

## Availability of Triazine Herbicides in Aged Soils Amended with Olive Oil Mill Waste

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Amendments are frequently added to agricultural soils to increase organic matter content. In this study, we examined the influence of alperujo, an olive oil mill waste, on the availability of two triazine herbicides, terbuthylazine and atrazine, in two different sandy soils, one from Sevilla, Spain, and the other from Minnesota. The effect of aging on herbicide sorption and bioavailability was also studied. Soils were amended with alperujo at a rate of 3–5% (w:w) in laboratory studies. Apparent sorption coefficients for the triazine herbicides were calculated as the ratio of the concentrations of each herbicide sequentially extracted with water, followed by aqueous methanol, at each sampling time. These data showed greater sorption of terbuthylazine and atrazine in amended soils as compared to nonamended soils, and an increase in the amount of herbicide sorbed with increasing aging time in nonamended soils. The triazine-mineralizing bacterium *Pseudomonas* sp. strain ADP was used to characterize triazine bioavailability. Less mineralization of the herbicides by *Pseudomonas* sp. strain ADP was observed in soils amended with alperujo, as compared to the unamended soils, and, despite the increase in sorption with aging in unamended soils, herbicide mineralization also increased in this case. This has been attributed to *Pseudomonas* sp. strain ADP first using alperujo as a more readily available source of N as compared to the parent triazines. In summary, addition of alperujo to the soils studied was shown to increase triazine herbicides sorption and hence to reduce its availability and potential to leach.

**KEYWORDS:** Triazine herbicides; sorption; bioavailability; *Pseudomonas* sp. strain ADP; soil; alperujo; olive oil mill waste; aging

### INTRODUCTION

As demonstrated by numerous studies in Europe and the United States, the use of pesticides involves a risk of potentially contaminating surface and groundwater (1–3). However, because pesticides often ensure an abundant and affordable food supply, the use of these products continues worldwide. For instance, in the United States, the amount of pesticides increased from 401 500 tons of active ingredient in 1992 to 443 700 tons in 1997. The amount of herbicides has also increased in the same period of time, to 207 600 tons of active ingredient in 1997, with atrazine the most widely used herbicide in crops (33 600 tons) (4). Also, over the last 10 years, the US-EPA has registered many new active ingredients and new uses of pesticides ([www.epa.gov/oppfead1/annual/2006/06annual-rpt.pdf](http://www.epa.gov/oppfead1/annual/2006/06annual-rpt.pdf)).

In Europe, the use of plant protection products increased from 50 000 tons in 1992 to 95 000 tons in 2002, after which it decreased to 85 000 tons in 2003. The use of herbicides has followed the same trend (5). The use of triazine herbicides, such as atrazine, cyanazine, simazine, and terbuthylazine, which are applied widely for weed control in a variety of crops including corn, sorghum, citrus orchards, olive groves, fruit trees, and grapes, has fallen from 5th to 12th place in a ranking of the most used chemicals in Europe. During 2003, 3624 tons of triazine herbicides was used in Europe, which represents 4.3% of the total of herbicides (5).

The decrease in triazine usage in Europe is chiefly due to legal provisions limiting the use of these products. Atrazine and simazine have been classified as priority substances by the European Parliament and the Council of the European Union in water policy Directive 2455/2001/EC. Triazine parent compounds and their microbial metabolites (the most important dissipation mechanism in soil is by microbial degradation) (6)

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have been detected in surface and ground waters in several European countries and in the United States (7–12).

It has been suggested that the use of organic amendments in soils can reduce the amounts of pesticide available for leaching to groundwater and for runoff to surface water (13). These amendments increase the organic matter (OM) content of the soil, enhancing herbicide sorption, and thereby decreasing availability for both transport and degradation processes. For instance, commercial peat used as a soil amendment increased herbicide sorption and decreased herbicide leaching as compared to humic acid-amended soil (14). Likewise, soil amended with sludge, urban waste compost, and composted straw have also been shown to increase atrazine sorption as compared to unamended soil (15, 16), and the latter additions were shown to decrease atrazine mineralization (16). It has also been reported that metribuzin has a lower mobility in soils amended with organic manure and with fly ash (17). Addition of organic amendments, however, does not protect against the leaching of highly water-soluble compounds or prevent the degradation of pH-sensitive compounds (18).

The extraction of oil from olives generates a large amount of organic waste. The impacts of liquid waste from olive oil extraction (13, 19), and the solid byproduct (alperujo) of the two-phase olive oil extraction process as organic amendments on pesticide leaching and degradation (20–22), have been recently studied. Cox et al. (13) reported that soils amended with olive-mill wastewater reduced or stopped leaching of the herbicides clopyralid (3,6-dichloropicolinic acid) and metamitron (4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one), respectively, in a clay soil, as compared to unamended soil. These authors speculated that an increase in sorption and degradation of the herbicides in the amended soil occurred via the reduction of soil porosity caused by the amendment. However, a decrease in imazaquin sorption was found in different soils amended with olive mill wastewater, except for a loamy sand soil, where herbicide sorption increased and leaching decreased (19).

