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Detection of Bisphenol A (BPA) in Plastic Bottles Using Vertical Cultivation at Various Temperatures

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Abstract

Polycarbonate plastics containing bisphenol A (BPA) used to manufacture drinking water bottles. Kurdistan region in northern Iraq is a developed area with increased pollution from plastic bottles. Trace amounts of BPA have been detected in bottled water samples. The absorption of BPA was measured with HPLC using a vertical cultivation system with Bulbs of the Allium Cepa plant planted in these plastic bottles with monitored growth. Vertical cultivation was found to have a low level of BPA in the plant cells, making it a safe cultivation method under specific climate conditions. The mean concentration of BPA in vertical cultivation is 0.19 ug/ml (3.8 ng for a 20 uL injection), and the Limit of Quantification (LOQ) is 0.63 ug/ml (12.7 ng for 20 uL injection). While Scanning Electron Microscope (SEM) shows that the concentrations are relatively low in water samples stored at room temperature compared to those exposed to direct sunlight (40˚C) and water bottle samples stored at (−4˚C), The correlation coefficients were found to be good (0.9992). SEM is used for plastic bottle samples stored at different temperatures. The images identify compound decay and explore the morphology of BPA in manufactured plastic materials.

Keywords

Vertical Cultivation, Allium, Bisphenol A, Plastic Bottles, Scanning Electron Microscope (SEM)

1. Introduction

Sulaimaniyah is one of the Kurdistan region's major cities that have developed significantly of [1]. The study area is classified as a separate Mediterranean-type continental interior and semi-arid climate; the summers are dry and extremely hot (average maximum temperature in July-August around 44˚C, and 5% humidity) with no rainfall, and the cool, rainy winters [2].

Apart from the infrastructure revolution, the growing needs of the civilians cannot be met directly by the government. One of these needs is access to a clean water network. This shortage has forced people to drink water only bottled water. The demand is enormous as the area's population is more than 5 million people [3]. All plastic bottles end up in landfills polluting the environment. Waste recycling is currently unavailable in the research area that compiles with environmental regulations [4], and improper solid waste dumping has an adverse impact on the environment [5]. The garbage is burned by the local authorities ignoring the fact that by burning the plastic, the BPA is released into the air and the soil polluting the environment enormously.

Solid waste is one of the significant environmental impact problems in developing countries. Approximately 3.5 million tons of MSW are generated daily globally [6]. Population growth, improving living standards after economic recovery and industrial activities are all primary reasons for a significant increase in the quantity of solid waste [7].

The daily per capita waste generation in the Sulaimaniyah governorate was 1.32 kg in 2023, a cumulative solid waste of about 1,325,800 tons, and the plastic portion (bottles and bags) accounted for 13.30% of the total waste [8].

Bisphenol A is a chemical compound widely utilized in several sectors, including synthetic polymers and specific applications in plastic containers, toys, and polycarbonate bottles. Microplastic ingestion and subsequent BPA are critical to the aquatic ecosystem's perceived harm and risk of pollution [9] [10].

Among others, it releases BPA at room temperature. At higher temperatures, the rate of rising is faster. A considerable volume of bisphenol-containing items is already in the environment, making them significantly more hazardous [10].

The maximum amount of BPA permitted in the human body is 50 μg/kg BW (body weight)/day, according to the United States Environmental Protection Agency. However, after re-evaluation, a temporary TDI of 5 μg/kg BW/day was re-set by the European food safety authority EFSA in 2014 [11] [12] [13].

Researchers have developed a series and comprehensive range of analytical techniques for the determination of BPA by researchers, such as, HPLC [14], GC-MS [15], HPLC-MS/MS [16], or UV, researchers have developed a series and comprehensive range of analytical techniques for determining BPA, including HPLC [14], GC-MS [15], HPLC-MS/MS [16], or UV spectroscopy. GC-MS and HPLC-MS are relatively highly cost, which greatly improve the qualitative ability of BPA and the detection sensitivity.

Standard BPA detection techniques include gas chromatography with mass spectrometry (GC-MS) and high-pressure liquid chromatography (HPLC) [17] [18] [19] [20]. In addition, SEM was used to characterise and examine the morphology of BPA in the produced materials [21] [22] [23].

There are international regulations to restrict Bisphenol A (BPA), as well as an updated process for assessing the toxicity of Bisphenol A (BPA) [24], however this control is not present in the research region.

