BISPHENOL A AND NONYLPHENOL TRANSFER TO VEGETABLES CULTIVATED ON CONTAMINATED SOIL

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EXTENDED ABSTRACT

A research program, financed by the Italian Ministry of Education, University and Research, started early 2013 to investigate the occurrence and fate of selected emerging contaminants in soil, water, sediments and food in Italy, in order to assess health risks and to develop control measures. Bisphenol A (BPA) and Nonylphenol (NP) are both emerging pollutants listed as endocrine disrupters, for which diet seems the major exposure route for humans. In this specific work, the transfer of BPA and NP to edible vegetables cultivated on polluted soil was investigated. Lettuce and tomatoes were selected due to their high worldwide per capita consumption rates.

Plants were cultivated in pots filled with agricultural soil artificially contaminated with BPA or 4-NP and in pots with uncontaminated soil (blank pots). After filling (time T_0), the pots were located in greenhouses, daily monitored and periodically watered. Lettuce was cultivated for about 55 d (T_1). Tomatoes were collected at different times (T_1 = 52 d, T_2 = 66 d, T_3 = 79 d and T_4 = 89 d) due to the different ripening time of the fruits. Soil samples were also collected, by coring the soil next to the plants. Commercial pre-washed lettuce, raw lettuce and tomatoes from conventional farming and organic farming were also bought at a local store and analyzed for BPA and 4-NP quantification.

Contamination of soil with BPA resulted in a very rapid (2 days) degradation of this contaminant and the production of two byproducts (p-HBA and p-HAP). No BPA was found at all sampling times in the vegetables from either the blank pots or the BPA pots. p-HBA was found at high concentrations in the vegetables from the contaminated pots; the concentration in the lettuce produced in the experiment was higher than in the commercial samples. Though p-HAP was found in some soil samples from the contaminated pots, it was never found in the vegetables.

As for 4-NP pots, a huge decrease in 4-NP concentration occurred in the contaminated soils between T_0 and T_1. Vegetables from the contaminated pots had high 4-NP concentrations. p-Cresol was the most frequently detected byproduct, which was found in the vegetables from the contaminated pots at concentrations higher than in the commercial samples. Phenol was found in the tomatoes from the contaminated pot, at concentrations higher than in the commercial tomatoes.

Keywords: bisphenol A, nonylphenol, cultivation, polluted soil, lettuce, tomato

1. INTRODUCTION

Emerging pollutants (EPs) are defined as any synthetic or naturally occurring chemical that is not commonly monitored in the environment. 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 plants cultivated on contaminated land or irrigated with reclaimed water, 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; Molnar et al. 2013).

EPs include the endocrine disrupter bisphenol-A (2,2-bis(4-hydroxyphenyl)propane, BPA) (Rykowska and Wasiak 2006) and the estrogen agonist nonylphenol (NP) (ECHA 2014). Actually, NP is a term used to refer to a wide group of isomeric compounds ($C_{15}H_{24}O$), among which the most produced and measured in the environment is 4-NP (USEPA 2010). For both BPA and NP, diet seems the major exposure route for humans (Christensen et al. 2012; USEPA 2010).

BPA concentrations in soils were found to span between 0.55 and 147 µg/kg on dry weight basis (d.w.), with the highest values generally found in agricultural fields amended with biosolids or irrigated with wastewater. BPA concentrations between 0.1 and 790 µg/kg fresh weight (f.w.) were found in food. As for NP, few studies are available about its 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).

In this work, the transfer of BPA and 4-NP to edible vegetables cultivated on polluted soil was investigated. Lettuce and tomatoes were selected due to their worldwide high per-capita consumption rates.

2. MATERIALS AND METHODS

Plants were cultivated in pots filled with agricultural soil (physicochemical properties reported in Table 1) artificially contaminated with BPA or 4-NP and in pots with uncontaminated soil (blank pots). In order to contaminate the soil, BPA or 4-NP were mixed, pulverized and homogenized 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 and 0.13 m^3 of soil for each tomato pot.

All pots were internally coated with unblanched cotton to avoid direct contact between the soil and the plastic walls. Pots for the lettuce growth were equipped with an incorporated water tank, from which water rose through the soil by capillarity, while pots for tomatoes had an automatic watering system ensuring enough water for plant growth and no percolation. The pots were located in greenhouses, where temperature and relative moisture were daily monitored, resulting in values of 28±6°C and 48±15% respectively.