When alperujo solids were used as an organic amendment in a sandy soil freshly treated with simazine, sorption was reported to increase, and leaching decrease, as compared to unamended soil (20, 21). Delgado-Moreno et al. (22) also found an increase in sorption and decrease in desorption of four triazine herbicides freshly applied to a soil amended with alperujo as compared to unamended soil; however, the magnitude of the increase in sorption decreased if the soil amended with alperujo had been aged for months prior to treatment with the triazines. Albarrán et al. (20, 21) also found that simazine dissipation in freshly treated soil was shown to be initially slower in the unamended soil as compared to amended soil, as characterized by solvent extractability, presumably due to increased sorption. However, after 30 d of incubation, simazine dissipation was faster in the unamended soil than in the amended soil, as shown in part by the decrease in simazine mineralization in the alperujo amended soil. Similarly, Farenhorst et al. (23) reported that there was less mineralization of 2,4-D (2,4-dichlorophenoxy acetic acid) in soils amended with biosolids or with manure as compared to the original soils.

In the studies described here, we examined the effect of alperujo amendment on the availability of terbuthylazine and atrazine, two triazine herbicides widely used for weed control, for transport, and for degradation in two sandy soils. The availability of parent triazine for transport was characterized using sequential solvent extraction techniques to determine sorption in freshly treated and aged unamended and amended soils. Characterization of bioavailability of freshly treated and

aged parent terbuthylazine and atrazine residues in alperujo-amended and unamended soils was facilitated by using *Pseudomonas* sp. strain ADP, a bacterium that can rapidly mineralize these two herbicides, but none of their metabolites.

## MATERIALS AND METHODS

**Soil, Organic Amendment, and Chemicals.** Two different sandy soils were used in this study, one from Sevilla, Spain (SP-U), and another from Becker, Minnesota (MN-U). The same soils were also amended with alperujo (SP-A and MN-A, respectively), the organic solid waste from the two-phase, olive oil extraction process. The SP-U soil was taken from the 0–15 cm depth from an olive grove located in Coria del Rio (Sevilla, Spain), air-dried, and sieved with a 2-mm mesh screen. Soil properties were as follows: pH 8.4, 0.36% organic carbon (OC), 20% clay, 9% silt, and 71% sand. The soil was classified as Typic Rhodoxeralf. The MN-U soil, which was classified as a Hubbard loamy sand, had a pH of 5.8, and contained 86.0% sand, 6.0% clay, and 1.5% OC. Neither soil had a history of triazine application in the previous 7–10 yrs. The soils were amended in the laboratory with alperujo to 2.78% OC in SP-A and 4.38% OC in MN-A. The properties of the alperujo used were: pH 5.8, 93.2% OM, 25 g kg<sup>-1</sup> dissolved organic carbon (DOC), and a C:N ratio of 18.3.

Terbuthylazine (*N*<sup>2</sup>-tert-butyl-6-chloro-*N*<sup>4</sup>-ethyl-1,3,5-triazine-2,4-diamine) (99% purity) was purchased from Riedel-de Haën (Seelze, Germany), and UL-ring-<sup>14</sup>C-labeled terbuthylazine (1.99 MBq mg<sup>-1</sup> specific activity; 94.3% radiochemical activity) was kindly supplied by Syngenta (Basel, Switzerland). Atrazine (6-chloro-*N*<sup>2</sup>-ethyl-*N*<sup>4</sup>-isopropyl-1,3,5-triazine-2,4-diamine) was purchased from Chem Service (West Chester, PA) (98% purity), and UL-ring-<sup>14</sup>C-labeled atrazine (1.63 MBq mg<sup>-1</sup> specific activity; 98.7% radiochemical purity) was purchased from Sigma-Aldrich (St. Louis, MO).

**<sup>14</sup>C-Herbicide Sorption in Aged Soil Studies by Analysis of Extractable Herbicides.** Unamended and alperujo-amended soils (10 g dry weight) were individually weighed into triplicate glass tubes, and methanolic terbuthylazine (0.6 mL) or atrazine (1.1 mL) solutions were added to each soil to a final triazine concentration of 1 µg g<sup>-1</sup> and 168.2 Bq g<sup>-1</sup>. After evaporation of the methanol (~1 h), distilled water was added to obtain a soil moisture content of 40% of the water-holding capacity. Glass wool was placed on the soil surface, and a vial containing 1 mL of 0.5 N NaOH was placed in the tube to trap <sup>14</sup>CO<sub>2</sub> resulting from the mineralization of <sup>14</sup>C-terbuthylazine or <sup>14</sup>C-atrazine. Tubes were stoppered, and soils were incubated in the dark at 25 ± 1 °C for 7, 14, and 28 days for terbuthylazine studies, and for 14 and 25 days for atrazine studies. The NaOH was replaced weekly, and a 1 mL aliquot was mixed with 6 mL of EcoLite scintillation cocktail. Radioactivity in vials was counted, for 5 min, using a Tri-Carb 1500 Packard liquid scintillation analyzer. Vials were kept in the dark overnight prior to counting to reduce chemiluminescence.

