F-048, in: H.V. Rectanus and P. Rodgers (Chairs). *Remediation of Chlorinated and Recalcitrant Compounds—2016.*
Tenth International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Palm Springs, CA; May 2016).
ISBN 978-0-9964071-1-3, ©2016 Battelle Memorial Institute, Columbus, OH, **www.battelle.org/chlorcon**.

Do Emerging Contaminants Translocate from Soil to Lettuce?

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ABSTRACT: Bisphenol A (BPA), nonylphenol (4-NP), benzophenone (BP), and benzophenone-3 (BP-3) are emerging contaminants (ECs) having the potential to enter the soil and food, and cause adverse effects in humans, wildlife, and the environment. In order to understand the potential translocation to edible vegetables and risk for humans due to their consumption, lettuce was cultivated for 54 d on artificially polluted soils and uncontaminated (blank) soil. BPA contamination in soil resulted in a very rapid degradation and no BPA was found in the vegetable. Lettuce from the 4-NP contaminated pot had higher concentrations compared to the vegetables from the blank pot; p-cresol was the most frequently detected 4-NP degradation product. BP and BP-3 concentrations in lettuce from the contaminated pot and the uncontaminated pot were similar.

INTRODUCTION

Emerging contaminants (ECs) are defined as any synthetic or naturally occurring chemical that is not commonly monitored in the environment. ECs include compounds from personal care products, industrial additives and byproducts, surfactants, etc. (Stuart et al., 2011). The most worrying consequence of their wide use and environmental diffusion is the increase in the possible exposure pathways for humans, such as the ingestion of food from plants cultivated on contaminated land or irrigated with reclaimed water, and the ingestion of meat/animal products from pasture on contaminated land, consumption of tap water from polluted groundwater or surface water (Weber et al., 2005).

Bisphenol-A (2,2-bis(4-hydroxyphenyl)propane, BPA) is listed as an endocrine disrupter (RIKZ 2001). Ingestion through contaminated food is the major exposure pathway for humans. Recommended reference dose (RfD) values for oral exposure are in the range of 10-50 μg/kg b.w./d (Rykowska and Wasiak 2006; USEPA 2014). BPA concentrations in soils were found to span between 0.55 and 147 μg/kg on dry weight basis (d.w.). BPA concentrations between 0.1 and 790 μg/kg fresh weight (f.w.) were found in food (Careghini et al., 2015). No data were found regarding BPA translocation from polluted soils to plants and vegetables.

Nonylphenol (NP) is a term used to refer to a wide group of isomeric compounds, among which the most produced and measured in the environment is 4-NP (USEPA 2010). NP is an estrogen agonist (ECHA 2014). Diet seems the major exposure route of NP for humans (USEPA 2010). Bakke (2003) proposed a RfD value of 100 μg/kg b.w./d, which should be protective for human health under chronic exposure, while the Danish Institute of Safety and Toxicology derived a preliminary value of 5 μg/kg b.w./d (DanishEPA 2000). Few studies are available regarding NP occurrence in soil, resulting in values between 0.01 and 2720 μg/kg d.w. Significant NP concentrations were found in various foods, with values between 0.1 and 100 μg/kg f.w. (Careghini et al., 2015), although no studies were found regarding NP translocation from soils to vegetables.

Benzophenones (BPs) include different compounds, among which diphenyl ketone (benzophenone, BP) and benzophenone-3 (2-hydroxy-4-methoxybenzophenone, BP3). BPs have adverse effects on reproduction and hormonal functions of fish (IARC 2010).

No data are available regarding BP carcinogenicity to humans, although they are classified as Group 2B substances, “possible carcinogenic to humans” (IARC 2010). The European Commission Scientific Committee on Food set a RfD for oral exposure of 10 μg/kg of body weight (b.w.)/d (EC 2005). BP and BP3 concentrations reported in literature for soils range between <0.1 and 16.55 μg/kg d.w. (Sánchez-Brunete et al., 2011). No concentration limits or guidelines have been proposed for benzophenones in soils. No studies are available about possible translocation of BPs to the edible parts of plants and vegetables in order to assess risk for humans due to their consumption (Careghini et al., 2015).