This study aims to establish a better approach to comparing BPA results in high-pressure liquid chromatography with SEM images at various temperatures, particularly in mono compound manufactured materials. Furthermore, to introduce the reuse of plastic bottles for vertical cultivation in private gardens and simultaneously explore whether this kind of cultivation would be safe for public health.

Bisphenol A (BPA)

Bisphenol A (BPA) is the common name for 2,2-(4,4'-dihydroxydiphenyl) propane, 4,4'-isopropyllidenediphenol, alternatively, 2,2'-bis (4-hydroxyphenyl) propane [25], an organic compound composed of two phenol rings connected by methyl bridge, with two methyl functional groups attached to the bridge [26]. It has been known since 1930 that BPA is an artificial estrogen, and its estrogen effect was used to promote industry profit [27]. Its essential properties include low vapor pressure, moderate water solubility, low volatility, and solid at room temperature [28] [29]. It is one of the highest volume chemicals produced worldwide, with product estimations of more than 7 million tons in 2019 that were emitted into the ocean [30]. Being an important industrial chemical, Bisphenol A is used as a material for producing phenol resins, polyacrylates, and polyesters but is mainly used as an intermediate in the production of polycarbonate (PC) plastics and epoxy resins. Polycarbonate plastics are widely used in daily life products like medical devices, food, and drink storage containers because they have high impact strength, hardness, toughness, transparency, and temperature resistance. In order to protect food and drinks from direct contact with metals, epoxy resins are also used in the internal coating of food and beverage cans. Children's toys may contain BPA, being used as an additive in other types of plastic [31] [32] [33] [34].

Although BPA is introduced extendedly in everyday life, supported by industry studies that showed various risks to human health [35] [36].

At a Glance, many people encounter toxic, cancer-causing chemicals through plastics, whether they're aware of the health risks or not. Plastic can harm our health at every stage of its lifecycle. For example, PVC or polyvinyl chloride, studies funded by government agencies showed that there is a wide range of effects on humans. BPA is an endocrine disruptor that can mimic the body's hormones [37] [38]. After entering the human body, BPA can disrupt normal cell function by acting as an estrogen agonist [37] [39], as well as an androgen antagonist [40], which may affect health. It has been suspected that BPA may be carcinogenic, potentially leading to the precursors of breast cancer [41]. In addition, exposure to BPA has been associated with chronic disease conditions in humans, such as cardiovascular disease and diabetes, and is a serum marker of liver disease [42] [43]. The ester bond linking BPA molecules in polycarbonate and resins are subject to hydrolysis, resulting in the leaching of BPA monomer even from new polycarbonate into the water at room temperature [33] [44] [45]. The heat and hydrolysis, such as that which occurs with the pasteurization and canning process, sterilizing, microwave heating, warming prior to serving, and washing of the containers, result in increased leaching of the BPA into the products that are consumed [46]. Now it is generally believed that consumer exposure occurs primarily via food in contact with BPA-containing materials, such as polycarbonate plastic baby bottles and table ware, plastic food containers and food and beverage cans lined with epoxy resins [35] [47]. It is also present in the air and drinking and other water sources. BPA leaches from the soil into fresh water, and plastic and metal waste disposal are a major contaminant in landfills. High levels of BPA in the atmosphere that were measured in many regions in southern Asia are considered to be related to the burning of plastics for waste disposal, a treatment that takes place also at the Kurdistan region [34].

Measured concentrations of BPA in human blood, urine and other tissues confirm that exposure is widespread in the human population.

2. Materials and Methods

Small plastic bottles of 330 ml were arranged in a vertical position. The narrow part with the cap was facing the ground, a hole of 3 cm in diameter was opened and 8 cm diameter opening was created on the other side. The lower half part of the bottle was painted black from the outside and a small piece of sponge was placed inside the cap, as shown in **Figure 1**. Small leak was created on every cap and then the bottles were placed vertically with the top of the one into the bottom of the other. Another bottle was cut in the middle and placed on the top. A bigger one was placed at the bottom of the last bottle to collect the excess water. Apart from the first and the last bottle of each row, the rest were filled with soil. According to [48], Allium plants need light soil; thus, a mixture of sand-turf-soil (1-1-1) was used for plantation. The sponge at the bottom prevented the leak from being blocked by the mixture and the black paint prevented the roots from coming in contact with the light, using the broader holes on the side the Allium bulbs were planted. Each vertical row consisted of one empty bottle on the top, five planted bottles in the middle and one empty bottle at the bottom. Totally 12 vertical rows were placed with 60 Allium bulbs. The watering was done by hand filling the top bottle. The water was moving gradually from top to bottom, watering the plants and the excess water was collected and reused.