At time 0 (immediately after solvent evaporation) and after each incubation period, soil samples were extracted by shaking with 20 mL of 0.01 N CaCl<sub>2</sub> for 20 h on a horizontal shaker, and centrifuged at 1500g for 30 min. The supernatant volume was measured, and a 1 mL aliquot was mixed with 6 mL of EcoLite scintillation cocktail. Radioactivity was measured as described above. The remaining aqueous supernatant was kept at 4 °C until it was extracted with dichloromethane (DCM) for analysis of parent herbicide.

The soil was subsequently extracted with 20 mL of methanol:water (80:20, v:v), by shaking for 20 h in a horizontal shaker. After the sample was centrifuged at 1500g for 30 min, the supernatant was removed, a 1 mL aliquot was mixed with 6 mL of EcoLite scintillation cocktail, and radioactivity was determined as described above. The remaining aliquot was analyzed by HPLC to quantify the organic-extractable parent herbicide as described below. The extracted soils were then frozen at -25 °C until they were later combusted to determine unextractable (bound) <sup>14</sup>C.

The methanol was removed from the aqueous methanol supernatant using a Zymark Turbo Vap II evaporator at 35 °C. The water extracts remaining and the supernatants of the 0.01 N CaCl<sub>2</sub> extractions were each extracted twice with DCM (1:4 DCM:aqueous, v:v). The two DCM

solutions from the 0.01 N CaCl<sub>2</sub> extractions were combined and evaporated using N<sub>2</sub> gas, just to dryness, in a water bath at 35 °C. The two DCM solutions from the aqueous methanol supernatants were combined and similarly processed. The residues were redissolved with 1 mL of acetonitrile (ACN):water (40:60, v:v) and analyzed by high performance liquid chromatography (HPLC), using a 1090 Hewlett-Packard high performance liquid chromatograph and a Waters Spherisorb C8 5 μm 150 mm × 4.6 mm i.d. column. The mobile phase, at a flow rate of 1 mL min<sup>-1</sup>, was a gradient of ACN and milli Q water, starting at 40% of ACN until minute 15 and changing to 70% ACN at minute 22. The injection volume was 50 μL.

For terbutylazine, fractions were collected from time 0 to 10 min, and then at each minute until minute 16, and another fraction from 16 to 22 min. For atrazine, fractions were collected from time 0 to 5 min, then each minute until 10 min and from 10 to 15 min. One milliliter aliquots of the fraction corresponding to terbutylazine and atrazine were mixed with 6 mL of EcoLite scintillation cocktail, and radioactivity was measured in the LSC to calculate the amount of herbicide in supernatants. One milliliter aliquots of the remaining fractions were also counted to enable calculation of the percentage of parent herbicide in the extracted <sup>14</sup>C. No standards were available to determine individual <sup>14</sup>C-metabolites in the solutions.

Sorption coefficients,  $K_d$  values, were calculated assuming that the methanol-extractable herbicide was the amount of herbicide sorbed by soil ( $C_s$ ) and that the water-extractable pesticide was the equilibrium concentration of herbicide in solution ( $C_e$ ) as in the batch equilibration method:  $K_d = C_s/C_e$ . The  $K_{oc}$  values were calculated as  $(K_d/OC) \times 100$ .

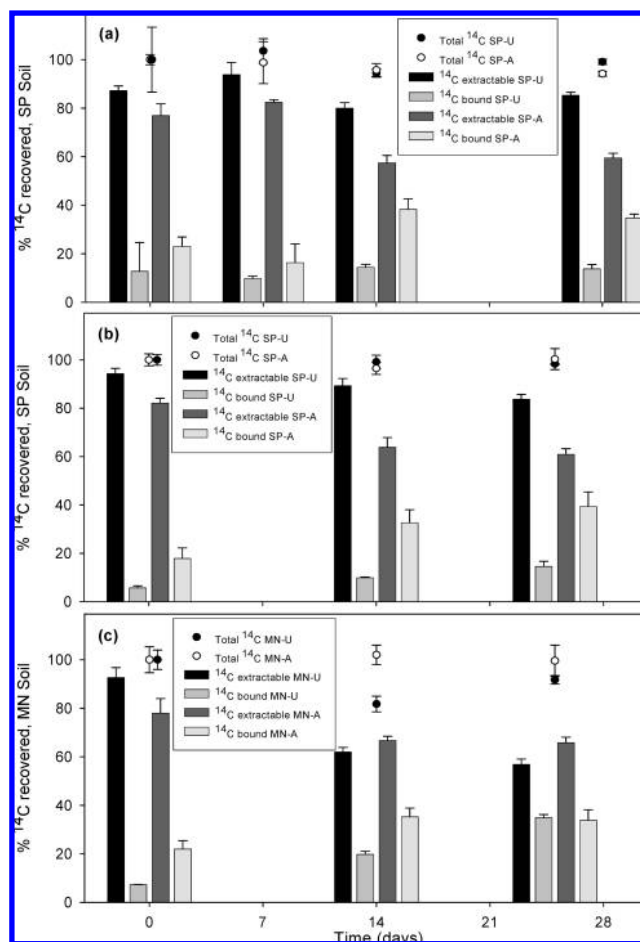
**Quantification of Unextractable (Bound) <sup>14</sup>C-Residues.** Before nonextractable [<sup>14</sup>C]terbutylazine or [<sup>14</sup>C]atrazine was determined, soil moisture was measured in the previous extracted soils. Soil samples, 0.33 g (dry weight) of each sample in triplicate, were combusted by using a Packard 307 sample oxidizer. The <sup>14</sup>CO<sub>2</sub> released was trapped in Carbo-Sorb E mixed with Permafluor V, and the <sup>14</sup>C was quantified by liquid scintillation counting (LSC) as described above.

