

## ORIGINAL ARTICLE

## Food Chemistry

# Elucidation of heavy metal content, phenolic profiles, and antioxidant activities of kale (*Brassica oleracea* L. var. *acephala*) and arugula (*Brassica eruca* L.) grown in urban gardens in Istanbul

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**Abstract:** This study was conducted to evaluate the effect of two distances: close (0–10 m) and far (60 m) from the heavy traffic roadside, at three different cultivation sites (MS: Mevlanakapi–Silivrikapi, SB: Silivrikapi–Belgradkapi, and BY: Belgradkapi–Yedikule kapi) along the road line. First, the phenolic compounds, antioxidant activity, and physicochemical properties in kale and arugula vegetables were examined. Second, heavy metal concentrations in vegetables, soil, and irrigated water were investigated. In both vegetables, the highest total phenolic content was detected in samples obtained from far distance in SB site (3880.3 mg/kg) for kale and in BY site (1459.9 mg/kg) for arugula, whereas the lowest content was found at the close distance in MS site for both kale (448.5 mg/kg) and arugula (586.4 mg/kg). The antioxidant activity values [mg Trolox/kg (dw)] ranged from 366.74 to 586.10 and 2349.00 to 3757.4 for kale and from 520.00 to 945.60 and 3323.00 to 5814.70 for arugula in 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl methods, respectively. The levels of Cd and Hg in kale and arugula and Fe content in arugula exceeded FAO/WHO permissible limits, making them unsafe for human consumption. Meanwhile, the Pb content in kale and arugula and Fe content in kale were observed to be within acceptable limits set by FAO/WHO. In the irrigated water, the Pb value was below the permissible limit, whereas the Cd value was above it and no Hg and Fe were detected. In the soil samples, the Pb and Fe values were below the limit, whereas the Cd and Hg values were higher.

**KEYWORDS**

antioxidant, heavy metals, phenolic compounds, urban agriculture, vegetable

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## 1 | INTRODUCTION

Vegetables are an important component of a healthy, balanced, and sustainable diet (Pojer et al., 2013). Brassicaceae (syn. Cruciferae) encompasses a substantial number of plant species, involving several familiar vegetables. Kale or black cabbage (*Brassica oleracea* L. var. *acephala*) and *Eruca sativa* (*Brassica eruca* L.) commonly known as rocket salad or arugula (roquette, rugula, rucoli, rucola, and colewort) are both leafy green vegetables belonging to the Brassicaceae family. According to the positive effects of these leafy vegetables on human health and the economical-agricultural income, they have recently been focused on several scientific research interests. They are a source of glucosinolates and contain several bioactive compounds, such as polyphenols, minerals, and vitamins. Moreover, minerals in vegetables have a positive influence on human health. However, some of essential micronutrients for daily consumption must not exceed more than a few milligrams per day (Ajasa et al., 2004). In addition, the consumption of some heavy metal-contaminated vegetables can also pose a threat to the human health (Khan et al., 2008).

Accelerated urbanization, industrial progress, rapid population growth, and the increasing number of vehicles in traffic are leading to environmental pollution, causing contamination of soil, water, and air. Furthermore, people are also entering urban farming to meet the ever-growing demand for the fresh crops (Tomno et al., 2020). In fact, the concentration of heavy metals in vegetables grown in cities is related to the location where the plants are cultivated. When plants are grown near pollution sources such as main roads, the risks of heavy metal accumulation may increase (Antisari et al., 2015). Contact with heavy metals may result in various adverse health impacts, including chronic and sub-chronic effects such as shortness of breath, neurotoxicity, mutagenicity, and teratogenicity as well as various types of cancers, depending on the type of the heavy metal. For instance, Cu, Pb, and Cd are associated with upper gastrointestinal cancer, which is responsible for almost 25% of all cancer-related deaths worldwide (Sayo et al., 2020).

Regarding the improvement of functional foods, the attention of researchers, consumers, and industrialists in the raw plant materials rich in bioactive compounds has risen. The beneficial impacts of Brassicaceae vegetables on health development have been relatively ascribed to their complex combination of phytochemicals that have the potential for antioxidant capacity. Among phytochemicals possessing antioxidant activity, phenolic compounds are one of the most significant classes. The varieties and concentrations of phenolic compounds differ significantly

among vegetables. For the purpose of separation and quantification of individual phenolic compounds, the high performance liquid chromatography (HPLC) is most frequently used, because of its high-separation capacity and relative simplicity (Huang et al., 2007).

There is limited information in the literature regarding the effect of the distance of kale and arugula cultivation sites from the road with the vehicular traffic on the profile of phenolic compounds, antioxidant capacity, and heavy metals accumulation in these vegetables. Therefore, the aim of this study was to compare the effect of the distance of the cultivation sites from the road with vehicular traffic on the profile of phenolic compounds by using the HPLC. Second, heavy metal accumulation in fresh kale and arugula samples was assessed by using atomic absorption spectrometry. Additionally, the antioxidant activity was measured by two different methods (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) [ABTS] and 2,2-diphenyl-1-picrylhydrazyl [DPPH]) and physicochemical properties of vegetable samples were also evaluated.

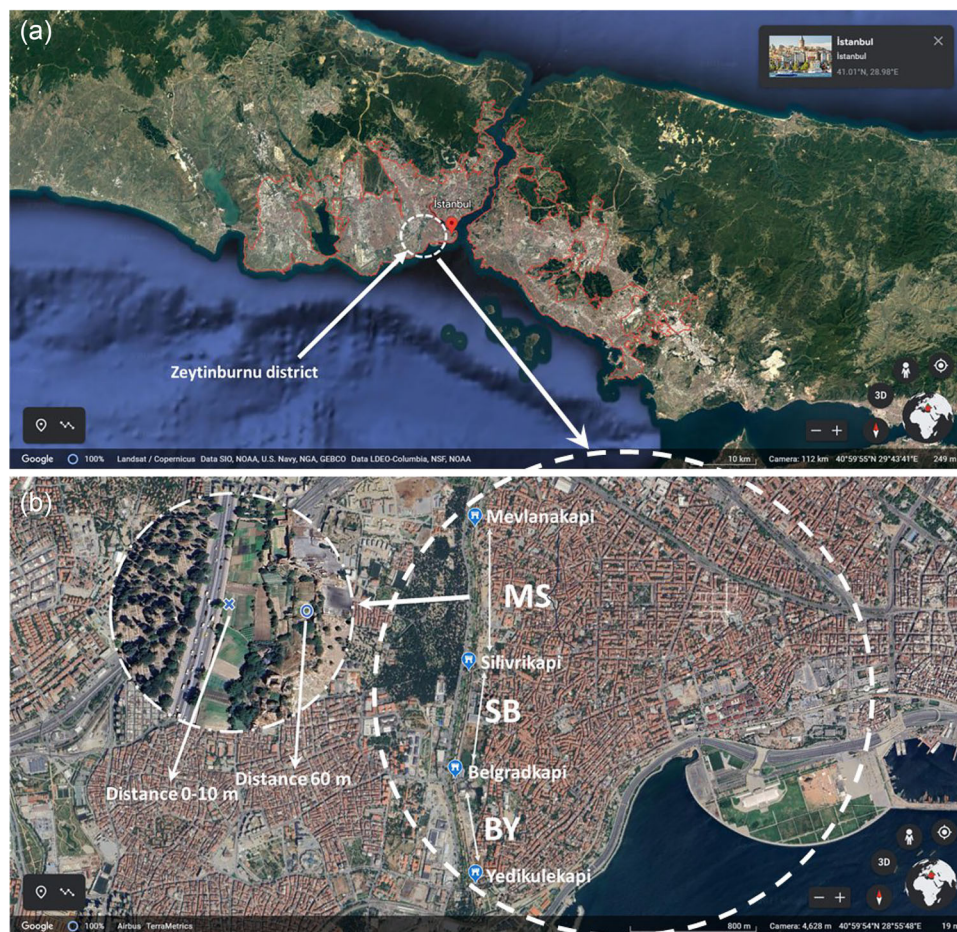
## 2 | MATERIALS AND METHODS

### 2.1 | Chemical and reagents

The chemicals used in the analyses, including methanol (HPLC grade), formic acid, sodium carbonate, and hydrochloric acid, were obtained from Merck, known for their HPLC grade solvents. Standards for phenolic compounds were provided by Sigma Chemical Co.

