Pose-Juan, E.; Marín-Benito, J.M.; Sánchez-Martín, M.J.; Rodríguez-Cruz, M.S. 2018. **Dissipation of herbicides after repeated application in soils amended with green compost and sewage sludge.** *Journal of Environmental Management*, 223: 1068-1077.

DISSIPATION OF HERBICIDES AFTER REPEATED APPLICATION IN SOILS AMENDED WITH GREEN COMPOST AND SEWAGE SLUDGE

Eva Pose-Juan, Jesús M Marín-Benito, María J Sánchez-Martín, M Sonia Rodríguez-
Cruz^{*}

Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC).
40-52 Cordel de Merinas, 37008 Salamanca, Spain

* Corresponding author: MS Rodríguez-Cruz. IRNASA-CSIC, 40-52 Cordel de Merinas, 37008 Salamanca, Spain; E-mail: msonia.rodriguez@irnasa.csic.es

Co-authors' E-mails: E Pose-Juan (evaposej@gmail.com); JM Marín-Benito (jesusm.marin@irnasa.csic.es); MJ Sánchez-Martín (mjesus.sanchez@irnasa.csic.es)

Present address: E Pose-Juan (Plant Protection Products Unit (DTEVPF), INIA, Ctra. de La Coruña, Km. 7.5, Madrid, Spain)

ABSTRACT

Certain agricultural practices, such as the repeated application of herbicides or organic amendments to soil, can influence herbicide dissipation. This research has studied the effects of two repeated applications of mesotrione, pethoxamid, and triasulfuron on their dissipation rates in unamended soil (S) and soil amended with green compost (S+GC) or sewage sludge (S+SS). The dissipation experiment has also included an evaluation of the adsorption of the three herbicides by soils and of changes in soil dehydrogenase activity (DHA). The adsorption of the three herbicides by amended soils (K_f range 0.83-2.98) was higher than by unamended soil (K_f range 0.20-0.64). The adsorption coefficients (K_d) of mesotrione and triasulfuron were higher for S+SS, while that of pethoxamid was higher for S+GC, but no relationship between values for the time to 50% degradation (DT_{50}) and adsorption coefficients could be determined. The repeated application of mesotrione decreased its dissipation rate in unamended soil (DT_{50} increased from 4.75 to 8.15 days) and amended soils (DT_{50} increased from 11.7 to 28.2 days in S+GC and from 17.7 to 37.9 in S+SS), whereas the repeated application of pethoxamid increased its dissipation rate in all the treatments, and the rate for triasulfuron increased only in amended soils. The highest DT_{50} values for pethoxamid (12.3 days) and triasulfuron (57.1 days) were in S+GC, and the lowest in S+SS (8.35 and 24.7 days). Soil DHA was stimulated by the presence of GC in the soil and by the first application of mesotrione. The second application of mesotrione and pethoxamid positively affected soil DHA, but this did not occur for triasulfuron. The repeated applications of herbicides and soil organic amendments have different effects on herbicide dissipation, adsorption, and soil DHA, and they should be taken into account when assessing soil quality and other potential environmental implications of pesticide use.

Keywords: herbicide; repeated application; soil organic amendment; dissipation; adsorption; dehydrogenase activity

1. Introduction

Soils in the Spanish region of “Castilla y Leon” belong to the Mediterranean area, where the organic matter (OM) content is low ($< 1\%$). The use of organic amendments in soils to increase the organic carbon (OC) content and improve their fertility and stability is becoming more frequent (Diacono and Montemurro, 2010). Furthermore, this practice could mean that pesticides are not lost through runoff or leaching because soil OC affects pesticide fate involving adsorption, transport and degradation processes with various chemical interactions (Rodríguez-Cruz et al., 2012). The soluble organic carbon (DOC) provided by organic amendments can also modify the adsorption and leaching of pesticides in soils (Marín-Benito et al., 2013; Álvarez-Martín et al., 2017). The influence of OC and DOC contents on the adsorption of pesticides with different characteristics has already been addressed (Marín-Benito et al., 2012; Gamiz et al., 2018; Paradelo et al., 2018)

Some authors have reported a lower adsorption of hydrophobic compounds by soils with DOC (Wanner et al., 2005). On the other hand, some studies have indicated that DOC could be adsorbed by the soil, increasing pesticide adsorption (Singh et al., 2003; Said-Pullicino et al., 2004; Rodríguez-Liebana and Peña, 2018).

Studies on the dissipation of pesticides in unamended and amended soils are usually carried out with the single application of one compound. However, the repeated application of individual pesticides to the same crop is a common agricultural practice, particularly in areas of intensive agriculture (horticultural crops, vine and fruit crops, etc.) (Singh et al., 2002; Fang et al., 2018). The repeated application of pesticides over a

long period can influence pesticide dissipation by accelerating biodegradation by adapted soil microorganisms, which may reduce or eliminate the pesticide's efficacy for controlling the target pest, disease or weed (Fang et al., 2015; Wang et al., 2015). On the other hand, pesticide residues could remain in the soil after repeated applications, having an effect on soil microbial function and structure (Singh et al., 2002; Xu et al., 2014; Wang et al., 2015). In some cases, repeated application can have a detrimental effect on the microbial community degrading the pesticide, significantly compromising its performance (Singh et al., 2002; Wang et al., 2015).

Three herbicides (mesotrione, pethoxamid, and triasulfuron) were used in this study. These herbicides have different characteristics and a broad spectrum of activity against weeds. They are extensively used worldwide, although there are only a few reports on the fate of these compounds in amended soils, and especially on their dissipation and effect on soil microbial communities (Pose Juan et al., 2015a, 2017).

Mesotrione (2-(4-mesyl-2-nitrobenzoyl) cyclohexane-1,3-dione) is a triketone herbicide used for the pre- and post-emergence control of broad-leaved weeds and annual grass weeds in maize (PPDB, 2017). It is weakly acid, non-volatile, and highly soluble in water (Dumas et al., 2017). Its anionic character means its adsorption to soil is low, indicating leaching potential (Dyson et al., 2002; Shaner et al., 2012; Mendes et al., 2016). Mesotrione is mostly degraded by microorganisms (Su et al., 2017). Most of the studies on the adsorption and degradation of mesotrione have been carried out in unamended soils (Dyson et al., 2002; Chen et al., 2012; Mendes et al., 2016). The influence of application dose, pH, temperature, soil moisture, soil OC content, soil texture, soil microorganisms and sunlight on its dissipation and soil microbial function and diversity has been mainly studied in unamended soils (Crouzet et al., 2010; Barchanska et al., 2016; Mendes et al., 2017; Su et al., 2017).

Pethoxamid (2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl) acetamide) is a chloroacetamide herbicide used for both grass and broadleaf weeds control in maize and soybean in pre-emergence and post-emergence. It is moderately soluble in water, and records low volatility and leachability (PPDB, 2017). This substance has only recently (2001) been included in Annex I of Directive 91/414/EEC. Hence there are few studies on its behaviour in soils, most of which are related to its toxicity or to its efficacy and selectivity (Dhareesank et al., 2005; Jursík et al., 2013). It records low adsorption, a medium level of leaching in soil, being considered a non-persistent herbicide in soil (Jursík et al., 2013; PPDB, 2017).

