Bisphenol A (BPA) Detection and Quantification in Plastic Bottles using Vertical Cultivation

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Abstract

Allium Cepa (Onion) is the most extensively produced vegetable in the Kurdistan region, with high production and resistance to environmental conditions. Considering its sensitivity to contaminants, it is commonly used for monitoring or testing environmental pollutants. Drinking water bottles made from polycarbonate plastics containing bisphenol A (BPA) are utilized. In the developing Kurdistan region of northern Iraq, there is an increasing issue of plastic bottle pollution. BPA traces have been found in bottled water samples. BPA release was assessed using HPLC in a vertical growing system with bulbs of the Allium Cepa plant placed in these plastic bottles and monitored growth. Vertical culture was discovered to have a low concentration of BPA in the plant cells than horizontal culture in soil, making it a safe growing method under certain climatic circumstances. The mean concentration of BPA in vertical cultivation is 0.19ug/ml (3.8ng for a 20uL injection), and the Limit of Quantification (LOQ) is 0.63ug/ml (12.7ng for a 20uL injection).

Keywords: HPLC, Bisphenol A, Vertical cultivation, Allium Cepa, plastic bottles.

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Introduction

Sulaimaniyah is one of the Kurdistan region's major cities that has developed significantly. 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 [1].

Allium Cepa (grain onion) is commonly cultivated in the Kurdistan Region considering the great production for obtaining plants with significant resistance to environmental conditions and impressive yield with diverse responses of plants to day length and temperature [2].

Allium Cepa has various advantages: its root growth dynamic is very sensitive to pollutants, it is commonly used for ecotoxicity testing, and it has been adopted for monitoring or testing environmental pollutants by the International Program on Plant Bioassays (IPPB) [2], [3].

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 has more than 5 million inhabitants [4]. All plastic bottles ultimately find their way to landfills, causing environmental pollution. Waste recycling is currently unavailable in the research area that compiles with environmental regulations, and improper solid waste dumping has a negative impact on the environment. The local authorities incinerate the garbage, disregarding the fact that this process releases BPA into the air and soil, resulting in significant environmental pollution.

Solid waste is one of the significant environmental impact problems in developing countries. Approximately 3.5 million tons of MSW are generated daily globally [5]. 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 [6]. The daily per capita waste generation in the Sulaimaniyah governorate was 1.32 kg in 2022, a cumulative solid waste of about 1,325,000 tons, and the plastic portion (bottles and bags) accounted for 13.30 % of the total waste [7]. 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 [8], [9]function and activity of endogenous hormones causing irregularity in the hypothalamus-pituitary-gonadal glands and also the pituitary-adrenal function. BPA has immuno-suppression activity and can downregulate T cells and antioxidant genes. The genotoxicity and cytotoxicity of BPA is paramount and therefore, there is an immediate need to properly detect and remediate its influence. In this review, we discuss the toxic effects of BPA on different metabolic systems in the human body, followed by its mechanism of action. Various novel detection techniques (LC-MS, GC-MS, capillary electrophoresis, immunoassay and sensors.

Among others, it releases BPA at room temperature. At higher temperatures, the rate of rise is faster. A considerable volume of bisphenol-containing items is already in the environment, making them significantly more hazardous [9] function and activity of endogenous hormones causing irregularity in the hypothalamus-pituitary-gonadal glands and also the pituitary-adrenal function. BPA has immuno-suppression activity and can downregulate T cells and antioxidant genes. The genotoxicity and cytotoxicity of BPA is paramount and therefore, there is an immediate need to properly detect and remediate its influence. In this review, we discuss the toxic effects of BPA on different metabolic systems in the human body, followed by its mechanism of action. Various novel detection techniques (LC-MS, GC-MS, capillary electrophoresis, immunoassay and sensors.

According to the United States Environmental Protection Agency (US EPA), the maximum amount of BPA permitted in the human body is 50 μg/kg BW (body weight)/day. 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 [10], [11], [12].

Standard BPA detection techniques include gas chromatography - mass spectrometry (GC-MS) and high-pressure

liquid chromatography (HPLC) [13], [14], [15], [16].

vertical cultivation is an alternative for cultivation or gardens with limited space. This study aimed to establish a better approach to comparing BPA results in high-pressure liquid chromatography, particularly in mono compound manufactured materials. and 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.

