CEST 2015
Proceedings of the 14th International Conference on Environmental Science and Technology
Rhodes, Greece, 3-5 September 2015

TRANSFER OF BENZOPHENONES FROM SOIL TO PLANTS

CAREGHINI A., CORTI M., MASTORGIO A., ROMELE L., SAPONARO S. and SEZENNA E.

Politecnico di Milano, DICA – Environmental Section, p.za Leonardo da Vinci 32, 20133, Milano (MI), Italy
E-mail: elena.sezenna@polimi.it

ABSTRACT

Benzophenones (BPs) include different compounds whose molecular structure is based on diphenyl ketone (benzophenone, BP). Some benzophenones have been reported to have estrogenic activity and some compounds have been classified as possibly carcinogenic to humans. Specific studies should be carried out about possible pollution of soils due to the use of biosolids and/or treated wastewater in agriculture practices, in particular to understand the transport of BPs to the edible parts of plants and risk for humans due to their consumption.

In this work, the transfer of BP and BP-3 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 BP or BP-3 and in pots with uncontaminated soil (blank pots). At the beginning of tests (time T_0) three soil samples from each pot were collected and analyzed. Lettuce was cultivated for about 55 d; at the end of this period (time T_1), the edible part of each plant was collected and analyzed. Tomatoes were collected at different times (T_1 = 54 d, T_2 = 70 d, T_3 = 83 d and T_4 = 92 d) due to the different ripening time of the fruits; in the BP-3 pot, tomatoes could be collected only at time T_3 and T_4, because no ripe fruits were available before. Soil samples were also collected at the different times, by coring the soil next to the lettuce plant roots or 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.

BP concentrations in the contaminated soils remained constant over time except for the tomato pot from time T_3, while BP-3 concentrations decreased over time. As for the vegetables, BP (at T_2) and BP-3 (at T_3 and T_4) in tomatoes were higher than in the Blank. BP and BP-3 concentrations found in the vegetables cultivated in this study were similar to those measured in the commercial vegetables, except for BP-3 in tomatoes at T_3.

Keywords: benzophenone, benzophenone-3, cultivation, polluted soil, lettuce, tomato

1. Introduction

Benzophenones (BPs) include different compounds, whose molecular structure is based on diphenyl ketone (benzophenone, BP). The physical-chemical properties and the environmental behavior of these compounds do not differ significantly from those of the parent compound BP, except for benzophenone-3 (2-hydroxy-4-methoxybenzophenone, BP-3), which is also the most commercialized compound of the group (León *et al*. 2010; Gago-Ferrero *et al*. 2012; Liu *et al*. 2012; Zhang *et al*. 2013).

BPs are used as flavor ingredients and additives for plastics, coatings and adhesive formulations. They are also used in laundry and household cleaning products and in the manufacture of insecticides and pharmaceuticals. BP-3 is commonly used worldwide as a UV filter in cosmetic formulations (Zhang *et al*. 2011; Liu *et al*. 2012).

BPs have adverse effects on reproduction and hormonal functions of fish (IARC 2010). No data are available on BPs carcinogenicity to humans, though they are classified as Group 2B substances, “possible carcinogenic to humans” (NTP 2006; IARC 2010). The European

Commission Scientific Committee on Food set a RfD for oral exposure of 10 µg/kg b.w./day (EC 2005). A limit concentration of 0.6 mg/kg in food was set for the sum of BP and 4-methylbenzophenone (IARC 2010).

The use of biosolids and/or treated wastewater in agriculture practices can cause pollution of soils. BP and BP-3 concentrations reported in the literature for this environmental medium range between <0.1 and 16.55 µg/kg d.w. (Jeon *et al*. 2006; Sánchez-Brunete *et al*. 2011). No concentration limits or guidelines have been proposed for benzophenones in soils. No studies are available about possible transfer of BPs to the edible parts of plants and vegetables in order to assess risk for humans due to their consumption.

In this work, the transfer of BP and BP-3 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 BP or BP-3 and in pots with uncontaminated soil (blank pots). In order to contaminate the soil, BP or BP-3 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.

