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Influence of Harvesting Method and Washing on the Presence of Pesticide Residues in Olives and Olive Oil

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The influence of the preliminary steps in olive oil production (harvesting and washing) on pesticide residues in olives and olive oil has been investigated. Analyses were performed by GC-MS/MS and revealed that endosulfan sulfate and two herbicides (diuron and terbuthylazine) were the most frequently found residues in olives and olive oil. The harvesting method has a decisive influence on herbicide concentrations found in olives. Thus, 16 and 48% of the olive samples harvested on the ground after falling from the tree presented concentrations higher than the maximum residue limit (MRL) for diuron and terbuthylazine, respectively. In olives harvested directly from the tree, diuron was not found at concentrations higher than MRL and terbuthylazine was found in only 10% of the samples. The washing step performed routinely in olive mills was effective in removing the superficial contamination by herbicides present in olives harvested on the ground. Nevertheless, even after washing, the olive oil obtained from ground olives showed herbicide residue concentrations higher than those obtained from tree olives.

KEYWORDS: Pesticide residues; olive washing; olive oil; olives harvest

INTRODUCTION

Virgin olive oil is obtained directly from the flesh of the olive fruit, and it is edible immediately after extraction if the raw material is of good quality. Attacks by pests and diseases and the presence of weeds make it necessary to apply pesticides to olive trees to ensure crop protection, which can leave residues on the drupes. The quantity of these residues depends mainly on the number of treatments, the degradation rate of the active ingredient, and the preharvest interval. Most pesticides are liposoluble, and because 5 kg of olives on average are needed to obtain 1 L of oil, a concentration effect could occur in the olive oil. Thus, maximum pesticide residues levels have been set by the European Union (*1*) and the Food and Agricultural Organization and the World Health Organization (FAO/WHO) Codex Committee (*2*) for olives.

Herbicides are by far the most used pesticides in olive farming, and their residues are detected frequently in olive oil together with residues from different insecticides (*3*). Different methods of analysis of pesticide residues in olive oil are described in the literature, most of them based on capillary gas chromatography (GC) (*4*) or high-performance liquid chromatography (HPLC) (*5*). Different detectors are used after a step of extraction and cleanup based on liquid−liquid partioning (*6*), solid-phase extraction (SPE) (*7*), gel permeation chromatography (GPC) (*8*), or matrix solid-phase dispersion (MSPD) (*9*). Methods for pesticide determination in olives available in the literature are scarce, and most of the reported methods are devoted to the analysis of only a few target compounds (*10*, *11*).

To date, there have been no studies conducted to evaluate the effect of two of the first steps in olive oil production (harvest and olives washing) on the presence and concentration level of pesticide residues in both olives and olive oil. Only one attempt to evaluate the effect of olive washing on pesticide residue levels in olives has been described (*12*). This work was restricted to insecticide residues, and olives were washed in the laboratory, not at the production facility.

There are many different methods of olive harvesting. The method used to harvest olives depends on cultural techniques, tree size and shape, and orchard terrain. These methods can be grouped into two categories: harvest from the tree and harvest on the ground (*13*). In this context, the harvesting method may be very relevant in the presence of pesticides residues in olives, especially herbicides that are applied to the soil. On the other hand, a previous step of cleaning is necessary in olive processing to obtain olive oil. After delivery to the oil mill, olives are filled in charges into a soil funnel and transported by a conveyor belt into a sucking device, where leaves, twigs, and other light matter are removed. Then a washing machine uses water to remove dust, sand, and soil. There are olive mills that manage separately olives harvested directly from the tree (tree olives) and those harvested on the ground (ground olives), but there are others that do not have separate processing lines for both types of olives.

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The goal of this work is to evaluate the efficacy of the washing step performed in the oil mills in removing pesticide residues from olives and the influence of the harvesting method, as well as the separate processing of tree and ground olives, on the presence of pesticide residues in olives and olive oil. For that purpose, olives before and after the washing step, water from the washing devices, and olive oil samples were collected in three different olive mills and analyzed by GC-MS/MS.

