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Effects of Ozone Treatment on the Degradation and Toxicity of Several Pesticides in Different Groups

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ABSTRACT

The effects of ozone treatment on the degradation and toxicity of nine pesticides were determined with different chromatographic techniques, using bubbled ozone and distilled water and two different buffer solutions as test media. The toxicity experiments were performed using *Daphnia magna,* a cladoceran fresh water flea. The results revealed that thiacloprid and acetamiprid can only be degraded by ozonation to a limited extent (max 2.6%). The other seven pesticides were successfully degraded by ozone. The degradation rates (%) were found to be 93, 99, 95, 99, 87, 98, and 85 for fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos, spiromesifen, clothianidin and thiamethoxam, respectively, after 5 minutes of ozone treatment in distilled water. The ozone treatment yielded reduced toxicity in fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos and spiromesifen. However, the degradation products of clothianidin and thiamethoxam were found to be more toxic than the pesticide itself. In general, the use of buffer solutions has no significant effect on pesticide degradation compared to water as an ozonation medium.

Keywords: Ozonation; *Daphnia magna;* Insecticide; Fungicide; Transformation; Toxic

Ozonlama İşleminin Farklı Gruplardaki Pestisitlerin Parçalanma ve Toksisitesi Üzerine Etkileri

ESER BİLGİSİ

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ÖZET

Ozonlama işleminin dokuz farklı pestisitin parçalanma ve toksisitesi üzerine etkileri farklı kromatografik tekniklerle incelenmiştir. Çalışmada ozon gazı baloncuk yöntemiyle su ve iki farklı tampon çözelti ortamında uygulanmıştır. Toksisite denemelerinde *Cladocera* takımında yer alan su piresi (*Daphnia magna)* kullanılmıştır. Bulgulara göre, ozonlama ile thiacloprid ve acetamiprid çok sınırlı bir şekilde (en çok % 2.6) parçalanabilirken diğer yedi pestisit

çok iyi parçalanmıştır. Saf suda 5 dakikalık ozonlama uygulamasıyla fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos, spiromesifen, clothianidin ve thiamethoxamın parçalanma oranları (%) sırasıyla 93, 99, 95, 99, 87, 98 ve 85 olarak bulunmuştur. Ozonlama işlemi fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos ve spiromesifende toksisitenin azalmasını sağlarken clothianidin ve thiamethoxamın parçalanma ürünlerinin toksisitesi başlangıç bileşiğinden daha fazla bulunmuştur. Genel olarak tampon çözelti kullanımı pestisitlerin parçalanmasında suya göre önemli bir farklılık oluşturmamıştır.

Anahtar Kelimeler: Ozon; *Daphnia magna;* İnsektisit; Fungusit; Bozunma; Toksik

1. Introduction

Pesticides are widely used pre-and post-harvest to meet the nutritional needs of a growing population, thus reducing product losses due to diseases, pests and weeds. Although pesticides have many benefits, their unconscious or intensive use during cultivation or storage has resulted in the presence of residual compounds or its degradation products in food products. In addition to contaminating food products, pesticide residues also create serious problems in the soil, on the ground, in surface water and in the air, therefore creating public and regulatory concern (Albanis et al 1998; Horvitz & Cantalejo 2014).

Pesticide residues on food products can be reduced through various methods, including washing with water or soaking in several chemical solutions, e.g. chlorine, ozone, hydrogen peroxide, salts, and detergents. The degradation mechanism of pesticides relies on oxidation, hydrolysis, reduction, photolysis, metabolism, temperature and pH (Bajwa & Sandhu 2014). Chemical oxidation is one of the most promising applications for destroying pesticides, chemical residues, and mycotoxins, converting non-biodegradable organic materials into biodegradable forms and reducing the microbial load of food products or water. Ozonation is considered to be one of the best variations of chemical oxidation. Ozone can be combined with hydrogen peroxide and UV radiation, which are other hydroxyl radical-based advanced oxidation techniques (Karaca & Velioglu 2007; Karaca et al 2010). The advantages of ozone as an oxidant are that it provides oxygen to the oxidizing medium and that no harmful substances are formed in this environment. Ozone is also used efficiently for industrial, domestic and drinking water purification (Wu et al 2007).