In this work, the translocation of BPA, 4-NP, BP, and BP3 to lettuce cultivated on polluted soil was investigated.

MATERIALS AND METHODS

Lettuce was selected due to its worldwide high per-capita consumption rate. Plants were cultivated in pots filled with agricultural soil (physical-chemical properties of interest in Table 1) artificially contaminated with BPA (CAS nr. 80-05-7, Sigma-Aldrich), 4-NP (CAS nr. 104-40-5, Sigma-Aldrich), BP (CAS nr. 119-61-9, Sigma-Aldrich) or BP3 (CAS nr. 131-57-7, Sigma-Aldrich), and in pots with uncontaminated soil (blank pots). In order to contaminate the soil, the pollutants were mixed and homogenized independently with 80 g of commercial fertilizer (Concime Blu, Verde Vivo), and then mixed with approximately 0.10 m^3 of soil for each lettuce pot. Based on mass balance, the expected concentrations in the contaminated soils were approximately: 120 μg/kg d.w. for BPA, 13000 μg/kg d.w. for 4-NP; 90 μg/kg d.w. for BP, and 70 μg/kg d.w. for BP3. Two days after filling the pots (time T0), three soil samples from each of them were collected by a corer and analyzed.

TABLE 1. Soil physical-chemical properties of interest before fertilization and contamination.

Physical-chemical property	Value (± standard deviation of *n* replicates)	Analytical method
Texture	Loamy sand (n=5)	ISO 11277:1998
Initial moisture	17.3±0.3 % (n=9)	ASTM D 2216-05
pH	5.3±0.5 (n=6)	ISO 10390:2005
Organic carbon content	1.70±0.08 % (n=6)	UNI EN 15169
Total nitrogen	1 g/kg d.w. (n=3)	ISO 11261:1995
Ammonia	1.5 mg N/kg d.w. (n=3)	ISO 11261:1995
Nitric nitrogen	2.0 mg N/kg d.w. (n=3)	ISO 11261:1995
Available phosphorous	32 mg P/kg d.w. (n=3)	ISO 11263:1994

Pots were equipped with an incorporated water tank, from which water rose through the soil by capillarity. The pots were located in greenhouses, where temperature and relative moisture were monitored daily, resulting in values of 28±6°C and 48±15%, respectively.

Lettuce (8 plants per pot) was cultivated in the pots for 54 d (T1); at the end of the period, the edible part of the plants was collected and analyzed; soil samples were also collected, coring the soil next to the plant roots.

Soil samples and lettuce were analyzed to quantify the pollutants and the degradation products reported in Table 2; for the 4-NP pot, p-cresol and phenol were also detected, and therefore quantified, although not explicitly mentioned in literature as 4-NP degradation products. After sampling, the vegetables were washed with deionized water, to remove the adhering soil particles.

Commercial lettuce and pre-washed bagged lettuce were also bought at a local store and analyzed to quantify the pollutants and their degradation products.

TABLE 2. Contaminants and degradation products analyzed in soil samples and lettuce. The overall coefficient of variance (CV) accounts for variability in the sample preparation procedure and the quantification step of the analyses.

Pollutant	Degradation products	Overall CV (%) (soil, lettuce)
BPA		25
	4-hydroxybenzoic acid (p-HBA)	29
	4-hydroxybenzaldehyde (p-HBAL)	29
	4-hydroxyacetophenone (p-HAP)	29
	(Spivack et al., 1994; Kang et al., 2006)	
4-NP		21
	p-HBA (Rozalska et al., 2010)	29
	p-cresol	25
	phenol	25
BP		21
	3-hydroxybenzophenone (3-HBP)	29
	4-hydroxybenzophenone (4-HBP)	29
	(Hayashi et al., 2006)	
BP3		29
	2,4-dihydroxybenzophenone (2,4-DHBP)	29
	4-HBP	29
	p-cresol	25
	(Gago-Ferrero et al., 2012; Liu et al., 2012)	

RESULTS AND DISCUSSION

Pollutant and degradation product concentrations in the analyzed soil samples and lettuce from the cultivation experiments are reported in Table 3.