The same soil mixture was used for planting Alliums in the cultivation table. Again 60 bulbs were planted near the vertical arrangement to discover possible differences in the plants' growth. For the laboratory data, BPA (>99%, CAS 80-05-7) was purchased from Sigma Aldrich (Germany). All other solvents used in extraction or chromatography were HPLC grade and purchased from Merck, Germany. Milli-Q water system was purchased from the USA (Millipore, Bedford, MA). In addition to standard laboratory materials, the following equipment are essential for the use of AFFINIMIP SPE cartridges:

Figure 1. Vertical cultivation in plastic boatel.

1) SPE vacuum manifold (Phenomenex, USA).

2) Mini vacuum pump.

The critical step is to follow the flow rate given in the protocol accompanied by AFFINIMIP SPE cartridges.

SEM is used for plastic bottle samples stored at different temperatures to identify compound decay and explore the morphology of BPA. The morphology of the BPA was characterized using SEM with an accelerating voltage CS3200 Tungsten 40 kV. Polymer samples were mounted on aluminum stubs using carbon tape and were gold coated.

2.1. Experimental

2.1.1. Sample Preparation and HPLC Analysis

Samples were received in good condition and stored in glass containers in a refrigerator under −2˚C before analysis.

Cold spring onions are put in a desiccator to get to room temperature before extraction process. Then, 25 g of spring onions, including the root system, and 50 ml of water/acetonitrile are shaken for 30 min in an orbital shaker at 150 rpm. After that, solids are removed with filtration through a filter paper (4 - 7 um), and the filtrates are centrifuged for 10 min at 4000 rpm. Supernatant solution is collected and filtered off through a filter paper as previously. Filtrates are diluted 1:1 with water to give the loading solution [49]. The clean-up method followed, as shown in Table 1.

The solution is collected in an amber glass vial (4 ml) and is evaporated until dryness under nitrogen or in a speed vac concentrator. During analysis, the dilute factors should be considered in calculations. The obtained eluent was filtered through a 0.22 um filter before injection. To avoid cross-contamination, all vials were not polycarbonate material [50].

Table 1. Sample clean-up method in HPLC.

Steps (Flow rate)	AFFINIMIP SPE BPA (100 mg/6 ml)
1) Equilibration with 2 drops/s	3 ml Methanol (2% Acetic acid) 3 ml Acetonitrile 3 ml Water
2) Loading (ml) 1 drop/2 s	20 ml of diluted filtrate
3) Washing of interferents (1 drop/s)	9 ml of ultrapure water 6 ml of Water-Acetonitrile (60:40, v/v)
4) Drying	Force the water down and out the bottom, apply vacuum for 30 s
5) Elution (1 drop/s)	3 ml of Methanol

2.1.2. HPLC Analysis Conditions

Chromatography analysis is performed using a Waters 2695 Alliance HPLC system with a 996-photodiode array detector (Waters, USA). BPA is monitored at 228 nm. Chromatographic separation is carried out with a C18 reversed-phase column (Symmetry RP 18,150 mm × 3.5 mm, 5 um) in gradient mode at a flow rate of 0.8 ml/min as shown in **Table 2**. HPLC data collection and manipulation were performed on a PC running Millenium (Waters, USA). The injected sample volume is 20 uL. The oven temperature was set at 25˚C.

3. Results Discussion

Six standard solutions (250 – 10,000 ng/ml) are tested to determine the linearity of BPA as shown in **Figure 2**.