A number of studies indicate that some pesticides are successfully degraded with ozone in aqueous solutions. Azinphos-methyl, captan and formenate can be degraded by a combination of ozone and chlorine. Dipping apples into 0.25 mg kg^{-1} ozonated water reduced the contents of these three pesticides by 75%, 72% and 46%, respectively. The maximum degradation rate of azinphos-methyl was 83% in a model system (Ong et al 1996). Hwang et al (2001) reported that mancozeb and ethylene-thiourea (ETU) in apples can be decreased by 56-97% with 1-10 mg kg^{-1} ozone, and the ETU was completely removed with 1 and 3 mg kg^{-1} ozone. In another study it was shown that an ozone wash with 3 mg kg^{-1} ozone was the most effective treatment for mancozeb and ETU removal. The authors also indicated that the degradation by-products of some organophosphate pesticides might be more toxic than the initial compound (Hwang et al 2002). Wu et al (2007) reported that 1.4 mg kg^{-1} ozone was effective to degrade 60-99% of 0.1 mg kg^{-1} diazinon, parathion, methyl-parathion and cypermethrin within 30 min. The degradation rate was highly dependent on the dissolved ozone, and the maximum removal was detected at 15-20 °C. Kim et al (2000) treated soybeans with 0.3 mg kg^{-1} ozonated water for 30 min and determined the changes on the carbendazim, captan, diazinon, fenthim, dichlorvos and chlorpyriphos residues. The ozone treatments destroyed residues better than water itself. A treatment temperature of 30 °C was found to be more effective than other tested

temperatures on the fenitrothion degradation by ozone in lettuce and cherry tomatoes, with relatively little effect on crop quality (Ikeura et al 2013). The ozone/UV/TiO_2 combination in tea leaves reduced the cypermethrin and malathion residues by 80% and 78%, respectively (Lin et al 2012). Five minutes of ozonation reduced the tetradifon residue 98.6% in lemons and 94.2% in grapefruits (Kusvuran et al 2012). The ozone flow at 500 mg kg^{-1} reduced chlorfluazuron and chlorothalonil residues 75% and 77%, respectively, in vegetables (Chen et al 2013). The storage of table grapes in an ozone atmosphere accelerated fenhexamid, cyprodinil and pyrimethanilin degradation (Karaca et al 2012). In olives ozonated water wash for 5 min reduced chlorpyrifos, beta-cyfluthrin, alpha-cypermethrin and imidacloprid contents by 38%, 50%, 55% and 61%, respectively (Kırış & Velioglu 2016).

Daphnia magna, a cladoceran fresh water flea, is an important bio indicator (Martins et al 2007) used in the evaluation of the toxicity levels of pesticides and pesticide degradation products in aquatic ecosystems (Sanchez-Bayo & Goka 2006). During the ozonation process, degradation products are formed but chemical compositions cannot be identified. In vivo and in vitro toxicity tests are important to specify the effects of degradation products on human and animal health.

Previous studies have demonstrated that most pesticides can be degraded with ozone treatment. However, ozonation may produce by-products caused by the reaction between ozone and the pesticide and, as shown in this paper, degradation products are formed but their chemical structure cannot be easily identified and this product can be more toxic than the initial product. The aim of this study is to reveal the effects of ozone treatment on the degradation and toxicity of nine pesticides belonging to six different groups.