BPA concentrations in the contaminated soil (BPA pot) were <3 μg/kg d.w. at all sampling times, as in the blank pots. However, two BPA degradation products (p-HBA and p-HAP) were found in the soil from the BPA pot at time T0, suggesting a very rapid BPA degradation in this matrix, as reported in Hurtado et al. (2016). No BPA (<8 μg/kg f.w.) was found in lettuce from either the blank pot or the BPA pot.

Figure 1 shows p-HBA concentrations measured in the analyzed samples from the BPA experiment. During harvesting, soil and lettuce from the blank pot exhibited p-HBA and p-HAP values always below the detection limit (<5 μg/kg d.w. in soil, <10 μg/kg f.w. in lettuce). p-HBA concentration in the soil from the BPA pot did not have a significant variation over time (5.5 - 12 μg/kg d.w.), but it was found at high concentration in lettuce (41 ± 14 μg/kg f.w.).

Figure 2 shows 4-NP concentrations measured in the analyzed samples from the 4-NP experiment. 4-NP concentration in the contaminated soil at the beginning of the experiment (T0) was significantly higher (approximately 11 mg/kg d.w.) than in the blank pot (38 μg/kg d.w.). A huge decrease in concentration occurred in the contaminated soil between T0 and T1, resulting in values of about 0.5 mg/kg d.w., while values below the detection limit (<3 μg/kg d.w.) where found in the soil from the blank pot. Lettuce from the contaminated pot had a high 4-NP concentration (174 ± 49 μg/kg f.w.) compared to lettuce from the blank pot (26 ± 4 μg/kg f.w.). As for 4-NP degradation products from the contaminated pot, only p-cresol was found in lettuce at a concentration (100 ± 60 μg/kg f.w.) significantly higher than in the lettuce from the blank pot (8 ± 1 μg/kg f.w.).

TABLE 3. Pollutant and degradation product concentrations (mean value ± standard deviation of *n* replicates) in the analyzed soil samples (μg/kg d.w.) and lettuce (μg/kg f.w.) from the cultivation experiments.

	Contaminated pots								*n*
	BPA		**p-HBA**		**p-HBAL**		**p-HAP**		
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	
T0	<3	-	12±3	-	<5	-	8.2±0.2	-	3
T1	<3	<8	5.5±0.6	41±14	<5	<10	<5	<10	7
	4-NP		**p-HBA**		**p-cresol**		**phenol**		
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	
T0	11033±2589	-	20±14	-	2.4±0.6	-	5±2	-	3
T1	514±457	174±49	<5	28±22	7±3	100±60	8±7	13±6	5
	BP		**3-HBP**		**4-HBP**				
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce			
T0	62±8	-	<2	-	<2	-			3
T1	67±18	56±19	<2	<4	<2	<4			7
	BP3		**2,4-DHBP**		**4-HBP**		**p-cresol**		
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	
T0	113±19	-	<5	-	<2	-	8±1	-	3
T1	<4	<5	<5	<10	<2	<4	<2	12±6	6
	Blank pots								*n*
	BPA		**p-HBA**		**p-HBAL**		**p-HAP**		
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	
T0	<3	-	<5	-	7±2	-	<5	-	3
T1	<3	<8	<5	<10	5±1	<10	<5	<10	3
	4-NP		**p-HBA**		**p-cresol**		**phenol**		
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	
T0	38±48	-	<5	-	8±1	-	3.6±0.7	-	3
T1	<3	26±4	<5	<10	<2	8±1	<2	33±5	3
	BP		**3-HBP**		**4-HBP**				
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce			
T0	7±3	-	<2	-	<2	-			6
T1	20±5	56±5	<2	<4	<2	<4			3
	BP3		**2,4-DHBP**		**4-HBP**		**p-cresol**		
	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	Soil	Lettuce	
T0	<4	-	<5	-	<2	-	7±1	-	3
T1	<4	13±9	<5	<10	<2	<4	7±1	11.3±0.6	3

BP and BP3 concentrations in the contaminated soils at the beginning of the experiment were significantly higher than those measured in the blank pot. During lettuce harvesting, no significant changes occurred at BP concentration in soil in the BP pot and in the blank pot. BP concentrations in lettuce from the contaminated and the uncontaminated pots were similar (Figure 3). BP3 concentration in the contaminated soil decreased over time down to a value below the detection limit. At any rate, lettuce from the BP3 pot and the blank pot had similar concentrations (Figure 4).