### 2.2 | Sampling of vegetables, water, and soil

The leaves of the studied vegetables, kale (*B. oleracea* L. var. *acephala*) and arugula (*B. eruca* L.), were harvested by local farmers from urban vegetable gardens located in Zeytinburnu district of Istanbul, Türkiye, in 2021. After harvesting, the leaves were immediately transported to the laboratory and stored in a cold room at 5°C in darkness. This area is characterized by heavy traffic and urbanization, stretching from Mevlanakapi to Yedikule kapi, near the historical city walls (Figure 1). The vegetable gardens were divided into three different sites: (1) Mevlanakapi to Silivrikapi (MS), (2) Silivrikapi to Belgradkapi (SB), and (3) Belgradkapi to Yedikule kapi (BY) along the coastline. Each of these three sites was further divided into two sites based on the distance from the roadside: close (0–10 m) and far (60 m). Soil samples were collected from



**FIGURE 1** Sampling sites adapted from Google Earth showing: (a) the location of Zeytinburnu district in Istanbul city of Türkiye and (b) sampling site stations in the 10. Yil Street of Yedikule area in Zeytinburnu district.

sites where vegetable samples were taken, both from the surface and at a depth of 25 cm. Soil samples were collected from five different random points in each site and mixed to form a single composite sample. Water samples were obtained from wells and ponds associated with the vegetable gardens. The vegetables were wrapped in cardboard and placed in plastic bags, whereas water samples were collected in sterile containers and stored in dark and cold conditions (4°C) until analysis. Three replicates were performed for samples collected from each site and distance.

### 2.3 | Physicochemical analysis

The dry matter (determined by oven drying method) (Anonymous, 1990), pH (measured using a digital pH meter, Mettler Toledo, Seven Compact TMS220, Schwerzenbach, Switzerland), and ash (determined using a muffle furnace at 550°C) (Ough & Amerine, 1988) in vegetable samples were analyzed. The color parameters, such as  $L^*$  (lightness),  $a^*$  (red/greenness), and  $b^*$  (yel-

low/blueness) values, were measured by using a colorimeter (Konica Minolta, Chroma Meter CR-5) at six locations on each vegetable sample (Cui et al., 2016).

## 2.4 | RP-HPLC Analysis of phenolic compounds and antioxidant capacity

### 2.4.1 | Extraction of phenolic compounds

Fresh kale and arugula samples, each weighing 5 g in triplicate, were diced. To each vegetable sample, 20 mL of methanol–water (85/15) mixture was added. The samples were vortexed, placed in an ultrasonic bath for 30 min, and then stirred under nitrogen gas for 2 h. After mixing under nitrogen gas, the samples were centrifuged at 4500 rpm for 10 min. After centrifugation, the samples were filtered through coarse filter paper. The vegetable extracts were concentrated to approximately 4 mL by evaporating the methanol using a rotary evaporator when reaching a temperature of 8°C (Amanpour et al., 2019). The obtained extracts were passed through 0.45  $\mu\text{m}$  membrane filters,

and the vegetable extracts were used for both antioxidant activity and HPLC analysis.

## 2.4.2 | Determination of phenolic compounds

The filtration of phenolic extracts was done using a 0.45 µm pore size polyvinylidene fluoride membrane filter prior to analyses.

The phenolic content of the vegetable extracts was determined using a Shimadzu LC 20 AD Prominence HPLC system (Shimadzu Corporation). The HPLC system was equipped with photodiode array detector model SPD-M20A, a pump system with an autosampler model SIL-20A HT, CTO-20A column heater, and DGU-20A5 degasser units. The column used for separation was C18 ODS-3 (250 × 4.6 mm I.D.) with a 5 µm packing (GL Sciences). The chromatographic conditions were arranged according to our previously published paper (Kocabey et al., 2016) for determination of phenolic compounds. Analytical standards (min 99% purity) [vanillic, syringic, 2,5-dihydroxybenzoic, rutin, *p*-coumaric, ferulic, sinapic, caffeic, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, and hesperidin] were purchased from Sigma-Aldrich. The phenolic compounds were quantified by comparison with peak areas of each standard. All analyses were made in triplicate.

## 2.4.3 | Evaluation of antioxidant capacity

The antioxidant potential of the samples was evaluated using the ABTS and DPPH methods. For the ABTS assay, the antioxidant capacities of the vegetables were assessed based on the decolorization of ABTS radical cation through an analysis method (Amanpour et al., 2019). The absorbance of the samples was measured at 734 nm and compared with the Trolox standard solutions, with results expressed as mg Trolox/kg of vegetables. DPPH radical scavenging activity was measured at 517 nm over an analysis method (Amanpour et al., 2019), as detailed in our previous research (Kocabey et al., 2016). The DPPH results were expressed as mg Trolox/kg of vegetables.

## 2.5 | Heavy metal analysis

### 2.5.1 | Heavy metal analysis in vegetables

After the samples were diced and dried in an oven, they were ground in a mortar. The ground samples were then burned in a muffle furnace at 550°C for 2.5 h. To the burned

samples, 3 mL of a 1/3 HCl solution was added. The samples were washed with 27 mL of distilled water through sieves and filtered using blue band filter paper. The filtered samples underwent heavy metal analysis using a Perkin Elmer Atomic Absorption Spectrometer AAnalyst 800 (AAS) (Uçar Sözmen et al., 2020).

### 2.5.2 | Heavy metal analysis in soil

Air-dried soil samples were passed through a 2 mm sieve. One gram of soil samples was weighed, and 15 mL of HCl and 5 mL of HNO<sub>3</sub> were added. The solution was dissolved at 300°C using a mixer. The dissolved samples were then filtered through blue band filter paper and transferred to 250 mL volumetric flasks. Ultrapure water was added to reach a total volume of 250 mL (Akyildiz & Karataş, 2018). After transferring the samples to 50 mL Falcon tubes, the heavy metal analysis of the samples was conducted using a Perkin Elmer AAS (Yaşar et al., 2016).

### 2.5.3 | Heavy metal analysis in water

For water samples, 100 mL was taken, and 1 mL of 65% HNO<sub>3</sub> solution was added to prevent microbial activity. After transferring the samples to 50 mL Falcon tubes, the heavy metal analysis of the samples was conducted using a Perkin Elmer AAS (Yaşar et al., 2016).

## 2.6 | Statistical analysis

All analyses were performed at least three times, and the mean values and standard deviations were calculated using Microsoft Excel software. The Mann–Whitney *U* test was used to compare mean values between two groups. Analysis of variance, Duncan's multiple comparison test, and hierarchical clustering analysis were used to find any significant differences between samples. Differences at both  $p < 0.05$  and  $p < 0.01$  were considered statistically significant. Statistical analysis was performed using SPSS 22 software package and R software (version 3.6.2 (2019-12-12)-CRAN).

## 3 | RESULTS AND DISCUSSION

### 3.1 | Physicochemical properties of kale and arugula vegetables

The mean values of physicochemical parameters, including dry matter, ash, pH, and color, in both kale and arugula

vegetable samples obtained from three different cultivation sites settled on the side of the road line (MS, SB, and BY) and two different distances from the road (0–10 and 60 m) through the period of the survey period were presented in Table 1. The values of dry matter and ash in kale samples ranged between 13.29%–19.11% and 2.17%–2.76%, respectively. Sikora and Bodziarczyk (2012) reported a dry matter value of 17.08% and an ash value of 2.11% in kale vegetables. In the case of arugula samples, the dry matter and ash values were found to range between 10.75%–12.59% and 1.92%–2.84%, respectively. In some studies, the highest ash value of 1.8% (Tassi et al., 2018) and the lowest ash value of 0.91% (da Silva Torres et al., 2006) in arugula (*E. sativa* L.) samples were reported. In the context of this study, the dry matter values in both kale and arugula vegetables aligned with results from other literature studies, whereas their ash values were slightly higher compared to other investigations. According to the analysis results, there was an increasing trend in pH values of both kale and arugula samples as the distance of the cultivation sites increased from the road. The carbon dioxide in the exhaust gas of vehicles in the traffic road may affect the metabolic activities in the vegetables, resulting in a rapid decrease in their pH values (Daniels et al., 1985).