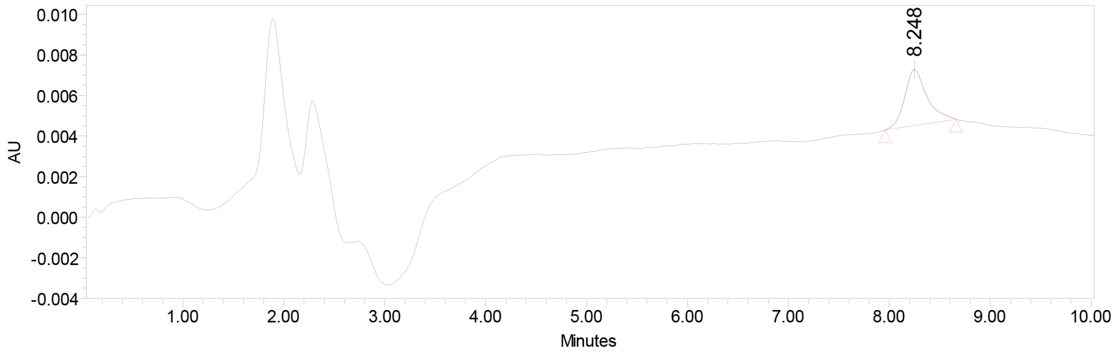
The peak area and concentration of BPA are subjected to regression analysis to calculate the calibration equation and correlation coefficient. The regression equation of BPA is $y = 0.0001x + 0.0805$ (correlation coefficient 0.999) as shown in **Figure 3**. The Limit of Detection (LOD) of BPA is 0.19 ug/ml (3.8 ng for 20 uL injection) and the Limit of Quantification (LOQ) is 0.63 ug/ml (12.7 ng for 20 uL injection).

3.1. BPA at Allium Cepa Plants

AFFINIMIP SEP ® SPE cartridges are selected after testing other SPE methods (OASIS SPE, etc.). MIPs are useful tools in sample preparation as analyze is entrapped in the polymer. Thus, MIPs offer high recoveries (>95%), repeatability and reproducibility. Here, we didn’t examine repeatability and reproducibility with spiked experiments. Of course, in case of method validation and robustness have to be examined by repeated experiments. **Figure 4** displays the chromatograms of the analyzed spring onions for BPA occurrence. BPA is detectable at an average level of 300 ng/g of Allium Cepa.

