0.09±0.02	0	0
16:0 2OH	0.75±0.08	0.82±0.23	0.45±0.17	0.28±0.14	0.28±0.08	0.11±0.05
16:0 3OH	0.38±0.23	0.84±0.22	0.26±0.25	0.41±0.05	0.14±0.11	0.27±0.08
20:0 3OH	0.42±0.15	0.18±0.04	0.26±0.16	0.26±0.07	0.31±0.12	0.24±0.11
cy 17:0	1.89±0.48	1.05±0.22	1.65±0.06	0.79±0.12	0.56±0.05	0.31±0.05
Total	**5.44±1.47**	**4.17±1.24**	**3.66±0.81**	**2.72±0.61**	**1.78±0.58**	**1.05±0.35**
Monounstaurated FA						
16:1ϖ5c	5.16±0.69	4.23±0.89	3.19±0.18	2.22±0.41	1.25±0.84	0.94±0.07
16:1ϖ7c	5.44±0.32	4.55±1.18	2.89±0.24	1.56±0.18	2.06±0.59	0.56±0.04
16:1ϖ9c	0.69±0.15	0.44±0.08	0.05±1.26	0	0	0
17:1ϖ8c	0.83±0.24	0.52±0.39	0.17±2.05	0	1.02±0.25	0.45±0.11
18:1ϖ9c	7.82±0.22	5.59±0.28	0.29±0.39	0	16.22±3.25	5.52±1.27
18:1ϖ9t	4.05±0.48	3.28±1.02	1.28±0.18	0.44±0.26	3.02±0.84	1.98±0.99
Total	**23.99±2.1**	**18.61±3.84**	**7.87±4.3**	**4.22±0.85**	**23.57±5.77**	**9.45±2.48**
Straight chain FA						
12:00	0.89±0.15	0	1.25±0.25	0.41±0.09	1.15±0.25	0.88±0.05
14:00	5.72±0.24	4.01±0.22	3.95±0.37	2.01±0.13	5.22±0.37	3.02±0.22
15:00	7.15±1.06	3.49±0.91	8.99±0.49	3.48±0.51	4.28±0.28	2.07±0.06
16:00	19.23±2.25	13.02±1.06	23.45±0.58	10.1±0.84	15.02±0.55	6.05±0.18
17:00	0.85±0.18	0.15±0.08	2.05±0.67	0.29±0.11	0	0
18:00	3.44±0.57	1.43±0.19	0.75±0.18	0.33±0.04	2.01±0.41	0.24±0.05
20:00	1.08±0.26	0.84±0.27	0.69±0.42	0.38±0.15	0	0.56±0.11
Total	**38.36±4.71**	**22.94±2.73**	**41.13±2.96**	**17±1.87**	**27.68±1.86**	**12.82±0.67**
Branched chain FA						
a13:0	0.26±0.05	0.38±0.21	0	0	0	0
i14:0	0.95±0.24	0.64±0.28	0.44±0.36	0.22±0.09	0.22±0.11	0
a15:0	6.11±0.08	1.28±0.41	3.05±0.15	2.03±0.15	4.1±0.52	0.29±0.11
i15:0	5.82±0.23	1.05±0.48	4.18±1.15	1.85±0.09	2.87±0.61	0.61±0.25
i16:0	4.05±1.05	2.29±0.32	2.99±0.82	0.28±0.18	1.15±0.25	0.87±0.29
a17:0	1.22±0.95	0.67±0.08	0.85±0.41	0	0.62±0.34	0.37±0.11
i17:0	1.96±1.14	0.94±0.15	1.04±0.33	0.56±0.03	0.48±0.17	0.41±0.23
Total	**20.37±3.74**	**7.25±1.93**	**12.55±3.22**	**4.94±0.54**	**9.44±2**	**2.55±0.99**
Polyunstaurated FA						
18:2ϖ6c	6.32±0.95	4.98±1.57	15.06±1.25	4.59±1.27	2.02±0.35	0.84±0.25
18:3ϖ6c	2.81±1.15	2.05±0.09	10.05±2.18	4.15±0.98	3.15±1.28	0.64±0.05
Total	**9.13±2.1**	**7.03±1.66**	**25.11±3.43**	**8.74±2.25**	**5.17±1.63**	**1.48±0.3**

*IBU: ibuprofen (50 mg·kg^{-1})

**AMT: amitrza (50 mg·kg^{-1})

In this study, specifically FAMEs with carbon length 10 – 20 were considered because according with literature, these are the dominant fatty acids in bacterial lipids (Cardinalli et al. 2015; Ibekwe and Kennedy, 1999). Among all samples analysis, palmitic acid (16:00, straight chain fatty acid), and polyunsaturated fatty acids (18:1ω9c and 18:2ω6c) were found in the main prevalent values from the studied experiments. They were detected in all pot experiments, usually in the highest content. Usually, straight chain fatty acids followed by monounsaturated fatty acids were detected in higher percentage in all experimental cases.