MATERIALS AND METHODS

Chemicals. Pesticide standards and triphenyl phosphate (TPP) were obtained from Riedel-de-Häen (Seelze-Hannover, Germany), with a purity of >99%. Stock and standard solutions (200 μg mL^{-1}) were prepared by weighing and dissolving in acetone; they were stored in a freezer (−18 °C). Working standard solutions were prepared in cyclohexane by appropriate dilution and then stored in a refrigerator (4 °C). HPLC quality grade solvents, acetone, cyclohexane, petroleum ether, hexane, and acetonitrile, as well as HPLC grade anhydrous Na_2SO_4, were obtained from Panreac (Barcelona, Spain).

Sampling. Olives and olive oil samples were collected in three olive mills located in Jaén, a province that accounts for ca. 40% of the Spanish olive oil production. Spain is the main olive oil producer in the world, with an average annual production of 700 000−800 000 Tm, reaching even 1 400 000 Tm in recent harvest years. According to the International Olive Oil Council (IOOC), this represents about 30% of the world production, followed by Italy, Greece, and Tunisia.

Two of the selected mills, namely, A and B, process separately tree and ground olives. They differ slightly in the washing process. In mill A, ground and tree olives are first sprayed with water in a continuous system and, then, only ground olives are washed in a washing device by immersion in water. Mill B washes both types of olives by immersion in water in washing devices. The third mill, namely, C, does not separate ground and tree olives and, thus, they are washed together by immersion.

Samples were collected during the two harvest periods 2003−2004 and 2004−2005 from the middle of December to the beginning of February every 2 weeks approximately.

Water Samples. Washing wastewater samples were collected at the inlet and outlet of the washing devices in 2.5 L amber glass bottles capped with Teflon-lined screw caps, transported to the laboratory, and kept at 4 °C and away from light for a short time until the analysis.

One hundred and one water samples were collected: 12 at the inlet of the washing device and 89 at the outlet (25 from ground olives, 5 from tree olives, and 12 from nonseparated olive washing devices).

Olives. About 1 kg olive samples were picked up before and after the washing process in plastic bags and then stored in a freezer until analysis. Two samples were collected in those mills that separate the fruit, one corresponding to tree olives and another one to ground olives. If the mill processed nonseparated fruit, only one sample was collected.

Ninety-four olive samples were collected, 47 of those samples corresponding to nonwashed olives (25 ground, 10 tree, and 12 nonseparated olives) and the same number corresponding to washed olives.

Olive Oil. Samples were collected in each of the olive mills directly from the storage tanks. One sample was taken each time in both processing lines in the case of olive mills that process tree and ground olives separately. In the case of the olive mill that does not separate both types of olives, only one olive oil sample was collected. A total of 33 oil samples were collected (15 from ground, 10 from tree, and 8 from nonseparated olives).

Olive oil was sampled in 100 mL amber glass bottles capped with Teflon-lined screw caps and kept refrigerated at 4 °C away from light before analysis.

Apparatus and Chromatography. A 12-port Visiprep SPE vacuum manifold and Supelclen C-18 SPE tubes packed with 500 mg of C18 were purchased from Supelco (Bellefonte, PA) and used for the solid-phase extraction of pesticides from water samples.

In the case of olives, a hammer mill (Talleres López, Priego de Cordoba, Spain) and an Ultra-Turrax T25 basic homogenizer (IKA-Werke, Schott Ibérica, S.A.) were used for the extraction procedure.

The GPC system comprised an L7110 LaChrom HPLC pump (Merck), two Waters Envirogel GPC cleanup columns, a guard column (19 × 150 mm), and a main cleanup column (19 × 300 mm). An L-7490 LaChrom RI detector (Merck), a fraction collector, and an autosampler (704 Varian ProStar) were used as well. The flow rate was set at 5.0 mL min^{-1}, and the mobile phase was ethyl acetate/cyclohexane (1:1). Between runs the flow rate was set at 0.5 mL min^{-1} to clean the GPC system and to avoid the drying of the columns.