3.2. Scanning Electron Microscopy (SEM)

Scanning Electron Microscope (SEM) is used for plastic bottle samples stored at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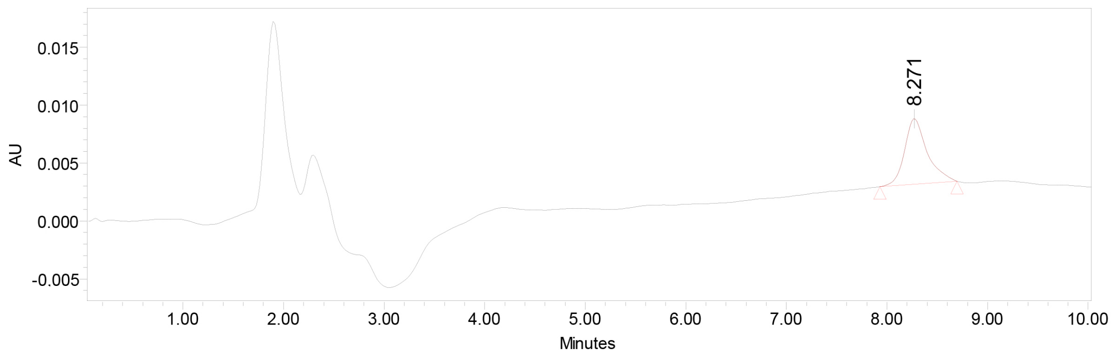
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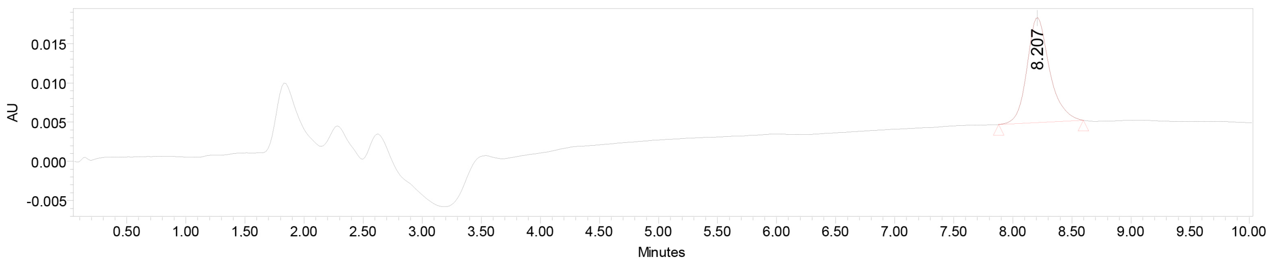
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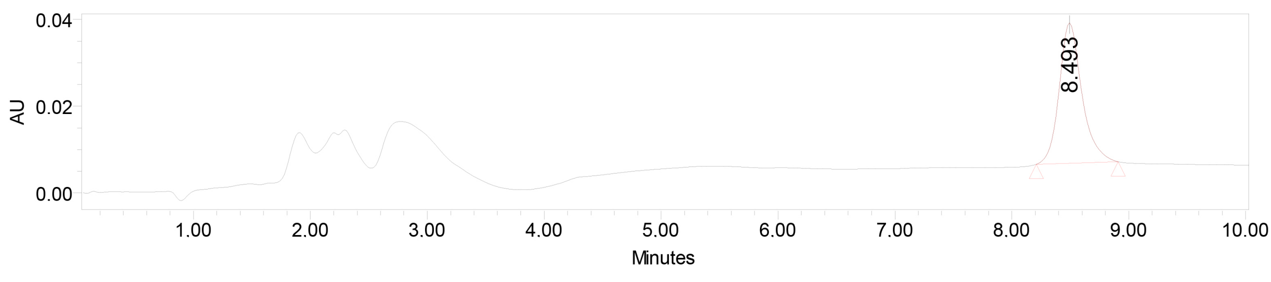
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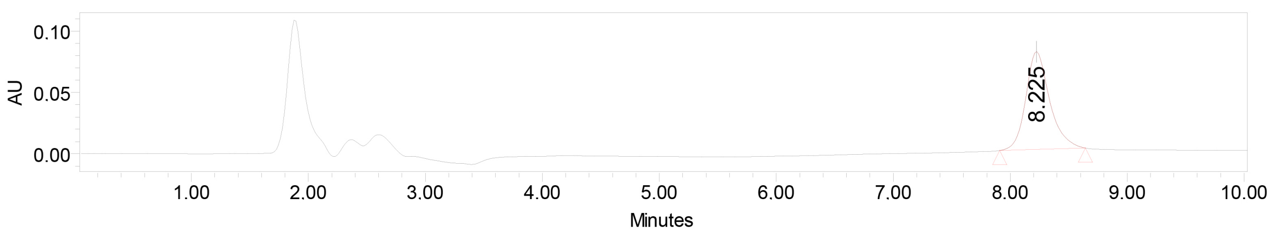
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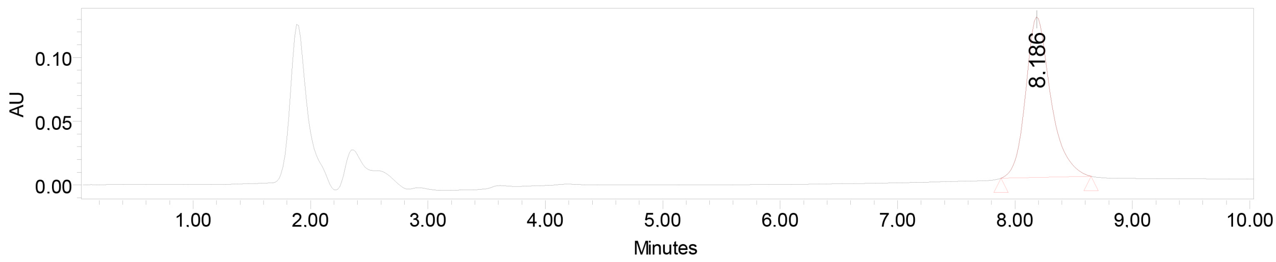


(f)

Figure 2. HPLC Chromatograms of calibration curve and samples. (a) Std. of 250 ng/ml, (b) Std. of 500 ng/ml, (c) Std. of 1000 ng/ml, (d) Std. of 2000 ng/ml, (e) Std. of 5000 ng/ml, (f) Std. of 10,000 ng/ml.