Two days after filling the pots (time T_0), three soil samples from each of them were collected by a corer and analyzed. Vegetable samples before cultivation were not analyzed due to insufficient amount of lettuce for chemical analysis and no tomatoes on the plants.

Physicochemical property	Value *(replicates)*	Analytical method
Texture	Loamy sand *(n=5)*	ISO 11277
Initial moisture	17.3±0.3 % *(n=9)*	ASTM D 2216-05
pH	5.3±0.5 *(n=6)*	Ministry decree 13 September 1999, III.1
Organic carbon content	1.70±0.08 % *(n=6)*	UNI EN 15169
Total nitrogen	1 g/kg d.w. *(n=3)*	Ministry decree 13 September 1999, VII.1
Ammonia	1.5 mg N/kg d.w. *(n=3)*	Ministry decree 13 September 1999, XIV.7
Nitric nitrogen	2.0 mg N/kg d.w.*(n=3)*	Ministry decree 13 September 1999, XIV.8
Available phosphorous	32 mg P/kg d.w.*(n=3)*	Ministry decree 13 September 1999, XV.3
Available potassium	112 mg K/kg d.w.*(n=3)*	Ministry decree 13 September 1999, XIII.5

Table 1. Physicochemical properties of the soil before fertilization and contamination.

Lettuce (8 plants per pot) was cultivated for 58 d in the blank pot, 56 d in the BPA pot and 51 d in the 4-NP pot. At the end of this period (time T_1), the edible part of each plant was collected and analyzed; soils samples were also collected, coring the soil next to the plant roots.

Tomatoes (6 plants per pot) were collected at different times (T_1 = 52 d, T_2 = 66 d, T_3 = 79 d and T_4 = 89 d) due to the different ripening time of the fruits. In the BPA pot, tomatoes could not be collected at time T_2, because no ripe fruits were available at that time. Soil samples were also collected at the different times, by coring the soil next to the plants from which tomatoes had been grabbed.

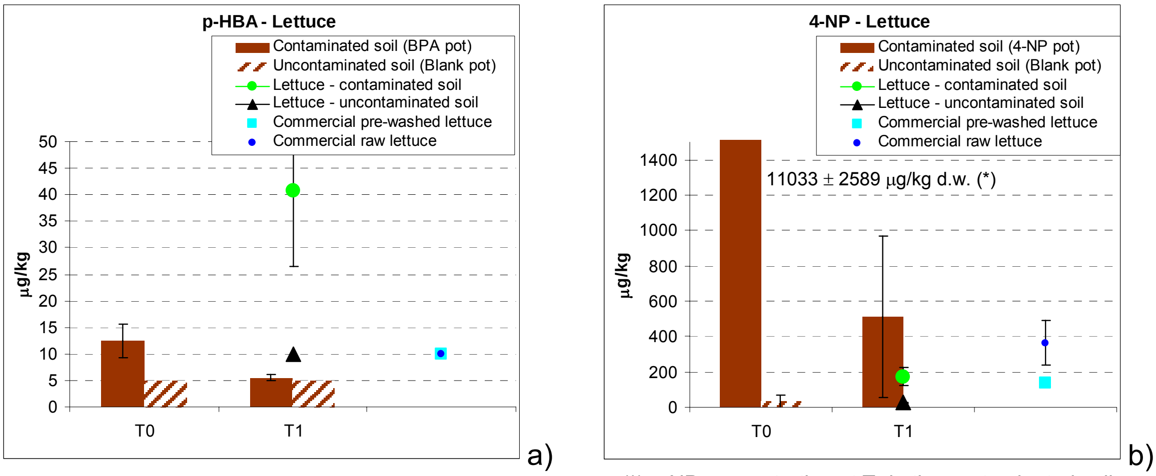
Commercial pre-washed lettuce, raw lettuce and tomatoes from conventional farming and organic farming were also bought at a local store and analyzed to quantify BPA and its byproducts (hydroxybenzoic acid p-HBA, hydroxybenzaldehyde p-HBAL, hydroxyacetophenone p-HAP), as well as 4-NP and its byproducts (p-HBA, 1,4-benzoquinone 1,4-BC, p-cresol, phenol).