Triasulfuron (1-[2-(2-chloroethoxy) phenylsulfonyl]-3-(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) urea is a sulfonylurea herbicide used in pre- and post-emergence for weed control in crops such as rice, wheat, soybean, sugar beet, and maize. It is a weak acid with low volatility, and high solubility in water and leachability (PPDB, 2017). To our knowledge, most of the studies developed so far have been on adsorption in unamended and amended soils (Said-Pullicino et al., 2004; Gennari et al., 2008; Pinna et al., 2009). Triasulfuron is mostly degraded by microorganisms and by chemical hydrolysis, and this degradation is influenced by soil OM and clay content, pH, fertilizers and amendments, and climatic conditions (Pose-Juan et al., 2017).

As part of the same research project, the authors have already conducted studies on the dissipation of mesotrione, pethoxamid, and triasulfuron applied in increasing doses to unamended and amended soils, and their effect on soil microbial communities (Pose-Juan et al., 2015a, 2015b, 2017). However, to our knowledge, no research is available on the dissipation of these herbicides in unamended and amended soils when repeated applications are carried out.

The aim of this work was therefore to evaluate the influence of two repeated applications (dose = 2 mg kg⁻¹ / application) of the herbicides mesotrione, pethoxamid, and triasulfuron on their dissipation in an unamended soil and this one amended with two organic amendments: green compost (GC) and sewage sludge (SS). To support this research, studies were simultaneously carried out to determine the following: (i) the adsorption of the three herbicides by unamended and amended soils, and (ii) soil dehydrogenase activity (DHA) as an indicator of soil microbial activity.

2. Materials and methods

2.1. Chemicals

Analytical standards of mesotrione (2-(4-mesyl-2-nitrobenzoyl) cyclohexane-1,3-dione; 99.9% a.i.), pethoxamid (2-chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenylprop-1-enyl) acetamide; 94.8% a.i.), and triasulfuron (1-[2-(2-chloroethoxy) phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea; 97.1% a.i.) from PESTANAL® were supplied by Sigma-Aldrich Química S.L. (Madrid, Spain). The characteristics of these compounds are presented in **Table 1** (PPDB, 2017).

HPLC grade acetonitrile, acetone, methanol and water were supplied by VWR International Eurolab (Spain). Calcium carbonate, 2,3,5-triphenyltetrazolium chloride (TTC) and 2,3,5-triphenylformazan (TPF) were supplied by Sigma-Aldrich Química S.L. (Madrid, Spain).

2.2. Organic residues and soil

Green compost (GC), which is comprised of pruning wastes from gardens and parks, was supplied by the Salamanca Local Authority (Salamanca, Spain). Sewage sludge (SS) from a domestic waste treatment plant and stabilized by anaerobic digestion

was supplied by Aqualia SA (Salamanca, Spain). Some characteristics of these organic residues were determined in samples previously air dried, homogenized and sieved (< 2 mm) (Table 2). The pH was determined in a residue/water suspension (1/2.5 w/v ratio). Organic carbon (OC) content was determined by oxidation (Walkley-Black method). Dissolved organic carbon (DOC) was determined in a suspension of residue (1/100 w/v ratio) in Milli-Q ultrapure water after residue shaking (24 h at 20°C), centrifugation (20 min at 10000 rpm), and filtering (Minisart NY 25 filter 0.45µm, Sartorius Stedim Biotech, Germany) using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) organic carbon analyzer. Total N was determined by the Kjeldahl method.

The soil used in all the experiments was taken from the surface horizon (0-30 cm) of an agricultural farm in Zamora (NW-Spain). It is a Typic Xerorthent (Soil Survey Staff, 2006), and its texture was classified as sandy loam (83.4% sand, 5.9% silt, and 10.7% clay). The amended soils were prepared in October 2012 by uniformly mixing soil with GC or SS *in situ* using an agricultural rotovator at a rate of 50 t ha⁻¹ (considering a soil depth of ~10 cm and a soil density of 1.3 g cm⁻³). As noted earlier, unamended soil is referred to as S, whilst soil amended with GC or SS is referred to as S+GC and S+SS, respectively. Undisturbed soil samples of all the treatments (~30 kg) were kept under environmental conditions in 60 x 40 x 25 cm trays on site at IRNASA (Salamanca, Spain) allowing drainage. After three months of incubation, fresh samples of unamended and amended soils were sieved (<2 mm) prior to their use in the adsorption and dissipation experiments. Characteristics of unamended and amended soils were determined using standard analytical methods (Sparks, 1996) and are included in Table 2. The DOC was determined in soil extracts (1/2 w/v ratio) as previously indicated for organic residues. The particle size distribution was determined using the pipette method. Clay minerals (smectite, illite and kaolinite) were qualitatively

identified in the soil clay fraction by X-ray diffraction using CuK α radiation on a Philips PW1710 diffractometer (Eindhoven, The Netherlands).

2.3. Dissipation studies

Initially, the herbicides were individually dissolved in acetonitrile to give a concentration of 1000 mg L⁻¹. Solutions of the herbicides were then prepared in Milli-Q ultrapure water, and a volume of 10 mL of appropriate concentration was spiked to 800 g of fresh weight of unamended or amended soils to give a concentration of 2 mg kg⁻¹ dry soil. Soil samples were incubated at 20 °C in the dark. The moisture content of the soil samples was previously adjusted to 40% of the maximum soil water-holding capacity, and it was maintained by adding sterile Milli-Q ultrapure water when necessary. Each soil treatment was prepared in duplicate. All the soils were thoroughly stirred with a sterilized spatula, and all the steps were performed in a sterile cabinet. Samples of the soils individually treated with mesotrione, pethoxamid, and triasulfuron were taken for pesticide analysis at different time intervals for 99, 43 and 144 days, respectively (according to the dissipation rates of each pesticide). A second treatment of the soils with the herbicides was applied at the same dose of 2 mg kg⁻¹ when the concentrations of each specific herbicide were between 30% and 70% of the amount initially added. This second treatment was applied to the soils previously treated with mesotrione, pethoxamid, and triasulfuron at 14, 9 and 35 days, respectively.

2.4. Extraction and determination of herbicides

Duplicate 5 g samples of each treatment (800 g of unamended or amended soil) were taken at each sampling time and shaken in glass tubes at 20 °C for 2 h (mesotrione and triasulfuron) or 24 h (pethoxamid) with 10 mL of methanol: water (1:1),

acetone:acetonitrile (1:1) or methanol: acetone (1:1) for mesotrione, pethoxamid, or triasulfuron extraction, respectively. The soil samples treated with mesotrione or triasulfuron were then sonicated for 1 h. In all cases, the samples were centrifuged at 5045 g for 15 min, and the herbicide extracts were passed through a Minisart NY 25 filter (Sartorius Stedim Biotech, Germany) to remove particles > 0.45 μm . For the determination of the herbicides, a volume of the extract was transferred to a glass vial for analysis. The recoveries of the extraction method were determined by spiking three unamended and amended soil samples with an analytical grade of each herbicide to a final concentration of 2 mg kg^{-1} , and then performing the extraction procedure as described above. The mean recovery values were > 70% for the three herbicides studied (Pose-Juan et al. 2015b, Pose-Juan et al., 2017).

Herbicides were quantified by HPLC with a diode array detector (DAD) and a mass spectrometer (MS) (Waters Associates, Milford, MA), using Empower software as the data acquisition and processing system. The analytical column was a Luna PFP (2) (150 $\times$ 4.6 mm i.d., 3.0 μm) (Phenomenex, Torrance, CA, USA). The mobile phase was 90:10 (v/v) acetonitrile/water (1% formic acid for mesotrione and triasulfuron, and 0.1% formic acid for pethoxamid). The flow rate of the mobile phase was 0.4 mL min^{-1} and the sample injection volume was 10 μL . Retention times were 5.6, 6.1, and 5.1 min, for mesotrione, pethoxamid, and triasulfuron, respectively.