BP, BP3, BPA and 4-NP concentrations found in the lettuce cultivated in this study were similar to those measured in the commercial vegetables (Table 4).

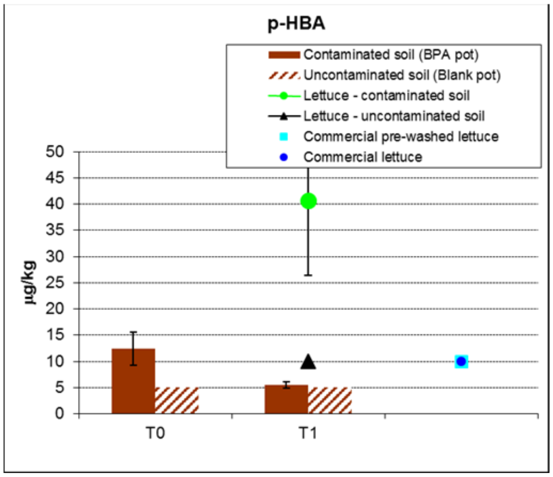

FIGURE 1. p-HBA concentrations in soil (μg/kg d.w.) and lettuce (μg/kg f.w.) from the BPA experiments and in the commercial samples. Values below the limit of quantification (LOQ) are shown as values equal to the LOQ.

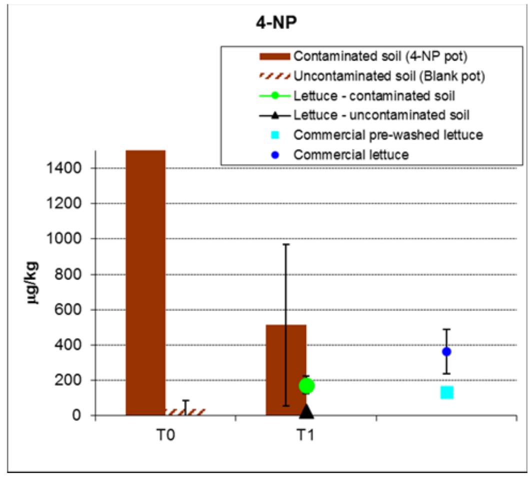

FIGURE 2. 4-NP concentrations in soil (μg/kg d.w.) and lettuce (μg/kg f.w.) from the 4-NP experiment and in the commercial samples. Values <LOQ are shown as values equal to the LOQ. 4-NP concentration at T0 in the contaminated soil is out of scale (11033 μg/kg d.w.).

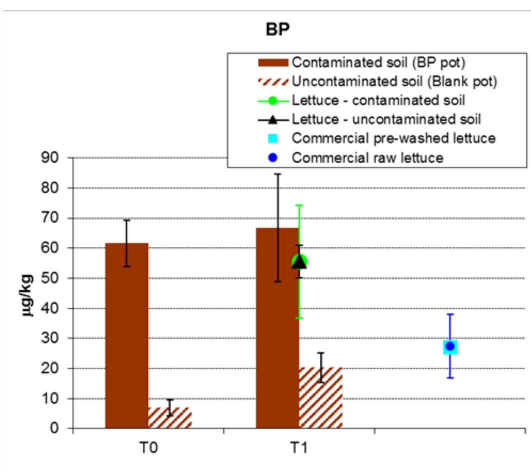

FIGURE 3. BP concentrations in soil (μg/kg d.w.) and lettuce (μg/kg f.w.) from the pots and in the commercial samples. Values <LOQ are shown as values equal to the LOQ.