In case of control samples, comparing FAMEs profile from the start of experiment (day 0) with data obtained from end of the experiment (day 30) it was observed that under CE-1 pot experiment conditions (optimal soil humidity) there were no significant changes. Similarly, in case of CE-2 pot experiment condition (excess rainfall events) the FAMEs profile has undergone at minor changes, usually branched fatty acids as 15:0, i15:0, a17:0 and 17:0 that are typical for gram positive bacteria, increased under rainfall events. As regards polyunsaturated fatty acid as 18:2ω6c, that is characteristic to fungal community, no changes in amount were observed. Also, it was observed that under CE-3 pot experiment considered decreased precipitation, the relative percentage of polyunsaturated fatty acids has increased, suggesting that fungal communities could be more drought tolerant than the bacterial communities (10:0 3OH and 12:0 3OH attributed usually as biomarkers of gram negative bacteria shifted negatively comparing data from day 0). These obtained data were consistent with findings of some previous studies (Hawkes et al., 2011; Gordon et al., 2008; Zhao et al., 2016).

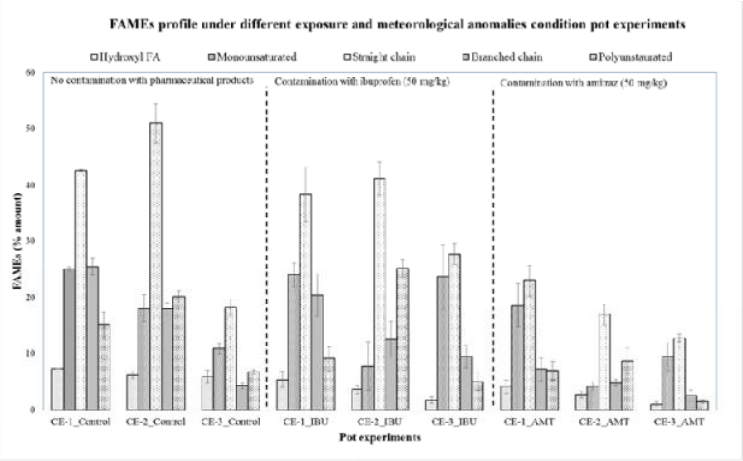
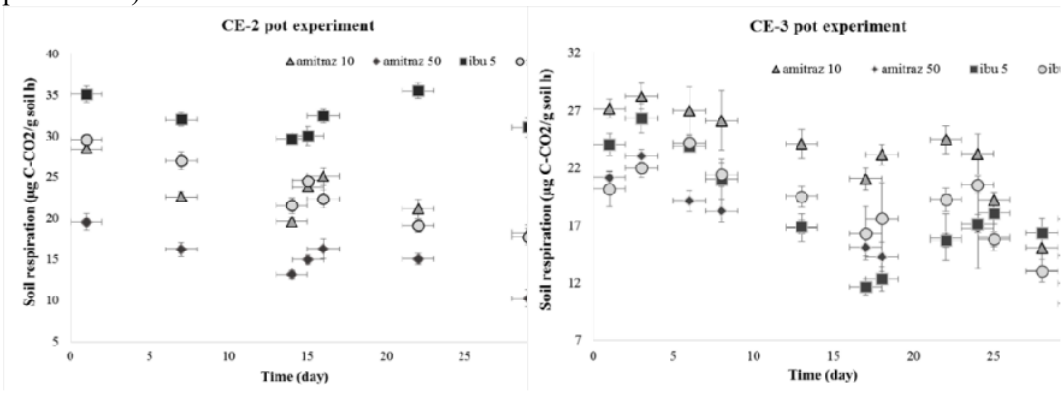


Fig. 1. FAMEs profile under different exposure experiments with pharmaceutical products considering different meteorological conditions (Error bars indicate standard deviation, n=3)

Comparing exposure experiments with ibuprofen (50 mg·kg^{-1}) and separately with amitraz (at 50 mg·kg^{-1}), Figure 1, with control samples (similar conditions but without contamination with pharmaceutical products), it was observed that in case of CE-1 (optimal soil humidity), CE-2 (excess rainfall events) and CE-3 (drought conditions) soil fatty acid profile presents minor changes in case of straight chain fatty

acids when contamination was performed with ibuprofen. In case of contamination experiment with amitraz, shifts of all FAME groups were observed under all experiments, those pharmaceutical products active substance could impact seriously soil microorganism communities, suggesting that active substance has pesticide action on several living organisms as case of amitraz that is considered also as a pesticide that acts against ticks, lice, mites, etc.