Quantitative analysis was performed using the peak area of the herbicides obtained from the total ion chromatogram (TIC) in SIM mode. The molecular ions (m/z) corresponding to mesotrione, pethoxamid, and triasulfuron in the positive ionization mode $[\text{M} + \text{H}]^+$ were 340.0, 296.8, and 402.8, respectively. Calibration was performed from 0.5 to 25 $\mu\text{g mL}^{-1}$ ($r \geq 0.99$), and the limit of detection (LOD) and limit of quantification (LOQ) were 0.0018 and 0.020 $\mu\text{g mL}^{-1}$, 0.0031 and 0.010 $\mu\text{g mL}^{-1}$, and

0.012 and 0.113 $\mu\text{g mL}^{-1}$ for mesotrione, pethoxamid, and triasulfuron, respectively.

2.5. Adsorption studies

The adsorption curves of pesticides for unamended and amended soils were obtained using the batch equilibrium technique. Duplicate soil samples (2.5 g for mesotrione and triasulfuron, and 5 g for pethoxamid) were equilibrated with 10 mL of a 0.01 M CaCl_2 Milli-Q ultrapure water solution of each herbicide at concentrations of 1, 5, 10, 15, 20 and 25 $\mu\text{g mL}^{-1}$. The soil/solution ratios were previously optimized. The suspensions were shaken at 20 °C for 24 h in a thermostated chamber, with intermittent shaking for 2 h at three-hour intervals. Preliminary experiments revealed that contact for 24 h was long enough for attaining equilibrium. The suspensions were subsequently centrifuged at 5045 g for 15–30 min, filtered, and the equilibrium concentration of each herbicide was determined by HPLC-MS, as indicated above. The amount of herbicide adsorbed was calculated as the difference between that initially present in the solution and that remaining after equilibration with the soil.

2.6. Soil dehydrogenase activity (DHA)

Soil dehydrogenase activity (DHA) was determined following the Tabatabai method (Tabatabai, 1994), which is based on the extraction and colorimetric determination of the intensely coloured 2,3,5-triphenylformazan (TPF) produced by reducing colourless triphenyltetrazolium chloride (TTC) in soils. Soil DHA was determined at the beginning of the dissipation experiments (0 days), and after 14, 36 and 99 days for the mesotrione experiment, 9 and 43 days for the pethoxamid experiment, and 35, 98 and 144 days for the triasulfuron experiment. These sampling times were set after the first and second herbicide application, at an intermediate time in

the second dissipation kinetics of each herbicide, and when the herbicides were almost completely dissipated or at the end of the incubation of soils with herbicides.

2.7. Data analysis

Herbicide adsorption data were fitted to the Freundlich equation, and adsorption and non-linearity coefficients, K_f ($\mu\text{g}^{1-n_f} \text{g}^{-1} \text{mL}^{n_f}$) and n_f , respectively, were obtained. Distribution coefficients, K_d (mL g^{-1}), were determined for equilibrium concentrations (C_e) of 5 and 15 $\mu\text{g mL}^{-1}$ (K_{d5} and K_{d15}) of each herbicide.

The dissipation kinetics for herbicides was fitted to a single first-order (SFO) kinetic model or first order multicompartment (FOMC or Gustafson and Holden) model. FOCUS work group guidance recommendations were followed for selecting the kinetic model that best describes the dissipation results (FOCUS, 2006). The coefficient of determination (r^2) and the chi-square (χ^2) test were calculated as indicators of goodness of fit. Values for the time to 50% dissipation (DT_{50}) were used to characterize the decay curves and compare variations in dissipation rates. The parameters of the kinetic models were estimated using the Excel Solver add-in package (FOCUS, 2006).

Standard deviation (SD) was used to indicate variability among replicates. Data were analysed by one-way or two-way ANOVA. Fisher's least significant difference (LSD) method was used, at a confidence level of 95%, to determine significant differences between means and evaluate the effects of the different treatments on DT_{50} , K_f and K_d and soil DHA values. SPSS Statistics v22.0 software for Windows (IBM Inc., Chicago, ILL, USA) was used.

3. Results and discussion

3.1. Adsorption studies

Fig. 1 includes the adsorption curves of mesotrione, triasulfuron, and pethoxamid for unamended (S) and amended soils (S+GC, and S+SS). The adsorption curves fitted the Freundlich equation with r^2 values in the range 0.80-0.98 ($p < 0.05$ -0.01), and the K_f , and n_f parameters determined from the linear form of this equation are included in **Table 3**.

The adsorption curves of the weak acid herbicides mesotrione and triasulfuron were, in general, non-linear, L-type. These herbicides were in an anionic form because the pH of soils is higher than the pKa of compounds (Table 2), and due to the presence of two plateaus the curves obtained were classified as sub-group 4 (Giles et al. (1960). Similar adsorption curves were obtained for other anionic compounds with different sorbents (Atay et al., 2002; Sánchez-Martín et al., 2008), with the second rise in the curves being attributed to the development of a fresh surface on which adsorption can occur, with the second plateau representing the complete saturation of this new surface (**Fig. 1**). The n_f values were in the range 0.68-0.90 and 0.70-1.27 for the adsorption of mesotrione and triasulfuron by unamended and amended soils, respectively. These values for mesotrione indicate a relatively higher affinity for all the soils at a low concentration, and a reduction in adsorption at a higher concentration (Pinna et al., 2014). The L-type curves of mesotrione could be due to herbicide adsorption on soil organic matter (Alekseeva et al., 2014). This behaviour was also observed for the adsorption of triasulfuron by amended soils, while it did not occur for the unamended soil (Table 3). The high water solubility of triasulfuron could prevent the herbicide's adsorption by the soil at low concentrations when herbicide compete with the water by soil surfaces as reported for some polar compounds (Stangroom et al., 2000).

The adsorption curve of pethoxamid for unamended soil was of S-type ($n_f > 1$), while its adsorption by S+SS and S+GC was close to linearity (C-type) ($n_f = 1.16$ and

0.96). These different types of curves are to be expected considering that pethoxamid is a compound with a slightly hydrophobic character, but with high water solubility (Table 1). Non-linear curves are generally obtained when specific interactions or different interaction mechanisms are involved in the adsorption of a compound by an adsorbent. This could occur here, considering the herbicides' characteristics and the changes in soil surface caused by the different amendments (Marín-Benito et al., 2009; Said-Pullicino et al., 2004).