**<sup>14</sup>C-Triazine Bioavailability to *Pseudomonas* in Aged Soil Studies.** In parallel studies, an experiment was conducted to determine herbicide bioavailability using *Pseudomonas* sp. strain ADP, an atrazine degrading bacterium. Alperujo-amended and unamended soils (10 g dry weight) were individually placed in tubes and treated with 0.6 mL of <sup>14</sup>C-labeled terbutylazine or 1.1 mL of <sup>14</sup>C-labeled atrazine solutions in the same way as was done in the sorption studies. At time 0, and after each incubation period (7, 14, and 28 d for terbutylazine in SP soil and 9, 17, and 26 d for atrazine in MN soil, and 10 and 26 d for atrazine in SP soil), soils were inoculated with a 1 or 2 mL suspension of *Pseudomonas* sp. strain ADP (final inoculum density  $1 \times 10^8$  or  $2 \times 10^8$  microorganisms g<sup>-1</sup> soil, respectively), and mixed thoroughly. Methods for growing and maintaining the organism have been previously reported (24, 25). Glass wool was placed on the soil surface, and a vial containing 1 mL of 0.5 N NaOH was placed in the tube to trap <sup>14</sup>CO<sub>2</sub> resulting from the mineralization of <sup>14</sup>C-terbutylazine or <sup>14</sup>C-atrazine. The NaOH in vials was replaced 24, 48, and 72 h after inoculation. A 1 mL aliquot of the NaOH solution was mixed with 6 mL of EcoLite scintillation cocktail, vials were kept in dark overnight, and radioactivity was measured using LSC as described above.

## RESULTS AND DISCUSSION

To characterize the availability of aged terbutylazine and atrazine residues for transport in alperujo-amended and unamended soils, the partitioning of terbutylazine and atrazine between aqueous extractable, solvent extractable, and bound residues, as a function of time, was determined. The bacterium *Pseudomonas* sp. strain ADP was also used to determine the bioavailability of aged terbutylazine and atrazine residues in unamended and alperujo-amended soils. To facilitate these experiments, <sup>14</sup>C-labeled triazine herbicides were used.

The mass balance of applied <sup>14</sup>C-triazines in the tested soils was excellent. The mass balance of <sup>14</sup>C from <sup>14</sup>C-terbutylazine

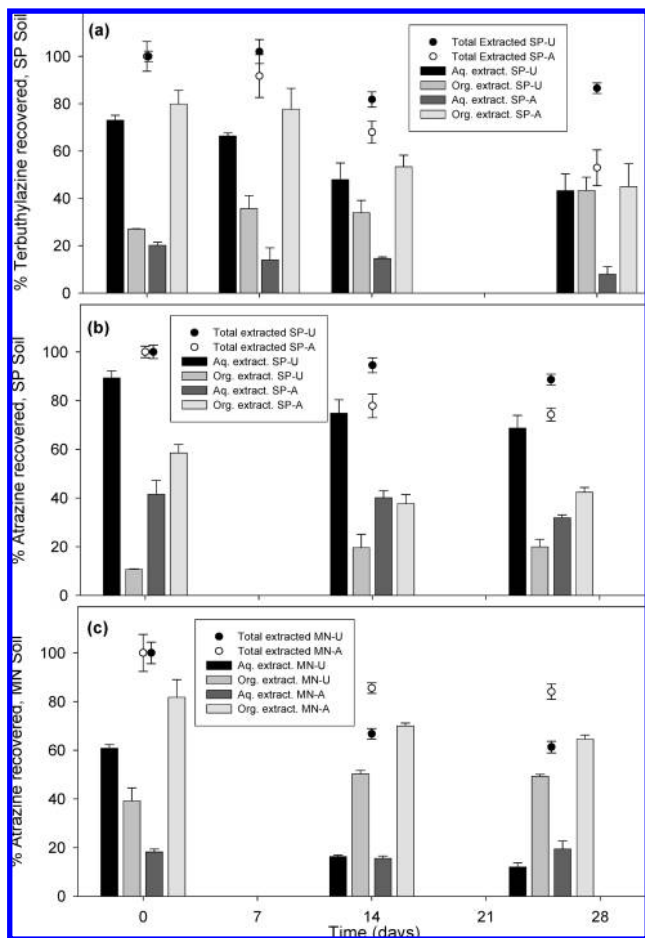


**Figure 1.** Mass balance of extractable and bound <sup>14</sup>C in unamended (U) and amended (A) soils: (a) from <sup>14</sup>C-terbutylazine in SP soil; (b) from <sup>14</sup>C-atrazine in SP soil; (c) from <sup>14</sup>C-atrazine in MN soil.

in SP-U and SP-A, averaged over all sampling times, was 99.3% and 97.2%, respectively (Figure 1a). Aging did not significantly affect the extractability of the terbutylazine <sup>14</sup>C-residues from SP-U; the ratio of total extractable to unextractable <sup>14</sup>C was about the same for the four sampling times, with >80% extractable and ≤15% unextractable (bound) <sup>14</sup>C at all sampling times. In contrast, terbutylazine <sup>14</sup>C-residues became increasingly bound with time in the SP-A soil; the ratio of extractable to unextractable residues decreased from 3.3 to 1.7 during the incubation period. Total extractable terbutylazine <sup>14</sup>C residues decreased from 76.9% to 59.5% during the 4 wk incubation period, while <sup>14</sup>C unextractable residues increased with time from 23.1% at time 0 to 34.8% after 4 wks of incubation.