***Incubation experiment – meteorological anomalies impact on soil microbial activities*:** Soil respiration is the most general and frequently used parameter for measuring the decomposition of organic compounds in soil and it depends on a wide range of biotic and abiotic factors from the field, that prevents comparison of the degree of soil microorganisms community functioning on its respiration level. Thus, for any assessment, it is important to be based on the proportional changes in activity in comparison with the control samples (Verma et al., 2010). In our case control, samples were exposed at similar incubation condition but without adding any pharmaceutical products (ibuprofen and amitraz) as soil contaminants. Comparing the obtained results, generally for all conditions soil respiration (μg $C\text{-}CO_2 \cdot g^{-1}$ soil $\cdot h^{-1}$) was higher in control samples, between the ranges of 12 – 35 % than when contamination with pharmaceutical products was performed (in the same incubation parameters).



a) **b)**

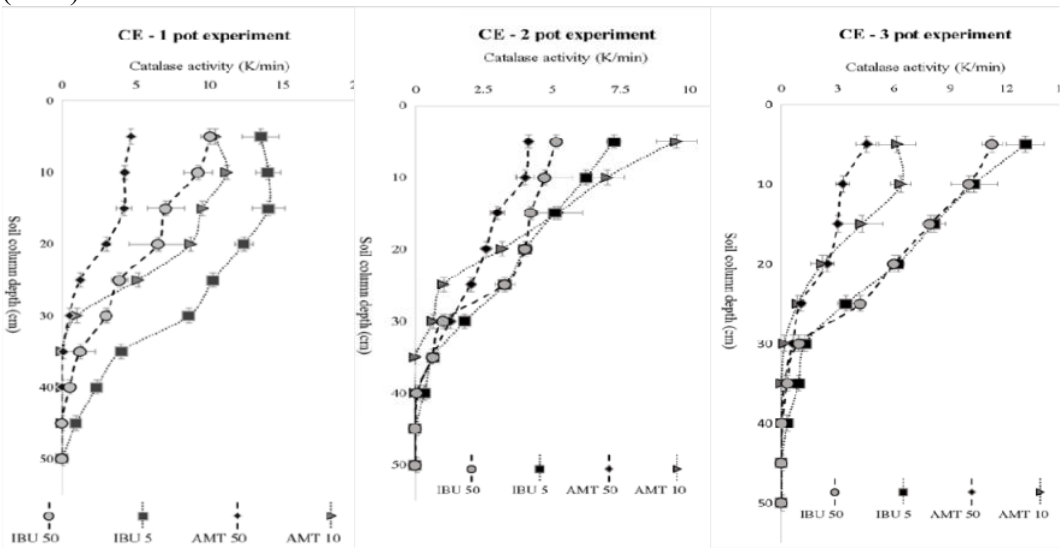
Fig. 2. Soil respiration variation when soil was contaminated with ibuprofen (5 $mg \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$) and amitraz (10 $mg \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$) and exposed at different meteorological conditions:

(a) CE-2 pot experiment: 18 °C for 30 days where watering was performed constantly for 7 days followed by a pause for 10 days after that similar watering process (as before mentioned) was resumed, thus simulating an excess rainfall events; (b) CE-3 pot experiment: 30 °C for 30 days where soil watering was performed twice (15 days) during the 30 days experiment, thus simulating drought condition. Error bars represent standard error, n = 3

Because soil microorganism communities are sensitive to water availability (Manzoni et al., 2012; Hanson et al., 2000), shifts in microbial composition could lead to changes in microbial communities associated processes and, consequent, changes in soil functioning and provided ecosystem services. Heterotrophic respiration through

soil microorganisms can account for 10 – 90 % of the CO_2 efflux from soils and substantially affects the atmospheric CO_2 concentration (Gordon et al., 2008). Increasing of precipitation can stimulate microbial respiration by increasing extracellular enzyme activities and the availability of substrates (Cheng et al., 2013). Analysing performed experiments data, these have showed that soil microorganisms communities function processes are closely related to meteorological and environmental conditions – see Figure 2 (a, b). Soil respiration decreased significantly when soil was exposed at higher amount of pharmaceutical products (amitraz and ibuprofen at 50 $mg \cdot kg^{-1}$), suggesting that active compounds of pharmaceutical products impact soil microorganisms community (Figure 2; CE-2, CE-3 pot experiment).