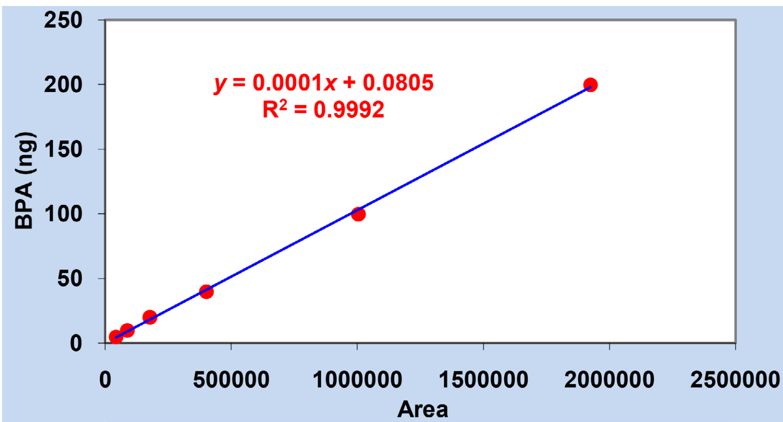


Figure 3. Correlation coefficient.

Table 2. HPLC Gradient system.

Time (min)	Acetonitrile (%)
0	40
15	95
20	95
23	40

different temperatures to identify compound decay and explore the morphology of BPA. The morphology of the BPA characterized using scanning electron microscopy (SEM) in a Caesium Imaging Analysis instrument, with an accelerating voltage CS3200 Tungsten 40 kV. Polymer samples were mounted on aluminum stubs using carbon tape and were gold coated, All SEM images are colored using scanning electron microscopy software (MountainsSEM). The results were shown in Figure 5 and Figure 6. Compared with Bisphenol A (BPA) plastic pollutant molecule and chemical structure [51].

4. Discussion

In both cultivations, there was not a significant difference in plant growth. The only difference that was observed was at the time of bulb germination, the bottle

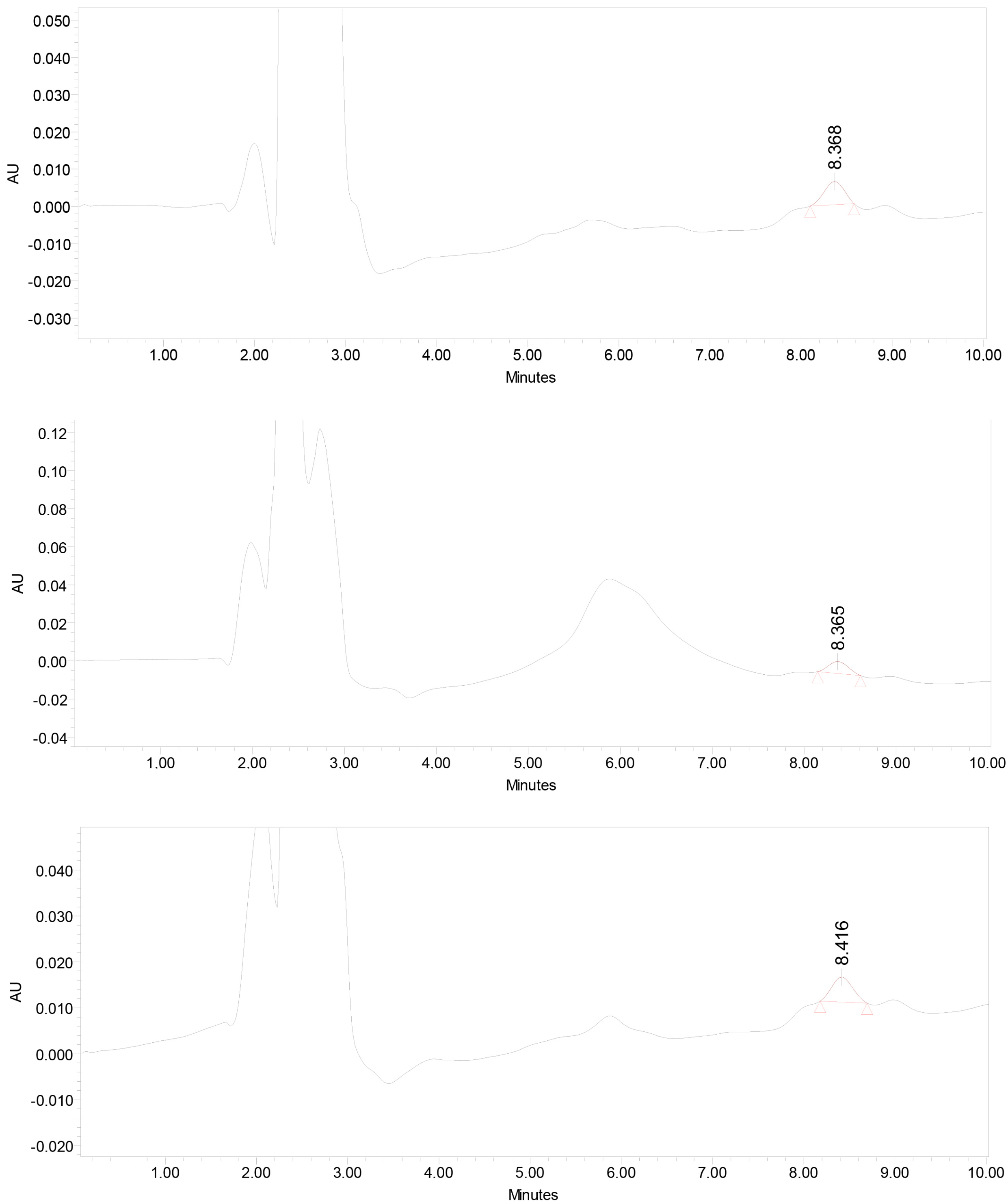
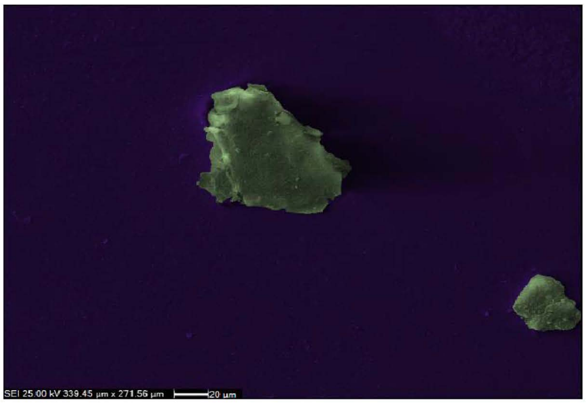
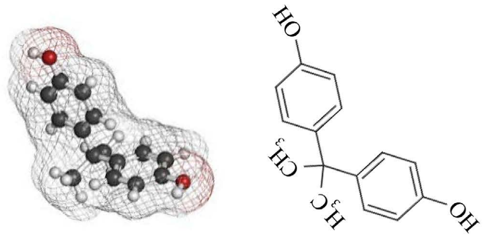