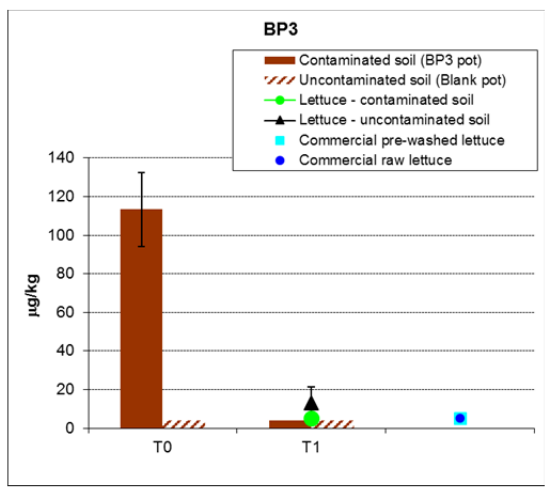

FIGURE 4. BP3 concentrations in soil (μg/kg d.w.) and lettuce (μg/kg f.w.) from the pots and in the commercial samples. Values <LOQ are shown as values equal to the LOQ.

Significant differences were found between the concentrations of p-HBA in lettuce from the BPA pot (41 μg/kg f.w.) and the blank pot (<10 μg/kg f.w.), as well as of p-cresol in the lettuce from the 4-NP pot (100 μg/kg f.w.) compared to the blank pot (8 μg/kg f.w.). p-HBA has been reported to have estrogenic properties (Pugazhendhi et al., 2005), but no RfD values can be found in literature. Many phenolic compounds, including creosols, are toxic, carcinogenic, mutagenic, and teratogenic (Autenrieth et al., 1991; Davì and Gnudi, 1999; Bruce et al., 2001; Sanders et al., 2009); a provisional RfD of 0.005 mg/kg b.w./d is reported in USEPA (1997) for p-cresol. Based on the average concentration found in lettuce cultivated on the contaminated soil for 4-NP (174 μg/kg f.w.) and p-cresol (100 μg/kg f.w.), and the average annual consumption rate of lettuce in Italy (6 kg f.w./y) (Piccinelli et al., 2010), the calculated chronic daily intake (CDI) via ingestion of this vegetable for a child of 15 kg are 0.19 μg/kg b.w./d for 4-NP and 0.11 μg/kg b.w./d for p-cresol. Compared to the RfD for oral exposure, the most critical situation is related to 4-NP, with a CDI to RfD ratio of 0.04, followed by p-cresol with a ratio of 0.02, although none of them represent a meaningful risk for humans.

TABLE 4. Pollutant and degradation product concentrations (mean ± standard deviation of three replicates) in the commercial lettuce (in μg/kg f.w.).

Degradation product	Hand-washed	Pre-washed bagged
2,4-DHBP	<10	<10
3-HBP	<4	<4
4-HBP	<4	<4
4-NP	363±125	135±21
BP	28±11	27±5
BP3	<5	<5
BPA	<8	<8
p-cresol	10.5±0.5	9±1
p-HAP	<10	<10
p-HBA	<10	<10
p-HBAL	<10	<10
phenol	8.95±0.3	<8

CONCLUSIONS

Contamination of soil with BPA resulted in a very rapid (2 d) degradation of this contaminant and the production of the degradation product p-HBA. No BPA was found in the lettuce from either the blank pot or the BPA pot. p-HBA was found at high concentrations in the lettuce from the contaminated pot; the concentration in the lettuce produced in the experiment was higher than in the commercial samples.

As for the 4-NP pot, a huge decrease in 4-NP concentration occurred in the contaminated soil between T0 and T1 (54 d). Lettuce from the contaminated pot had high 4-NP concentrations. p-Cresol was found in the lettuce from the contaminated pot at concentrations much higher than in the vegetable samples from the blank pot and in the commercial samples.

BP concentrations in the contaminated soil remained constant over time, while BP3 concentrations decreased over time. Neither the two pollutants nor their degradation products were found in lettuce from the contaminated pots at higher values than in lettuce from the blank pot.

Based on the maximum concentrations found in lettuce cultivated on the contaminated soils, the major risk for oral ingestion (lettuce consumption) is related to 4-NP, whose concentration results in a chronic daily intake to RfD ratio of 0.04.

ACKNOWLEDGMENTS

This work was financially supported by the Italian Ministry of Education, University and Research in the National Research Program 2010-2011 "Emerging contaminants in air, soil, and water: from source to the marine environment", without any involvement in the research conduction.

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