Thus it was evidenced that alteration of soil temperature and watering conditions impact soil respiration. This could be attributed to microbial tolerability and nutrients availability under rapid changes of meteorological conditions as precipitation and temperature gradient, the relative bacterial and fungal dominance could be impacted by precipitation. On the other hand, with slow increases of precipitation gradient, microbial respiration was enhanced (CE-2 and CE-3 pot experiment). It was observed that shift in microbial composition could lead to changes in microbial respiration before rainfall events, increasing on the following days after the rainfall events, and, in this way, the control of soil moisture on microbial activity that affects CO2 production via soil respiration could be concluded. These data were found to be in accordance with data presented by Coleman et al., (2002), Cheng et al., (2013) and Manzoni et al., (2013).



a) **b)** **c)**

Fig. 3. Soil catalase activity variation when soil was contaminated with ibuprofen (5 $mg \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$) and amitraz (10 $mg \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$) and exposed at different meteorological conditions: (1.) column experiment CE-1: simulating optimal soil humidity; (2.) column experiment CE-2: simulating an excess rainfall events; and (3.) column experiment CE-3: simulating drought condition. Error bars represent standard error of the mean, n = 3

Meteorological and anthropogenic changes impact soil biological and biochemical interactions. Soil enzymatic activities are sensitive indicators of such changes. One could be considered that the soil pollution oxidative stress in soil microorganism communities is an important factor because it is likely that growth and reproduction could be inhibited and/or even mortality of organisms could occur subsequently in several cases. The enzymes as catalase and dehydrogenase are known for their sensitivity and enhanced activity when exposed to pollutants (Roberts and Thomas, 2006; Verlicchi et al., 2010) and for this reason they were therefore chosen as markers for oxidative stress (Esterhuizen-Londt et al., 2016). These extracellular enzymatic activities were used as an indicator for possible pharmaceuticals breakdown because they were previously reported to catalyse electron oxidation of pharmaceuticals to the radical species (Esterhuizen-Londt et al., 2016; Ressems et al., 1998).

Through our experimental data, contamination of soil with pharmaceutical products as ibuprofen and amitraz at different amount (5, 10 and 50 $mg \cdot kg^{-1}$, respectively) modified the biochemical parameters as catalase and dehydrogenase activity of the soil samples (see Figure 3 – a, b, c) with a range between 10 – 62 %, if we compare the results with the control experiment pot. Changes in catalase and dehydrogenase activity value were observed even when contamination experiments were performed with low amount of pharmaceuticals (5 and 10 50 $mg \cdot kg^{-1}$). Soil enzymatic activity behaviour changed either negatively as case of catalase, either positively as case of dehydrogenase. In addition, simulated meteorological conditions as soil poor or excessive watering and temperature also influence strongly soil catalase and dehydrogenase activity. Through CE-1 experiment when normal humidity condition was assured to soil samples, catalase variation with depth was slower than in the other two cases (excess soil watering or poor soil watering). Higher amount of catalase at top soil was detected in case of CE-1 and CE-3 pot experiments. Usually, a more pronounced declining trend of catalase was registered when soil samples were exposed at higher amount of pharmaceutical compounds, especially in case of contamination with amitraze (Figure 3). Decline in catalase activity can be a response to oxidative stress, due to inhibition of enzyme synthesis by increased formation of reactive oxygen species (Esterhuizen-Londt et al., 2016).

As considering data obtained for dehydrogenase activities, the amount increased even with 50 % when exposure experiments was performed, if we compare these data with control pot experiments. Our findings in this sense are in accordance with those reported in literature (Jastrzebska, 2011; Achuba and Peretiemo-Clark, 2008)

Pharmaceutical pathways: Pharmaceutical products presence in environment and their retention in several environmental compartments for different time interval depends on a large scale of variables as soil characteristics, soil type, climate conditions and, not ultimately, by pharmaceutical products characteristics. Their degradation in soil environment is driven in most cases by soil microorganisms communities. Also, a large scale of pharmaceutical products is considered as being susceptible to enzymatic transformation reactions. However, pharmaceuticals can accumulate in soil environment and further impact soil microorganism communities, thus impacting either positively or negatively soil microorganism communities.