BPA, 4-NP and their byproducts were extracted from soil and chopped vegetables (15 g) with ethyl acetate (25 ml) in a sonication bath (15 min, three times), after acidification with phosphoric acid 50% v/v (1 ml) and addition of Celite 545. A clean-up step was performed by a QuEChERS method, using Supel™ QuE PSA/C18/ENVI-Carb (AC). The organic phase with the analytes of interest was recovered after phase separation, and filtration on Whatman 1PS Phase Separator Paper to remove residual water. A final volume of 1 ml was obtained by evaporation under nitrogen. N,O-bis(trimethylsilyl)trifluoroacetamide with 1% of trimethylchlorosilane were used for silylation at ambient temperature (22 °C) for 24 hours, before Triple Quadrupole GC-MS/MS analysis in Multiple Reaction Monitoring mode.

3. RESULTS

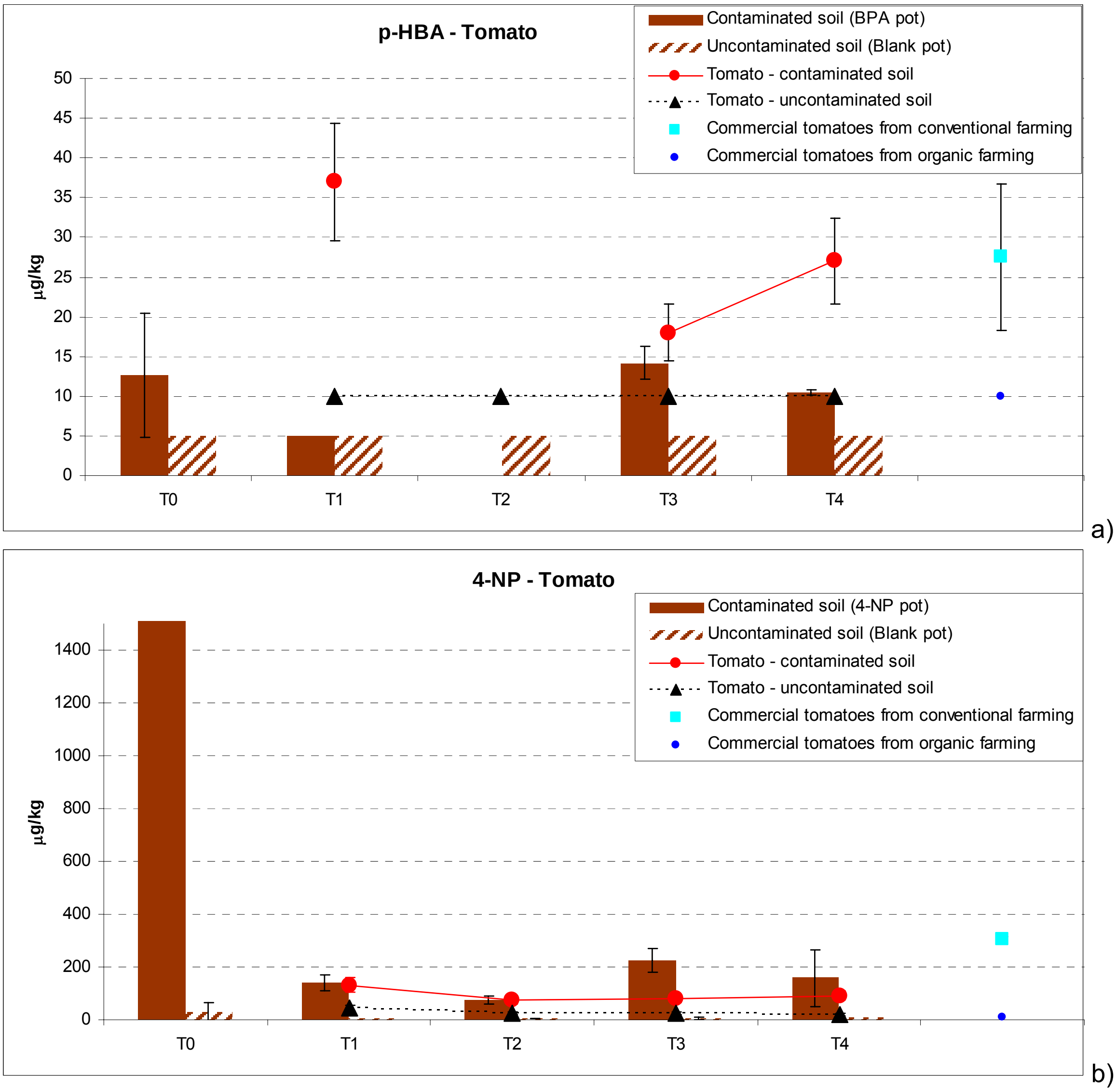
BPA concentrations in the contaminated soils (lettuce and tomato BPA pots) were <3 μg/kg d.w. at all sampling times, as in the blank pots. However, two BPA byproducts (p-HBA and p-HAP) were found in the soils from the BPA pots at time T_0 (two days after their preparation), suggesting a very rapid BPA degradation in this matrix. No BPA (<8 μg/kg f.w.) was found at all sampling times in the vegetables from either the blank pots or the BPA pots. Therefore, BPA results will not be further discussed in the following.