For the GC-MS/MS analysis, a Varian 3400 gas chromatograph, fitted with a Saturn 2000 ion trap mass spectrometer from Varian Instruments (Walnut Creek, CA) was employed. It was equipped with an 8200 autosampler, a 1079 split/splitless temperature-programmable injector port operated in the splitless mode, a Varian fused silica capillary column (30 m × 0.25 mm i.d.), coating CP-SIL 5CB and film thickness 0.25 μm, and a Varian fused-silica uncoated (2 m × 0.25 mm i.d.) precolumn. The ion trap mass spectrometer operated in the electronic impact (EI) mode, and the MS/MS option was used. The carrier gas used was helium (purity = 99.999%) at a flow rate of 1 mL min^{-1}. The injector temperature was programmed from 70 °C (held for 0.5 min at 70 °C) to 330 °C at 100 °C/min to desorb the pesticides retained in the carbofrit inside the insert after the vaporization of the solvent. The column was programmed from 70 °C (held for 2 min) to 180 °C at 25 °C/min (held for 10 min), from 180 to 240 °C at 4 °C/min, and from 260 to 280 °C at 30 °C/min (held for 1 min). The temperature for the manifold, transfer line, and trap were 50, 270, and 200 °C, respectively. The emission current was 80 μA, and the axial modulation amplitude voltage was 4.0 V.

Extraction Procedures for Water, Olives, and Olive Oil Samples. *Water Samples.* Pesticides were extracted from the washing water samples by using a procedure previously developed in our laboratory (*14*). Washing water samples were first filtered until the sample remained transparent. One liter of washing water was slowly passed through a C_{18} cartridge under vacuum. Afterward, the pesticides were eluted from the solid phase with dichloromethane. The eluate was filtered over anhydrous Na_2SO_4 and evaporated to dryness, and the residue was dissolved with 1 mL of cyclohexane.

Olive Oil. The method of extraction and cleanup applied to the multiresidue analysis of olive oil samples was developed in our laboratory as well (*15*). Two grams of olive oil was dissolved in 10 mL of *n*-hexane saturated in acetonitrile. The solution was extracted three times with 10 mL of acetonitrile saturated in *n*-hexane. The extracts were combined and concentrated to dryness. To separate the pesticides from the triglyceride matrix, the residue was dissolved in 10 mL of GPC mobile phase (ethyl acetate/cyclohexane, 1:1). Five milliliters of the extract was injected into the GPC, and the eluate was collected between 15 and 20 min. The eluate fraction was concentrated to dryness in a rotary evaporator, and the residue was dissolved with 1 mL of cyclohexane for the chromatographic analysis.

Table 1. Positive Results in Olive and Olive Oil Samples

	olives			olive oil		
pesticide	ground, $n = 50$[a]	tree, $n = 20$	nonseparated, $n = 24$	ground, $n = 15$	tree, $n = 10$	nonseparated, $n = 8$
diuron	46	13	20	15	10	6
α-HCH	6	2	4	0	0	1
simazine	2	1	1	5	2	2
dimethoate	2	0	0	0	0	0
atrazine	5	0	1	0	0	0
β-HCH	6	2	1	0	0	0
terbuthylazine	42	13	24	15	10	7
lindane	2	1	2	0	0	0
carbaryl	2	1	3	0	0	0
chlorpyrifos	2	0	0	5	2	2
α-endosulfan	7	10	2	2	2	0
dieldrin	2	0	0	0	0	0
β-endosulfan	2	0	0	0	0	0
endosulfan sulfate	26	9	16	9	9	5

[a] n = total number of analyzed samples.

Table 2. Analytical Parameters of the Methods

	recovery (% ± RSD[a])		LD[b] (μg kg^{-1})		LQ[c] (μg kg^{-1})	
pesticide	olives	olive oil	olives	olive oil	olives	olive oil
diuron	82 ± 9	98 ± 7	0.1	0.5	1.2	5.0
terbuthylazine	136 ± 11	101 ± 5	0.1	0.5	0.2	1.0
endosulfan sulfate	130 ± 6	90 ± 5	2.0	0.5	5.0	1.2

[a] RSD, relative standard deviation. [b] LD, limit of detection. [c] LQ, limit of quantitation.

Table 3. Obtained Results for Nonwashed Olives (Milligrams of Pesticide per Kilogram of Fresh Crushed Olives)[a]