The green color of leafy vegetables is an important quality characteristic perceived by consumers (Colonna et al., 2016). In kale samples, the highest  $L^*$  value was found in the samples grown at a distance of 60 m in the MS site (41.25), whereas the lowest value was found in the samples grown at a distance of 60 m in the BY site (34.87). Although the highest  $a^*$  value in the kale samples was found in the samples grown at a distance of 0–10 m in the BY site (−7.0), the lowest value was found in the samples grown at a distance of 0–10 m in the MS site (−4.10). Similarly, the highest  $b^*$  value was found in the samples grown at a distance of 0–10 m in the BY site (11.2) in the kale samples, whereas the lowest value was found in the samples grown at a distance of 0–10 m in the MS site (9.13). In a study carried out to analyze the color of kale, it was determined that the  $L^*$  value was between 36.48 and 45.44,  $a^*$  value between −8.26 and −7.54, and  $b^*$  value between 13.93 and 20.32 (Nunes et al., 2009). The values of  $L^*$ ,  $a^*$ , and  $b^*$  in kale samples under the study shown in Table 1 were in agreement with the literature results (Nunes et al., 2009). In the case of arugula vegetable samples, the highest  $L^*$  value was determined in the samples grown at a distance of 0–10 m in the SB site (41.58), whereas the lowest value was obtained in the samples grown at a distance of 0–10 m in the MS site (28.32). The highest  $a^*$  value was determined in the arugula samples grown at a distance of 60 m in the SB site (−9.36), whereas the lowest value was determined in the samples grown at a distance of 0–10 m in the MS site (−4.71). Similarly, the highest  $b^*$  value was

found in the samples grown at a distance of 60 m in the SB site (21.73), whereas the lowest value was found in the samples grown at a distance of 0–10 m in the MS site (10.98). In a study conducted in Italy on 10 different kinds of leafy vegetables, the average  $L^*$ ,  $a^*$ , and  $b^*$  values in arugula were found to be 48.0, −2.2, and 26.5, respectively (Colonna et al., 2016). In another study examining the color values in arugula, it was reported that  $L^*$  values ranged from 42.02 to 43.50,  $a^*$  values from −15.05 to −19.90,  $b^*$  values from 19.86 to 22.98 (Tuncay, 2011). It was determined that the  $L^*$  values in arugula were between 42.68 and 43.83,  $a^*$  values between −16.74 and −17.77, and  $b^*$  values between 24.91 and 26.77 (Oztekin et al., 2019). Although the results for arugula samples demonstrated in Table 1 in the current study were similar to the literature (Colonna et al., 2016; Oztekin et al., 2019) in terms of  $L^*$  and  $b^*$  values, it was found that they were between the range of −2.2 (Colonna et al., 2016) and −19.90 (Tuncay, 2011) in terms of  $a^*$  value.

### 3.2 | Characterization of phenolic compounds in kale and arugula vegetables and effect of distances from the traffic road in different cultivation sites on these compounds

Regarding the profile of the phenolic compounds in vegetable samples, a total of 12 and 11 compounds were identified and quantified in kale and arugula samples, respectively (Table 2). As can be seen in Table 2, the most dominant phenolic compound quantitatively in kale vegetables is *trans*-caffeic acid (1246.8–2797.5 mg/kg), followed by rutin, 4-dihydroxybenzoic acid, and quercetin-3-*O*-glucoside; and in arugula vegetables is quercetin-3-*O*-glucoside (382.2–871.1 mg/kg), followed by vanillic acid, caftaric acid, and hesperidin. Most of those compounds have already been identified by former studies on both vegetables (Bianchi et al., 2024; Fusari et al., 2020; Li et al., 2018; Seong et al., 2016). Total phenolic content in kale and arugula vegetables ranged from 448.5 to 3880.3 mg/kg in kale samples and from 586.4 to 1459.9 mg/kg in arugula samples. According to the literature, these amounts are in conformity with those affirmed in several former investigations in kale and arugula (Korus, 2011; Seong et al., 2016; Zappia et al., 2019). Although the outcomes demonstrated that the amount of total phenolic compounds slightly diminished as the distance of vegetable cultivation site from the road in MS and SB sites decreased, whereas it was slightly raised in BY site, the differences of the total phenolic compounds in kale samples were not significant depending on the distance from the vehicle road ( $p < 0.05$ ). On the contrary, the differences of the total phenolic compounds in kale samples were found to be

TABLE 1 Physicochemical properties of kale and arugula vegetables.

Physicochemical properties	Distance (m)	Kale			Arugula			
		MS	SB	BY	MS	SB	BY	
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Dry matter (%)	0–10	15.94 ± 0.85 <sup>a</sup>	13.85 ± 0.96 <sup>a</sup>	13.76 ± 0.76 <sup>a</sup>	12.59 ± 0.4 <sup>a</sup>	12.02 ± 0.4 <sup>a</sup>	11.44 ± 3.5 <sup>a</sup>	0.132
	60	19.11 ± 0.10 <sup>a</sup>	13.29 ± 1.03 <sup>a</sup>	15.31 ± 1.29 <sup>a</sup>	12.26 ± 0.6 <sup>a</sup>	10.75 ± 0.6 <sup>a</sup>	12.42 ± 2.8 <sup>a</sup>	0.472
<b>p</b>		<b>0.003**</b>	0.532	0.149	0.452	<b>0.042*</b>	0.727	
Total ash (%)	0–10	2.19 ± 0.00 <sup>a</sup>	2.76 ± 0.02 <sup>a</sup>	2.38 ± 0.09 <sup>a</sup>	2.05 ± 0.15 <sup>b</sup>	2.84 ± 0.2 <sup>a</sup>	2.13 ± 0.12 <sup>a</sup>	0.878
	60	2.18 ± 0.06 <sup>a</sup>	2.17 ± 0.05 <sup>a</sup>	2.28 ± 0.24 <sup>a</sup>	2.19 ± 0.15 <sup>a</sup>	2.22 ± 0.03 <sup>a</sup>	1.92 ± 0.10 <sup>a</sup>	0.976
<b>p</b>		0.925	<b>0.004**</b>	0.623	0.462	0.06	0.195	
pH	0–10	6.060 ± 0.01 <sup>b</sup>	6.12 ± 0.01 <sup>a</sup>	6.18 ± 0.01 <sup>a</sup>	6.24 ± 0.01 <sup>c</sup>	6.30 ± 0.01 <sup>b</sup>	6.36 ± 0.01 <sup>a</sup>	<b>0.001**</b>
	60	6.090 ± 0.01 <sup>c</sup>	6.15 ± 0.01 <sup>b</sup>	6.21 ± 0.01 <sup>a</sup>	6.27 ± 0.01 <sup>c</sup>	6.33 ± 0.01 <sup>b</sup>	6.39 ± 0.01 <sup>a</sup>	<b>0.001**</b>
<b>p</b>		<b>0.021*</b>	<b>0.021*</b>	<b>0.021*</b>	<b>0.021*</b>	<b>0.021*</b>	<b>0.021*</b>	
<b>Color values</b>								
<b>L</b>	0–10	35.78 ± 3.15 <sup>a</sup>	39.61 ± 2.21 <sup>a</sup>	40.30 ± 1.01 <sup>a</sup>	28.32 ± 2.68 <sup>a</sup>	41.58 ± 2.15 <sup>b</sup>	36.65 ± 2.58 <sup>b</sup>	<b>0.002**</b>
	60	41.25 ± 1.76 <sup>b</sup>	37.76 ± 0.14 <sup>ab</sup>	34.87 ± 1.73 <sup>b</sup>	38.98 ± 1.08 <sup>b</sup>	41.47 ± 1.09 <sup>a</sup>	36.48 ± 0.43 <sup>c</sup>	<b>0.002**</b>
<b>p</b>		0.058	0.221	0.058	<b>0.003*</b>	0.943	0.916	
<b>a</b>	0–10	-4.10 ± 1.18 <sup>a</sup>	-4.90 ± 0.02 <sup>a</sup>	-7.0 ± 0.34 <sup>b</sup>	-4.71 ± 0.53 <sup>b</sup>	-6.66 ± 0.97 <sup>a</sup>	-7.03 ± 0.53 <sup>a</sup>	<b>0.014*</b>
	60	-6.13 ± 0.25 <sup>a</sup>	-6.22 ± 0.09 <sup>a</sup>	-5.64 ± 0.52 <sup>a</sup>	-7.34 ± 0.67 <sup>b</sup>	-9.36 ± 0.51 <sup>a</sup>	-6.69 ± 0.81 <sup>b</sup>	<b>0.007**</b>
<b>p</b>		<b>0.044*</b>	<b>0.001**</b>	<b>0.019*</b>	<b>0.013*</b>	<b>0.013*</b>	0.578	
<b>b</b>	0–10	9.13 ± 0.11 <sup>a</sup>	9.77 ± 1.09 <sup>a</sup>	11.2 ± 1.36 <sup>a</sup>	10.98 ± 2.68 <sup>a</sup>	14.44 ± 0.16 <sup>a</sup>	13.40 ± 1.32 <sup>a</sup>	0.116
	60	9.88 ± 0.37 <sup>a</sup>	9.30 ± 0.21 <sup>a</sup>	9.78 ± 0.73 <sup>a</sup>	14.3 ± 1.347 <sup>b</sup>	21.73 ± 1.69 <sup>a</sup>	14.07 ± 1.35 <sup>b</sup>	<b>0.007**</b>
<b>p</b>		<b>0.028*</b>	0.509	0.193	0.125	<b>0.002**</b>	0.57	

Note: It refers to the area between Mevlanakapi and Siliirikapi (MS), between Siliirikapi and Belgradkapi (SB), and between Belgradkapi and Yedikule kapi (BY) coastline. The statistics of the results in the 0–10 and 60 m distances from the road line on the sites are the results of the Mann–Whitney *U* test. Analysis of variance and Duncan's multiple comparison test were used to find significant differences in the results of the three different sites (MS, SB, and BY).  $\bar{X} \pm SD$ ; \*Results are the means of three repetitions performed in the laboratory; <sup>a</sup>SD standard deviation. Different letters on the same line are statistically different: \*significant differences at  $p \leq 0.05$ ; \*\*significant differences at  $p \leq 0.01$ .

