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Isolating and identifying microbiological contamination and evaluating the quantity of polycyclic aromatic hydrocarbons (PAHs) in dried figs

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Abstract

Human, industrial, and agricultural activities pollute foods, including dried fruits in local markets, with many chemical pollutants, including PAHs, and microorganisms. This study measured polycyclic aromatic hydrocarbons in dried fruits and isolated and identified bacteria and fungi from dried figs. Samples of dried figs, each weighing 100 grammes, were collected from local markets in Tikrit and dispatched to the laboratory for the assessment of chemical and microbiological contamination in dried fruits. Upon the emergence of fungal colonies, fungi were isolated by culturing on SDA medium with a sterile loop to obtain pure fungal isolates from the colony's periphery. Bacterial isolation was determined through cultural, microscopic, and biochemical identification methods. Subsequently, the analysis and quantification of PAHs and HPLC. The current study found that *Escherichia coli* bacteria constituted the largest percentage of bacterial contaminants (37%), followed by Staphylococcus (33%), while *Aspergillus nigar* mold constituted the largest percentage of fungal contaminants (23%), and Candida constituted 7% of the total microbial isolates in Dried fig samples, represent the chromatograms of polyaromatic hydrocarbons (PAHs) in random samples of the studied dried figs. The average Phenanthrene compound in dried figs was (11.57ppb), but the average Naphthalene (0.03ppb), Benzo[b]fluoranthene (10.57ppb), Chrysene (13.66ppb), and Benzo[ghi]perylene recorded an average of 0.39ppb.

Conclusion: In this study, 12 Priority PAHs were studied in dried fruits obtained from local markets, and certain PAHs were found at significant levels in dried figs.

Key words: PAHs, dried figs, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus*.

Introduction

Food pollution is defined as the introduction of harmful microorganisms or chemical substances into food that make it unfit for human consumption [1].

Drying is the most ancient technique of food preservation that diminishes the water activity of food, preventing the growth of yeasts, molds, and bacteria at water activity levels of (0.90, 0.88, 0.87) accordingly. Consequently, by reducing water activity through drying, microbes are rendered incapable of growth [2].

Drying not only prevents the growth of germs but also stops other moisture-induced processes that cause deterioration, preserving the nutritional value and other quality aspects of the original product [3].

Dried figs contain many concentrated nutrients and are rich in antioxidants and fiber, which makes eating dried figs beneficial for the health of the body, including California figs and Turkish figs [4].

Dried figs contain a higher concentration of

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carbohydrates and sugars, the calories in dried figs are higher than those in fresh figs, as they amount to 371 calories per cup of dried figs, while they amount to 111 calories per cup of fresh figs One of the nutritional benefits of dried figs is that they contain many vitamins and minerals, including *vitamin C, k, B1, B2, B6, Ca, k, Mg and others [5]. Many microorganisms, such as bacteria and fungi, were isolated from dried and fresh figs [6].

Polycyclic aromatic hydrocarbons (PAHs) are toxic and mutagenic pollutants found in the environment, which can be absorbed by plants and accumulate in them [7]. They are organic compounds that contain carbon (C) and hydrogen (H) only, consisting of more than one aromatic ring combined through two or more carbon atoms [8]. PAHs are considered complex organic compounds and are classified as persistent organic pollutants (POPs). Many microorganisms were isolated from fruits and vegetables, and the highest percentage was *E. coli*, Staphylococci, and other types that caused poisoning, which leads to diarrhea and vomiting [9-12]. Therefore, staphylococcal food poisoning does not result from ingesting the bacteria, but from ingesting toxins in food that are produced by the bacteria [13-15]. This study aimed to estimate polycyclic aromatic hydrocarbons levels in dried fig fruits, as well as the isolation and identification of microorganisms (bacteria and fungi) from dried fig fruits.

Materials and Methods

Sample collection

Samples of dried figs were obtained from the local markets of Tikrit city, each weighing 100 grammes, and were subsequently placed in sterile bags that were securely closed and labelled. The samples were moved to the laboratory to identify chemical and microbial contamination in dried fruits.

Isolation, Identification, and Purification of Microbiology from the collected samples

The collected dried fig samples were transferred to the microbiology laboratory in Tikrit city, and 25 grams of each sample were grinded in 225 ml of Peptone water [16]. A series of consecutive decimal dilutions was performed under sterile conditions. 1 ml was transferred from the tubes to perform a series of dilutions, where 1 ml of each dilution was transferred to the plates, and then the media was poured. Incubation was carried out at 30 °C for 24 hours for bacteria and seven days for fungi. After incubation, colonies appeared, and the colonies were purified by re-cultivation [17]. After the appearance of fungal colonies, fungi were isolated by culturing them on SDA medium using a sterile loop to isolate pure fungal isolates from the far edge of the colony. This process was done near a Bunsen burner to prevent contamination. After that, they were incubated for 3 to 5 days at a temperature of 25 °C. Then, fungal isolates were identified based on taxonomic characteristics [18]. The diagnosis of bacterial isolation is based on Cultural identification [19], microscopic identification [20], and six Biochemical identification tests utilized for each sample.