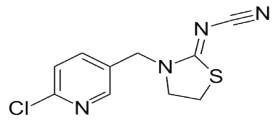
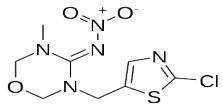
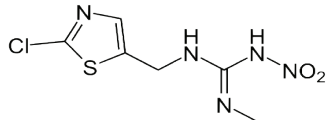
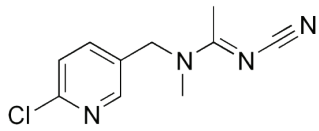
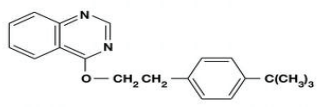
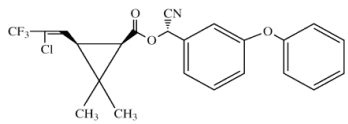
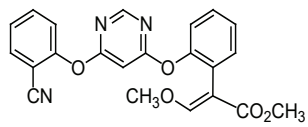
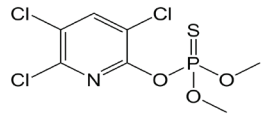
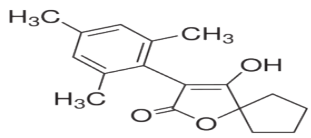
2. Material and Methods

Names, sources, functions, sub-groups, purities, stock solution concentrations (approx. 10000 mg kg^{-1}), open structures and the chromatographic method of tested pesticides were given in Table 1. Test solutions were freshly prepared by dissolving the pesticides with a few drops of dimethylsulfoxide (fenazaquin, clothianidin, thiacloprid and acetamiprid) or acetonitrile (lambda cyhalothrin, azoxystrobin, chlorpyrifos, spiromesifen and thiamethoxam) and then diluting the solution to its final volume using ASTM Type 2 high-purity water (TKA Scientific, Niederelbert, Germany) and citrate-buffer solutions (10^{-3} M at pH 5.5 and pH 6.5). Because of the high dilution rate and the use of control solutions in the toxicity experiments, the use of acetonitrile did not result in any problems. Each test solution of pesticides was diluted to concentration of 10 mg kg^{-1} from the stock solutions.

2.1. Ozonation apparatus and procedure

Ozone was produced from air using a corona discharge ozone generator (OG-20, Opal, Turkey), with a production capacity of 20 g h^{-1} of ozone. The pesticide solutions were ozonated in 30 mL Falcon test tubes; alternatively, in cases where large volumes of solvent were needed because of a pesticide's low toxicity, 250 mL glass bottles (Isolab, Boro 3.3, Wertheim, Germany) were used for the pesticide degradation and toxicity tests. Tube (or bottle) caps had a hole in the centre, allowing a tube to be passed through. The tubing was connected to a stainless steel solvent inlet filter (10 µm pore size) (Fisher Scientific, Schwerte, Germany). The filter was kept at the bottom of the tube (or bottle) during the ozonation process, allowing efficient ozone diffusion in the liquid phase. The caps also had 6-8 other small holes around the centre hole to permit the release of ozone. The ozone flow was adjusted to be 600 mL min^{-1} with a "Riteflow" flowmeter, size 2, (Bel-Art Products, Pequannock, USA). All ozonation processes were performed at 15 °C, using a cooling water bath (Polyscience, USA). After the ozonation was complete, the reaction was stopped using 10 µL of 5.2 g L^{-1} Difco neutralizing buffer (Cat. No. 236210, Becton, Dickinson and Sparks, USA). All experiments were conducted in triplicate in a fume hood.

Table 1- Chemical structures and properties of the studied pesticides

Active substance/ sources	*Function/ sub-group*	*Purity (%)/ stock solution used (mg kg^{-1})*	*Open structure*	*Analysis method*
Thiacloprid/ BAYER	Insecticide/ neonicotinoid	99.7/ 10000		HPLC
Thiamethoxam/ SYNGENTA	Insecticide/ neonicotinoid	99.7/ 20000		HPLC
Clothianidin/ BAYER	Insecticide/ neonicotinoid	99.7/ 10000		HPLC
Acetamiprid/ AGRO-BEST	Insecticide neonicotinoid	99.0/ 10000		HPLC
Fenazaquin/ AGRO-BEST	Acaricide/METİ	96.0/ 10000		HPLC
Lambda cyhalothrin/ DR. EHRENS-TORFER	Insecticide/ Pyrethroid	98.0/ 10000		GC/µECD GC/MS
Azoxystrobin/ SYNGENTA	Fungicide/ methoxyacrylate	98.0/ 10000		HPLC
Chlorpyrifos/ DR. EHRENS-TORFER	Insecticide/ organophosphate	98,5/ 10000		GC/µECD GC/MS
Spiromesifen/ BAYER	Insecticide & acaricide/tetronic acid derivative	99.9/ 10000		LC-MS/MS