The results for atrazine in SP soil were similar to those for terbutylazine. The average mass balance of <sup>14</sup>C from <sup>14</sup>C-atrazine in SP-U and SP-A was 99.2% and 98.9%, respectively (Figure 1b). The percentage of total atrazine <sup>14</sup>C residues extracted decreased with aging in both unamended and amended SP soil, from 94.3% to 83.7% in SP-U after 26 d, and from 82.2% to 60.9% during the same period in the SP-A soil, respectively. Bound atrazine <sup>14</sup>C residues increased with aging, from 5.7% to 14.6% in SP-U and from 17.8% to 39.4% in SP-A after nearly 4 wks. The ratio of total extractable to unextractable <sup>14</sup>C decreased in both unamended and amended soils, from 17 to 6 after the 26 d incubation period in SP-U, and from 5 to 2 in the SP-A soil.

The average amount of <sup>14</sup>C from <sup>14</sup>C-atrazine recovered, averaged over all sampling times, for MN-U and MN-A was 91.2% and 100.5%, respectively (Figure 1c). The ratio between



**Figure 2.** Aqueous-extractable and organic-extractable herbicide in unamended (U) and amended (A) soils: (a) terbutylazine in SP soil; (b) atrazine in SP soil; (c) atrazine in MN soil.

extractable and unextractable  $^{14}\text{C}$ -atrazine residues decreased from 13 to 2 in the MN-U soil after the 26 d incubation period; however, the ratio only slightly decreased (3.5 to 1.9) in the MN-A soil. Extractable  $^{14}\text{C}$  residues in the MN-U decreased with aging, from 92.7% to 56.9% after 26 d, and from 78.0% to 65.8% in the MN-A soil during the same time period. Bound atrazine  $^{14}\text{C}$  residues increased in both soils, from 7.3% to 34.9% in MN-U and from 22.0% to 33.8% in MN-A during the 26 d incubation period.

The amount of terbutylazine and atrazine in the extractable  $^{14}\text{C}$  residues was determined by HPLC. Less terbutylazine was recovered from the amended SP soil after 4 wks (50% of applied) as compared to unamended soil (90% of applied) (Figure 2a). After 26 d of incubation, atrazine recovery also was lower in amended SP soil (75% of applied recovered) as compared to that in unamended soil (90% recovered) (Figure 2b). The recovery of atrazine from MN soil was slightly lower as compared to the SP soil; after 26 d 60% and 85% of applied atrazine was recovered from unamended and amended MN soils, respectively (Figure 2c). The decrease in recovery of both herbicides may be attributed to microbial degradation during the incubation period. Although there was no attempt to determine metabolites in this study, we did determine that  $^{14}\text{C}$ -terbutylazine and  $^{14}\text{C}$ -atrazine mineralization by soil microorganisms was <0.1% of the initially applied herbicides in all tested amended and unamended soils (data not shown). The lack of mineralization of triazines in numerous soils has been shown by others. For example, insignificant amounts of atrazine were reported to

be mineralized in five of six soils (three unamended soils and three amended with treated wastewater), and the percentage of atrazine mineralized in those soils only slightly increased with increasing aging time (26).

The decrease in recovery of parent herbicide may also be due to an increase in the amounts of unextractable herbicides bound to soil components due to a soil aging effect, similar to that reported by Gevaio et al. (27). Bound pesticide residues tend to lose their biological activity with longer residence times in soil, becoming more resistant to degradation and extraction. Numerous studies have reported that the formation of bound residues by organic chemicals is enhanced after amendment of soils with organic matter (16, 28). The large amount of total  $^{14}\text{C}$  recovered during the 4 wk incubation period (Figure 1), however, suggests that microbial degradation of the herbicides, and formation of metabolites, is the likely cause of the decreased recovery of parent herbicide in our studies.

Since 60–90% and 50–85% of the applied herbicides remained in unamended and alperujo-amended soils after aging, the quantity of the remaining herbicides available for transport and degradation was determined at selected times during the incubation. To do this, the herbicides were sequentially extracted with 0.01 N  $\text{CaCl}_2$ , followed by extractions with aqueous methanol. The amounts of aqueous extractable (readily available) herbicides decreased with incubation time in unamended SP and MN soils, while the amounts of organic solvent extractable (sorbed herbicide) increased (Figure 2). Aqueous extractable terbutylazine in SP-U soil decreased from 73% of initially applied to 43% after 4 wks, whereas the amount extracted with organic solvent increased from 27% to 43% (Figure 2a). Aqueous extractable atrazine also decreased after 26 d of incubation (from 89.3% of initially applied to 68.7% in SP-U, and from 60.8% to 12.1% in MN-U) (Figure 2b,c). The amount of solvent extractable atrazine increased by 10% in both SP-U and MN-U after the 25 d incubation period (from 10.7% to 20.0%, SP-U; from 39.2% to 49.3% in MN-U) (Figure 2b,c).