Figure 1 (lettuce) and Figure 2 (tomato) shows p-HBA and 4-NP concentrations measured in the analyzed samples.



(*) 4-NP concentration at T_0 in the contaminated soil.

Figure 1. p-HBA (a) and 4-NP (b) concentrations in soil (µg/kg d.w.) and lettuce (µg/kg f.w.) from the pots, and in the commercial samples.



(*) 4-NP concentration at T_0 in the contaminated soil.

Figure 2. p-HBA (a) and 4-NP (b) concentrations in soil (µg/kg d.w.) and tomatoes (µg/kg f.w.) from the tomato pots, and in the commercial samples.

9318 ± 4967 µg/kg d.w. (*)

During harvesting, soils and vegetables from the Blank pots 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 vegetables). p-HBA concentration in the soils from the BPA pots did not have a significant variation overt time (5 - 14 μg/kg d.w.), but it was found at high concentrations in the vegetables (lettuce: 41 ± 14 μg/kg f.w., tomatoes: 18 - 37 μg/kg f.w.). As for p-HAP in the soil samples from the BPA pots, it could be quantified at T_0 (10 ± 4 μg/kg d.w.) and in the tomato pot at T_3 and T_4 (9 μg/kg d.w.), while it was never found in the vegetables (<10 μg/kg f.w.).

4-NP concentrations in the contaminated soils (4-NP pots) at the beginning of the experiment (T_0) were significantly higher (approximately 10 mg/kg d.w.) than those measured in the Blank pots (32 ± 35 μg/kg d.w.). A huge decrease in concentration occurred in the contaminated soils between T_0 and T_1, resulting in values of about 0.5 mg/kg d.w. in the lettuce pot and 0.2 mg/kg d.w. in the tomato pot, while values close to the detection limit (3 μg/kg d.w.) where found in the soils from the Blank pots. Vegetables from the contaminated pots had high 4-NP concentrations (lettuce: 174 ± 49 μg/kg f.w., tomatoes: 74 - 132 μg/kg f.w.) compared to the vegetables from the Blank pots (lettuce: 26 ± 4 μg/kg f.w., tomatoes: 19 - 45 μg/kg f.w.). As for 4-NP byproducts, p-cresol was the most frequently detected, which was found in lettuce (100 ± 60 μg/kg f.w.) and tomatoes (139 - 632 μg/kg f.w.) from the contaminated pots, while phenol was found in the tomatoes from the 4-NP pot (up to 220 μg/kg f.w. at time T_4).

5. CONCLUSIONS

Contamination of soil with BPA resulted in a very rapid (2 days) degradation of this contaminant and the production of two byproducts (p-HBA and p-HAP). No BPA was found at all sampling times in the vegetables from either the blank pots or the BPA pots. p-HBA was found at high concentrations in the vegetables from the contaminated pots; the concentration in the lettuce produced in the experiment was higher than in the commercial samples. Though p-HAP was found in some soil samples from the contaminated pots, it was never found in the vegetables.

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