2.2. Analysis of pesticides

The concentration of thiacloprid, acetamiprid, clothianidin and thiamethoxam measured with HPLC (Shimadzu, Japan) consisted of a LCX-20AD pump, SPD-M20A diode array detector (DAD), DGU 20A5-E degasser, and a CTO-10ASVP column oven. The working temperature and injection volume were 25 ºC and 20 µL, respectively, and the detection wavelength was 242 nm for thiacloprid and acetamiprid, 267 nm for clothianidin and 252 nm for thiamethoxam. A mixture of methanol water (50:50 v v^{-1} for thiacloprid and acetamiprid; 60:40 v v^{-1} for clothianidin and thiamethoxam) at a flow rate of 0.5 mL min^{-1} was used as the mobile phase under isocratic conditions. Samples were filtered through a 0.45 µm PTFE filter (Millipore, Millex-LCR) and injected directly. The concentrations of fenazaquin and azoxystrobin were measured using an Agilent (1100) HPLC system that consisted of a gradient elution pump and a DAD detector. The working temperature, injection volume and detection wavelengths were 25 ºC, 20 µL, and 216 nm (fenazaquin) and 254 nm (azoxystrobin), respectively. A mixture of acetonitrile water (90:10 v v^{-1} for fenazaquin; 37:63 v v^{-1} for azoxystrobin) at a flow rate of 1.0 mL min^{-1} was used as the mobile phase under isocratic conditions. A Nucleosil (Phenomenex, Torrance, CA, USA) or Inertsil (GL Sciences, Japan) column (C18, 5 µm; 250 mm × 4.6 mm) was used in the HPLC analyses. The reductions in the pesticide contents were calculated from the reduction of the peak areas after ozone treatments.

The concentrations of lambda cyhalothrin and chlorpyrifos were measured using a GC/µECD. The Agilent (6890N GC) system consisted of an Agilent 6890 series auto sampler and a fused-silica capillary column HP-5 (30 m x 0.25 mm ID and 0.25 µm film thickness) (Chrom Tech., Apple Valley, MN, USA). The split ratio was 50:1 and the carrier gas was 99.999% helium at 1 mL min^{-1}. The working temperature, detector temperature and injection volume were 250 ºC, 300 ºC and 1 µL, respectively. The column program was 70 ºC (2 min) to 280 ºC at 25 ºC min^{-1} (7 min). The detector's make-up gas was nitrogen (99.999%) at 59 mL min^{-1}. The total analysis time was 17.40 min. For the GC analyses, samples were treated as follows: 10 g of sample was weighed into 50 mL centrifuge tube and 10 mL of acetonitrile, 4 g of anhydrous $MgSO_4$ and 0.5 g of NaCl were added. Tube was shaken immediately for 2 min. The extract was than centrifuged at 5000 rpm for 5 min. A 1 µL aliquot of supernatant was then injected into the GC/µECD. The concentration of the spiromesifen was measured under isocratic conditions using LC-MS/MS equipment (Waters, TQD Triple Quadrupole Mass Spectrometer) and an Acquity UPLC BEH column (C18, 2.1 × 100 mm × 1.7 µm) (Waters, USA). The working conditions are shown in Table 2.