1.1 Bisphenol A (BPA)

Bisphenol A (BPA) C15-H16-O2 is the common name for 2,2-(4,4′-dihydroxy diphenyl) propane, 4,4′-isopropylidene diphenol, alternatively, 2,2′-bis (4-hydroxyphenyl) propane [17], an organic compound composed of two phenol rings connected by methyl bridge, with two methyl functional groups attached to the bridge [18]. It has been known since 1930 that BPA is an artificial estrogen, and its estrogen effect was used to promote industry profit [19]. Its essential properties include low vapor pressure, moderate water solubility, low volatility, and solid at room temperature [20], [21]. 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 [22]either as macro- or microplastics. In this article, an overview is given of the presence of marine plastic debris globally and its potential to reach remote locations in combination with an analysis of the oceanic long-range transport potential of organic additives present in plastic debris. The information gathered shows that leaching of hydrophobic substances from plastic is slow in the ocean, whereas more polar substances leach faster but mostly from the surface layers of the particle. Their high content used in plastic of several percent by weight allows also these chemicals to be transported over long distances without being completely depleted along the way. It is therefore likely that various types of additives reach remote locations with plastic debris. As a consequence, birds or other wildlife that ingest plastic debris are exposed to these substances, as leaching is accelerated in warm-blooded organisms and in hydrophobic fluids such as stomach oil, compared to leaching in water. Our estimates show that approximately 8100–18,900 t of various organic additives are transported with buoyant plastic matrices globally with a significant portion also transported to the Arctic. For many of these chemicals, long-range transport (LRT. 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 producing polycarbonate (PC) plastics and epoxy resins. Polycarbonate plastics find extensive applications in various everyday items such as medical devices and food and beverage storage containers. This is due to their exceptional qualities including high impact strength, hardness, toughness, transparency, and resistance to temperature changes. Additionally, to safeguard food and beverages from direct contact with metal, epoxy resins are employed as inner coatings for food and beverage cans. BPA can also be present in children's toys as an additive, used in different types of plastics. [23] [24] [25], [26].

Although BPA is introduced extendedly in everyday life, supported by industry studies that showed various risks to human health [27], [28], studies funded by government agencies showed a wide range of effects on humans. BPA is an endocrine disruptor that can mimic the body's hormones [29], [30]. After entering the human body, BPA can disrupt normal cell function by acting as an estrogen agonist [29], [31], as well as an androgen antagonist [32], which may affect health. It has been suspected that BPA may be carcinogenic, potentially leading to the precursors of breast cancer [33]. 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 [34], [35]. The ester bond linking BPA molecules in polycarbonate and resins is subject to hydrolysis, resulting in the leaching of BPA monomer even from new polycarbonate into the water at room temperature [25], [36], [37]. 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 [38]. 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 [27], [39]. 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 [26]. Measured concentrations of BPA in human blood, urine and other tissues confirm that exposure is widespread in the human population.

Materials and Methods

Small plastic bottles of 330ml were arranged in a vertical position. The narrow part with the cap was to wording the ground, a hole of 3cm in diameter was opened and 8cm 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. A 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 bottles of each row, the rest were filled with soil. According to [40], The genus Allium is characterised by herbaceous geophyte perennials with true bulbs, some of which are borne on rhizomes, and an onion or garlic odor and flavor. 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. A total of 12 vertical rows were placed with 60 paired Allium bulbs in each bottle outdoors in the Autumn season, to ensure that a minimum of one of them will be successfully cultivated. The watering was done by hand-filling the top bottle. The water moved gradually from top to bottom, watering the plants and the excess water was collected and reused.

