Abstract

BACKGROUND: Bisphenol A (BPA) is a chemical widely used in polycarbonate plastics, epoxy resins. BPA is an endocrine disruptor. Residue of BPA in agricultural environments is a major concern. The objective of this study was to understand the characteristics of the uptake and distribution of BPA and its metabolites introduced into the agricultural environment to crops, and to use it as basic data for further research on reduction of BPA in agricultural products.

METHODS AND RESULTS: This study established the analysis method of BPA and its metabolites in soil and crops, and estimated the intake of BPA and its metabolites from lettuce (*Lactuca sativa*) grown in sandy loam and loam soil, which are representative soils in Korea. The two major metabolites of BPA were 4-hydroxyacetophenone (4-HAP) and 4-hydroxybenzoic acid (4-HBA). BPA, 4-HAP and 4-HBA have been analyzed by using liquid chromatography tandem mass spectrometry (LC-MS/MS). These substances were detected in sandy loam and loam soil, indicating that certain portions of BPA were converted to 4-HAP and 4-HBA in the soil; however, it was observed that only 4-HBA migrated to lettuce through the roots into crops.

CONCLUSION: The uptake residues showed the BPA and 4-HAP were not detected in lettuces grown on sandy loam (SL) and loam (L) soil treatments that were applied with of 10 ng/g, 50 ng/kg and 500 ng/g of BPA. However, the 4-HBA was detected at the level of 7 ng/g and 11 ng/g in the lettuce grown in sandy loam and loam soil that were treated with the 500 ng/g of BPA, respectively, while the 8 ng/g of 4-HBA was measured in the lettuce cultivated in the loam that was treated with 100 ng/g of BPA. This result presents that the BPA persisting in the soil of the pot was absorbed through the lettuce roots and then distributed in the lettuce leaves at the converted form of 4-HBA, what is the oxidative metabolite of BPA.

Key words: Bisphenol A, 4-hydroxyacetophenone, 4-hydroxybenzoic acid, Uptake
Introduction

Globally, agro-food hazards caused by environmental pollution are diverse. Accordingly, the types of hazardous substances subject to management are expanding, while the standards for each hazardous substance are being increased. The need for management to ensure the safety of agricultural products is emerging through the establishment of a new method for analyzing hazardous chemicals, assessment of the pollution level of the relevant substances in the agricultural environment, and identification of crop absorption.

Bisphenol A (BPA) is a chemical that is widely used in polycarbonate plastic containers, epoxy resins, and flame retardants in daily life. BPA is a well-known endocrine disruptor. BPA causes hypertension, weight gain, insulin resistance, and hypothyroidism in the human body. In the U.S., BPA was detected in the urine of more than 93% of adults. It has been found that experimental animals exposed to low levels intensify diabetes, breast cancer, prostate cancer, sperm count, reproductive problems, early maturity, obesity, and neurological problems [1-3].

BPA is present in a wide range of environments such as air, water, and soil. Residues of BPA in agricultural environments have been a concern [4, 5]. [5] reported that the content of BPA was from 0.05 to 0.18 µg/L and 0.1 to 34.0 µg/kg in irrigation water and basin soil in domestic agricultural reservoirs, respectively. In 2008, [6] reported that BPA was not only detected at a maximum of 147 µg/kg (dry weight) in unmodified cropland soil and at 87 µg/kg (d.w.) in the area improved with biological solids, but also detected at 0.55 to 147 µg/kg (d.w.) in crop cultivation land to utilize a wastewater irrigation. In 2010, [7] reported that 30.2 µg/kg (d.w.) of BPA was detected at agricultural land irrigated with wastewater for 90 years in California, USA. This result implicates BPA did not accumulate in the soil. Therefore, it is valuable to understand a pattern of uptake and distribution of BPA and its metabolites at the level of 10 µg/kg ~ 100 µg/kg since 30.2 µg/kg (d.w.) of BPA was detected at agricultural land irrigated with wastewater for 90 years in California, USA [7]. BPA analysis method in the agricultural environment has been established and the degree of contamination is being evaluated. Studies related to agro-food safety such as BPA uptake into crops and risk assessment is, however, insufficient. Therefore, the analysis method of BPA and its metabolites in soil and crops was established in the present study, and the uptake of BPA and its metabolites as lettuce grown in sandy loam and loam soils that are representative soils of Korea. The data can be useful for research for BPA pollution assessment and management. This study aims to evaluate the uptake of BPA in the lettuce at the level of 10, 50, 100 and 500 ppb in the sandy loam and loam soils.