TABLE 2 Phenolic compounds and antioxidant activities in kale and arugula vegetables.

Phenolic compounds	Kale				Arugula			
	Distance (m)	MS	SB	BY	MS	SB	BY	P
		Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	
<b>Phenolic acids</b>								
Sinapic acid	0–10	23.8 ± 2.22 <sup>a</sup>	21.0 ± 1.60 <sup>a</sup>	22.5 ± 0.94 <sup>a</sup>	25.0 ± 15.1 <sup>a</sup>	37.7 ± 5.32 <sup>a</sup>	24.1 ± 0.3 <sup>a</sup>	0.077
	60	18.0 ± 0.8 <sup>b</sup>	25.8 ± 1.18 <sup>a</sup>	16.1 ± 0.0 <sup>c</sup>	<b>0.001**</b>	44 ± 0.07 <sup>a</sup>	33.5 ± 10.4 <sup>a</sup>	15.3 ± 0.4 <sup>b</sup>
<b>P</b>		0.07	0.076	<b>0.011*</b>	0.211	0.662	<b>0.002**</b>	
<i>t</i> -Caffeic acid	0–10	n.d. <sup>c</sup>	1246.8 ± 317 <sup>b</sup>	2120 ± 107 <sup>a</sup>	n.d.	n.d.	n.d.	–
	60	n.d. <sup>c</sup>	2797.5 ± 603 <sup>a</sup>	2026 ± 234 <sup>b</sup>	<b>0.001**</b>	n.d.	n.d.	n.d.
<b>P</b>		–	0.084	0.656	–	–	–	–
Chlorogenic acid	0–10	73.9 ± 10.4 <sup>b</sup>	n.d. <sup>c</sup>	149 ± 62.3 <sup>a</sup>	n.d.	n.d.	n.d.	–
	60	91.7 ± 7.61 <sup>a</sup>	n.d. <sup>c</sup>	63.6 ± 1.08 <sup>b</sup>	<b>0.001**</b>	n.d.	n.d.	n.d.
<b>P</b>		0.19	–	0.193	–	–	–	–
Caftaric acid	0–10	n.d.	n.d.	n.d.	35.4 ± 0.57 <sup>b</sup>	35.3 ± 6.89 <sup>b</sup>	63.9 ± 2.33 <sup>a</sup>	<b>0.001**</b>
	60	n.d.	n.d.	n.d.	39.0 ± 12.4 <sup>ab</sup>	29.1 ± 7.64 <sup>b</sup>	53.8 ± 11.3 <sup>a</sup>	<b>0.019*</b>
<b>P</b>		–	–	–	0.721	0.486	0.340	–
Ferulic acid	0–10	n.d.	n.d.	n.d.	23.7 ± 2.36 <sup>b</sup>	25.4 ± 0.29 <sup>b</sup>	39.3 ± 0.47 <sup>a</sup>	<b>0.001**</b>
	60	n.d.	n.d.	n.d.	23.0 ± 3.81 <sup>b</sup>	26.1 ± 6.01 <sup>ab</sup>	32.9 ± 2.28 <sup>a</sup>	<b>0.019*</b>
<b>P</b>		–	–	–	0.856	0.884	0.061	–
<i>p</i> -Coumaric acid	0–10	n.d.	n.d.	n.d.	n.d. <sup>b</sup>	n.d. <sup>b</sup>	41.6 ± 0.68 <sup>a</sup>	<b>0.001**</b>
	60	n.d.	n.d.	n.d.	n.d. <sup>a</sup>	n.d. <sup>a</sup>	40.4 ± 4.47 <sup>b</sup>	<b>0.001**</b>
<b>P</b>		–	–	–	–	–	0.750	–
4-Dihydroxybenzoic acid	0–10	0 <sup>a</sup>	230.7 ± 32.4 <sup>b</sup>	238.7 ± 10.1 <sup>b</sup>	n.d.	n.d.	n.d.	–
	60	0 <sup>a</sup>	293.5 ± 18.8 <sup>b</sup>	233.8 ± 94.2 <sup>b</sup>	<b>0.001**</b>	n.d.	n.d.	–
<b>P</b>		–	0.141	0.948	–	–	–	–
2,5-Dihydroxybenzoic acid	0–10	n.d.	n.d.	n.d.	15.5 ± 0.05 <sup>a</sup>	15.4 ± 0.24 <sup>a</sup>	25.1 ± 0.14 <sup>b</sup>	<b>0.001**</b>
	60	n.d.	n.d.	n.d.	14.7 ± 0.68 <sup>b</sup>	17.7 ± 1.32 <sup>a</sup>	15.0 ± 0.22 <sup>b</sup>	<b>0.002**</b>
<b>P</b>		–	–	–	0.246	0.141	<b>0.001**</b>	–
Syringic acid	0–10	n.d. <sup>c</sup>	34.9 ± 13.2 <sup>b</sup>	63.03 ± 0.16 <sup>a</sup>	n.d. <sup>c</sup>	26.8 ± 28.9 <sup>a</sup>	11.7 ± 0.6 <sup>b</sup>	<b>0.001**</b>
	60	n.d. <sup>c</sup>	97.3 ± 10.1 <sup>a</sup>	56.87 ± 1.14 <sup>b</sup>	n.d. <sup>b</sup>	8.7 ± 4.33 <sup>a</sup>	7.72 ± 0.42 <sup>b</sup>	<b>0.002**</b>
<b>P</b>		–	<b>0.034*</b>	<b>0.017*</b>	–	0.475	<b>0.017*</b>	–
Vanillic acid	0–10	n.d.	n.d.	n.d.	n.d. <sup>b</sup>	n.d. <sup>b</sup>	188 ± 6.60 <sup>a</sup>	<b>0.001**</b>
	60	n.d.	n.d.	n.d.	n.d. <sup>b</sup>	n.d. <sup>b</sup>	114.2 ± 6.6 <sup>a</sup>	<b>0.001**</b>
<b>P</b>		–	–	–	–	–	<b>0.028*</b>	–

(Continues)

TABLE 2 (Continued)