Table 5

Pharmaceuticals degradation under various incubation conditions

Contamination with pharmaceutical products – CE-1 pot experiment				
Soil depth (cm)	IBU 5 mg·kg^{-1}	IBU 50 mg·kg^{-1}	AMT 10 mg·kg^{-1}	AMT 50 mg·kg^{-1}
	Detected amount (µg·kg^{-1} d.w) ± SD			
5	78.31 ± 1.25	133.28 ± 0.22	157.25 ± 0.38	265.25 ± 2.55
10	69.25 ± 0.57	134.15 ± 0.09	148.51 ± 0.25	189.54 ± 3.84
15	70.49 ± 0.69	132.35 ± 0.15	138.11 ± 4.56	162.33 ± 1.25
20	68 ± 0.78	129.11 ± 0.41	133.25 ± 1.54	143.25 ± 0.96
25	61.16 ± 1.05	102.15 ± 0.25	118.26 ± 4.11	109.99 ± 3.25
30	54.95 ± 0.55	93.25 ± 0.39	100.62 ± 1.84	87.15 ± 1.47
35	48.88 ± 1.25	88.16 ± 0.47	89.77 ± 2.56	78.25 ± 2.08
40	45.53 ± 1.55	74.55 ± 0.64	81.45 ± 3.25	74.26 ± 3.25
45	41.24 ± 0.93	72.17 ± 0.18	76.25 ± 1.15	71.65 ± 1.24
50	39.06 ± 0.78	69.56 ± 0.63	62.25 ± 0.89	70.89 ± 1.65
Contamination with pharmaceutical products – CE-2 pot experiment				
Soil depth (cm)	IBU 5 mg·kg^{-1}	IBU 50 mg·kg^{-1}	AMT 10 mg·kg^{-1}	AMT 50 mg·kg^{-1}
	Detected amount (µg·kg^{-1} d.w) ± SD			
5	98.15 ± 1.25	145.15 ± 0.25	145.05 ± 0.65	305.15 ± 1.57
10	95.18 ± 0.59	135.09 ± 0.54	128.02 ± 3.22	274.25 ± 2.55
15	87.18 ± 0.77	141.12 ± 3.24	105.62 ± 1.45	298.14 ± 3.15
20	83.57 ± 1.02	118.19 ± 1.85	98.52 ± 2.55	262.84 ± 2.25
25	75.26 ± 0.64	126.51 ± 0.68	84.29 ± 3.64	215.36 ± 3.15
30	69.05 ± 1.11	105.01 ± 0.95	81.02 ± 2.15	188.61 ± 1.25
35	63.54 ± 0.95	95.24 ± 1.22	76.25 ± 1.18	174.25 ± 2.65
40	59.54 ± 0.58	82.34 ± 2.75	69.58 ± 4.56	151.34 ± 1.57
45	54.55 ± 1.22	84.35 ± 2.28	66.29 ± 1.54	144.02 ± 3.25
50	52.39 ± 1.05	80.14 ± 0.45	63.25 ± 2.05	140.39 ± 2.18
Contamination with pharmaceutical products – CE-3 pot experiment				
Soil depth (cm)	IBU 5 mg·kg^{-1}	IBU 50 mg·kg^{-1}	AMT 10 mg·kg^{-1}	AMT 50 mg·kg^{-1}
	Detected amount (µg·kg^{-1} d.w) ± SD			
5	115.67 ± 1.16	198.15 ± 0.59	268.54 ± 2.36	405.25 ± 4.66
10	105.65 ± 0.98	199.02 ± 1.25	266.15 ± 2.99	401.28 ± 3.59
15	100.22 ± 2.84	195.12 ± 2.07	251.24 ± 3.15	385.26 ± 4.45
20	95.18 ± 3.25	190.22 ± 3.47	241.82 ± 3.74	325.55 ± 2.16
25	96.17 ± 1.28	187.76 ± 0.95	228.14 ± 4.75	302.11 ± 1.15
30	93.45 ± 1.14	183.28 ± 1.25	199.28 ± 1.85	287.15 ± 2.05
35	90.06 ± 0.58	177.28 ± 0.55	187.51 ± 3.25	267.18 ± 4.25
40	87.05 ± 1.27	171.65 ± 0.62	168.89 ± 0.95	265.58 ± 1.22
45	86.28 ± 1.59	168.25 ± 1.25	166.14 ± 1.25	235.55 ± 0.98
50	82.46 ± 2.15	165.74 ± 0.59	161.27 ± 0.68	221.51 ± 2.39