The K_f values for the adsorption of the anionic herbicides mesotrione and triasulfuron by unamended and amended soils fell within the ranges 0.64-1.12 and 0.61-2.66, respectively, while it was 0.20-2.98 for the adsorption of the non-ionic herbicide pethoxamid. The adsorption of the more hydrophobic herbicide pethoxamid by the unamended soil was lower than that of the anionic herbicides. However the adsorption by the amended soils increased for the three herbicides due to their higher OC content. In general, this effect was also recorded for the distribution coefficients (K_d) determined at equilibrium concentrations of 5 and 15 $\mu\text{g mL}^{-1}$ to compare the adsorption capacity of herbicides by soils, because the n_f values of the adsorption curves were different to 1. The K_{d5} and K_{d15} values for the herbicide mesotrione and K_{d5} for the herbicide triasulfuron were higher in S+SS than in S+GC, although these values were higher for pethoxamid in S+GC (**Table 3**). The lower pH of S+SS could increase the adsorption of anionic herbicides observed in some cases by this soil together with the higher OC and DOC contents, which could give rise to the formation of new hydrophobic surfaces, as observed for other compounds (Álvarez-Martín et al., 2016). However, the K_{d15} values were in general lower than the K_{d5} values for the most hydrophilic compounds (mesotrione and triasulfuron), and higher for pethoxamid (more hydrophobic) in agreement with the adsorption curves obtained. These curves showed a plateau for

hydrophilic herbicides representing the complete saturation of adsorbent surface with the increase of herbicide concentration. This behaviour could be explained taking into account that different adsorption mechanism could be involved for the adsorption of the anionic herbicides and the non-ionic herbicide pethoxamide as reported previously (Rodríguez-Cruz et al., 2009).

A correlation analysis between the adsorption parameters and the soil characteristics was carried out to extend the information on the influence of soil parameters on the adsorption of these herbicides. OC and DOC contents and pH were the parameters considered in the analysis because they are the parameters influencing the adsorption of organic compounds with different characteristics (Stangroom et al., 2000; Rodríguez-Cruz et al., 2012). The CEC of soils was also reported as a factor influencing the adsorption of ionic compounds. However in this study CEC were not considered in the analysis because a highly significant relationship between CEC and OC contents of soils was found ($r=0.993$).

For mesotrione, the results reveal no significant correlation of K_f values with any soil parameters. However, there was a positive relationship (not significant because of the low number of samples examined) between the distribution coefficients (K_{d5} and K_{d15}) and soil OC content. This indicates the influence of soil OC content in the adsorption when the concentration of mesotrione in solution increased ($C_i \geq 10 \mu\text{g mL}^{-1}$). A positive, significant relationship was observed between K_{d15} values and soil DOC contents ($r = 0.99$, $p < 0.1$), while no significant correlation was observed between any adsorption coefficients and soil pH. Mesotrione is an ionic compound, and relationships between adsorption and soil pH have been reported (Dyson et al., 2002). The influence of OC and clay mineral contents and CEC have also been reported (Dyson et al., 2002; Shaner et al., 2012; Alekseeva et al., 2014; Pinna et al., 2014; Mendes et al., 2016).

The results for triasulfuron also indicate the influence of OC and DOC contents on its adsorption by soils, although positive relationships were only observed at low concentrations of herbicide in solution ($C_i = 1\text{--}5 \mu\text{g mL}^{-1}$), that is, between K_f or K_d and soil OC and DOC contents, significant in the case of K_f values and OC contents ($r = 0.99$, $p < 0.1$). A negative but non-significant correlation between K_d and soil pH was also obtained. Data on the influence of soil pH and soil OC, DOC and humic acid contents on the adsorption of triasulfuron by unamended and amended soils have been also reported (Said-Pullicino et al., 2004; Pinna et al., 2009). There is a higher adsorption of this herbicide by unamended soil than by the amended soils at higher concentrations of triasulfuron in solution ($C_i \geq 20 \mu\text{g mL}^{-1}$), which could indicate that the clay fraction and the smectite mineral present in this soil is also important for adsorption, as observed for other ionic and water-soluble compounds (Azejjel et al., 2008).

For pethoxamid, increased adsorption was obtained by the amended soils independently of the initial herbicide concentration in solution. Adsorption was higher by S+GC, with lower OC content, than by S+SS, and a non-significant correlation was observed between the adsorption coefficients and soil parameters. The higher DOC content in S+SS than in S+GC could explain this behaviour by pethoxamid, considering the contrary effect of DOC on the adsorption reported for some hydrophobic compounds (Marín-Benito et al., 2009, 2012).

3.2 Dissipation studies

Fig. 2 shows the dissipation curves of mesotrione, triasulfuron and pethoxamid after the first and second repeated applications of 2 mg kg^{-1} in unamended and amended soils. The data for the residual concentrations of herbicides as a function of time were

fitted to the SFO kinetic model ($r^2 \geq 0.95$ for mesotrione, $r^2 \geq 0.98$ for pethoxamid, and $r^2 \geq 0.96$ for triasulfuron) to calculate the dissipation rate constants (k) and DT₅₀ values. These kinetic parameters for the first application of the herbicides were calculated from dissipation curves between 0 and 14, 35 or 9 days of mesotrione, triasulfuron, and pethoxamid application, respectively. The percentages of the residual amounts of herbicides at the end of the first application were between 30% and 70%. The second herbicide application was then carried out, and the kinetic parameters were calculated from dissipation curves between 14 and 99 days, 35 and 144 days and 9 and 43 days for mesotrione, triasulfuron, and pethoxamid, respectively. The percentages of residual amounts of herbicides at the end of the second application were < 25% (**Fig. 2**).

The DT₅₀ values of mesotrione after the first application were 4.75, 11.7, and 17.7 days in S, S+GC, and S+SS, respectively, and they increased to 8.15 (S), 28.2 (S+GC), and 37.9 (S+SS) days after the second application of the herbicide (Table 4). These DT₅₀ values agree with the wide range of DT₅₀ values (4.5-34 days) reported in the literature for mesotrione dissipation in different unamended soils (Dumas et al., 2017; PPDB, 2017). These results indicate a significant increase in DT₅₀ values (up to $\approx$ 2.5 times) in all the treatments after the second application (LSD = 4.80, $p < 0.05$), and a reduction in the dissipation rates of mesotrione, or inhibited degradation when it was repeatedly applied. This is contrary to accelerated degradation expected due to microbial adaptation as usually reported by repeated application of herbicides (Celis et al., 2015), although a similar effect was also obtained when higher doses of mesotrione were applied in the unamended and amended soils. The effect on the decrease in dissipation by the repeated application of the herbicide was less significant than the application of the highest single dose of herbicide (2-50 mg kg⁻¹) in similar soils, as in that case DT₅₀ increased up to five times (Pose-Juan et al., 2015b).

The dissipation of mesotrione was faster in S than in S+GC and in S+SS before and after the repeated application of the herbicide consistent with the highest K_d adsorption coefficients by the amended soils, as indicated above (Table 3). Su et al. (2017) have reported that the use of farmyard manure as soil amendment decreases the mesotrione degradation rate due to higher herbicide adsorption by soils. Furthermore, the adsorption of mesotrione by these soils after repeated application could increase with time, with slower dissipation rates if the mass transfer from the sorbed phase to the solution is limited, as previously reported (Shaner et al., 2012). Similarly, the slower dissipation rates of other pesticides after repeated application in soils were also attributed to the toxicity of the increased amount of pesticide or their degradation products for soil microorganisms or the lack of easily degradable OM in the soil (Singh et al., 2002; Celis et al., 2015).