Material and Methods

Reagents and Materials

The BPA standard material used in this study was purchased from Sigma-Aldrich (USA). Distilled water, acetonitrile, dichloromethane, and methanol in HPLC grade were purchased from Tedia (USA). Hydrochloric acid, ammonium acetate, sodium chloride, and sodium sulfate anhydrous were purchased from Junsei Chemical (Japan). Wagner pots (NF-5/φ174.6×φ160.4×197.5 mm) for cultivating the lettuce and the Ecospin 3180C (NFEC-429 W×655 D×1080 H mm) for liquid drying were purchased from Asone (Osaka, Japan) and Hanil Science Medical (Daejeon, Korea), respectively. The AOAC QuEChERS method was utilized to determine BPA in lettuce for evaluation of crop absorption performance. The extraction kit used was KRIAT (Korea)’s AnA-QTM QuEChERS pouch (6.0 g magnesium sulfate, 1.5 g sodium acetate). The purification kit was the AnA-QTM QuEChERS tube (150 mg magnesium sulfate, 50 mg primary secondary amine). Equipment for pretreatment of lettuce samples included centrifuge (3,500 rpm, Combi-514R, Hanil Science, Korea), ultra-high speed centrifuge (13,000 rpm, Legendmicro 17, Thermo Co., USA), vacuum rotary concentrator (RV-10, IKA, Germany), a separating filter head shaker (SR-2W, Taisec Co. Japan), and shaker (Geno/Grinder, SPEX® SamplePrep Inc., USA). LC-MS/MS (QTrap 4500, ABSciex Co., USA) was used for analysis of BPA (Table 1). The two kind of soil samples came from Eunseong and Yongin location to cultivate the lettuces. The physicochemical properties of soils was described in Table 2.

Soil Correction and Lettuce Cultivation for Uptake of BPA to Lettuce

The sandy loam and loam soils had been collected from the upland soil and submerged soil on May 10, 2017. An aliquot of 100 mL of 0.1, 0.5, 1.0 and 5.0 µg/g of BPA was treated in the 3 Kg of sandy loam soil and
Uptake and Distribution of Bisphenol A and Its Metabolites in Lettuce Grown in Sandy Loam and Loam Soil

### Table 1. Physicochemical properties of bisphenol A and its metabolites

| Compounds           | Bisphenol A | 4-hydroxyacetophenone (4-HAP) | 4-hydroxybenzoic acid (4-HBA) |
|---------------------|-------------|--------------------------------|-------------------------------|
| Formula             | ![Formula](image1) | ![Formula](image2)             | ![Formula](image3)             |
| IUPAC name          | 4,4’-dihydroxy-2,2’-diphenylpropane | 1-(4-hydroxyphenyl)ethanone     | 4-hydroxybenzoic acid         |
| CAS No.             | 80-05-7     | 99-93-4                        | 99-96-7                       |
| Molecular Formula   | C₁₄H₁₀O₂    | C₆H₈O₂                         | C₇H₈O₃                        |
| Molecular weight    | 228.29      | 136.15                         | 138.12                        |

### Table 2. Physicochemical properties of cultivated soils

| Soils      | Particle size distribution (%) | pH (1:5 H₂O) | OM<sup>a</sup> (g/kg) | CEC<sup>b</sup> (cmol/kg) | Texture         |
|------------|--------------------------------|--------------|------------------------|---------------------------|-----------------|
| Eumseong   | 83.2 9.6 7.2                   | 6.6          | 20                     | 8.5                       | Sandy loam (SL) |
| Yongin     | 45.7 38.1 16.3                 | 6.5          | 39                     | 18                        | Loam (L)        |

<sup>a</sup> Organic matter content, <sup>b</sup> Cation exchange capacity

Fig. 1. Lettuce before transplanting after lettuce sowing.

loam soil, respectively (May 15, 2017) to give concentrations 10, 50, 100 and 500 ppb. The treated soil was naturally dried at room temperature for four weeks. The lettuce (*Lactuca sativa*, Variety: Jeogchima, Asia Seed Co., Ltd) was directly sown on Jun 2, 2017 and transplanted to the Wagner pots containing BPA-treated soil on Jun 12, 2017 (Fig. 1). The lettuce was harvested after two months (Fig. 4). Each treatment was in triplicate.