Figure 4. HPLC chromatograms of the analyzed Allium cepa for BPA.

cultivation occurred after four days while the soil cultivation occurred after 13 days. This difference occurred because of the higher temperature in the root zone of bottles due to the sun heating. The plastic raised the bottles' soil temperature, germinating the bulbs faster than those in the ground soil. The rate of

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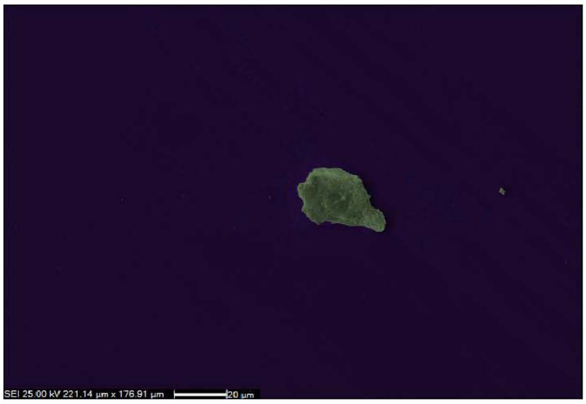

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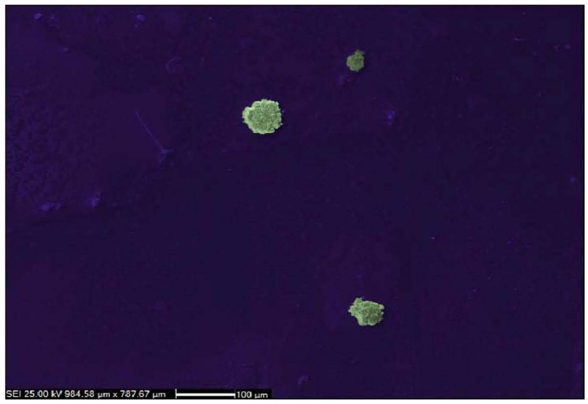
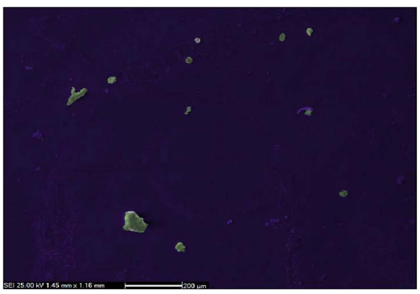
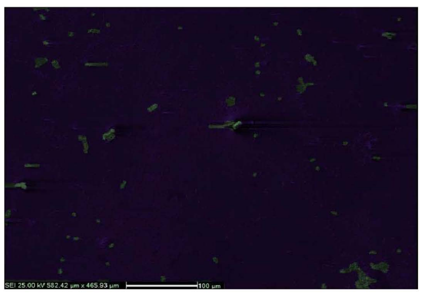