			pesticide concentration (mg kg^{-1})		
	olive mill	sample	diuron	terbuthyl-azine	endosulfan sulfate
ground	A	1	0.396	0.303	ND[b]
		2	0.331	0.296	0.005
		3	0.191	0.239	0.003
		4	0.141	0.162	0.007
		5	0.139	0.257	0.004
		6	0.039	0.051	ND
		7	0.018	0.023	0.004
		8	0.009	ND	ND
		9	0.008	0.284	ND
		10	0.007	0.013	ND
		11	0.003	0.023	ND
		12	ND	<LQ[c]	ND
	B	13	0.661	0.560	ND
		14	0.253	0.321	0.011
		15	0.129	0.152	0.002
		16	0.073	0.101	ND
		17	0.067	0.011	0.007
		18	0.062	0.018	0.002
		19	0.049	0.061	0.012
		20	0.018	0.013	0.006
		21	0.011	ND	ND
		22	0.010	<LQ	ND
		23	0.007	0.004	0.050
		24	ND	ND	0.016
		25	ND	<LQ	0.002
tree	A	26	0.020	0.024	0.005
		27	0.016	0.046	ND
		28	0.009	0.015	0.003
		29	0.003	0.023	0.012
		30	0.003	<LQ	0.008
	B	31	0.025	0.059	0.010
		32	0.025	0.037	0.035
		33	<LQ	ND	ND
		34	ND	ND	0.012
		35	ND	ND	0.005
nonseparated	C	36	0.120	0.116	ND
		37	0.032	<LQ	<LQ
		38	0.033	0.013	0.007
		39	0.016	0.049	ND
		40	0.011	0.027	0.004
		41	0.011	0.018	ND
		42	0.009	<LQ	0.006
		43	0.003	0.012	0.011
		44	<LQ	<LQ	<LQ
		45	<LQ	<LQ	ND
		46	ND	0.007	ND
		47	ND	0.004	ND

[a] Samples for each group and mill have been ordered by decreasing concentration of diuron for better visualization. [b] ND, not detected. [c] LQ, limit of quantitation.

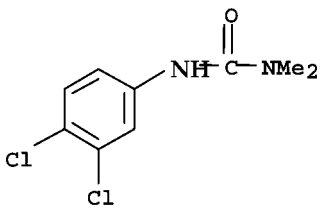
Olives. Olives (including the seeds) were first crushed by means of a metallic olive crusher. Afterward, a 100 g portion was weighed in a glass tube, and 50 g of anhydrous Na_2SO_4 was added. The sample was then extracted with 100 mL of petroleum ether by homogenizing with the Ultra-Turrax device. The extract was decanted, filtered over anhydrous Na_2SO_4, and transferred to a 250 mL round-bottom flask. The process was repeated twice again, homogenizing the residue with two portions of 50 mL of petroleum ether. The extracts were combined, and the organic solvent was evaporated. The so-obtained oil was cleaned up following the above-described procedure for olive oil.

Statistical Analysis. A two-tailed Wilcoxon signed-rank test was used to judge the statistical significance of pesticide washing. Analysis of data was performed using WinStat, the statistics add-in for Microsoft Excel.

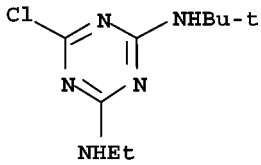
RESULTS AND DISCUSSION

The analytical methods employed had been previously optimized for the determination of 32 pesticides, including those more intensively used in olive trees cultivars in the region of Jaén (*14*, *15*). **Table 1** shows the number of samples in which pesticide residues were encountered. Only 14 pesticides were detected in the analyzed samples, which included olives collected before and after washing in the mill and olive oil. The herbicides diuron and terbuthylazine, as well as endosulfan sulfate (a degradation product of the insecticide endosulfan), have been encountered in most of the analyzed olives and olive oil samples. Other residues have been found less frequently. Thus, α-endosulfan, α-HCH, and β-HCH were detected in 20, 13, and 9% of the olives analyzed, but concentrations were lower than the limit of quantitation (LQ) in most cases. Moreover, these residues are rarely detected in the olive oil. Only those pesticides more frequently found at concentrations above the quantification limit, namely, diuron, terbuthylazine, and endosulfan sulfate, will be considered for a more detailed comparison. The main parameters of the methods for determination of these compounds in olives and olive oil are summarized in **Table 2**.