The DT_{50} values of triasulfuron after the first application were 20.4 (S), 57.1 (S+GC), and 24.7 (S+SS) days, and these values were 22.9 (S), 33.2 (S+GC), and 21.6 (S+SS) days after the second application. The DT_{50} values obtained are consistent with other values reported in unamended soils (15-48 days) (EFSA, 2015). DT_{50} values were similar in S and S+SS, and increased significantly in S+GC after the first and second applications ($LSD = 6.58$, $p < 0.05$) (**Table 4**). The repeated application of herbicide in the S+GC soil enhanced the dissipation rate of triasulfuron, while the rate in S or S+SS was less affected. Consequently, the dissipation rates were unrelated to the adsorption of this herbicide by the soils, which was higher in S+SS (Table 3). The dissipation of triasulfuron did not follow a similar trend when the effect of higher doses of herbicide was evaluated (Pose-Juan et al., 2017). DT_{50} values of triasulfuron increased by around twice for doses applied up to 50 mg kg^{-1} in both S+GC and S+SS. The behaviour of triasulfuron was different to the other anionic herbicide mesotrione because its

dissipation was slower than that of mesotrione after the first and second repeated applications. The higher adsorption of triasulfuron by unamended and amended soil than that of mesotrione could explain its lower bioavailability to soil microorganisms in spite of its characteristics (low hydrophobic character and high water solubility).

The DT_{50} values for pethoxamid after the first application were 11.9, 12.3, and 8.35 days in S, S+GC, and S+SS, respectively, and they decreased up to 8.05 days (S), 10.0 days (S+GC), and 6.75 days (S+SS) after the second application ($p < 0.05$) (**Table 4**). The DT_{50} values for the dissipation of pethoxamid applied at both repeated doses in unamended soil fell within the range of the DT_{50} values reported (6-14.8 days) (PPDB, 2017). The DT_{50} values indicated an increase in the dissipation rate by the repeated application of the herbicide. These results are consistent with previous findings for other pesticides after repeated applications, which were explained by accelerated degradation due to microbial adaptation leading to faster metabolism (Celis et al., 2015). The dissipation rate of pethoxamid in S+GC was the lowest after the first and second applications, while the highest dissipation rate was in S+SS. The DT_{50} values obtained could be explained by the higher adsorption constants of pethoxamid for S+GC than those for S or S+SS (Table 3). The low DT_{50} values in S+SS could be explained by its high content in DOC. The herbicide could be adsorbed by DOC, and the bioavailability for degradation could be enhanced if it remains adsorbed in solution, as indicated for other pesticides (Marín-Benito et al., 2012). The dissipation of pethoxamid was faster than that of the anionic herbicides mesotrione and triasulfuron, indicating the different bioavailability of the herbicides in the soil to be degraded by microorganisms. The apparent rapid dissipation of pethoxamid in amended soils could be explained by its adsorption in a more irreversible form over time after the application due to its hydrophobic character (Dhareesank et al., 2016).

No significant correlations between DT₅₀ values and soil pH or OC content were observed for any one the three herbicides, possibly due to the low number of soil samples considered in this study.

3.3. Soil dehydrogenase activity (DHA)

Soil DHA was determined in soils with different treatments, and **Fig. 3** shows the effects of the amendments (GC or SS) and herbicides (mesotrione, pethoxamid, and triasulfuron) at different incubation times after the repeated application of herbicides.

In soil without herbicides (control soils) the application of amendments to soil stimulated DHA in the case of GC, but the application of SS did not increase the mean values of DHA with respect to S (**Fig. 3**, different upper-case letters denote significant differences within treatments for unamended and amended soils untreated and treated with herbicides). The soil DHA enhancement in S+GC was expected due to the increase in soil OC and the addition of a new microbial population with this amendment, as reported previously for soils amended with different organic residues (Marín-Benito et al., 2012; Pose-Juan et al., 2015b, 2017). However, the DHA values in S+SS either decreased or were similar to those of S, although OC content in this soil also increased. Similar results have been reported in previous works, being explained by a possible toxic effect of SS for soil microorganisms (Mingorance et al., 2014; Pose-Juan et al., 2015a)

The effects of herbicides on soil DHA were variable. Mesotrione stimulated soil DHA in unamended and amended soils after the application of the first dose, but this effect did not occur for the herbicides triasulfuron (in any soil) or pethoxamid (in S and S+GC) (**Fig. 3**, different lower-case letters denote significant differences between sampling times for untreated or soil treated with herbicides within the same unamended

or amended soil). Soil DHA increased initially with the application of the second dose of mesotrione after 14 days and of pethoxamid after 9 days in the unamended and amended soils, but DHA did not increase (or decrease) for triasulfuron after 35 days (second sampling time in **Fig. 3**), indicating a positive or negative effect of the herbicides on soil microbial activity. At the end of the soil-amendment-herbicide incubation period, DHA decreased in both amended soils treated with mesotrione (99 days), in S+SS treated with pethoxamid (43 days), and in S+GC treated with triasulfuron (144 days) (**Fig. 3**).

These findings are consistent with previous results in which soil DHA was stimulated following the application of mesotrione at the recommended dose (Kacynski et al., 2016; Pose-Juan et al., 2015b). However, Borowik et al. (2017) and Kacynski et al. (2016) have reported that mesotrione applied in herbicide mixtures of mesotrione + terbuthylazine + S-metolachlor and mesotrione + S-metolachlor inhibit DHA when the herbicides are applied at doses higher than the recommended field rate. It has been reported accordingly that the application of herbicides mixtures to soil can inhibit DHA (Kacynski et al., 2016; Wyszowska et al., 2016).

The influence of herbicides on DHA was corroborated by the relationship between the amounts of herbicides present in the different soils and the DHA values measured at the different sampling times. A positive significant relationship was obtained for mesotrione in S+GC ($r=0.716$, $p < 0.05$) and S+SS ($r=0.909$, $p < 0.01$) or when all the soils were considered ($r=0.615$, $p < 0.01$). For pethoxamid, there was a positive significant relationship between herbicide amounts and DHA values in S+SS ($r=0.902$, $p < 0.02$), and for triasulfuron this positive relationship was only significant in S ($r=0.747$, $p < 0.05$), and hardly significant in S+GC ($r=0.618$, $p \leq 0.1$). The results corroborate the stimulatory effect of the repeated application of mesotrione on DHA,

especially in amended soils, where mesotrione was dissipated at a slower rate after the second application. However, the positive effect of the herbicides pethoxamid and triasulfuron on DHA was only significant for some individual soil samples because dissipation was faster after the second application, or because of a possible toxic effect of the herbicide on soil microorganisms (Sofa et al., 2012).

4 CONCLUSION

The repeated application of herbicides in soils leads to dissipation effects that differ to those observed with just a single application. For mesotrione, this repeated application had an inhibitory effect on its dissipation rate in the unamended and amended soils studied, and the herbicide could become more persistent or increase its accumulation in amended soils. However, repeated applications of pethoxamid and triasulfuron accelerated the dissipation of these compounds in amended soils through the possible formation of bound residues over time, decreasing the bioavailability of the compound to be degraded. In this case, faster dissipation could lead to a loss of efficacy. The dissipation mechanism of herbicides could be explained by the adsorption constants determined, which indicate the highest adsorption by amended soils. On the other hand, soil DHA was stimulated by the application of GC, but not by SS. The first and second application of mesotrione stimulated soil DHA, while only the second application of pethoxamid increased DHA values, and DHA was not stimulated for any application of triasulfuron. Therefore, the repeated application of herbicides in soils amended with organic residues can modify their dissipation and affect soil microbial activity depending on the type of herbicide and the characteristics of the amended soil. These results should be taken into account when assessing soil quality and other potential environmental implications of pesticide use.