### Sample Preparation for BPA and Metabolites in Lettuce

A 5 g of lettuce (*Lactuca sativa*) sample was taken and transferred to a 50 mL centrifuge tube, and then a 10 mL of acetonitrile was added and shaken for 5 min. A 6.0 g of magnesium sulfate and 1.5 g of sodium acetate were added, mixed for 2 min, and then centrifuged at 3,500 rpm for 10 min to separate the acetonitrile and water layers. A 1 mL of the separated acetonitrile was aliquoted and placed in a 2 mL centrifuge tube to which a 25 mg of primary-secondary amine and 150 mg of magnesium sulfate were added, followed by centrifugation at 10,000 rpm for 2 min, and the supernatant was transferred to a 2 mL vial after filtration using a syringe filter (0.22 µm). A 2 µL was injected into LC-MS/MS to measure the area of the peak displayed on the chromatogram, and the concentration was calculated by a standard calibration curve.

### Sample Preparation for the Analysis of BPA and Metabolites in Lettuce—Cultivated Soil

A 50 g of soil sample was taken in an Erlenmeyer flask to which a 100 mL of acetone was added, and shaken at 180 rpm for 1 hour. The extract was filtered under reduced pressure, and the container and residue were washed with 50 mL of extra acetone, transferred to a 500 mL separatory funnel and a 200 mL of distilled water was added. The pH of the solution was adjusted to approximately 3.0-3.5 with 0.1 M HCl. A 50 mL of methylene chloride was added and shaken for 5 min. The organic solvent layer was passed through an anhydrous sodium sulfate. The remaining water
layer was re-extracted with 50 mL of methylene chloride followed by anhydrous sodium sulfate dehydration. The extracts were combined, and then concentrated with a rotary vacuum concentrator at 40°C. The extract residues were re-dissolved in 5 mL of de-ionized water : methanol (2:8, v/v) mixed solution and transferred to a GC vial. A 2 µL of this solution was injected into LC-MS/MS. The concentration was calculated by a standard calibration curve.

Uptake of BPA and Its Metabolites into Lettuce

Loam and sandy loam soils, which are representative soils for crop cultivation in Korea, were collected from the farmhouse used as lettuce cultivation soil. Each soil was treated with 10, 50, 100, and 500 µg/kg of BPA, and the soil was naturally dried for 4 weeks. During that time, lettuce was grown to evaluate the absorption of lettuce. It was cultivated as a lettuce seedling that can be planted. Thereafter, lettuce seedlings were planted in each soil treated with BPA by concentration, and then the BPA concentration according to the growth degree was confirmed. Lettuces grown for 2 months were harvested and BPA in crops and cultivated soil was analyzed.

Results and Discussion

Establishment of Analytical Method for BPA and Metabolites in Lettuce and Soil

An amount of 101 mg of BPA (purity 99.0%), 4-HAP (purity 99.0%), and 4-HBA (purity 99.0%) standards was weighed and dissolved individually in 100 mL of 80% aqueous methanol to prepare the 100 mg/L stock solution. The stock solution was diluted with 80% aqueous methanol to prepare a series of working standard solutions of 5.0 to 200.0 µg/L.

One mL of each standard solution was taken, dried with an Ecospin 3180 C (NFEC-429 W×655 D×1080 H mm) and then re-dissolved in 1 mL of the untreated sample solution that was then fortified into the lettuce and soil sample.