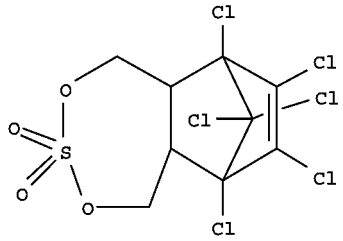
The triazine terbuthylazine and the phenylurea diuron are herbicides widely used in olive cultivars and they remain largely in the topsoil, controlling a wide range of weeds. Endosulfan is an organochlorine nonsystemic insecticide and acaricide used for the control of insects and mites on a very wide range of crops including olives. Their structures are shown below:



Diuron



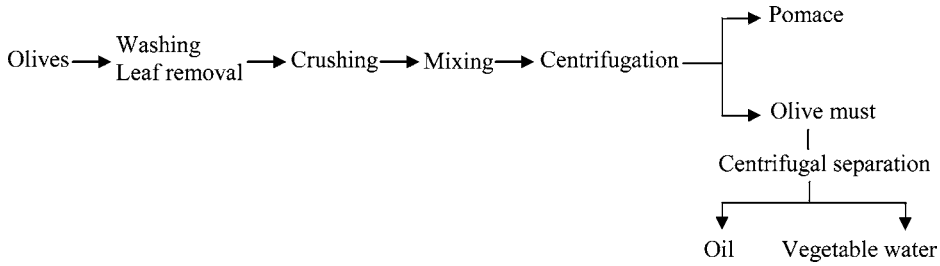
Terbuthylazine



Endosulfan Sulfate

The influence of the harvesting method will be considered first. To do this, olive samples collected in the olives mills before processing and those harvested using different methods were analyzed. Olive harvesting methods can be grouped into two categories:

Scheme 1



Harvest from the Tree (Tree Olives): Hand Picking (Raking). The fruit falls onto nets spread under the tree canopies and is placed in bins when all olives have been picked from the tree. This method demands considerable human labor, and the cost has resulted in the appearance of *machine harvest*. It is performed by shakers, which are attached to the trunk and scaffold branches.

Harvest on the Ground (Ground Olives). These are methods of harvesting the olives off the ground once they have dropped naturally. Usually a circular piece of ground under the tree crown is swept and well-trodden. The olives are periodically gathered by mean of brushes or suction equipment and then pass through suitable machines that remove leaves, twigs, and impurities.

Table 3 shows the obtained results for olive samples collected before processing in the oil mill for ground, tree, and nonseparated olives. Regarding ground olives, diuron was quantified in 22 of 25 analyzed samples. In 4 samples the found amount was higher than the maximum residue limit (MRL) fixed in the European Union (0.2 mg kg^{-1}). Terbuthylazine residues were quantified in 19 samples and detected at concentration levels below the LQ in 3 samples. The obtained results were above the MRL (0.05 mg kg^{-1}) for 12 samples. From the obtained results for tree olives it is interesting to note that found levels were significantly lower. Regarding diuron, all analyzed samples were below the MRL and the maximum found amount was nearly 10 times lower than the MRL. For terbuthylazine only the concentration of one sample (0.059 mg kg^{-1}) is slightly above the limit. Endosulfan sulfate was quantified in 14 of 25 ground olive samples and in 8 of 10 tree olive samples, concentration values being in the same range.

These results suggest that herbicide residues are mainly due to contamination when the olives come in contact with the soil after falling from the tree. Consequently, levels found in olives that have been in contact with herbicide residues present in the soil (ground olives) are significantly higher than levels found in olives that did not come in contact with the soil (tree olives). In contrast, endosulfan sulfate levels do not significantly differ between ground olives and tree olives. Insecticide endosulfan is not used frequently in olive cultivations in the studied area of Jaén, but it may be off-farm transported by air when it is released directly to the atmosphere during application in cotton. Due to its high liphophillic character, endosulfan is incorporated into the olives and, thus, contamination must occur when olives are still on the tree. Once in the olive, endosulfan undergoes oxidation to the equally or more toxic endosulfan sulfate, a process that is mediated essentially by microorganisms and influenced by the moisture content and pH, for example.

Table 3 also shows results of olive samples from an olive mill that does not separate olives harvested via different procedures. It should be noted that in the case of nonseparated olives the harvesting method is not known. In general, low levels of pesticides were found in these samples. Only one sample exceeded the MRL for terbuthylazine. Because this mill does not consider the origin of the olives, it could happen that most of the sampled olives had been harvested from the tree. In addition, a dilution effect could be observed when olives were mixed because ground olives have the highest levels, whereas