In contrast to the unamended soils, both aqueous and organic extractable terbutylazine decreased with time in amended SP soil (from 20% to 8.0%, and from 80% to 45%, respectively) (Figure 2a). The amounts of both aqueous and organic extractable atrazine also decreased with time, by 10–16% in SP-A (from 41.5% to 31.9% for aqueous extractable), and from 58.5% to 42.4% for solvent extractable (Figure 2b). In contrast, aqueous extractable atrazine in MN-A did not vary significantly during the 25 d incubation period (from 18.2% to 19.4%), whereas organic extractable decreased with time (from 81.8% to 64.6%) (Figure 2c).

The availability of herbicides for transport has been traditionally characterized by determination of aqueous extractable herbicide or by calculation of sorption coefficients,  $K_d$  values, or Freundlich coefficients,  $K_f$  and  $1/n_f$  values. The  $K_d$  values (Table 1) were calculated using the quantity of herbicides measured in the aqueous (solution phase) and in organic extracts (sorbed phase) at each incubation time. The terbutylazine sorption  $K_d$ , averaged over the 4 wk incubation period, was higher by a factor of 8 in the amended SP soil, as compared to unamended SP soil. In amended soils (SP-A and MN-A), the  $K_d$  values for atrazine were also higher as compared to the  $K_d$  values in the unamended soils, except for the MN soil after the 25 d incubation period. The greater sorption in amended soils was attributed to the higher content of OC (2.78% OC in SP-A, vs 0.36% OC in SP-U; and 4.36% OC in MN-A, vs 1.50%

**Table 1.**  $K_d$  and  $K_{oc}$  Values Calculated for Unamended (U) and Amended (A) Spanish (SP) and Minnesota (MN) Soils after Different Aging Times

triazine	soil	incubation time (d)							
		$K_d$ (mL g <sup>-1</sup> )				$K_{oc}$ (mL g <sup>-1</sup> )			
		0	7	14	28 <sup>a</sup>	0	7	14	28 <sup>a</sup>
terbutylazine	SP-U	1.0 ± 0.0	1.4 ± 0.3	2.0 ± 0.6	2.8 ± 0.8	278 ± 7	389 ± 72	556 ± 157	778 ± 216
	SP-A	10.7 ± 0.9	12.1 ± 2.0	10.0 ± 1.2	22.2 ± 0.5	385 ± 32	435 ± 71	360 ± 43	799 ± 17
atrazine	SP-U	0.3 ± 0.0	0.6 ± 0.0		0.9 ± 0.1	83 ± 8	167 ± 0		250 ± 16
	SP-A	3.9 ± 0.7	2.6 ± 0.3		3.6 ± 0.2	140 ± 25	94 ± 11		129 ± 6
	MN-U	1.7 ± 0.3	7.9 ± 0.2		10.7 ± 1.4	113 ± 18	527 ± 11		713 ± 93
	MN-A	11.6 ± 1.1	11.7 ± 0.6		8.8 ± 1.5	265 ± 26	266 ± 14		201 ± 35

<sup>a</sup> Atrazine incubation was 25 d.

OC in MN-U), as has been widely reported by many authors (29–32). Delgado-Moreno et al. (22) also observed an increase in  $K_f$  values for four triazine herbicides, including terbuthylazine, in soil freshly amended with olive-mill waste as compared to unamended soil. However, if the soil was preincubated with the organic amendment for 1–3 months, there was no difference in sorption for amended and unamended soils (22).

Aging, or residence time, of the herbicides in soil, caused an increase, with time, in the apparent  $K_d$  and  $K_{oc}$  values for terbuthylazine in both unamended and amended SP soils. In SP-U, the  $K_d$  increased by a factor of 2.8 after 4 wk of incubation and by a factor of 2.1 in the SP-A soil during the same period of time (Table 1). Atrazine  $K_d$  and  $K_{oc}$  values also increased with aging time in the unamended SP-U soil by a factor of 3.0, and by a factor of 6.5 for the MN-U soil. However, in the amended soils (SP-A and MN-A), the atrazine  $K_d$  and  $K_{oc}$  values did not change significantly during the incubation period. Similar results were reported by Johannesen and Aamand (33) who calculated  $K_d$  in aged soils from the pore-water content of <sup>14</sup>C and found an increase in sorption of atrazine and terbuthylazine in unamended soil with aging time. Similar increases in sorption with incubation time have also been observed for a variety of classes of herbicides, including the sulfonaminocarbonyltriazolinone herbicides (34). In contrast, Menasseri et al. (35) did not observe an increase in sorption of dicamba in either amended or unamended soil with increased aging time.