Phenolic compounds	Distance (m)	Kale			Arugula			P
		MS	SB	BY	MS	SB	BY	
		Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	
Total phenolic acids	0–10	97.7 ± 8.93 <sup>c</sup>	1533.4 ± 238.7 <sup>b</sup>	2593.1 ± 112 <sup>a</sup>	99.6 ± 12.81 <sup>b</sup>	140.6 ± 29.51 <sup>b</sup>	394.5 ± 5.72 <sup>a</sup>	<b>0.001**</b>
	60	109.7 ± 4.95 <sup>c</sup>	3214.1 ± 447.7 <sup>a</sup>	2396.4 ± 233 <sup>b</sup>	121.1 ± 13.25 <sup>b</sup>	115.1 ± 21.05 <sup>b</sup>	279.3 ± 25.31 <sup>a</sup>	<b>0.001**</b>
	<b>P</b>	0.127	0.050	0.275	0.050	0.275	0.050	
<b>Flavonoids</b>								
Hesperidin	0–10	21.7 ± 3.57 <sup>a</sup>	12.74 ± 8.5 <sup>a</sup>	7.06 ± 3.92 <sup>a</sup>	19.6 ± 3.13 <sup>b</sup>	25.4 ± 6.86 <sup>b</sup>	50.9 ± 9.34 <sup>a</sup>	<b>0.001**</b>
	60	9.20 ± 4.97 <sup>a</sup>	15.78 ± 1.23 <sup>a</sup>	6.47 ± 4.15 <sup>a</sup>	23.0 ± 7.69 <sup>b</sup>	15.4 ± 7.13 <sup>b</sup>	62.5 ± 8.57 <sup>a</sup>	<b>0.001**</b>
	<b>P</b>	0.669	0.433	0.761	0.614	0.290	0.325	
Quercetin-3-O-glucoside	0–10	106.3 ± 11.2 <sup>a</sup>	82.8 ± 9.38 <sup>b</sup>	90.1 ± 2.22 <sup>ab</sup>	382.2 ± 67.7 <sup>b</sup>	464.2 ± 81.5 <sup>b</sup>	601.4 ± 50.2 <sup>a</sup>	<b>0.004**</b>
	60	264.9 ± 266.3 <sup>a</sup>	107.4 ± 10.4 <sup>a</sup>	156.5 ± 13.5 <sup>a</sup>	462.3 ± 195 <sup>b</sup>	391.2 ± 181 <sup>b</sup>	871.1 ± 123 <sup>a</sup>	<b>0.005**</b>
	<b>P</b>	0.489	0.130	0.560	0.638	0.655	0.103	
Kaempferol-3-O-glucoside	0–10	156.8 ± 29.4 <sup>a</sup>	127.0 ± 16.6 <sup>a</sup>	152.4 ± 3.4 <sup>a</sup>	85.0 ± 55.8 <sup>ab</sup>	69.9 ± 11.0 <sup>b</sup>	137.8 ± 8.2 <sup>a</sup>	<b>0.028*</b>
	60	78.3 ± 5.46 <sup>b</sup>	100.4 ± 7.17 <sup>a</sup>	76.9 ± 2.57 <sup>b</sup>	69.7 ± 18.9 <sup>b</sup>	45.5 ± 20.9 <sup>b</sup>	165.1 ± 21 <sup>a</sup>	<b>0.001**</b>
	<b>P</b>	0.065	0.174	<b>0.002**</b>	0.748	0.282	0.229	
Myricetin	0–10	48.1 ± 8.08 <sup>a</sup>	32 ± 19.7 <sup>a</sup>	21.5 ± 12.0 <sup>a</sup>	n.d.	n.d.	n.d.	–
	60	23.25 ± 0.03 <sup>c</sup>	44.5 ± 3.53 <sup>a</sup>	27.0 ± 0.0 <sup>b</sup>	n.d.	n.d.	n.d.	–
	<b>P</b>	0.100	0.472	0.584	–	–	–	
Rutin	0–10	n.d. <sup>c</sup>	339.7 ± 38.6 <sup>b</sup>	444.8 ± 30.8 <sup>a</sup>	n.d. <sup>c</sup>	57.5 ± 0.5 <sup>b</sup>	100.2 ± 20.5 <sup>a</sup>	<b>0.001**</b>
	60	n.d. <sup>a</sup>	312.1 ± 31.7 <sup>a</sup>	205.8 ± 8.41 <sup>a</sup>	n.d. <sup>c</sup>	50.3 ± 18.8 <sup>b</sup>	81.9 ± 16.2 <sup>a</sup>	<b>0.001**</b>
	<b>P</b>	–	0.914	<b>0.009**</b>	–	0.645	0.427	
Luteolin	0–10	n.d. <sup>c</sup>	47.1 ± 0.82 <sup>b</sup>	155.3 ± 34.1 <sup>a</sup>	n.d.	n.d.	n.d.	–
	60	n.d. <sup>c</sup>	69.4 ± 1.93 <sup>a</sup>	44.3 ± 4.78 <sup>b</sup>	n.d.	n.d.	n.d.	–
	<b>P</b>	–	<b>0.004**</b>	<b>0.045*</b>	–	–	–	

(Continues)

TABLE 2 (Continued)

Phenolic compounds	Distance (m)	Kale			Arugula			p
		MS	SB	BY	MS	SB	BY	
		Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	
Total flavonoids	0–10	345.8 ± 42.18 <sup>c</sup>	641.3 ± 67.52 <sup>b</sup>	883.7 ± 45.67 <sup>a</sup>	486.8 ± 89.5 <sup>b</sup>	617 ± 70.7 <sup>b</sup>	890.3 ± 62.3 <sup>a</sup>	<b>0.002**</b>
	60	393.3 ± 187 <sup>a</sup>	663.2 ± 239.1 <sup>a</sup>	523.3 ± 88.9 <sup>a</sup>	555 ± 156.9 <sup>b</sup>	502.4 ± 161.2 <sup>b</sup>	1180.6 ± 119.6 <sup>a</sup>	<b>0.002**</b>
	<b>p</b>	0.513	0.827	0.050	0.513	0.275	0.050	
<b>Stilben</b>								
Resveratrol	0–10	4.99 ± 0.62 <sup>a</sup>	3.60 ± 0.36 <sup>b</sup>	3.88 ± 0.19 <sup>b</sup>	n.d.	n.d.	n.d.	–
	60	2.30 ± 0.12 <sup>a</sup>	3.00 ± 1.56 <sup>a</sup>	2.53 ± 1.60 <sup>a</sup>	n.d.	n.d.	n.d.	–
	<b>p</b>	<b>0.027*</b>	0.650	0.361	–	–	–	
Total phenol	0–10	448.5 ± 51.5 <sup>c</sup>	2178.3 ± 306.5 <sup>b</sup>	3480.8 ± 66.5 <sup>a</sup>	586.4 ± 102.4 <sup>b</sup>	757.6 ± 100.2 <sup>b</sup>	1284.8 ± 56.57 <sup>a</sup>	<b>0.001**</b>
	60	505.3 ± 193 <sup>b</sup>	3880.3 ± 686 <sup>a</sup>	2922.2 ± 324 <sup>a</sup>	676.1 ± 170.1 <sup>b</sup>	617.5 ± 182.3 <sup>b</sup>	1459.9 ± 144.9 <sup>a</sup>	<b>0.002**</b>
	<b>p</b>	0.513	0.050	0.050	0.513	0.275	0.127	
<b>Antioxidant activity (mg Trolox/kg)</b>								
ABTS	0–10	461 ± 1.7 <sup>b</sup>	574.7 ± 9.5 <sup>a</sup>	567 ± 0.5 <sup>a</sup>	610 ± 2.6 <sup>c</sup>	614.7 ± 1.37 <sup>b</sup>	945.6 ± 1.2 <sup>a</sup>	<b>0.001**</b>
	60	366.7 ± 2.4 <sup>c</sup>	586.10 ± 4.0 <sup>a</sup>	445.7 ± 4.6 <sup>b</sup>	586.4 ± 5.1 <sup>b</sup>	652.1 ± 2.71 <sup>a</sup>	520 ± 1.6 <sup>c</sup>	<b>0.001**</b>
	<b>p</b>	<b>0.001**</b>	0.259	<b>0.001**</b>	<b>0.028*</b>	<b>0.003**</b>	<b>0.001**</b>	
DPPH	0–10	2867 ± 1 <sup>c</sup>	3437 ± 9 <sup>b</sup>	3450 ± 2 <sup>a</sup>	3691 ± 13 <sup>b</sup>	3634 ± 27.4 <sup>c</sup>	5814.7 ± 15 <sup>a</sup>	<b>0.001**</b>
	60	2349 ± 9.6 <sup>c</sup>	3757.4 ± 15 <sup>a</sup>	2771 ± 8.3 <sup>b</sup>	3547 ± 13.5 <sup>b</sup>	4089 ± 6.51 <sup>a</sup>	3323.0 ± 0.1 <sup>c</sup>	<b>0.001**</b>
	<b>p</b>	<b>0.001**</b>	<b>0.001**</b>	<b>0.001**</b>	<b>0.008**</b>	<b>0.002**</b>	<b>0.001**</b>	

Note: It refers to the area between Mevlanakapi and Siliirikapi (MS), between Siliirikapi and Belgradkapi (SB), and between Belgradkapi and Yedikule kapi (BY) coastline. The statistics of the results in the 0–10 and 60 m distances from the road line on the sites are the results of the Mann–Whitney *U* test. Analysis of variance and Duncan's multiple comparison test were used to find significant differences in the results of the three different sites (MS, SB, and BY).  $\bar{X} \pm SD$ :  $\bar{X}$  Results are the means of three repetitions performed in the laboratory;  $SD$  standard deviation. Different letters on the same line are statistically different: \*significant differences at  $p \leq 0.05$ ; \*\*significant differences at  $p \leq 0.01$ . n.d., not detected.

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

significant depending on the different sites where the vegetables were cultivated ( $p < 0.05$ ). In the case of arugula samples, though the findings exhibited that the amount of total phenolic compounds was slightly reduced as the distance of vegetable cultivation site from the road in MS and BY sites decreased, whereas it was slightly raised in SB site, the differences of the total phenolic compounds in arugula samples were not significant depending on the distance from the vehicle road ( $p < 0.05$ ). In contrast, the differences of the total phenolic compounds in arugula samples were found to be significant depending on the different sites where the vegetables were cultivated ( $p < 0.05$ ). The levels of phenolic compounds can be changed depending on environmental conditions and also availability of pesticide content used during cultivation (Quan et al., 2008). Moreover, it was reported that the amount of phenolic compounds and phenylpropanoid metabolism may increase under different environmental and stress conditions (Quan et al., 2008). Although the results of a study (Otlés & Yalcin, 2012) were in-line with the present study findings, the results of another study (Qayoom Mir et al., 2009) were not. The difference between the results of the current study and the literature can be related to the decrease of the concentration of phenolic compounds in vegetables after taking a role in defense mechanisms against mechanical or biological stresses due to infections caused by bacteria, viruses, and fungi (Vermerris & Nicholson, 2007).