Several methods were reported for the analysis of either the parent BPA or BPA and its analogues using GC-MS/MS or LC-MS/MS [4, 8, 9]. However, only one method was found to be suitable for the analysis of BPA and its metabolites (4-HAP and 4-HBA) using HPLC-DAD [10]. Since the LC-MS/MS based method is popular owing to the high selectivity and sensitivity, a new method was developed for the simultaneous analysis of BPA and its metabolites in lettuces using LC-MS/MS in the present study. BPA and its metabolites were carried out on LC-MS/MS (AB Sciex Co. USA) with Aquity UPLC H-Class (Waters Co., USA) system consisting of a TQ 4500 detector equipped with a binary pump and an Capcell Core-C18 (150 mm L × 2.1 mm ID × 2.7 µm, Shiseido Co., Japan) housed in a thermostatic compartment at 40°C. The mobile phase was set gradient elution starting from 70% MeOH (A) with 30% 10 mM ammonium acetate in water (B), holding at 85% A for 5 min and ending at 70% A after 5 min at a constant flow rate of 0.2 mL/min. The injection volume was 2 µL. The MS was operated in electrospray ionization mode. The drying gas was nitrogen at a flow rate of 10.0 L/min at 350 uC, creating a nebulizing pressure of 40 psi. The ion spray voltage was set at 4.5 kV. Mass spectra were collected in negative ion mode with selected ion monitoring of m/z fragments at 131.2 amu with a fragmentation voltage of 150 V (Table 3). The BPA, 4-HAP and 4-HBA were identified by comparison of retention times and MS spectra against BPA and its metabolites (Fig. 3).

A calibration curve was prepared based on the peak
Table 3. Selected reaction monitoring (SRM) condition of bisphenol A and its metabolites

| Compounds                        | Precursor ion | Product ion | Dwell<sup>a</sup> (msec) | DP<sup>b</sup> (V) | CE<sup>c</sup> (eV) | CXP<sup>d</sup> (eV) |
|----------------------------------|---------------|-------------|--------------------------|------------------|------------------|-------------------|
| Bisphenol A (BPA)                | 227.0         | 211.9       | 150                      | -75              | -24              | -15               |
|                                  | 227.0         | 132.9       | 150                      | -75              | -32              | -9                |
| 4-hydroxyacetophenone (4-HAP)    | 134.8         | 92.0        | 150                      | -60              | -30              | -7                |
|                                  | 134.8         | 120.0       | 150                      | -60              | -22              | -9                |
| 4-hydroxybenzoic acid (4-HBA)    | 136.8         | 105.0       | 150                      | -5               | -6               | -3                |
|                                  | 136.8         | 93.0        | 150                      | -5               | -8               | -9                |

<sup>a</sup> Dwell time means it accumulated counts time (dwell time/integration time), <sup>b</sup> Declustering potential, <sup>c</sup> Collision energy, <sup>d</sup> Collision cell exit potential

Fig. 3. Total ion current chromatograms of LC-Qtrap MS for standard solution of BPA (Bisphenol A), 4-HBP (4-hydroxyacetophenone) and 4-HBA (4-hydroxybenzoic acid) (0.1 μg/mL in 80% MeOH).
Table 4. Average recoveries of Bisphenol A, 4-Hydroxyacetophenone and 4-Hydroxybenzoic acid in lettuce grown at loam and sand loam soil

| Analyte    | Sample          | Fortification (ng/g) | % recovered ± standard deviation (SD) | LOQ\(^a\) (ng/g) |
|------------|-----------------|----------------------|--------------------------------------|------------------|
| BPA\(^b\)  | Lettuce         | 10 / 50              | 117.6 ± 2.1 / 114.1 ± 7.6             | 5                |
|            | Sandy loam,     | 1.0 / 5.0            | 114.8 ± 1.5 / 116.6 ± 3.4             | 0.5              |
|            | Loam            | 1.0 / 5.0            | 109.7 ± 0.9 / 108.3 ± 3.0             | 0.5              |
| 4-HAP\(^c\) | Lettuce         | 10 / 50              | 106.5 ± 4.8 / 111.6 ± 4.5             | 5                |
|            | Sandy loam,     | 1.0 / 5.0            | 111.3 ± 0.7 / 116.6 ± 1.3             | 0.5              |
|            | Loam            | 1.0 / 5.0            | 100.2 ± 4.1 / 105.7 ± 1.6             | 0.5              |
| 4-HBA\(^d\) | Lettuce         | 10 / 50              | 110.4 ± 19.4 / 109.2 ± 3.4            | 5                |
|            | Sandy loam,     | 1.0 / 5.0            | 110.8 ± 8.9 / 101.2 ± 3.6             | 0.5              |
|            | Loam            | 1.0 / 5.0            | 99.6 ± 20.0 / 110.0 ± 3.4             | 0.5              |