2.3. Toxicity tests

A total of 10 neonates (age <24 h) attained from the original culture were exposed to pesticide. There was no feeding during the test. The toxicity was expressed by the median lethal concentration, that is, the dose required to kill half of the daphnid members of LC_{50} (median lethal concentration) exposure. After 24h-48h the live *D. magna* were counted. Exposure to the different concentrations was carried out in triplicate. LC_{50} values were calculated using the regression line obtained by plotting the concentration against the death percentage on a probit scale, and the results were assessed with probit analysis (SPSS 22.0v.). (Fikirdeşici et al 2012). A total of 10 neonates (age <24 h) obtained from the original culture were exposed to five different concentrations of clothianidin (120000, 125000, 130000, 135000, 140000 µg L^{-1}); thiamethoxam (70000, 110000, 150000, 190000, 230000 µg L^{-1}); fenazaquin (1, 5, 9, 13, 17 µg L^{-1}); lambda cyhalothrin (0.001, 0.005, 0.009, 0.013, 0.017 µg L^{-1}); azoxystrobin (50, 100, 150, 200, 250 µg L^{-1}); chlorpyrifos (0.3, 0.6, 0.9, 1.2, 1.5 µg L^{-1}); and spiromesifen (1, 1.2, 1.4, 1.6, 1.8 µg L^{-1}).

2.4. Statistical analysis

Experimental results were expressed as the means±standard errors. Two-way ANOVA was performed using SPSS for Windows (ver. 10.1, USA). Significant differences between the means

Table 2- LS-MS/MS working conditions for the determination of spiromesifen

Equipment/ Column	Water, Model TQD Triple Quadrupole Mass Spectrometer Acquity UPLC BEH C18 2.1 x 100 mm x 1.7 µm					
Mobil phases	A1: 2 mM ammonium format containing MeOH: water (10:90) B1: 2 mM ammonium format containing MeOH: water (95:5) A2: MeOH: water (50:50) B2: MeOH: acetonitrile (50:50) Weak needle wash and seal wash: water: MeOH (95:5) Strong needle wash: MeOH					
Inlet programme:	Gradient Time Flow %A %B - 0.45 90 10 1.00 0.45 90 10 12.00 0.45 5 95 13.00 0.45 5 95 13.05 0.45 90 10 16.00 0.45 90 10 Seal wash: 5 min, run time: 15 min, max pressure: 1500 psi					
Autosampler	Full loop Wash solvents: 600 µL weak, 300 µL strong Column temp.: 50 ºC Sample temp.: 10 ºC					
Injection volume:	20 µL					
Tune parameters:	Polarity: ES+ or ES-; capillary (kV): 1.00; cone (V): compound dependent; extractor (V): 3; RF lens (V): 0.1; source temp.: 130 ºC Desolvation temp.: 400 ºC; cone gas flow (L h^{-1}): 50; desolvation gas flow (L h^{-1}): 900; LM1 resolution: 14; HM1 resolution: 14; ion energy 1: 0,5; entrance: 50; collision: compound dependent; exit: 50 LM2 resolution: 14; HM2 resolution: 14; ion energy 2: 0.8; gain: 1.0; multiplier (V): 650					
Spiromesifen MRM parameters						
Spiromesifen (ESI+)	Parent (m/z)	Daughter1 (m/z)	Daughter2 (m/z)	Cone (V)	Collision1 (V)	Collision2 (V)
	371.20	255.15	273.15	23	24	8

were determined using Duncan's multiple range test. Differences were considered significant at $P<0.05$. All of the experiments were performed in triplicate.

3. Results and Discussion

3.1. Thiacloprid and acetamiprid degradation by ozone treatment

The results revealed that thiacloprid degradation with ozone was quite difficult and the degradation rate was never more than 2.6% at 10 minutes of treatment time (Table 3). To determine the effects of unpractically longer treatment times, the samples were ozonated for 20 minutes and the degradation rate only reached 5% (not shown in the table). Buffer use yielded a non-significant degradation rate ($P>0.05$) as compared to treatment in water. Because the thiacloprid degradation was much lower than the expected level (20-25%) it was, therefore, not subjected to toxicity tests.