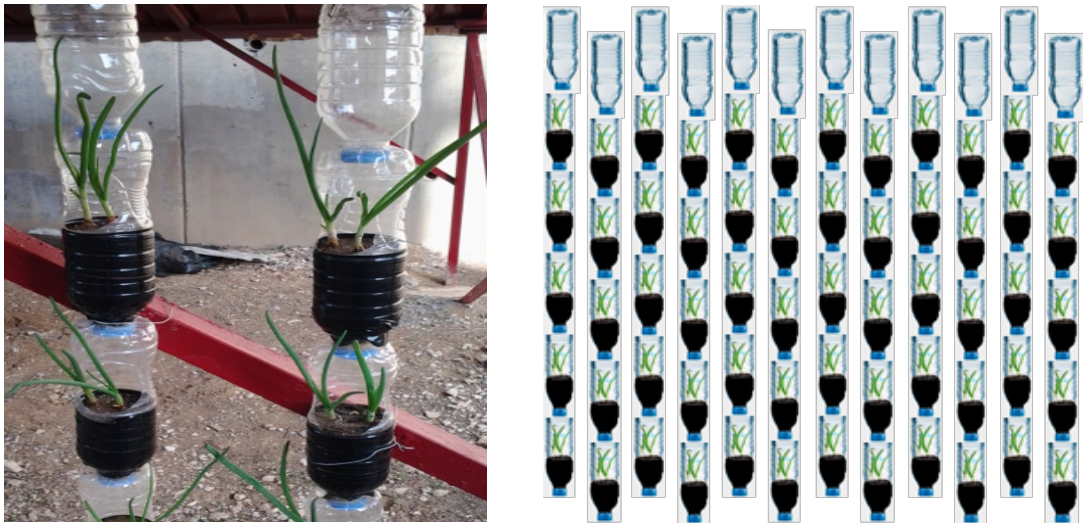
The same soil mixture was used for planting Alliums in the cultivation table, 60 paired bulbs were planted near the vertical arrangement to discover possible plant growth differences. For the laboratory data, BPA (>99%, CAS 80-05-7) was purchased from Sigma Aldrich (Germany). All other solvents used 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 is essential for the use of AFFINIMIP SPE cartridges (solid phase extraction):

(i) SPE vacuum manifold (Phenomenex, USA).

(ii) Mini vacuum pump.

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

Fig.1 Vertical cultivation in plastic boatel.



HPLC Sample Preparation and Analysis

Samples were received in good condition and stored in glass containers in a refrigerator under -2 oC before analysis.

Cold spring onions are put in a desiccator to get to room temperature before extraction process. Then, 25g of spring onions, including the root system, and 50ml of water/acetonitrile are shaken for 30min in an orbital shaker at 150rpm. After that, solids are removed with filtration through a filter paper (4-7um), and the filtrates are centrifuged for 10min at 4000rpm. 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 [41]. The clean-up method followed, as shown in the table below.

The solution is collected in an amber glass vial (4ml) and is evaporated until dry 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 free from polycarbonate material or polymers[42].

Table 1. Sample Clean-up Method in HPLC

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

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 228nm. Chromatographic separation is carried out with a C18 reversed-phase column (Symmetry RP 18 150mm x 3.5mm, 5um) in gradient mode at a flow rate of 0.8ml/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 oC.