IBU represent ibuprofen while AMT represent amitraz

In our days, pharmaceutical products could be present in water systems, run-off, soil, and not finally in produced food products. There is more clear the reason of growing concern about identifying and understanding the mechanisms controlling the fate and pathways of pharmaceutical products, especially in soil environment (being threat for non-target organisms, biota and a possible emergent contaminant for different environmental compartments), under challenges of meteorological anomalies as a consequence of climate change.

The fundamental processes that determine the fate of pharmaceutical compounds while they infiltrate through soil are mainly biodegradation and sorption (Carr et al., 2011, b; Teng et al., 2012; Hoyett et al., 2016). At present, there are a myriad of pharmaceutical products which are differentiated through the multivarious physical and chemical properties that they may possess. Because of that, their fate even under same environmental and meteorological conditions, differs strongly between pharmaceutical compounds and that could be a reason why these compounds are often found in different environmental compartments. Although the international literature concerning pharmaceutical products presence in environment has begun to grow over the years and, more recent researches are published and continue to be published in our moments, there are many questions remaining about the fate and effects of these possible emerging contaminants. Through our column incubation experiment, it was observed that both pharmaceutical products, ibuprofen and amitraz decrease with time and soil depth (Table 5). Higher degradation rate was achieved when soils were exposed at lower amount of pharmaceutical products. Also, under soil watering conditions pharmaceutical products amount decrease more seriously, with 50 – 60 % in both contamination cases of studied pharmaceutical compounds.

Also, it was observed that under simulated drought condition, the studied pharmaceutical products decreased with a smaller tendency, between the ranges of 23 – 42 % with poore degradation especially, in case of ibuprofen.

CONCLUSIONS

Understanding how soil microorganism communities are affected by meteorological anomalies is an essential aspect of predicting soil functional responses to future climate change and, the consequences of those responses for the soil provided ecosystem services. Under this study, it was observed that soil microorganism abundance suffers changes under simulated meteorological anomalies. According with FAMEs analyses, specifically considering polyunsaturated FAs as 18:2ω6c attributed as fungal biomarkers, we could conclude that soil fungal community responds directly to rainfall anomalous events, predominating under simulated drought conditions and being less abundant during excess rainfall events simulation. Similarly, considering bacterial communities FAMEs profile (Gram-positive: 15:0, i15:0, a17:0, 17:0; Gram negative: 10:0 3OH, 12:0 3OH), their abundance during different weathering conditions suffered changes without respecting a concrete pattern for all FAMEs groups profile, so, it is recommendable in future to follow the changes between the timescale at which soil microbial communities experience meteorological fluctuations for a larger period and their ability to respond to future environmental changes.

Soil respiration is influenced by several environmental and climatic conditions, thus to avoid such scenarios of variations soil respiration measures were performed in similar conditions both for control as well contamination pot experiments. Given the differences in soil respiration pattern under different environmental and simulated meteorological conditions, we could suppose that soil respiration could be enhanced through precipitation but this positive relationship will not persist through excessive rainfall or flooding.

Organic compounds as pharmaceutical products mineralization also vary between differences of soil, environment and climate conditions. Therefore,

pharmaceutical products decomposition and oxidation time depend strongly with its physicochemical properties (solubility, log K_{OW}, pKa, etc.) as well of surrounding environment physicochemical characteristics (temperature, pH, microorganism community abundance, moisture, etc). Soil respiration that could be considered as a sensitive parameter that receives changes from the earliest moment, showed a decrease tendency when soil was exposed at higher amount of pharmaceuticals. Also, differences of soil respiration amount were observed with differences of pharmaceutical products. The soil respiration and catalase activity decreased with increasing the incubation period. As regards pharmaceutical products fate under column experiment, soil watering facilitates pharmaceutical compounds degradation. Poorest degradation tendency of ibuprofen was observed when drought condition was simulated. In case of amitraz this wasn't observed. However, changes in meteorological conditions as temperature, precipitation pattern and soil microbial community abundance could impact seriously pharmaceutical products fate and degradation rate in real environment.

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