The acetamiprid degradation was found to be even more difficult, and a very long treatment time (20 minutes) yielded only 0.22% degradation in

Table 3- Effects of ozonation time and media on the degradation (%) of pesticides[a] (initial pesticide concentration and volume in ozonation media 10 mg kg^{-1} and 20 mL, respectively)

Pesticides	*Ozonation time (min)*	*Ozonation media*		
		Water	*Buffer (pH 5.5)*	*Buffer (pH 6.5)*
Fenazaquin	2	91.26±0.61^{Aa}	94.56±0.18^{Aa}	93.67±0.12^{Aa}
	5	93.38±0.21^{Aa}	96.46±0.29^{Aa}	95.70±0.32^{Aa}
Lambda cyhalothrin	1	79.21±1.74^{Ab}	82.22±0.03^{Ab}	73.21±2.40^{Aa}
	2	97.00±0.39^{Ba}	95.80±0.37^{Ba}	94.93±0.05^{Ba}
	5	98.62±0.62^{Ba}	99.19±0.16^{Ba}	98.61±0.1^{Ca}
Azoxystrobin	2	93.71±0.67^{Ac}	89.76±0.48^{Ab}	95.43±0.21^{Aa}
	5	94.68±0.17^{Ab}	95.02±0.33^{Bb}	97.76±0.15^{Ba}
	10	98.82±0.14^{Ba}	98.19±0.28^{Ca}	99.24±0.04^{Ca}
Chlorpyrifos	2	93.25±0.15^{Ac}	67.05±1.63^{Ab}	24.90±1.84^{Aa}
	5	98.96±0.04^{Bc}	72.14±0.67^{Bb}	96.85±0.32^{Ba}
	10	99.00±0.09^{Bb}	86.99±0.29^{Ca}	98.70±0.24^{Bb}
Spiromesifen	2	76.70±0.13^{Aa}	94.05±0.02^{Ab}	78.49±0.20^{Aa}
	5	86.93±0.26^{Ba}	99.00±0.03^{Bb}	82.05±0.08^{Ba}
	10	99.07±0.02^{Cb}	99.72±0.05^{Bb}	90.98±0.15^{Ca}
Clothianidin	2	88.17±0.06^{Ab}	67.24±6.92^{Ab}	66.32±2.87^{Aa}
	5	98.45±0.02^{Ba}	96.91±0.58^{Ba}	93.12±0.86^{Ba}
	10	99.08±0.02^{Ba}	99.16±0.04^{Ba}	99.16±0.46^{Ba}
Thiamethoxam	2	71.15±1.85^{Aa}	61.46±1.06^{Aa}	61.44±0.40^{Aa}
	5	85.36±0.23^{Ba}	81.44±0.02^{Ba}	79.00±0.03^{Ba}
	10	97.50±0.07^{Ca}	92.76±0.03^{Ca}	92.79±0.12^{Ca}
Thiacloprid	2	1.4±1.07^{Aa}	1.6±1.03^{Aa}	0.80±0.23^{Aa}
	6	2.6±1.14^{Ba}	0.43±0.13^{Aa}	1.41±1.85^{Aa}
	10	2.6±0.20^{Ba}	0.47±0.37^{Aa}	2.07±0.12^{Aa}
Acetamiprid	20	0.22±0.01^{a}	nd	1.28±0.18^{b}

[a], data expressed as the means ±SE of triplicate experiments. For a specific pesticide, different letters in a column are shown in uppercase (A-C) or in a row in lower case (a-c), indicating a statistically significant difference ($P<0.05$); nd, not detected.

water and 1.28% in pH 6.5 buffer; toxicity tests were also not completed for acetamiprid.