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

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

Figure 1: Lettuce (a) and tomato (b) pots in the greenhouses during cultivation.

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 (Figure 1), where temperature and relative moisture were daily monitored, resulting in values of 28±6°C and 48±15% respectively.

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.

Lettuce (8 plants per pot) was cultivated for 58 d in the blank pot, 56 d in the BP pot and 51 d in the BP-3 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 = 54 d, T_2 = 70 d, T_3 = 83 d, T_4 = 92 d and T_5 = 112 d) due to the different ripening time of the fruits. In the BP pot, tomatoes could not be collected at time T_4, because no ripe fruits were available at that time; the same occurred in the BP-3 pot at time T_1, T_2 and T_5. 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 BP and its byproducts (benzhydrol, 3-hydroxy benzophenone, 4-hydroxy benzophenone), as well as BP-3 and its byproducts (2,4-dihydroxybenzophenone, p-cresol).

BPs 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 70 °C for 30 minutes, before Triple Quadrupole GC-MS/MS analysis in Multiple Reaction Monitoring mode.

3. Results

Figure 2 (lettuce) and Figure 3 (tomato) shows BP and BP-3 concentrations measured in the analyzed samples. BP and BP-3 concentrations in the contaminated soils at the beginning of the experiment (BP T_0 and BP-3 T_0) were significantly higher than those measured in the blank pots (Blank T_0).