ACKNOWLEDGEMENTS

This work was supported by the “Junta de Castilla y León” (project CSI264A12-1). EPJ thanks to the Ministry of Science and Innovation for her postdoctoral “Juan de la Cierva” contract (JCI-2011-10150). JMMB thanks to the Ministry of Economy and Competitiveness for their “Juan de la Cierva-Incorporación” contract (IJCI-2014-19538). We would like to thank Aqualia SA and local authorities of Salamanca for their collaboration.

REFERENCES

- Alekseeva, T., Kolyagin, Y., Sancelme, M., Besse-Hoggan, P., 2014. Effect of soil properties on pure and formulated mesotrione adsorption onto vertisol (Limagne plane, Puy-de-Dôme, France). *Chemosphere* 111, 177-183.
- Álvarez-Martín, A., Rodríguez-Cruz, M.S., Andrades, M.S., Sánchez-Martín, M.J., 2016. Application of a biosorbent to soil: a useful method for controlling water pollution by pesticides. *Environ. Sci. Pollut. Res.* 23, 9192–9203.
- Álvarez-Martín, A., Sánchez-Martín, M.J., Ordax, J.M., Marín-Benito, J.M., Rodríguez-Cruz, M.S., 2017. Leaching of two fungicides in spent mushroom substrate amended soil: Influence of amendment rate, fungicide ageing and flow condition. *Sci. Total Environ.* 584-585, 828-837.
- Atay, N.Z., Yenigun, O., Asutay, M., 2002. Sorption of anionic surfactants SDS, AOT and cationic surfactant Hyamine 1622 on natural soils. *Water Air Soil Pollut.* 136, 55-67.
- Azejjel, H., Aatouf, N., Draoui, K., Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., 2008. Influence of soil properties on the adsorption of two ionisable herbicides by

525 Moroccan soils. *Fresenius Environ. Bull.* 17, 1627-1633.

526 Barchanska, H., Kluza, A., Krajczewska, K., Maj, J., 2016. Degradation study of
527 mesotrione and other triketone herbicides on soils and sediments. *J. Soils Sediments*
528 16, 125-133.

529 Borowik, A., Wuszkowska, J., Kucharski, J., Bacmaga, M., Tomkiel M., 2017.
530 Response of microorganisms and enzymes to soil contamination with a mixture of
531 terbuthylazine, mesotrione and S-metolachlor. *Environ. Sci. Pollut. Res.* 24, 1910-
532 1925.

533 Celis, R., Gámiz, B., Adelino, M.A., Cornejo, J., Hermosín, M.C., 2015. Effect of
534 formulation and repeated applications on the enantioselectivity of metalaxyl
535 dissipation and leaching in soil. *Pest Manag. Sci.* 71, 1572-1581.

536 Chen, X., Wu, Q., Chen, W., Han, L., 2012. Dissipation and residues of the herbicide
537 mesotrione in maize and soil in open field. *Bull. Environ. Contam. Toxicol.* 88, 772–
538 775.

539 Crouzet, O., Batisson, I., Besse-Hoggan, P., Bonnemoy, F., Bardot, C., Poly, F.,
540 Bohatier, J., Mallet, C., 2010. Response of soil microbial communities to the
541 herbicide mesotrione: A dose-effect microcosm approach. *Soil Biol. Biochem.* 42,
542 193-202.

543 Dhareesank, A., Kobayashi, K., Usui, K., 2005. Phytotoxic activity of pethoxamid in
544 soil under different moisture conditions. *Weed Biol. Manag.* 5, 197–202.

545 Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil
546 fertility. A review. *Agron. Sustain. Dev.* 30, 401-422.

547 Dumas, E., Giraudo, M., Goujon, E., Halma, M., Knhili, E., Stauffert, M., *et al.*, 2017.
548 Fate and ecotoxicological impact of new generation herbicides from the triketone
549 family: An overview to assess the environmental risks. *J. Hazard. Mater.* 325, 136-

550 156.

551 Dyson, J.S., Beulke, S., Brown, C.D., Lane, M.C.G., 2002. Adsorption and degradation
 552 of the weak acid mesotrione in soil and environmental fate implications. *J. Environ.*
 553 *Qual.* 31, 613–618.

554 EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the
 555 pesticide risk assessment of the active substance triasulfuron. *EFSA J.* 13(1), 3958,
 556 78pp.

557 Fang, H., Lian, J., Wang, H., Cai, L., Yu, Y., 2015. Exploring bacterial community
 558 structure and function associated with atrazine biodegradation in repeatedly treated
 559 soils. *J. Hazard. Mater.* 286, 457–465.

560 Fang, H., Han, L., Zhang, H., Deng, Y., Ge, Q., Mei, J., Long, Z., Yu, Y. 2018. Repeated
 561 treatments of ciprofloxacin and kresoxim-methyl alter their dissipation rates,
 562 biological function and increase antibiotic resistance in manured soil. *Science of the*
 563 *Total Environment* 628–629, 661–671.

564 FOCUS, 2006. Guidance Document on Estimating Persistence and Degradation
 565 Kinetics from Environmental Fate Studies on Pesticide in EU Registration, Report of
 566 the FOCUS Work Group on Degradation Kinetics. EC Document Reference
 567 Sanco/10058/2005 version 2.0, 434 pp.

568 Gámiz, B., Hermosín, M.C., Celis, R., 2018. Appraising factors governing sorption and
 569 dissipation of the monoterpene carvone in agricultural soils. *Geoderma* 321, 61–68.

570 Gennari, M., Abbate, C., Baglieri, A., Négre M., 2008. Fate and degradation of
 571 triasulfuron in soil and water under laboratory conditions. *J. Environ. Sci. Health B*
 572 43, 498-505.

573 Giles, C.H., McEwan, J.H., Nakhwa, S.W., Smith, D., 1960. Part XI. A system of
 574 classification of solution adsorption isotherms, and its use in diagnosis of adsorption

575 mechanisms and in measurement of specific surface areas of solids. J. Chem. Soc.
576 111, 3973–3993.

577 Jursík, M., Soukup, J., Holec, J., Andr, J., Hamouzová, K., 2015. Efficacy and
578 selectivity of pre-emergent sunflower herbicides under different soil moisture
579 conditions. Plant Protect. Sci. 51, 214-222.

580 Kaczynski, P., Lozowicka, B., Hrynko, I., Wolejko, E., 2016. Behaviour of mesotrione
581 in maize and soil system and its influence on soil dehydrogenase activity. Sci. Total
582 Environ. 571, 1079-1088.

583 Sparks, D.L., 1996. Methods of Soil Analysis. Part 3-Chemical Methods. Soil Science
584 Society of America, Inc., Madison, WI.

585 Marín-Benito J.M., Sánchez-Martín, M.J., Andrades, M.S., Pérez-Clavijo, M.,
586 Rodríguez-Cruz, M.S., 2009. Effect of spent mushroom substrate amendment of
587 vineyard soils on the behavior of fungicides: 1. Adsorption-desorption of
588 penconazole and metalaxyl by soils and subsoils. J. Agric. Food Chem. 57, 9634–
589 9642.

590 Marín-Benito, J.M., Andrades, M.S., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S.,
591 2012. Dissipation of fungicides in a vineyard soil amended with different spent
592 mushroom substrates. J. Agric. Food Chem. 60, 6936-6945.

593 Marín-Benito, J.M., Brown, C.D., Herrero-Hernández, E., Arienzo, M., Sánchez-
594 Martín, M.J., Rodríguez-Cruz, M.S., 2013. Use of raw or incubated organic wastes as
595 amendments in reducing pesticide leaching through soil columns. Sci. Total Environ.
596 463-464, 589–599.