Thiacloprid and acetamiprid pesticides are not persistent in the environment because of a high water solubility (the water solubility and log octanol-water partition coefficient (log K_{OW}) of the active ingredient at 20 °C is 185 mg kg^{-1} and 1.26, respectively), resulting in the potential contamination of surface water following rainfall (EPA 2003). Krohn & Hellpointner (2002) reported that thiacloprid was stable in water between pH 5.0 and 9.0 for a relatively long period of time. Acetamiprid is relatively non-persistent and although it is mobile, rapid degradation will reduce its potential to leach to groundwater. Pitam et al (2013) reported that acetamiprid is stable in acidic and neutral conditions compared to alkaline conditions. The stability of thiacloprid and

acetamiprid in water was also indicated elsewhere (EPA 2002; 2003; Cernigoj 2007), so it is important to develop a successful method for thiacloprid and acetamiprid degradation.

3.2. Clothianidin and thiamethoxam degradation by ozone treatment

Two and 5 minutes of ozone treatment reduced the clothianidin content 88% and 98%, respectively. Extending the treatment time from 5 min to 10 min had no effect on clothianidin degradation in water. The degradation rates were 71 and 85% for thiamethoxam at 5 and 10 min, respectively (Table 3) (Figure 1). Therefore, 2 minutes of ozone treatment yielded a significant reduction for both of these two pesticides and no extended treatment times were needed. However, ozone treatment increased the toxicity for these two pesticides (Table 4) because of the formation of new compounds. The degradation products of thiamethoxam were considerably (approximately 2.7 times) more toxic than the original compound. The toxicity experiments on these two pesticides were repeated numerous times and the same results were always obtained. As indicated by Hwang et al (2002), the degradation products may have a higher toxicity than the active ingredients themselves. Thus, "the risk from pesticides in the diet cannot be completely removed by ozonation" unless the breakdown products are proven safe (Karaca & Velioglu, 2007). Degradation of clothianidin and thiamethoxam by ozone cannot be recommended because of the higher toxicity of the degradation products.

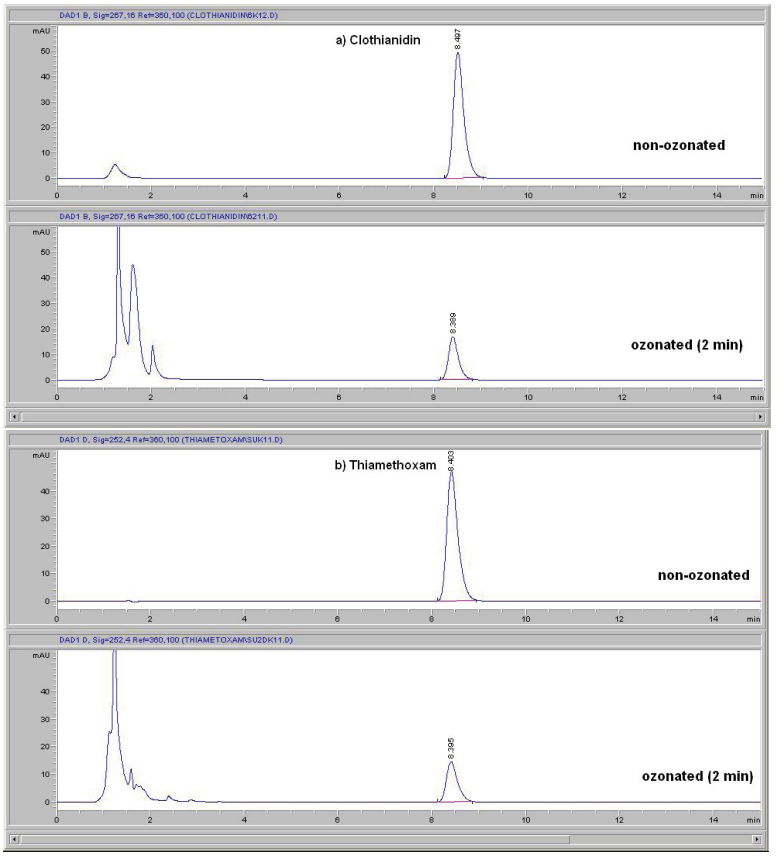


Figure 1- Effects of ozone treatment on the degradation of clothianidin (a) and thiamethoxam (b)

Table 4- The effects of ozonation on the acute pesticide toxicity on *Daphnia magna* (48 hours, 20 mL)