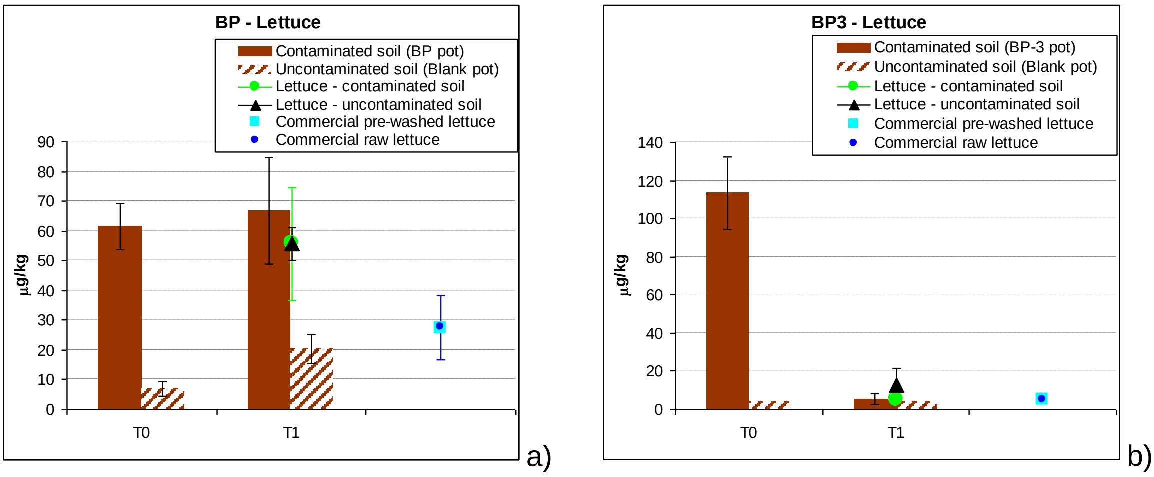
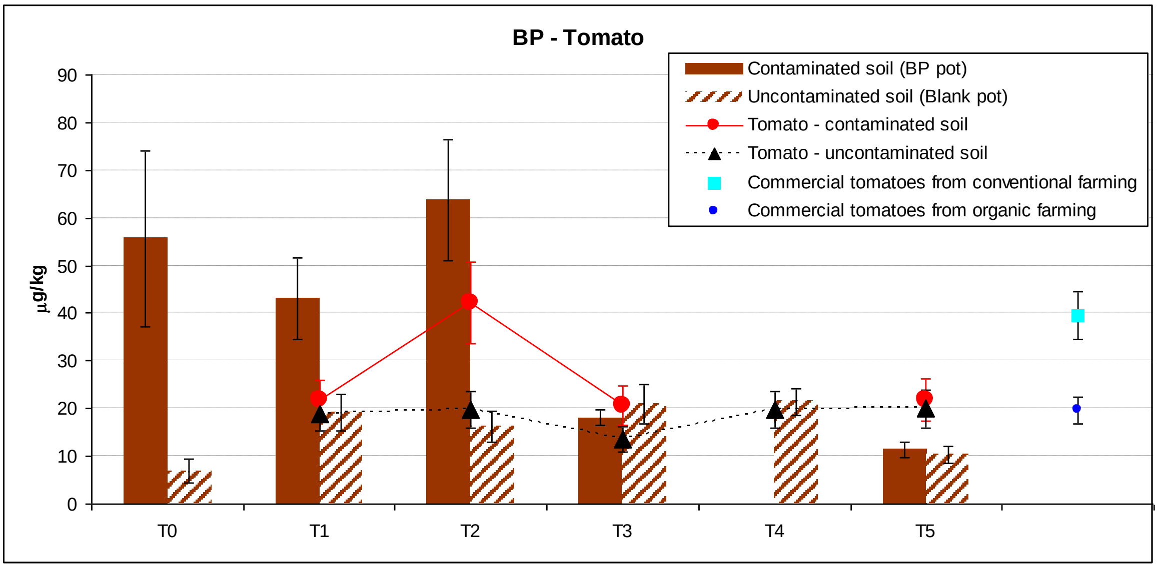
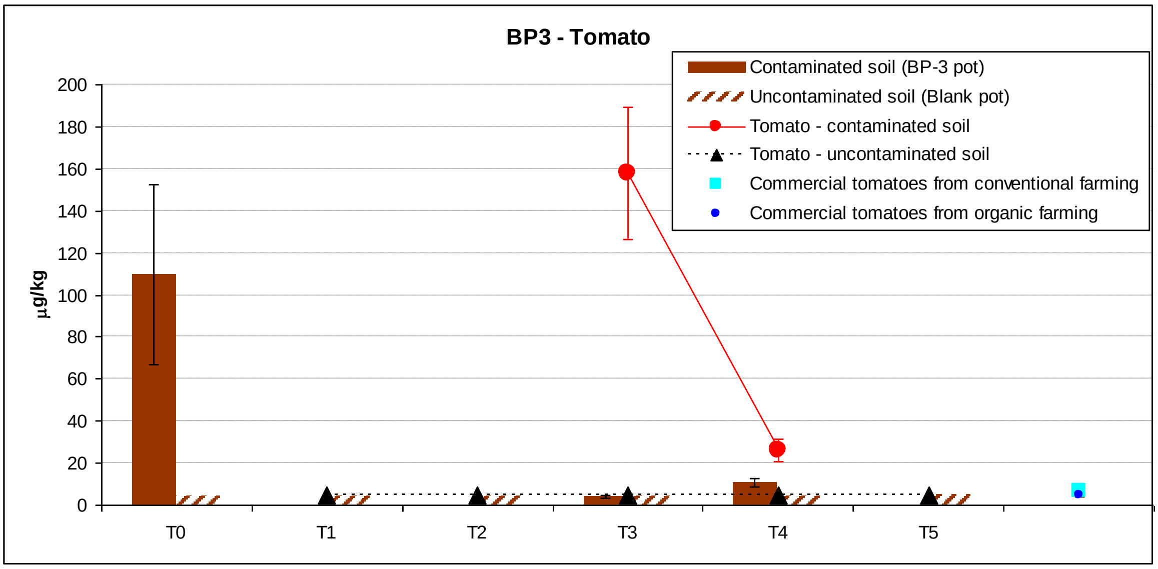


Figure 2: BP (a) and BP-3 (b) concentrations in soil (µg/kg d.w.) and lettuce (µg/kg f.w.) from the pots, and in the commercial samples.



a)



b)

Figure 3: BP (a) and BP-3 (b) concentrations in soil (µg/kg d.w.) and tomatoes (µg/kg f.w.) from the tomato pots, and in the commercial samples.