Table 4. Obtained Results for Washed Olives

			pesticide concentration (mg kg^{-1})		
	olive mill	sample[a]	diuron	terbuthyl-azine	endosulfan sulfate
ground	A	1	0.020	0.037	ND[b]
		2	0.008	0.027	0.003
		3	0.009	0.026	0.003
		4	0.052	0.044	0.009
		5	0.008	0.016	0.003
		6	<LQ[c]	ND	ND
		7	0.031	0.066	ND
		8	0.043	ND	ND
		9	0.016	0.135	ND
		10	<LQ	<LQ	ND
		11	ND	<LQ	ND
		12	<LQ	0.013	<LQ
	B	13	0.007	0.006	ND
		14	0.007	0.004	0.012
		15	ND	<LQ	ND
		16	<LQ	0.006	ND
		17	0.029	0.011	0.009
		18	0.057	ND	ND
		19	0.051	0.046	0.018
		20	<LQ	0.009	ND
		21	0.020	<LQ	ND
		22	0.013	ND	<LQ
		23	0.004	ND	0.012
		24	0.004	0.009	0.006
		25	ND	<LQ	ND
tree	A	26	0.001	<LQ	ND
		27	ND	<LQ	ND
		28	0.004	0.003	0.004
		29	<LQ	0.004	0.011
		30	0.004	0.005	0.008
	B	31	ND	ND	0.021
		32	0.021	0.088	0.008
		33	<LQ	<LQ	0.040
		34	0.011	0.035	0.040
		35	ND	<LQ	<LQ
nonseparated	C	36	0.071	0.059	ND
		37	0.005	0.008	0.007
		38	0.009	0.003	0.004
		39	0.025	0.072	0.004
		40	0.063	0.084	ND
		41	0.068	0.044	0.004
		42	<LQ	ND	ND
		43	<LQ	<LQ	<LQ
		44	0.003	ND	ND
		45	<LQ	0.022	ND
		46	<LQ	0.012	ND
		47	ND	<LQ	ND

[a] Sample numbering is the same as in **Table 3**. [b] ND, not detected. [c] LQ, limit of quantitation.

Table 5. Results of Wilcoxon Signed-Rank Test for Significance of Washing in Reducing Pesticide Residues in Olives

	pesticide	*P* value	significant ($P < 0.05$)
ground	diuron	0.0056	yes
	terbuthylazine	0.0009	yes
	endosulfan sulfate	0.0640	no
tree	diuron	0.0929	no
	terbuthylazine	0.4838	no
	endosulfan sulfate	0.6744	no
nonseparated	diuron	1.0000	no
	terbuthylazine	0.4148	no
	endosulfan sulfate	0.7353	no

tree olives have the lowest ones, but if both are mixed together the final levels would be lower.

Olive Washing Efficacy. Once the olives are brought to the olive mills, they go through different processes that can be summarized in the **Scheme 1** (*16*). Olives harvested via different methods can be processed together or separately depending on the olive mill.

Table 4 shows the results obtained in the analysis of ground, tree, and nonseparated olives after they have been washed in the olive mill. The most remarkable finding is the reduction of herbicide residue concentrations that were found in ground olives when compared to nonwashed olives (**Table 3**). Only two samples exceeded the MRL for terbuthylazine and none of them for diuron. The Wilcoxon signed-rank test was used to compare pesticide concentrations between unwashed and washed olives. This nonparametric test is an adequate alternative to the paired *t* test in this case for two reasons: first, because samples are not independent and, second, because the large number of very small residue levels skews the distributions toward zero. To compute the Wilcoxon signed-rank, the absolute values of the nonzero differences between unwashed and washed olives are ranked from lowest to highest. Then, a sign is given the rank on the basis of the original sign of the difference. The negative and positive ranks are then summed separately, and the lower of the values ignoring the sign is compared to a table value and the two-tailed probability (*P*) is computed. Results are shown in **Table 5**. Statistical analysis using the Wilcoxon signed-rank test gave *P* values of less than the null hypothesis value of 0.05, indicating that there is a significant difference between the washed and unwashed samples for the herbicides diuron and terbuthylazine in ground samples.