Among the detected individual phenolic groups, phenolic acids were the dominant class of phenols, followed by flavonoids in kale samples, whereas flavonoids were the prevailing class, followed by phenolic acids in arugula samples. Phenolic acid content changed from 97.7 to 3214 mg/kg in kale samples and from 99.6 to 394.5 mg/kg in arugula samples. It has been determined that the kale vegetables cultivated at a distance of 60 m in the SB site were the richest one in terms of the content of total phenolic acids (3214.1 mg/kg), whereas the samples cultivated at a distance of 0–10 m in the MS site were the lowest one (97.7 mg/kg). In the case of arugula vegetables, the total content of phenolic acids collected from BY site with 0–10 m distance possessed the highest amount (39.5 mg/kg), whereas from MS site with 0–10 m distance possessed the lowest amount (99.6 mg/kg). The differences of the content of the total content of phenolic acids like total phenolic compounds in both vegetable samples were not significant depending on the distance from the vehicle road ( $p < 0.05$ ). On the contrary, the differences of the total phenolic acids in both vegetables were found to be significant depending on the different sites where they were cultivated ( $p < 0.05$ ). The exposure of vegetables to biotic and abiotic stress and the variety of seeds can change the amount of phenolic acid (Rashmi & Negi, 2020).

The content of flavonoids was altered from 345.8 to 883.7 mg/kg in kale samples and from 486.8 to 1180.6 mg/kg in arugula samples. Although the highest total content of flavonoids (883.7 mg/kg) was found in kale samples grown at a distance of 0–10 m in the BY site, it was determined that the samples grown at a distance of 0–10 m in the MS site had the lowest content of flavonoids (345.8 mg/kg). Statistically, there were no significant differences in the total content of flavonoids obtained from kale vegetables cultivated at a distance of 60 m from the road in all three different sites settled on the side of the road line (MS, SB, and BY), whereas there were significant differences in samples cultivated at a distance of 0–10 m from the road in all three different sites ( $p < 0.05$ ). Although there was a slight difference in the total flavonoids content of kale vegetables collected from two different distances in all different sites, there were not significant differences between them ( $p < 0.05$ ). In the case of arugula vegetables, although the highest total content of flavonoids (1180.6 mg/kg) was found in samples cultivated at a distance of 60 m in the BY site, it was determined that the samples cultivated at a distance of 0–10 m in the MS site showed the lowest content of flavonoids (486.8 mg/kg). The differences of the total content of flavonoids in arugula vegetables were not significant depending on the distance from the vehicle road ( $p < 0.05$ ). On the contrary, the differences of the total content of flavonoids in arugula vegetables were statistically found to be significant depending on the different sites where they were cultivated ( $p < 0.05$ ). Most of the detected flavonoids under study have already been reported by former investigations on both vegetables (Ayaz et al., 2008; Fusari et al., 2020; Li et al., 2018; Seong et al., 2016).

To evaluate the possibility of distinguishing the samples, the hierarchical clustering heatmap analysis (Figure 2) was carried out to elucidate the influence of two different distances including close (0–10 m) and far (60 m) from the heavy traffic roadside in the three different cultivation sites (MS, SB, and BY) stretched on the side of the road line (Figure 1) on in the light of phenolic profile of vegetable samples. According to phenolic compounds, a total of 12 and 11 variables (phenolic compounds) were selected for the heatmap analysis in kale and arugula vegetables, respectively. Raw data were used to build a heatmap and the color-coding to represent the values taken by a variable in a hierarchy (Figure 2). The data of both vegetable samples from the different sites are indicated in the color scale (class) in the heatmap (Figure 2): 0 (MS-10 m), 1 (MS-60 m), 2 (SB-10 m), 3 (SB-60 m), 4 (BY-10 m), and 5 (BY-60 m). Six classes for each type of vegetable, both kale (Figure 2a) and arugula (Figure 2b), were generated column wise and highlighted with hierarchical clustering. Indeed, vegetable samples obtained from different growing sites of the heavy traffic roadside were clearly divided into

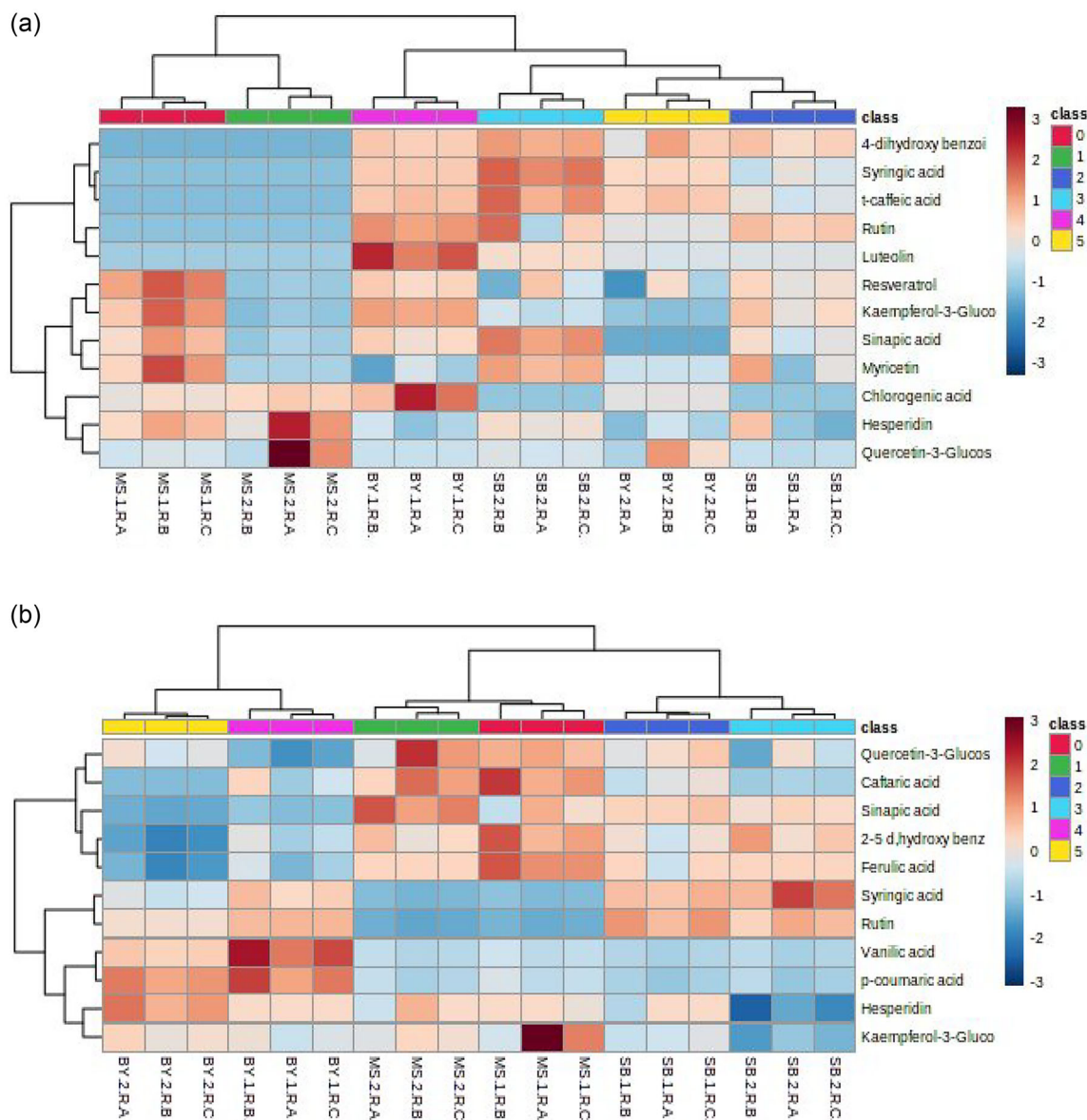


FIGURE 2 Hierarchical clustering heatmap of quantified phenolic compounds (mg/kg) in the kale (a) and arugula (b) vegetables.

six separate classes (from 0 to 5 classes shown in Figure 2) based on the composition of their phenolic compounds. As can be seen in the results of both kale and arugula samples based on phenolic compounds in the heatmap (Figure 2), vegetables grown in far (60 m) from the heavy traffic roadside for the kale vegetables in the MS-60 m and SB-60 m sites and for the arugula vegetables in BY-60 m and MS-60 m sites stretched on the side of the road line, demonstrated higher phenolic concentrations than in close (10 m) sites. Moreover, both kale and arugula vegetables grown in all different six sites were classified in different classes in the heatmap based on the concentrations of their phenolic compounds.