\(^a\) LOQ: Limit of quantitation, \(^b\) BPA; Bisphenol A \(^c\) 4-HAP; 4-Hydroxyacetophenone, \(^d\) 4-HBA; 4-Hydroxybenzoic acid

Fig. 4. Lettuce elapsed one month after transplanting lettuce. A; Control of lettuce in sandy loam (SL) soil, B; 10 ng/g-fortified SL soil, C; 50 ng/g-fortified SL soil, D; 100 ng/g-fortified SL soil, E; 500 ng/g-fortified SL soil, F; Control of lettuce in loam (L) soil, G; 10 ng/g-fortified L soil, H; 50 ng/g-fortified L soil, I; 100 ng/g-fortified L soil, J; 500 ng/g-fortified L soil.

area on the chromatogram displayed by injecting a certain amount (10 µL) into the LC-MS/MS. The linearity of the calibration curve and the reliability of the analysis result showed a determination coefficient \(R^2\) > 0.99 or greater. The limit of quantitation of BPA and its metabolites under the analytical method were 5 ng/g for lettuce and 0.5 ng/g for soil, respectively, while the minimum detection amount was 0.01 ng. One mL of the standard solution containing 10 and 50 ng/g of BPA, 4-HAP, and 4-HBA, respectively, was accurately added to 5 g of lettuce sample and uniformly mixed. The average recovery was in the range of 106.5 ~ 117.6%. To 50 g of each untreated soil, one mL of the standard solution containing 10 and 50 ng/g of BPA, 4-HAP, and 4-HBA, respectively, was accurately added and uniformly mixed. The average recoveries ranged from 101.2% to 116.6% in sandy loam soil and 99.6% to 110.0% in loam soil (Table 4). The recovery percentage of BPA, 4-HAP, and 4-HBA were in acceptable range (60-120%) according to the Codex Alimentarius (CAC/GL 90-2017).

Uptake of BPA and Its Metabolites into Lettuce

In this study, both loam and sandy loam soils were collected from farms and dried, which were then fortified with BPA at 10, 50, 100 and 500 ng/g in each soil. Lettuce was grown for 2 months in the BPA treated soil. The lettuce grew faster in the sandy loam (upland soil) than in the loam soil (paddy soil) (Fig. 3). In the resulted uptake residue, 7.0 ng/g of 4-HBA was detected in lettuce grown in sandy loam treated with 500 ng/g of BPA, while 8.0 ng/g and 11.0 ng/g of 4-HBA were detected in lettuce grown in loam soil treated with 100 ng/g and 500 ng/g of BPA, respectively (Table 5). The results were similar to the previous study where rapid microbial degradation of BPA was found in the soil and water environment [11].

A small amount of BPA, 4-HAP, and 4-HBA was still detected in the soil after full growth of lettuce. The
Uptake and Distribution of Bisphenol A and Its Metabolites in Lettuce Grown in Sandy Loam and Loam Soil

2.80 and 4.38 ng/g, respectively. This means that un-

of BPA, resulted the parent residues of 1.43, 1.58,

Table 6. Residues of BPA, 4-HAP and 4-HBA in the sandy loam (SL) soil and loam (L) soil

| Crop Treatments | BPA Residue (ng/g)±standard deviation (SD) | 4-HAP Residue (ng/g)±standard deviation (SD) | 4-HBA Residue (ng/g)±standard deviation (SD) | LOQ (ng/g) |
|-----------------|------------------------------------------|---------------------------------------------|---------------------------------------------|-----------|
| Lettuce cultivated in SL soil | Control: <5.0, BPA 10 ppm: 5.0±0.0, BPA 50 ppm: 5.0±0.0, BPA 100 ppm: 5.0±0.0, BPA 500 ppm: 5.0±0.0 | Lettuce cultivated in L soil | Control: <5.0, BPA 10 ppm: 5.0±0.0, BPA 50 ppm: 5.0±0.0, BPA 100 ppm: 5.0±0.0, BPA 500 ppm: 5.0±0.0 | LOQ: 0.5 |
| SL 100 ppm | 4.96±0.06 | 6.0±0.6 | 5.0±0.0 | 0.5 |
| 500 ppm | 7.4 9.4 7.2 | 8.0±1.2 5 | 8.0±1.2 5 |