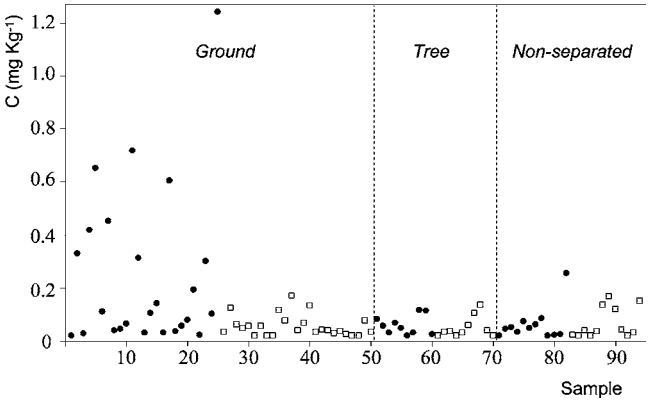


Figure 1. Total pesticide content distribution in ground, tree, and nonseparated olives: (●) nonwashed olives; (□) washed olives. (Total pesticide content = sum of diuron, terbuthylazine, and endosulfan sulfate concentrations.) Note that sample numbering is random within a group.

Table 6. Obtained Results for Washing Wastewater Samples

			pesticide concentration (μg L^{-1})		
	olive mill	sample[a]	diuron	terbuthyl-azine	endosulfan sulfate
ground	A	1	10.32	16.13	ND[b]
		2	2.93	1.12	ND
		3	12.79	21.41	ND
		4	6.24	11.95	ND
		5	7.13	5.01	ND
		6	3.66	3.76	ND
		7	13.25	19.09	ND
		8	7.07	11.04	ND
		9	8.40	9.35	ND
		10	23.70	18.83	ND
		11	19.83	15.36	ND
		12	5.96	2.23	ND
	B	13	5.07	1.71	ND
		14	0.30	0.02	ND
		15	3.84	0.04	ND
		16	13.05	0.28	ND
		17	13.40	11.33	ND
		18	9.42	2.21	ND
		19	5.35	1.15	ND
		20	39.40	28.49	ND
		21	1.90	0.32	ND
		22	1.24	1.13	ND
		23	4.07	0.81	ND
		24	34.17	19.02	ND
		25	8.60	0.11	ND
tree	A	26			
		27			
		28			
		29			
		30			
	B	31	0.22	0.22	ND
		32	0.23	0.03	ND
		33	0.38	0.06	ND
		34	1.36	1.72	ND
		35	0.27	0.91	ND
nonseparated	C	36	3.18	2.09	ND
		37	2.04	0.80	ND
		38	16.16	2.66	ND
		39	1.98	1.91	ND
		40	4.46	3.25	ND
		41	2.81	2.37	ND
		42	7.09	27.54	ND
		43	5.40	3.39	ND
		44	2.92	10.75	ND
		45	1.53	2.81	ND
		46	2.16	1.76	ND
		47	2.26	3.31	ND

[a] Sample numbering is the same as in **Table 3**. [b] ND, not detected.

The effect of olive washing in the presence of residues in olives can be also visualized in **Figure 1**. It is observed how washing decreases significantly pesticide residue levels in ground olives. The influence of washing is not so clearly observed in tree olives. In the case of nonseparated olives it is interesting to point out that some washed olives present higher residue levels than unwashed ones. This may be due to contamination of residue-free tree olives during the washing process if highly contaminated ground olives had been previously washed. The washing devices are cleaned, and water (about 20 m^3) is totally replaced at the beginning of the working day. With this procedure, about 160 000 kg olives may be washed before the water is replaced; therefore, high contamination levels are reached in some occasions.

The washing step does not influence the concentration levels of endosulfan sulfate in all types of olives (see **Table 5**). This

Table 7. Obtained Results for Olive Oil Samples[a]

	olive mill	sample	pesticide concentration (mg kg^{-1}) diuron	terbuthylazine	endosulfan sulfate
ground	A	1	0.170	0.150	<LQ[b]
		2	0.140	0.140	<LQ
		3	0.130	0.130	<LQ
		4	0.119	0.175	ND[c]
		5	0.053	0.103	0.019
		6	0.046	0.146	ND
		7	0.035	0.196	ND
		8	0.014	0.014	ND
	B	9	0.220	0.150	0.040
		10	0.180	0.140	0.030
		11	0.130	0.080	0.054
		12	0.108	0.113	ND
		13	0.104	0.138	ND
		14	0.060	0.079	0.048
		15	0.033	0.049	0.031
tree	A	16	0.050	0.070	<LQ
		17	0.037	0.098	0.041
		18	0.015	0.031	0.023
		19	0.013	0.072	ND
		20	0.013	0.045	0.019
	B	21	0.080	0.090	0.090
		22	0.039	0.021	0.074
		23	0.029	0.062	0.017
		24	0.021	0.081	0.066
		25	0.013	0.011	0.014
nonseparated	C	26	0.100	0.120	ND
		27	0.100	0.100	<LQ
		28	0.080	0.120	<LQ
		29	0.051	0.104	0.023
		30	0.037	0.118	0.016
		31	0.025	0.077	0.011
		32	0.018	0.068	ND
		33	ND	0.050	0.010