### 3.3 | Antioxidant activities of kale and arugula

The results of antioxidant activities of vegetables are presented in Table 2. As can be seen in Table 2, the DPPH method indicated better antioxidant activity of phenolic compounds in all vegetable samples than the ABTS method, as the former method provided higher values. Significant differences were observed among the samples in terms of antioxidant activity determined by the ABTS method, except for the samples of kale cultivated in the SB site, depending on the distance to the road and the site where they were cultivated ( $p < 0.05$ ). Similarly,

significant differences were found among the samples in terms of antioxidant activity determined by the DPPH method, depending on the distance to the road and the site where they were cultivated ( $p < 0.05$ ). In arugula samples, significant differences were also observed among the samples in terms of antioxidant activity determined by the ABTS and DPPH methods, depending on the distance to the road and the site where they were cultivated ( $p < 0.05$ ). The highest antioxidant capacity value determined by the ABTS and DPPH methods in kale was found in samples grown at a distance of 60 m in the SB site, whereas the lowest value was observed in samples grown at a distance of 60 m in the MS site. It was observed that the antioxidant capacity increased as the distance to the road decreased in kale samples from the BY and MS sites. In arugula samples, the highest antioxidant capacity value determined by the ABTS and DPPH methods was found in samples from the BY site at a distance of 0–10 m, whereas the lowest value was observed in samples grown at a distance of 60 m in the BY site. Similarly, it was also observed that the antioxidant capacity increased as the distance to the road decreased in arugula samples from the BY and MS sites. Additionally, another important finding is that while the total phenolic compounds and antioxidant capacity values of kale vegetables showed correlated changes, no correlation was observed between the total phenolic compounds and antioxidant activity values in arugula samples.

### 3.4 | Heavy metal concentrations in kale and arugula vegetables

The heavy metal concentrations in the leaf tissues of vegetables are shown in Table 3. Significant differences were found among the Cd, Pb, Hg, and Fe values of kale samples for both distances from the road in all different sites, except for Pb values of SB and BY sites as well as for Fe values of BY site ( $p < 0.05$ ). In the case of arugula samples, significant differences were found among the Cd, Pb, Hg, and Fe values of arugula samples based on the two different distances from the road and the three different cultivation sites settled on the side of the road line ( $p < 0.05$ ). In a study, examining the possible changes in heavy metal pollution in soils surrounding the Antakya-Cilvegözü international highway and the plants grown in these soils based on the distance from the road in Hatay province of Türkiye, it was found that the values of Fe, Cu, Mn, and Zn in plant samples exhibited random variation, whereas the values of Pb, Cd, and Ni decreased as the samples moved away from the road in both plant and soil samples (Özkan, 2017). The similarity can be observed between the random variation in Fe values of vegetable

samples and the decrease in Pb values of kale samples with increasing distance from the road, as reported by Özkan (2017). In another study, evaluating the effect of distance from the road on the enrichment of pollutants in garden plants, the heavy metal concentration in leaf tissues of plants grown near the road (10 m away) was compared with those grown in a more distant area (60 m away). It was found that Ni, Sn, and Zn accumulated more in pumpkin samples at a distance of 10 m from the road, whereas the concentration of Ba was higher in pumpkin samples at a distance of 60 m from the road (Antisari et al., 2015). The results obtained from this study do not correspond to the findings of Antisari et al. (2015), where most of the heavy metals in plants increased as the distance from the road decreased. This discrepancy is believed to be due to the difference in pesticide and fertilization practices used in vegetables grown at different distances from the road.

The Cd contents in the sampling sites ranged between 4.39–55.11 and 11.11–32.90 mg/kg, respectively, for kale and arugula, exceeding World Health Organization (WHO) permissible limits (0.5 mg/kg) (Food and Agriculture Organization/World Health Organization [FAO/WHO], 2003), indicating that the both vegetables cultivated in these sites were unsafe for human consumption concerning Cd. The reason why the values obtained in the study are much higher than the WHO set limit values can be due to the traffic density, the pesticides used while growing vegetables, artificial fertilization, and the climatic conditions in which the vegetables are grown (Seven et al., 2018). The concentration of Pb in vegetable samples ranged between 0.14–0.24 and 0.07–0.32 mg/kg, respectively, for kale and arugula, and were observed to be within WHO acceptable limits (2 mg/kg) (FAO/WHO, 2003) for both vegetables. These results demonstrated that both vegetables were safe for human consumption regarding Pb content. The concentrations of Hg in vegetables were differed between 2.56–22.38 and 4.70–20.62 mg/kg, respectively, for kale and arugula, exceeded WHO permissible limits (0.03 mg/kg), (FAO/WHO, 2003), revealing that both vegetables cultivated in these sites were unsafe for human consumption relating Hg content. The obtained values of Hg under the study were found to be above the limit value reported by WHO (Table 3). The highest Fe concentration revealed in kale samples was 28.65 mg/kg, whereas the lowest was 1.10 mg/kg. These concentrations were observed to be within WHO permissible limits (30 mg/kg) (FAO/WHO, 2003) for kale. Although it was observed that the Fe value of kale increased in parallel with the increasing distance from the road in the MS and BY sites, it was observed that the Fe value increased as the distance to the vehicle road decreased in the SB site (Table 3). It was deter-

TABLE 3 Heavy metal concentrations in kale and arugula vegetables.

Heavy metals	Distance (m)	Kale			Arugula			<sup>a</sup> FAO/WHO limit (FAO/WHO, 2003)
		MS	SB	BY	MS	SB	BY	
		Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	p	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	p	
<b>Cd</b>	0–10	16.55 ± 0.35 <sup>b</sup>	4.39 ± 0.21 <sup>c</sup>	48.69 ± 0.28 <sup>a</sup>	16.50 ± 0.26 <sup>a</sup>	32.9 ± 0.17 <sup>b</sup>	16.65 ± 0.0 <sup>a</sup>	<b>0.001**</b>
	60	7.97 ± 1.2 <sup>c</sup>	14.98 ± 0.32 <sup>b</sup>	55.11 ± 0.4 <sup>a</sup>	27.16 ± 0.57 <sup>c</sup>	24.1 ± 0.56 <sup>b</sup>	11.11 ± 0.4 <sup>a</sup>	<b>0.001**</b>
	<b>p</b>	<b>0.010**</b>	<b>0.001**</b>	<b>0.008**</b>	<b>0.002**</b>	<b>0.002**</b>	<b>0.003**</b>	
<b>Pb</b>	0–10	0.19 ± 0.01 <sup>b</sup>	0.24 ± 0.02 <sup>a</sup>	0.17 ± 0.01 <sup>b</sup>	0.19 ± 0.00 <sup>c</sup>	0.15 ± 0.01 <sup>b</sup>	0.13 ± 0.01 <sup>a</sup>	<b>0.001**</b>
	60	0.14 ± 0.00 <sup>a</sup>	0.17 ± 0.02 <sup>a</sup>	0.14 ± 0.03 <sup>a</sup>	0.32 ± 0.00 <sup>a</sup>	0.07 ± 0.02 <sup>c</sup>	0.23 ± 0.01 <sup>b</sup>	<b>0.001**</b>
	<b>p</b>	<b>0.032*</b>	0.092	0.361	<b>0.001**</b>	<b>0.040*</b>	<b>0.010**</b>	2
<b>Hg</b>	0–10	10.41 ± 1.62 <sup>b</sup>	2.56 ± 0.03 <sup>c</sup>	15.51 ± 1.27 <sup>a</sup>	4.72 ± 0.82 <sup>b</sup>	18.9 ± 3.11 <sup>a</sup>	4.7 ± 0.07 <sup>b</sup>	<b>0.001**</b>
	60	22.38 ± 0.79 <sup>a</sup>	7.08 ± 0.13 <sup>b</sup>	3.71 ± 0.18 <sup>c</sup>	20.62 ± 0.25 <sup>a</sup>	5.30 ± 0.62 <sup>c</sup>	6.63 ± 0.15 <sup>b</sup>	<b>0.001**</b>
	<b>p</b>	<b>0.011*</b>	<b>0.001*</b>	<b>0.006*</b>	<b>0.001*</b>	<b>0.026*</b>	<b>0.004*</b>	0.03
<b>Fe</b>	0–10	1.20 ± 0.57 <sup>b</sup>	11.3 ± 1.27 <sup>a</sup>	1.10 ± 0.57 <sup>b</sup>	48.35 ± 1.20 <sup>c</sup>	333.2 ± 1.7 <sup>a</sup>	84.5 ± 0.14 <sup>b</sup>	<b>0.001**</b>
	60	28.65 ± 1.49 <sup>a</sup>	5.05 ± 0.92 <sup>b</sup>	2.05 ± 0.78 <sup>c</sup>	70.5 ± 0.28 <sup>b</sup>	85.5 ± 0.14 <sup>a</sup>	25.2 ± 0.35 <sup>c</sup>	<b>0.001**</b>
	<b>p</b>	<b>0.002**</b>	<b>0.03*</b>	0.297	<b>0.002**</b>	<b>0.001**</b>	<b>0.001**</b>	30