(c)

Figure 5. BPA particles from the interior side of the plastic water bottle.



(a)



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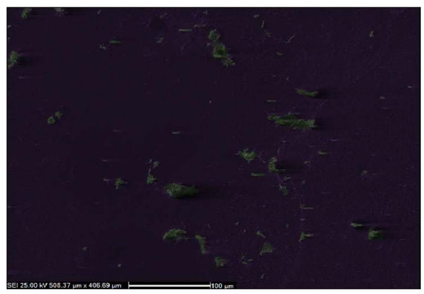

(c)

Figure 6. Scanning Electron Microscope (SEM) shows (a) BPA concentrations are relatively low in water samples stored at room temperature (b) high BPA concentrations exposed to direct sunlight (40˚C) and (c) water bottle samples stored in the refrigerator (−4˚C).

growth was the same after two months of cultivation. The weight of both cultivars was almost the same, with 25 gr for each plant. The morphology of mono-compound produced plastic materials samples in SEM images have the same molecular structures as BPA as presented in the Figure 5.

BPA Concentration

The Tolerable Daily Intake (TDI) for Allium was defined by at 0.0 5 mg/kg body weight (bw) per day [52], which is progressively higher than the Allium concentrations that were discovered. Even though the concentrations found are low, it is significant to consider that the daily intake refers to the total amount of BPA that an animal can intake, which includes many other sources of products that consist of BPA like water, air, and foods. Especially in the areas where the wastes are burned, the BPA concentrations in the air and underground water are high [53]. In the study area, the garbage is burned as it is mentioned above; therefore, the habitants' surcharge with BPA from the air. The low amount of BPA at the plant might be due to the climate conditions. It is reported that the higher rate of BPA migration from the plastic to the container occurs at high temperatures and sunlight exposure [54] [55]. During the experiment, the temperatures were low, from 2˚C to 16˚C, and the exposure to sunlight was less than 1 hour per day. Thus, the impact of these climate conditions did not affect the migration of BPA to the soil. Furthermore, a very significant issue that affects the BPA concentration is that phytoremediation reduces the detected amount of it. The plants have the ability to transform the BPA into other forms that are harmless to human health [56] [57] [58]. In addition, some microorganisms can metabolism the BPA concentration in the soil, causing a non-detected concentration in the plant cells [59]. SEM images as shown in **Figure 6** indicate that the higher rate of BPA from plastic occurs at refrigerator temperatures and samples exposed to sunlight [60] [61] [62].

Low temperatures construct a challenging environment for most materials, and plastics are no exception. Most plastics at room temperature present their common properties of flexibility (a low Young's modulus) and high resistance to cracking but when the temperature drops this can change rapidly and many common plastics become brittle with low failure stresses [63].

Plastics consist of long chain molecules that are entangled with one another. The degree of entanglement varies with the length and exact structure of the polymer molecule, long chains in most polymers at moderate temperatures are able to slither over one another and the material is flexible. As the temperature is decreased, most polymers begin to stiffen up and they go through what is known as the "glass transition" to become effectively glassy solids with all the properties of glass, becoming exceedingly hard and brittle. Thus, BPA levels release in water increases [63].

5. Conclusions

Although the concentrations of BPA at the cultivated Allium cepa were significantly low, nevertheless further investigation needs to be done under different climate conditions to assure that there is no health risk. The first results present a hopeful way of reusing plastic bottles, but at this point it is not safe to admit that there is no risk of BPA leach. Therefore, it is proposed the use of this vertical arrangement of cultivation only for decorative purposes, planting small flower plants and the cultivation of low growing vegetables during the winter time.

Although BPA is present in plastic water bottles, the concentration rises when the bottles are stored at cold temperatures or in direct sunlight.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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