During lettuce harvesting, no significant changes occurred at BP concentration in soil in the BP pot and in the Blank pot. As for tomatoes, BP concentration in soil in the Blank pot remained constant over time, while in the BP pot a significant decrease occurred from time T_3. BP concentrations in the vegetables from the contaminated and the uncontaminated pots were similar, with the exception of tomatoes picked up from the BP pot at time T_2, which was higher than in the Blank. Benzhydrol was detected in soils from the BP pots (lettuce and tomato) at concentrations up to 18 µg/kg d.w. This byproduct was found in tomatoes at time T_2 and T_3 (3.5 µg/kg f.w.), as well as 3-hydroxy benzophenone at T_2 (6.5 µg/kg f.w.).

BP-3 concentrations in the contaminated soils decreased over time down to values close to the detection limit in both lettuce and tomato pots. At any rate, lettuce from the BP-3 pot and the Blank pot had similar concentrations, while higher values were found in the tomatoes from the BP-3 pot at time T_3 and T_4 compared to the Blank pot.

4. Conclusions

BP concentrations in the contaminated soils remained constant over time except for the tomato pot from time T_3, while BP-3 concentrations decreased over time. As for the vegetables, BP and

BP-3 in tomatoes at some sampling times were higher than in the Blank. BP and BP-3 concentrations found in the vegetables cultivated in this study were similar to those measured in the commercial vegetables, except for BP-3 in tomatoes at T_3.

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".

REFERENCES

1. EC (2005), Provisional list of monomers and additives notified to European commission as substances which may be used in the manufacture of plastics or coatings intended to come into contact with foodstuff. SANCO D3/AS D(2005). Technical report. European Commission Health & Consumer Protection Directorate-general.
2. Gago-Ferrero P., Badia-Fabregat M., Olivares A., Piña B., Blánquez P., Vicent T., Caminal G., Díaz-Cruz M. and Barceló D. (2012), Evaluation of fungal- and photo-degradation as potential treatments for the removal of sunscreens BP3 and BP1, Sci. Total Environ., **427-428**, 355-363.
3. IARC (2010), Benzophenone. IARC Monographs 101. International Agency for Research on Cancer.
4. Jeon H., Chung Y. and Ryu J. (2006), Simultaneous determination of benzophenone-type UV filters in water and soil by gas chromatography–mass spectrometry, J. Chromatogr. A, **1131**, 192-202.
5. León Z., Chisvert A., Tarazona I. and Salvador A. (2010), Solid-phase extraction liquid chromatography–tandem mass spectrometry analytical method for the determination of 2-hydroxy-4-methoxybenzophenone and its metabolites in both human urine and semen, Anal. Bioanal. Chem., **398**, 831-843.
6. Liu Y.S., Ying G.G., Shareef A. and Kookana R.S. (2012), Biodegradation of the ultraviolet filter Benzophenone-3 under different redox conditions, Environ. Toxicol. Chem., **31(2)**, 289-295.
7. NTP (2006), Toxicology and carcinogenesis studies of benzophenone (CAS No. 119–61–9) in F344/N rats and B6C3F1mice (feed studies). NTP TR 533. Technical Report. National Toxicology Program, U.S. Department of Health and Human Services. NIH Publication no. 06-4469.
8. Sánchez-Brunete C., Miguel E., Albero B. and Tadeo J (2011), Analysis of salicylate and benzophenone-type UV filters in soils and sediments by simultaneous extraction cleanup and gas chromatography–mass spectrometry, J. Chromatogr. A, **1218**, 4291- 4298.
9. Zhang T., Sun H., Qin X., Wu Q., Zhang Y., Ma J. and Kannan K. (2013), Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: Partitioning between blood and urine as well as maternal and fetal cord blood, Sci. Total Environ., **461-462**, 49-55.
10. Zhang Z., Ren N., Li Y.F., Kunisue T., Gao D. and Kannan K. (2011), Determination of Benzotriazole and Benzophenone UV Filters in Sediment and Sewage Sludge, Environ. Sci. Technol. **45**, 3909-3916.