[a] Samples for each group and mill have been ordered by decreasing concentration of diuron for better visualization. [b] LQ, limit of quantitation. [c] ND, not detected.

fact is probably due to the adsorption of this compound on the wax of the fruit surface due to its marked lipophilic character as suggested by Cabras et al. for other insecticides (*12*). The same reason could be responsible for herbicide residues that were not removed by washing. Only the superficial contamination with herbicides that mainly occurred for ground olives could be eliminated by washing.

Washing wastewater samples from the three olive mills were also analyzed, and results (see **Table 6**) confirm the above-mentioned conclusions. In addition, water samples were analyzed in the inlet of the washing devices to make sure they were residue free. Diuron and terbuthylazine are detected in all water samples from ground olive washing. Concentration levels ranged between 0.3 and 39.40 μg L^{-1} for diuron and between 0.04 and 28.49 μg L^{-1} for terbuthylazine. In the case of water samples from tree olives, only results for olive mill B are available because tree olives in olive mill A were not washed by immersion in a washing device, but just sprayed with water in a continuous system. Concentration levels found in water from tree olive washing are significantly lower (0.22−1.36 and 0.03−1.72 μg L^{-1} for diuron and terbuthylazine, respectively). Washing water from nonseparated olives shows residue levels similar to water from ground olives (1.53−16.16 μg L^{-1} for diuron and 0.80−27.54 μg L^{-1} for terbuthylazine). This fact also supports the hypothesis that residue-free olives can be contaminated during the washing step in olive mills where different types of olives are nonseparated. Endosulfan sulfate was not detected in any of the washing water samples.

Residues in Olive Oil. A series of olive oil samples were collected in the three studied olive mills to show the possible influence of the method used to harvest the olives on the presence of pesticide residues in the obtained olive oil. For comparison purposes, it would have been desirable to analyze the olive oil obtained in the olive mill from the same batch as the analyzed olives. However, this is difficult to achieve in an industrial plant because thousands of kilograms of olives are processed continuously. Thus, samples were collected at storage tanks. Results are shown in **Table 7** (note that sample numbers do not match those in **Tables 3**, **4**, and **6** and, thus, only comparison of the overall results may be performed). The three pesticides more frequently found in olives were also found in olive oil. A fast comparison between residue levels found in washed olives and olive oil reveals a concentration effect in the oil. This fact makes sense considering that 5 kg of olives on average are needed to obtain 1 L of oil and, thus, a concentration effect is expected to occur in the olive oil, especially in the case of lipophilic pesticides. Furthermore, as expected from the results obtained from olives, mean values found for the two herbicides in oil from tree olives are lower than in both nonseparated and ground olives. Oil from nonseparated olives shows intermediate herbicide concentrations between tree and ground olives, whereas endosulfan sulfate residues do not follow this pattern. It should also be mentioned that none of the analyzed oil samples obtained from ground and nonseparated olives could be sold as "extra virgin olive oil" (the highest quality olive oil) because they did not fulfill quality criteria, such as acidity, established by the European Union for this category (*17*, *18*).

Conclusions. Two herbicides (diuron and terbuthylazine) and the degradation product of the insecticide endosulfan (endosulfan sulfate) are the most frequently found residues in the analyzed olives and olive oil samples. The presented results demonstrate the decisive influence of the harvesting method in the concentration of herbicide residues in olives and olive oil. Olive farmers are encouraged to apply good agricultural practices including harvest from the tree if good-quality olive oil is intended to be produced. Furthermore, in those cases in which climatic conditions cause olives to fall it has been demonstrated that washing can effectively remove superficial contamination with herbicides. However, contamination of residue-free olives can also happen at the production facility. In conclusion, olives harvested from the ground should always be processed separately from those harvested from the tree to preserve the quality of the oil obtained from the latter.

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