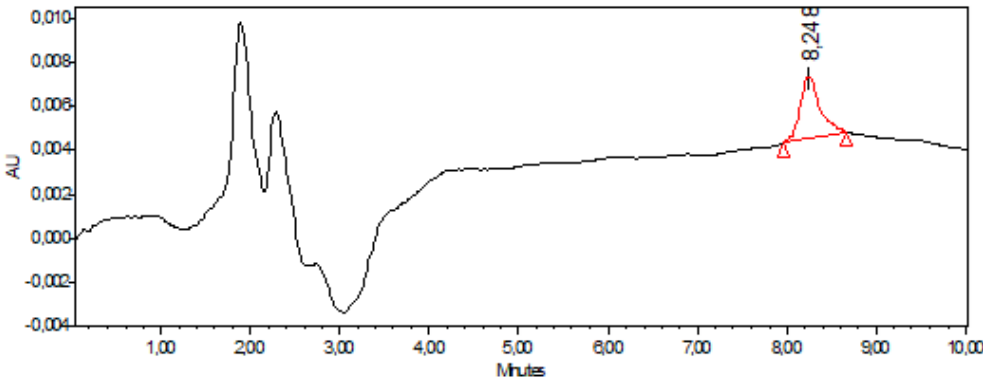
Table 2: HPLC Gradient system

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

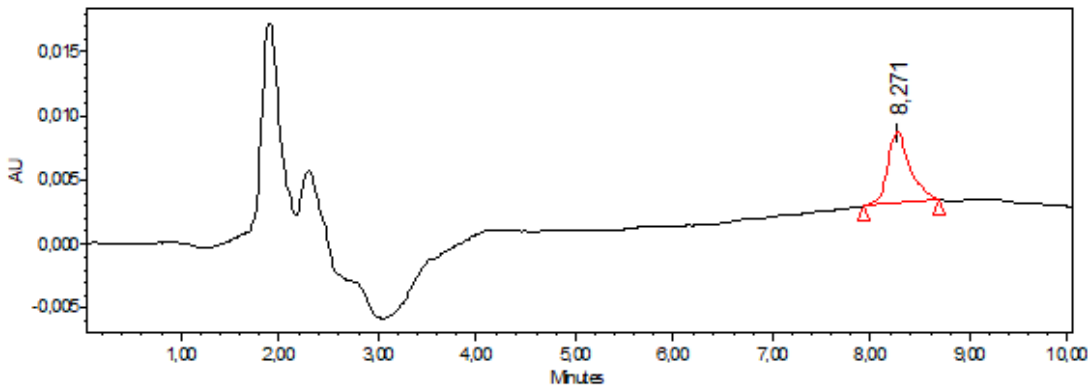
Results

Six standard solutions (250 - 10.000ng/ml) are tested to determine the linearity of BPA as shown in Figure 2.

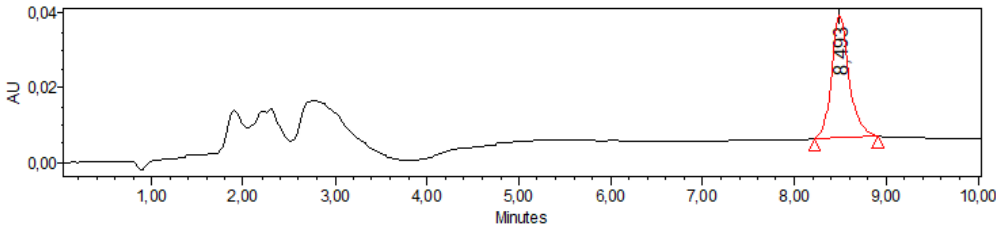
Std. of 250ng/ml



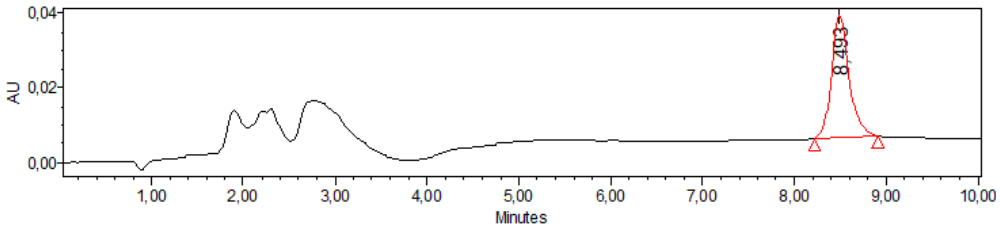
Std. of 500ng/ml



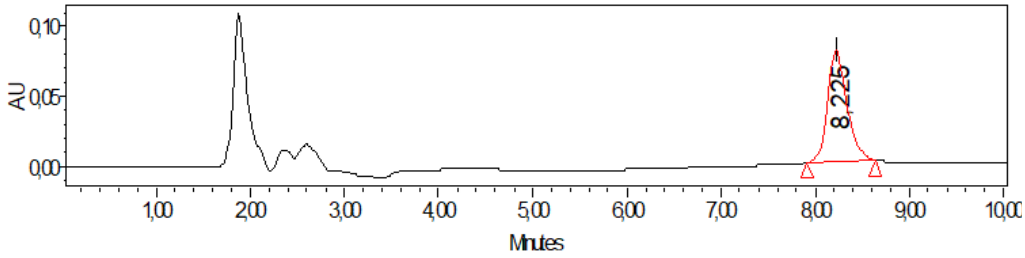
Std. of 1000ng/ml



Std. of 2000ng/ml



Std. of 5000ng/ml



Std. of 10000ng/ml

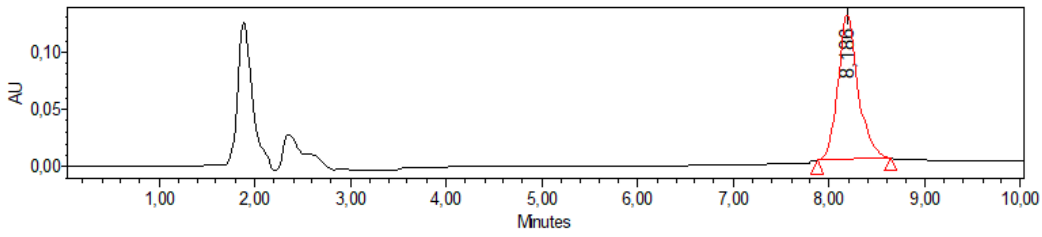


Fig 2. HPLC Chromatograms of calibration curve and samples.