597 Mendes, K.F., Reis, M.R., Inoue, M.H., Pimpinato, R.F., Tornisielo, V.L., 2016.
598 Sorption and desorption of mesotrione alone and mixed with S-metolachlor +
599 terbuthylazine in Brazilian soils. Geoderma 280, 22-28.

600 Mendes, K.F., Martins, B.A.B., Reis, M.R., Pimpinato, R.F., Tornisielo, V.L., 2017.
 601 Quantification of the fate of mesotrione applied alone or in a herbicide mixture in
 602 two Brazilian arable soils. *Environ. Sci. Pollut. Res.* 24, 8425-8435.

603 Mingorance, M.D., Rossini Oliva, S., Valdés, B., Pina Gata F.J., Leidi E.O., Guzmán
 604 I., Peña A., 2014. Stabilized municipal sewage sludge addition to improve properties
 605 of an acid mine soil for plant growth. *J. Soil Sediment* 14, 703–712.

606 Paradelo, R., Conde-Cid, M., Martín Abad, E., Nóvoa-Muñoz, J.C., Fernández-Calviño,
 607 D., Arias-Estévez, M., 2018. Retention and transport of mecoprop on acid sandy-
 608 loam soils. *Ecotox. Environ. Safety* 148, 82–88.

609 Pinna, M.V., Giunta, F., Nardi, S., Pusino, A., 2009. Effects of a municipal sewage
 610 sludge amendment on triasulfuron soil sorption and wheat growth. *J. Agric. Food*
 611 *Chem.*, 57, 11249–11253.

612 Pinna, M.V., Roggero, P.P., Seddaiu, G., Pusino, A., 2014. Soil sorption and leaching of
 613 active ingredients of Lumax[®] under mineral or organic fertilization. *Chemosphere*
 614 111, 372-378.

615 Pose-Juan, E., Igual, J.M., Curto, N., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S.,
 616 2015a. Mesotrione dissipation and response of soil microbial communities in a soil
 617 amended with organic residues. *Spanish J. Soil Sci.* 5, 12-25.

618 Pose-Juan, E., Sánchez-Martín, M.J., Herrero-Hernández, E., Rodríguez-Cruz, M.S.,
 619 2015b. Application of mesotrione at different doses in an amended soil: Dissipation
 620 and effect on the soil microbial biomass and activity. *Sci Total Environ* 536, 31-38.

621 Pose-Juan, E., Igual, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2017.
 622 Influence of herbicide triasulfuron on soil microbial community in an unamended
 623 soil and a soil amended with organic residues. *Front. Microbiol.* 8, 378.

624 PPDB, 2017. Pesticide Properties Database, University of Hertfordshire.
 625 <http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm>.
 626 Rodríguez-Cruz, M.S., Valderrábano, M., del Hoyo, C., Sánchez-Martín, M.J., 2009.
 627 Physicochemical Study of the Sorption of Pesticides by Wood Components. J.
 628 Environ. Qual. 38, 719–728.

629 Rodríguez-Cruz, M.S., Herrero-Hernández, E., Ordax, J.M., Marín-Benito, J.M.,
 630 Draoui, K., Sánchez-Martín, M.J., 2012. Adsorption of pesticides by sewage sludge,
 631 grape marc, spent mushroom substrate and by amended soils. (2012). Int. J. Environ.
 632 Anal. Chem. 92, 933-948.

633 Rodríguez-Liébana, J.A., Peña, A., 2018. Adsorption-desorption of dimethenamid and
 634 fenarimol onto three agricultural soils as affected by treated wastewater and fresh
 635 sewage sludge-derived dissolved organic carbon. J. Environ. Manage. 217, 592–599.

636 Said-Pullicino, D., Gigliotti, G., Vella, A.J., 2004. Environmental fate of triasulfuron in
 637 soils amended with municipal waste compost. J. Environ. Qual. 33, 1743–1751.

638 Sánchez-Martín, M.J., Dorado, C., Rodríguez-Cruz, M.S., 2008. Influence of clay
 639 mineral structure and surfactant nature on the adsorption capacity of surfactants by
 640 clays. J. Hazard. Mater. 150, 115-123.

641 Shaner, D., Brunk, G., Nissen, S., Westra, P., Chen, W., 2012. Role of soil sorption and
 642 microbial degradation dissipation of mesotrione in plant-available soil water. J.
 643 Environ. Qual. 41, 170-178.

644 Singh, B.K., Walker, A., Wright, D.J., 2002. Degradation of chloropyrifos, fenamiphos,
 645 and chlorothalonil alone and in combination and their effects on soil microbial
 646 activity. Environ. Toxicol. Chem. 21, 2600-2605.

647 Singh, N., Megharaj, M., Gates, W.P., Churchman, G.J., Anderson, J., Kookana, R.S.,
 648 Naidu, R., Chen, Z., Slade, P.G., Sethunathan, N., 2003. Bioavailability of an

organophosphorus pesticide, fenamiphos, sorbed on an organo clay. J. Agric. Food Chem. 51, 2653-2658.

Su, W., Hao, H., Wu, R., Xu, H., Xue, H., Lu, C., 2017. Degradation of mesotrione affected by environmental conditions. Bull. Environ. Contam. Toxicol. 98, 212-217.

Soil Survey Staff, 2010. Keys to Soil Taxonomy, 11th ed. USDA-Natural Resources Conservation Service, Washington DC.

Sofo, A., Scopa, A., Dumontet, S., Mazzatura, A., Pasquale, V., 2012. Toxic effects of four sulphonylureas herbicides on soil microbial biomass. J. Environ. Sci. Health B 47, 653-659.

Stangroom, S.J., Lester, J.N. Collins. C.D., 2000. Abiotic behaviour of organic micropollutants in soils and the aquatic environment. A review: I. Partitioning. Environ. Technol. 21, 845-863.

Tabatabai, M.A., 1994. Soil enzymes, in *Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties*, ed. by Weaver RW, Soil Science Society of America, Madison, Wisconsin, pp. 903–947.

Wang, F., Zhu, L., Wang, X., Wang, J., Wang, J., 2015. Impact of repeated applications of metalaxyl on its dissipation and microbial community in soil. Water Air Soil Pollut. 226: 430.

Wanner, U., Führ, F., Burauel, P., 2005. Influence of the amendment of corn straw on the degradation behaviour of the fungicide dithianon in soil. Environ. Pollut. 133, 63-70.

Wyszkowska, J., Monika, T., Baćmaga, M., Borowik, A., Kukarski, J., 2016. Response of microorganisms and enzymes to soil contamination with a mixture of pethoxamid terbuthylazine. Environ. Earth Sci. 75, 1285.

Xu, J., Zhang, Y., Dong, F., Liu, X., Wu, X., Zheng, Y., 2014. Effects of repeated

674 applications of chlorimuron-ethyl on the soil microbial biomass, activity and
675 microbial community in the greenhouse. Bull. Environ. Contam. Toxicol. 92, 175–
676 182.

Figure captions

Fig. 1. Adsorption curves of mesotrione, triasulfuron and pethoxamid by unamended soil and soil amended with green compost (GC) or sewage sludge (SS) fitted to the Freundlich equation. C_s ($\mu\text{g g}^{-1}$) is the amount of adsorbed herbicide; C_e ($\mu\text{g mL}^{-1}$) is the herbicide's equilibrium concentration in the solution. Error bars represent the standard deviation of the C_s mean value ($n=2$).