Sorption is one of the main processes that affects the fate of pesticides in soil and water (36), and sorption–desorption interactions of pesticides with soil determine the availability of these organic compounds for plant uptake, transport, and microbial degradation in soil (37). Equilibrium sorption estimates ( $K_d$  and  $K_{oc}$  values) can be used to predict the mobility of pesticides in soil (38, 39).

Transport models typically use  $K_d$  or  $K_{oc}$  values to describe pesticide retention by soil (40). The accuracy of the sorption estimates can be more important than the choice of transport model in correctly simulating pesticide leaching (40), emphasizing the need for evaluating pesticide sorption in aged soils. Aging effects on sorption can also impact mobility classifications of pesticides. For instance, mobility classification based on a unitless retardation factor (RF) (41), which in turn is a function of  $K_d$ , would change from moderately mobile to moderately immobile for terbuthylazine and atrazine in the unamended soils. In contrast, aging would not have an effect on amended MN soil.

*Pseudomonas* sp. strain ADP, a triazine mineralizing bacterium (24), has previously been used to determine the bioavailability of selected triazines in soil (42, 43). In freshly treated soil, atrazine and terbuthylazine mineralization by *Pseudomonas* sp. strain ADP has been shown to be inversely related to soil sorption (i.e., less mineralization was seen with increased

sorption) (42). Atrazine bioavailability has been reported to decrease in aged soil, as evidenced by decreased mineralization of atrazine by *Pseudomonas* ADP in soils aged for different lengths of time soil prior to inoculation with *Pseudomonas* ADP (43). In the same study with a subsurface soil, atrazine mineralization was significantly reduced when the aging time exceeded 1 d, whereas no differences were observed between mineralization rates of samples aged 7–30 d. In a similar study done using a surface soil, atrazine mineralization decreased as a function of increased aging time; however, no effect of aging on atrazine mineralization was observed for an aquifer sediment (33).

In the above-mentioned studies on aging effects on atrazine mineralization, however, there were no independent measurements of the amounts of atrazine present in the aged soils. Since *Pseudomonas* sp. strain ADP has been shown to be able to mineralize, within hours, the same amount of atrazine and simazine in aged soils that was extractable from the same aged soils using aqueous methanol (25, 44), it may be possible that the reported decrease in mineralization of <sup>14</sup>C-atrazine by *Pseudomonas* ADP (33, 42, 43) was due to decreased amounts of atrazine present with aging, as opposed to decreased availability of the aged residues to the degrading microorganism. Although there are studies on bioavailability of pesticides in soils of different organic matter content, we are aware of no research that has used a specific degrading bacterium to study the effects of organic amendments, such as alperujo, on bioavailability of aged atrazine and terbuthylazine residues.

To examine the influence of organic amendments on bioavailability of aged pesticide residues in more detail, *Pseudomonas* sp. strain ADP was added to aged, alperujo-amended, and unamended soils, and <sup>14</sup>C mineralization was followed over time. In unamended SP soil (SP-U), the amount of <sup>14</sup>C-terbuthylazine mineralized by *Pseudomonas* ADP increased from 6% to 11% of applied chemical at the end of a 28 d incubation period (Table 2). Even though *Pseudomonas* sp. strain ADP has been shown to be less efficient at mineralizing terbuthylazine as compared to atrazine (33, 42), the amount of <sup>14</sup>C-terbuthylazine mineralized by *Pseudomonas* sp. strain ADP was less than expected considering the amount of water extractable terbuthylazine (>70% of applied terbuthylazine) (Figure 2a). In the case of <sup>14</sup>C-atrazine, the total amount of herbicide mineralized by the bacterium was also lower than expected at time 0, particularly because >90% of the atrazine was aqueous extractable. Increasing the inoculation density of *Pseudomonas* sp. strain ADP in the SP-U soil from  $1 \times 10^8$  to  $2 \times 10^8$  organisms g<sup>-1</sup> nearly doubled the amount of atrazine mineralized at time 0, from 5% to 8% of applied. The average amount of atrazine mineralized in unamended MN soil (MN-U) by the lower density inoculum during the 17 d incubation period (23% of applied) was slightly higher than when MN-U

**Table 2.**  $^{14}\text{C}$  Herbicide Mineralized by *Pseudomonas* sp. Strain ADP in Unamended (U) and Amended (A) Spanish (SP) and Minnesota (MN) Soils during a 28 day Incubation

triazine	soil	inoculum density (organisms $\text{g}^{-1}$ )	herbicide mineralized during incubation (% of applied)			
			$T = 0$ d	$T = 7-10$ d <sup>a</sup>	$T = 14-17$ d <sup>b</sup>	$T = 26-28$ d <sup>c</sup>
terbutylazine	SP-U	$1 \times 10^8$	$5.7 \pm 1.6$	<1	$5.9 \pm 0.5$	$11.1 \pm 3.1$
	SP-A	$1 \times 10^8$	<1	<1	$1.0 \pm 0.5$	<1
atrazine	SP-U	$1 \times 10^8$	$5.3 \pm 1.4$	$34.4 \pm 11.2$		
	SP-U	$2 \times 10^8$	$8.2 \pm 1.6$	$12.0 \pm 2.5$		$30.8 \pm 11.3$
	SP-A	$1 \times 10^8$	<1	$1.7 \pm 0.9$		
	SP-A	$2 \times 10^8$	<1	$3.5 \pm 1.4$		$25.2 \pm 9.8$
	MN-U	$1 \times 10^8$	$13.3 \pm 0.3$	$29.0 \pm 12.6$	$23.1 \pm 2.6$	
	MN-U	$2 \times 10^8$	$18.3 \pm 5.1$		$19.0 \pm 11.8$	$15.4 \pm 5.0$
	MN-A	$1 \times 10^8$	<1	<1	<1	
	MN-A	$2 \times 10^8$	<1	<1	<1	<1