Note: It refers to the area between Mevlanakapi and Silivrikapi (MS), between Silivrikapi and Belgradkapi (SB), and between Belgradkapi and Yedikule kapi (BY) coastline. The statistics of the results in the 0–10 and 60 m distances from the road line on the sites are the results of the Mann–Whitney *U* test. Analysis of variance and Duncan's multiple comparison test were used to find significant differences in the results of the three different sites (MS, SB, and BY).  $\bar{X} \pm SD$ ;  $\bar{X}$ : Results are the means of three repetitions performed in the laboratory;  $SD$ : standard deviation. Different letters on the same line are statistically different: \*significant differences at  $p \leq 0.05$ ; \*\*significant differences at  $p \leq 0.01$ .

<sup>a</sup>Heavy metal limit values accepted by WHO in plants (mg/kg) (FAO/WHO, 2003).

mined that the kale Fe values obtained in the study were similar to some studies in the literature and were below the limit value reported by WHO samples (FAO/WHO, 2003; Thavarajah et al., 2016; Yıldırım et al., 2019). In the case of arugula vegetables, concentration of Fe was changed between 25.20 and 333.2 mg/kg. These outcomes demonstrated that all arugula samples exceeded WHO permissible limits (30 mg/kg) (FAO/WHO, 2003) and were unsafe for human consumption with regard to Fe content, except for Fe level in the sample of the BY site at a 60 m distance from the road (25.20 mg/kg). The arugula Fe values obtained in the study overlap with some studies in the literature and were found to be above the limit value reported by WHO (FAO/WHO, 2003; Güven, 2019).

### 3.5 | Heavy metal concentrations in the soil and irrigation water

The mean concentrations of Cd, Pb, Hg, and Fe heavy metals in water used for irrigating cultivation sites of both studied vegetables were displayed in Table 4. There was a statistically significant difference in Cd and Pb values in irrigation water samples from the vegetable growing sites ( $p < 0.01$ ). The Cd values in the irrigation water of the vegetables obtained in the study were higher than those reported in other studies (Ngweme et al., 2020; Tomno et al., 2020). When comparing the heavy metal values in the irrigation water of the analyzed vegetable samples with the FAO permissible heavy metal limits in water for the vegetable irrigation (FAO, 1985) (Table 4), it was found that the Pb value was below the limit, while the Cd values were above the limit, and there were no Hg and Fe heavy metals detected in the irrigation water under the study. Therefore, the water was not contaminated with Pb, Hg, and Fe and was safe for agricultural use.

The average heavy metal content in soil samples differed in the order Fe > Cd > Hg > Pb (Table 4). In fact, there was a significant difference in Cd, Pb, Hg, and Fe values in the soil samples collected from the studied different vegetable growing sites ( $p < 0.01$ ). In a study, in the agricultural soils of Zhejiang and Jiangsu provinces, the reported values of heavy metals were ranged from 28.80 to 36.79 mg/kg for Pb, from 0.18 to 0.23 mg/kg for Cd, and from 0.07 to 0.12 mg/kg for Hg (Yang et al., 2020). The acquired outcomes for the Pb and Fe heavy metals in soil samples were below acceptable limits (Council of the European Communities [CEC], 1986) and were safe for agricultural use. However, the Cd and Hg values were higher than those reported in the literature (CEC, 1986).

TABLE 4 Heavy metal concentrations in soil and irrigated water.

Heavy metals	Soil			Water			<sup>b</sup> Limits in water (FAO, 1985)
	MS	SB	BY	MS	SB	BY	
	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	Mean ± SD (mg/L)	Mean ± SD (mg/L)	Mean ± SD (mg/L)	
Cd	32.375 ± 0.41 <sup>c</sup>	51.175 ± 0.62 <sup>a</sup>	39.510 ± 0.16 <sup>b</sup>	0.055 ± 0.0 <sup>a</sup>	0.022 ± 0.0 <sup>c</sup>	0.029 ± 0.0 <sup>b</sup>	0.01
Pb	4.061 ± 0.38 <sup>c</sup>	17.216 ± 0.02 <sup>a</sup>	13.27 ± 0.16 <sup>b</sup>	0.006 ± 0.0 <sup>b</sup>	0.004 ± 0.0 <sup>c</sup>	0.007 ± 0.0 <sup>a</sup>	5.0
Hg	6.932 ± 0.83 <sup>b</sup>	19.45 ± 0.90 <sup>a</sup>	5.08 ± 0.13 <sup>b</sup>	n.d.	n.d.	n.d.	-
Fe	4137.5 ± 65 <sup>b</sup>	3022.5 ± 22.5 <sup>b</sup>	2760 ± 17.5 <sup>b</sup>	n.d.	n.d.	n.d.	5.0

Note: It refers to the area between Mevlanakapi and Siliirikapi (MS), between Siliirikapi and Belgradkapi (SB), and between Belgradkapi and Yedikule kapi (BY) coastline. The statistics of the results in the 0–10 and 60 m distances from the road line on the sites are the results of the Mann–Whitney *U* test. Analysis of variance and Duncan's multiple comparison test were used to find significant differences in the results of the three different sites (MS, SB, and BY).  $\bar{X} \pm SD$ ; <sup>x</sup>Results are the means of three repetitions performed in the laboratory; <sup>SD</sup> standard deviation. Different letters on the same line are statistically different: \*significant differences at  $p \leq 0.05$ ; \*\*significant differences at  $p \leq 0.01$ .

Abbreviation: n.d., not detected.

<sup>a</sup>Guidelines values from the 86/278/EEC Directive for agricultural soils with pH 6–7 (CEC, 1986).

<sup>b</sup>Acceptable limits by Food and Agriculture Organization (FAO, 1985).

## 4 | CONCLUSION

This research studied, for the first time, the concentrations of micronutrients such as beneficial bioactive compounds and harmful heavy metal contaminations in kale and arugula vegetable samples grown in very heavy traffic roadside of Istanbul, Türkiye. The site where kale and arugula vegetables were grown and the distance from vehicle traffic had a significant impact on the phenols in the studied vegetables. Additionally, the findings showed that the Cd and Hg contents in both kale and arugula, and Fe content in arugula exceeded FAO/WHO permissible limits, indicating that these vegetables cultivated in these sites were unsafe for human consumption, whereas Pb content in both kale and arugula and Fe content in kale were observed to be within FAO/WHO acceptable limits. The results of the heavy metal analyses in the irrigation water samples showed that the water was not contaminated with Pb, Hg, and Fe and was safe for agricultural use, whereas the Cd value was above the permissible FAO limit. In the soil samples taken as part of the current study, the Pb and Fe levels were below the permissible limit and were safe for agricultural use, whereas the Cd and Hg levels were above. Regarding these findings, consumers of these vegetables from these sites may be exposed to possible heavy metal adverse impacts on human health. The vegetable heavy metal contamination can be clarified by the vehicle and motorcycle emissions around. Therefore, the outcomes from this investigation deeply advise the relocation of such sites to decline the pollution accumulations of vehicles and motorcycles on vegetables.

### AUTHOR CONTRIBUTIONS

**Armin Amanpour:** Conceptualization; supervision; visualization; investigation; methodology; writing—original draft; project administration; funding acquisition; data curation; validation. **Beril Coskun:** Visualization; methodology; validation; formal analysis; writing—review and editing. **Hilal Kanmaz:** Formal analysis; investigation; data curation; validation. **Busra Kaya Turan:** Data curation; investigation; validation; formal analysis. **Meltem Soylu:** Investigation. **Fatma Celik:** Investigation. **Ali Adnan Hayaloglu:** Supervision; investigation; software; formal analysis; funding acquisition; writing—review and editing; writing—original draft.

### CONFLICT OF INTEREST STATEMENT

No, there are no conflicts of interest.

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