Fig. 2. Dissipation kinetics of mesotrione, triasulfuron and pethoxamid in unamended soil and soil amended with green compost (GC) or sewage sludge (SS). The initial time of each kinetics is indicated as T_0 -1st and T_0 -2nd for the first and second herbicide application, respectively. Error bars represent the standard deviation of the mean value ($n=2$).

Fig 3. Soil dehydrogenase activity (DHA) in unamended and amended soils, untreated (control) and treated with mesotrione, triasulfuron and pethoxamid at different incubation times after the repeated application of herbicides. The second herbicide application is indicated with an arrow. Error bars represent the standard deviation of the mean ($n=2$). Different upper case letters denote significant differences between treatments for unamended and amended soils untreated and treated with herbicides and different lower case letters denote significant differences between sampling times for untreated or treated soil with herbicides within the same unamended or amended soil).

Table 1. Chemical structure and physicochemical properties of herbicides.

Common name IUPAC name	Chemical structure	WS ^a (mg L ⁻¹)	Log K _{ow} ^b	pKa	Koc ^c (mL g ⁻¹)	DT ₅₀ ^d (days)	GUS ^e index
Mesotrione (2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione)		160-1500	0.11	3.12	122	5-19.6	2.69
Triasulfuron (1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea)		32-815	-0.59	4.64	60	38-59	5.12
Pethoxamid (2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl)acetamide)		400	2.96	-	211	6-14.8	1.34

^aSolubility in water at 20 °C and pH 5-7, ^bOctanol/water partition coefficient at 20 °C and pH 7, ^cAdsorption coefficient normalized to organic carbon content. ^dTime to degradation 50% of compound, ^eGustafson mobility index (Data taken from PPDB, 2017).

Table 2. Characteristics of organic residues and unamended and amended soils.

	Green compost (GC)	Sewage sludge (SS)	Soil (S)	S+GC	S+SS
pH	7.08	6.73	6.29	7.05	6.16
OC (%)	8.06	27.0	0.49	1.50	2.20
DOC ^a (mg g ⁻¹)	1.02	21.7	0.05	0.07	0.68
N (%)	0.79	4.76	0.04	0.18	0.35
C/N	10.2	5.67	12.2	8.33	6.28
CEC ^b (cmol _c kg ⁻¹)	18.7	64.5	4.38	6.87	7.95
Na (cmol _c kg ⁻¹)	0.27	1.22	0.03	0.03	0.02
K (cmol _c kg ⁻¹)	56.4	4.99	0.66	2.78	0.78
Ca (cmol _c kg ⁻¹)	28.9	24.5	6.96	17.2	10.8
Mg (cmol _c kg ⁻¹)	3.83	14.2	2.40	3.16	4.33

^aDissolved organic carbon. ^b Cationic exchange capacity.

Table 3. Freundlich adsorption constants (K_f and n_f) and distribution coefficients (K_d) of mesotrione, triasulfuron and pethoxamid by unamended soil (S) and soil amended with green compost (S+GC) or sewage sludge (S+SS).

Soil	$K_f \pm SD^a$	$n_f \pm SD$	r^2	$K_d5^b \pm SD$	$K_d15^c \pm SD$
Mesotrione					
S	0.64 $\pm$ 0.02a	0.85 $\pm$ 0.01	0.81	0.51 $\pm$ 0.01a	0.43 $\pm$ 0.01a
S+GC	1.12 $\pm$ 0.10a	0.68 $\pm$ 0.04	0.81	0.67 $\pm$ 0.01ab	0.47 $\pm$ 0.02a
S+SS	0.83 $\pm$ 0.11a	0.90 $\pm$ 0.04	0.87	0.71 $\pm$ 0.07b	0.64 $\pm$ 0.01b
LSD (p<0.05)	0.51			0.19	0.06
Triasulfuron					
S	0.61 $\pm$ 0.08a	1.27 $\pm$ 0.09	0.92	0.94 $\pm$ 0.08a	1.26 $\pm$ 0.20a
S+GC	1.53 $\pm$ 0.06b	0.84 $\pm$ 0.12	0.81	1.18 $\pm$ 0.20ab	0.99 $\pm$ 0.28a
S+SS	2.66 $\pm$ 0.11c	0.70 $\pm$ 0.02	0.80	1.65 $\pm$ 0.10b	1.19 $\pm$ 0.19a
LSD (p<0.05)	0.39			0.62	1.02
Pethoxamid					
S	0.20 $\pm$ 0.06a	1.68 $\pm$ 0.44	0.94	0.59 $\pm$ 0.03a	1.26 $\pm$ 0.11a
S+GC	2.98 $\pm$ 0.77b	0.96 $\pm$ 0.10	0.94	2.79 $\pm$ 0.19c	2.67 $\pm$ 0.14b
S+SS	1.49 $\pm$ 0.05ab	1.16 $\pm$ 0.05	0.98	1.92 $\pm$ 0.11b	2.28 $\pm$ 0.27b
LSD (p<0.05)	2.01			0.58	0.84

^aSD, standard deviation (n=2). ^bDistribution coefficient for an equilibrium concentration of 5 $\mu\text{g mL}^{-1}$. ^cDistribution coefficient for an equilibrium concentration of 15 $\mu\text{g mL}^{-1}$. For each herbicide, K_f , K_d5 or K_d15 values followed by different letter are significantly different (p < 0.05).

Table 4. Kinetics parameters for the degradation of mesotrione, triasulfuron and pethoxamid after two repeated applications (1 and 2) in unamended and amended soils obtained from fitting kinetics to a single first order (SFO) model ($C=C_0 e^{-kt}$).

Soil	Application	k (days ⁻¹)	DT ₅₀ (days)	r ²	χ ²
Mesotrione					
S	1	0.145	4.75 ± 0.2a	0.99	2.3
	2	0.085	8.15 ± 0.8ab	0.97	13.5
S+GC	1	0.059	11.7 ± 2.2b	0.97	5.2
	2	0.025	28.2 ± 0.9d	0.99	6.3
S+SS	1	0.039	17.7 ± 1.5c	0.95	5.9
	2	0.018	37.9 ± 0.5e	0.98	6.0
LSD (p<0.05)			4.80		
Triasulfuron					
S	1	0.034	20.4 ± 0.7a	0.97	7.4
	2	0.030	22.9 ± 0.1a	0.99	10.5
S+GC	1	0.012	57.1 ± 3.0c	0.97	1.8
	2	0.021	33.2 ± 2.3b	0.96	11.6
S+SS	1	0.028	24.7 ± 1.3a	0.98	5.1
	2	0.032	21.6 ± 0.2a	0.97	14.1
LSD (p<0.05)			6.58		
Pethoxamid					
S	1	0.058	11.9 ± 0.8cd	0.99	0.2
	2	0.086	8.05 ± 0.4a	0.99	4.5
S+GC	1	0.057	12.3 ± 0.2d	0.98	1.6
	2	0.069	10.0 ± 0.2bc	0.99	3.5
S+SS	1	0.083	8.35 ± 0.6ab	0.99	1.7
	2	0.102	6.75 ± 0.1a	0.99	7.9
LSD (p<0.05)			1.88		

For each herbicide, DT₅₀ values followed by different letter are significantly different (p < 0.05).

Fig. 1.

Fig. 2.

Fig. 3.