LOQ: Limit of quantitation

Table 6. Residues of BPA, 4-HAP and 4-HBA in the sandy loam (SL) soil and loam (L) soil

| Soils | Treatment | BPA Residue (ng/g)±standard deviation (SD) | 4-HAP Residue (ng/g)±standard deviation (SD) | 4-HBA Residue (ng/g)±standard deviation (SD) | LOQ (ng/g) |
|-------|-----------|------------------------------------------|---------------------------------------------|---------------------------------------------|-----------|
| Control | <0.5, 1.0, 1.0, 1.0 | 0.65±0.00 0.5 | 0.65±0.00 0.5 | 0.65±0.00 0.5 | 0.65±0.00 0.5 |
| SL | BPA 10 ppm: 5.0±0.0, BPA 50 ppm: 5.0±0.0, BPA 100 ppm: 5.0±0.0, BPA 500 ppm: 5.0±0.0 | 4.96±0.06 | 6.0±0.6 | 5.0±0.0 | 0.5 |
| 500 ppm | 7.4 9.4 7.2 | 8.0±1.2 5 | 8.0±1.2 5 |

LOQ: Limit of quantitation

calculated amount of BPA residues were 0.81, 3.77, 5.04 and 11.2 ng/g in the sandy loam treated with 10, 50, 100, and 500 ng/g of BPA, respectively (Table 6). Similarly, the detected concentrations of 4-HAP were 0.80, 1.21, 1.25 and 1.51 ng/g, respectively, in the soil with the same treatment of BPA. 4-HBA acid was detected at 0.81, 0.65 and 0.59 ng/g in sandy loam treated with 10, 100, and 500 ng/g of BPA, respectively. On the other hand, the loam soil treated with 10, 50, 100, and 500 ng/g of BPA, resulted the parent residues of 1.43, 1.58, 2.80 and 4.38 ng/g, respectively. This means that unlike sandy loam, when BPA was treated at a high concentration, a small amount of residues remained. Volatilization is one of the factors responsible for chemical and biological degradation and plant uptake of organic contaminants [11]. When the loam soil samples containing 50, 100, and 500 ng/g of BPA were treated, 0.76, 0.76, and <0.5 ng/g of 4-HAP were detected, respectively (Table 6). However, unlike lettuce grown in loam, the concentration of 4-HBA was lower than the limit of detection. It is estimated that the translocation of 4-HBA to crops may be more likely as in sandy loam than in loam soils.

[12] reported that in a chemical absorption test using soybeans and tomatoes, if the log Kow value is 4.8 or higher, absorption into crops does not occur. The distribution of BPA in the crops is greatly affected by the route of exposure. However, the log Kow of BPA is 3.40 and the water solubility is 300 mg/L. BPA can be sufficiently absorbed when exposed to the roots [13, 14]. In the uptake study of BPA, [4] found that the highest concentrations of BPA residues (53.9%) were
observed in lettuce roots and the lowest concentrations were in stems (7.1%) and remaining intermediate residues (39.0%) was observed in lettuce leaves [15]. [16] reported that the 2% of total treatment-amount was detected in the roots using the water convolvulus but it was not detected in leaves or stems, and most of the absorbed BPA was rapidly metabolized in plants through the BPA absorption experiment.

On the other hand, in the absorption transition experiment using C\textsuperscript{14}-labelled BPA under nutrient culture conditions, the metabolites were detected in the culture medium. On the basis, [17] concluded that metabolites were not formed in the plant, but metabolites were formed in the culture medium and then absorbed into the plant.

In conclusion, the results of this study indicated that most of the residues in the soil for lettuce cultivation were BPA. BPA in soil was absorbed through the lettuce roots and metabolized to HBA.

**Note**

The authors declare no conflict of interest.

**Acknowledgement**

This work was supported by the Research Program for Agricultural Science & Technology Development (Project No. PJ010896 & PJ01364103), Korea in 2020.

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