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= 0.0001 x+0.0805 (correlation coefficient 0.999), as shown in Figure 3. The limit of detection (LOD) of BPA is 0.19ug/ml (3.8ng for 20uL injection), and the limit of quantification (LOQ) is 0.63ug/ml (12.7ng for 20uL injection)

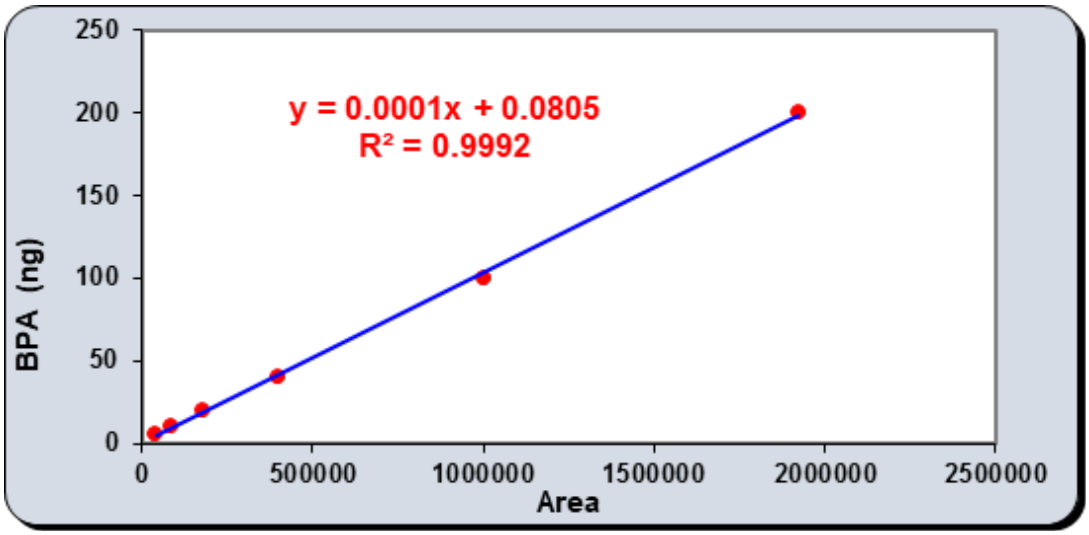
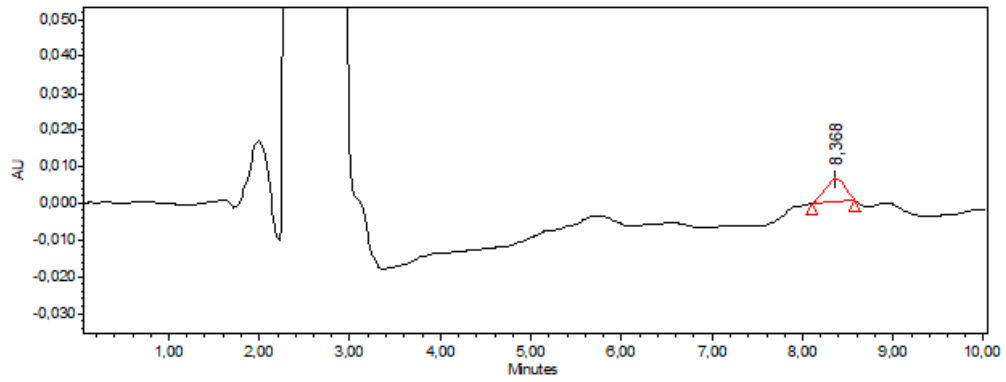


Fig. 3 correlation coefficient

Concentration of BPA in Planted Allium Cepa

AFFINIMIP SEP ® SPE cartridges are selected after testing other SPE methods (OASIS SPE, etc). MIPs are useful tools in sample preparation as analysis is entrapped in the polymer (BPA). Thus, MIPs offer high recoveries (>95%), repeatability and reproducibility. Method validation, robustness has 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.