	LC_{50} (µg L^{-1})		*Toxicity reduction coefficient by ozonation*	*Ozonation time (sec)*	*Ozonated pesticide concentration (mg L^{-1})*
Pesticides	*Non-ozonated*	*Ozonated*			
Fenazaquin	1623	14662	9.03	60	10
Lambda cyhalothrin	0.003	0.010	3.33	120	10
Azoxystrobin	88317	197258	2.23	120	80
Chlorpyrifos	0.220	1.037	4.70	30	10
Spiromesifen	1338	1954	1.46	1200	50
Clothianidin	132403	120000	increased toxicity	120	100
Thiamethoxam	213316	77868	increased toxicity	300	100
Thiacloprid	Very low degradation by ozone. Toxicity experiments were not performed.				
Acetamiprid	Very low degradation by ozone. Toxicity experiments were not performed.				

3.3. Fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos and spiromesifen degradation by ozone treatment

Fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos and spiromesifen (Figure 2) were perfectly degraded with the application of ozone (Table 3). Degradation rates in 5-min ozonated samples in water solutions were between 87 and 99% for all of the five pesticides. Even 2 minutes of treatment time yielded > 90% degradation of all pesticides, except for spiromesifen (approximately 77%).

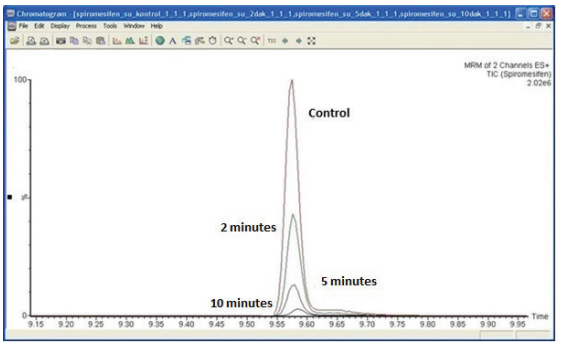


Figure 2- A sample LC-MS/MS chromatogram for spiromesifen degradation using ozone

When considering 5 min of treatment time, changing the ozonation media from water to a buffer solution did not significantly change the degradation rates of fenazaquin and lambda cyhalothrin. The use of a buffer revealed some changes on the other three pesticides; however, in all samples, the degradation rates were more than 80% (except in chlorpyrifos, which was 72% at a pH 5.5 buffer). This finding indicates that these pesticides can be successfully degraded by ozone without the need of buffer use. Ozone treatment also significantly reduced the toxicity. Reduction rates varied between 1.46 and 9.03 times depending on the pesticide tested. The degradation mechanism of azoxystrobin was probably based on a hydroxyl radical attack (Calza et al 2006; Lofrano et al 2010). This study indicated that azoxystrobin was more prone to degradation under ozone conditions than other methods (UV, photo catalytic process, etc.) (Calza et al 2006). In contrast to our study, Lozowicka et al (2014) reported that only 48.9% of azoxystrobin decomposed in 5 min in their ozonation study.

4. Conclusions

Ozone is a strong oxidizing agent and can degrade some pesticides successfully. Among the nine pesticides tested in this study, thiacloprid and acetamiprid can only be degraded to an insignificant extent (max 2.6%). Clothianidin and thiamethoxam can be easily degraded by ozone; however, their degradation products were found to be more toxic than the initial products. Other pesticides, namely,

fenazaquin, lambda cyhalothrin, azoxystrobin, chlorpyrifos and spiromesifen, were easily degraded by ozone, and their toxicities were significantly reduced. It seems that chemical composition is a more significant factor than the chemical group on the degradation rate and toxicity. Thiacloprid, acetamiprid, clothianidin and thiamethoxam all belong to the same chemical group (neonicotinoids); however, the first two pesticides were not degraded by ozone, and the latter two were degraded easily. When investigating pesticide degradation by ozone, the toxicity of some degradation products must be seriously considered because of their higher toxicity, as shown in this study.

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