<sup>a</sup>  $T = 7$  d terbutylazine SP,  $T = 10$  d atrazine SP,  $T = 9$  d atrazine MN. <sup>b</sup>  $T = 14$  d terbutylazine,  $T = 17$  d atrazine. <sup>c</sup>  $T = 28$  d terbutylazine,  $T = 26$  d atrazine.

soil was inoculated with twice as many microorganisms and incubated for 26 d (15% of applied).

The amount of atrazine mineralized in SP-U at time 0 was also low in comparison with other studies of atrazine and simazine mineralization by *Pseudomonas* sp. strain ADP, but increased to comparable levels as has been previously reported by the end of the incubation period (25, 33, 43, 44). An increase in atrazine mineralization was observed in MN-U soil with incubation. Jacobsen et al. (42) also observed less mineralization of terbutylazine as compared to atrazine; in both cases, the amount of herbicide mineralized by the bacteria increased at the end of the aging period.

The amount of labeled herbicide mineralized by *Pseudomonas* sp. strain ADP in alperujo-amended soils was much less than that seen in the unamended soils, <1% of applied at every aging time, except for atrazine in SP-A soil after 10 or 26 d incubation. These results indicate that olive mill waste used as organic amendment reduces either bioavailability or degradation of terbutylazine and atrazine in soil. Masaphy and Mandelbaum (26) reported a significant decrease in atrazine mineralization rates in soils irrigated with treated wastewater as compared to the soils not irrigated. They attributed this decrease in mineralization to a combination of factors, the increase in sorption and complexation of atrazine with the OM in the soil with aging, and the increase in N of soil by the wastewater irrigation.

The low rate of triazine mineralization by *Pseudomonas* sp. strain ADP in these studies may be due to the microorganism's use of inorganic sources of N preferentially over herbicide-derived N, thereby reducing triazine degradation. Laboratory studies have previously shown that inorganic nitrogen inhibits the ability of *Pseudomonas* ADP to degrade atrazine (45). It is possible that once the inorganic sources of N were depleted by microbial activity during the incubation in the unamended soils, particularly in SP soil, the *Pseudomonas* sp. strain ADP then used the triazines as a N source. It is suggested that in amended soil, alternate sources of N (alperujo C:N ratio was relatively high, 18.3) were not depleted during the incubation, resulting in very little triazine mineralization (<1% of applied triazine). However, the reported relationship between available N and atrazine mineralization is contradictory. Shapir et al. (46) found that atrazine mineralization activity of *Pseudomonas* sp. strain ADP was not negatively influenced by the addition of wastewater and sludge as amendments.

It appears that bioavailability of triazines in aged soil is a complex process that may be difficult to characterize. While Barriuso et al. (25) reported a correlation between the amounts of  $^{14}\text{C}$ -residues in water and organic extracts and bioavailable residues, and proposed that this may help to

determine the bioavailability of other triazines, we could not use this correlation in our studies due to the low mineralization of herbicide by *Pseudomonas* sp. strain ADP in comparison with the amounts of extractable  $^{14}\text{C}$ . Jacobsen et al. (42), using a two compartment model on terbutylazine mineralization and sorption data, suggested that terbutylazine mineralization in soil inoculated with *Pseudomonas* sp. strain ADP was initially controlled by the rate of mineralization by *Pseudomonas* sp. strain ADP, followed by the sorption of terbutylazine. They further suggested that in soils with high biodegrading potential, sorption was the limiting parameter for terbutylazine mineralization, whereas in soils with low biodegrading potential, the limiting parameter was the number of degrading microorganisms. However, the present research indicates that the N status of the soil may be equally of importance. More work is needed on the relationship between the availability of inorganic N in soil and triazine mineralization by *Pseudomonas* sp. strain ADP.

In conclusion, addition of alperujo, a solid olive-mill waste, as an organic amendment to sandy soils from Spain and Minnesota resulted in reduced availability of two triazine herbicides, terbutylazine and atrazine, as compared to unamended soils. In the Spanish soil, terbutylazine sorption increased with aging in both amended and unamended soils. Aging/amendment interactions appear to be triazine dependent, and atrazine sorption increased with aging in both unamended Spanish and Minnesota soils. Although atrazine sorption was greater in alperujo-amended soils, as compared to unamended soils, aging had no effect on atrazine sorption in amended soils. Addition of alperujo decreased mineralization of both herbicides by *Pseudomonas* sp. strain ADP as compared to unamended soils. The mineralization of the herbicides in unamended soil increased with aging, possibly because of decreased readily available inorganic N in the soils during incubation.

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