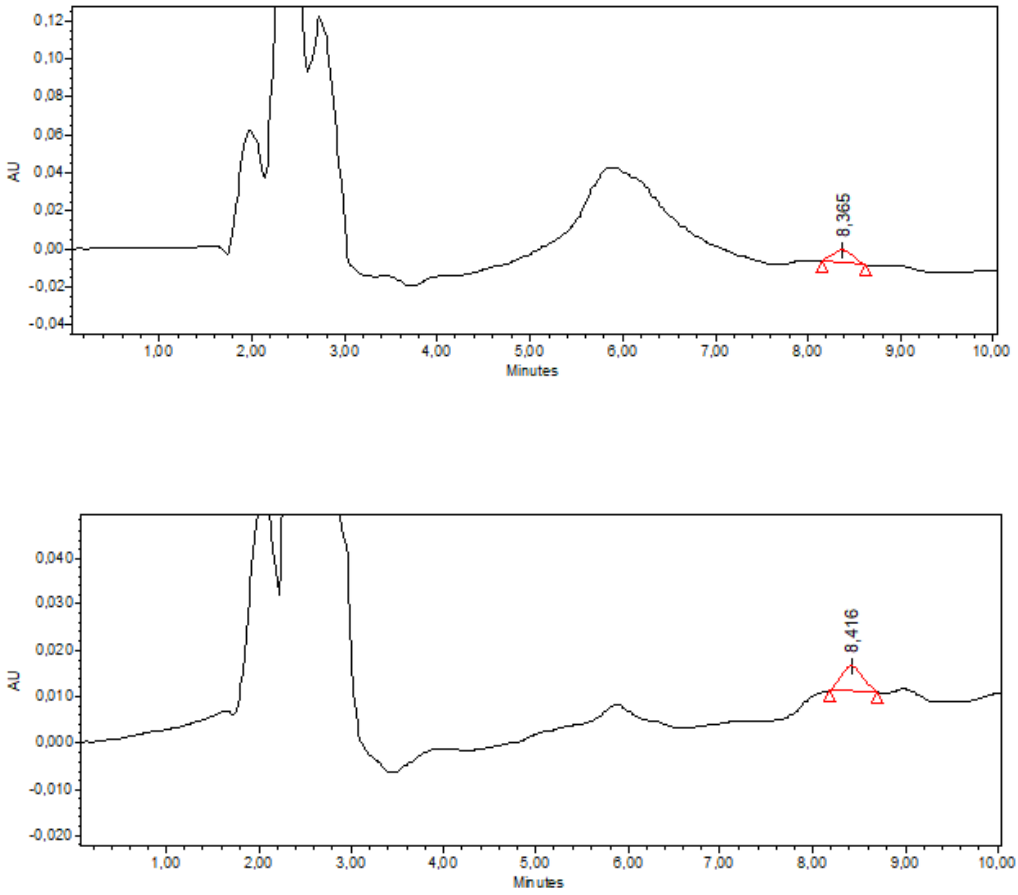


Fig. 4 HPLC chromatograms of the analyzed Allium Cepa for BPA

[43]2-bis (4-hydroxyphenyl set a total Tolerable Daily Intake (TDI) of BPA is 0.05 mg/kg body weight (bw)/day, which is gradually higher than the Allium concentrations that were found in current study The mean concentration of BPA in vertical cultivation is 0.19ug/ml (3.8ng for a 20uL injection), and the limit of quantification (LOQ) is 0.63ug/ml (12.7ng for a 20uL injection).. 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 [44]. In the study area, the garbage is burned, as it is mentioned above; therefore, the habitants surcharged 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 [45],[46]. During the experiment, the temperatures were low, from 2 oC to 16 oC, and the exposure to sunlight was less than 1 hour per day. Thus, the impact of these climatic conditions did not affect the migration of BPA to the soil. Furthermore, a very significant factor 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 [47], [48] and [49]. In addition, some microorganisms can metabolism the BPA concentration in the soil, causing a non-detected concentration in the plant cells [50].

Conclusion

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 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 because of the sun heating. The plastic raised the bottles' soil temperature, germinating the bulbs faster than those in the ground soil. The rate of growth was the same after two months of cultivation. The weight of both cultivars was almost the same, with 25gr for each plant.

Although the concentrations of BPA at the cultivated Allium Cepa were significantly low,further investigation needs to be done under different climate conditions to ensure 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 to use this vertical arrangement of cultivation only for decorative purposes, planting small flower plants and cultivating low-growing vegetables during the winter time.

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