Analysis and measurement of PAHs using HPLC

Many techniques have been used to isolate and analyze PAHs compounds in various environmental pollution fields, the most common of which is chromatography techniques. Chromatography is a physical method of analysis and separation that involves the use of two phases, one of which is stationary and has a relatively large surface area, and the other is mobile (Mobile Phase), which moves through the stationary phase and usually contains the samples to be examined.

The US Environmental Protection Agency has identified widely applied global techniques for analyzing PAHs compounds, the most accurate of which is high-performance liquid chromatography (HPLC).

Statistical analysis

The raw data was first analyzed with the SPSS test, and the significant differences were determined using the Chi square and with significance levels at <0.05.

Results:

The current project showed a wide range of bacterial and fungal growth in dried fruits after planting them on different media, where *Escherichia coli* bacteria constituted the largest percentage of bacterial contaminants (37%), then Staphylococcus (33%), while *Aspergillus nigar* mold showed the largest percentage of fungal contaminants (23%), as well as Candida constituted (7%) of the total microbial isolates as shown in Table (1).

Essential techniques utilised for the identification of E. coli. The results indicated that the bacteria were lactose fermenters, producing pink, smooth, shiny, and sharply defined colonies on MacConkey differential agar medium, which contains bile salts and crystal violet dye. This medium facilitates the growth of Gram-negative bacteria, including the Enterobacteriaceae family, while inhibiting Gram-positive bacteria, as illustrated in the figure (1A). The colonies grown on Blood agar medium were all hemolytic with complete hemolysis of the β-hemolysis type, indicating the production of hemolysin enzyme as shown in Figure 1 B.

Gramme staining was conducted by preparing a smear from a colony cultured on McConkey agar medium for 48 to 20 hours. The bacterial cells manifested as short rods, Gram-negative, and non-spore producing, as illustrated in the figure. (2).

Figure (3) and Table (2) illustrate that biochemical tests were conducted on all isolates, with all demonstrating positive results for the catalase test, and negative results for both the oxidase and urease tests. The results of the IMVC tests indicated that the bacteria were positive for the indole test, positive for the methyl red test, positive for the motility test, and negative for the citrate test.

Table (1): Type of bacterial and fungal isolation with percentage

Type of bacterial and fungal isolation	Number of isolation
Escherichia coli	11(%37)
Staphylococcus aureus	10(33%)
Aspergillus nigar	7(23%)
Candida albicans	2(7%)
Total	30(100%)

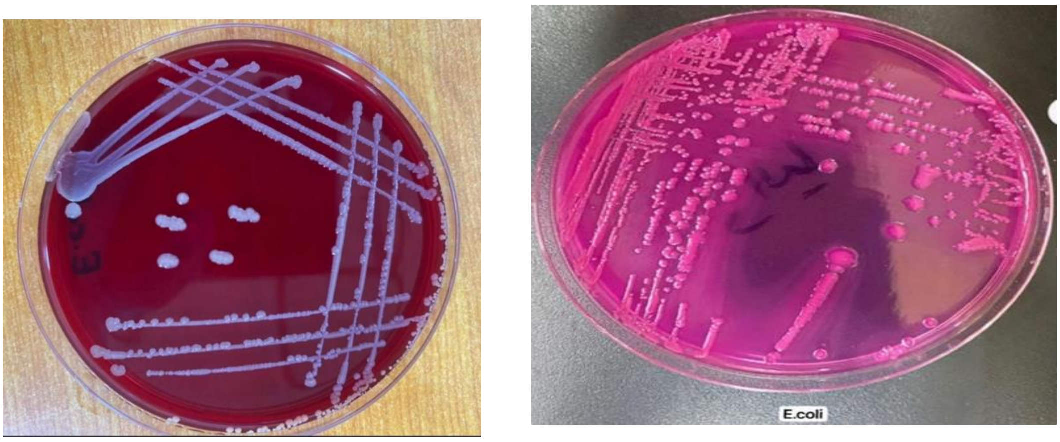

Figure (1): Morphological characteristics of E. coli colonies on various diagnostic media. Pink colonies on MacConkey agar, B- Opalescent haemolytic colonies on Blood agar

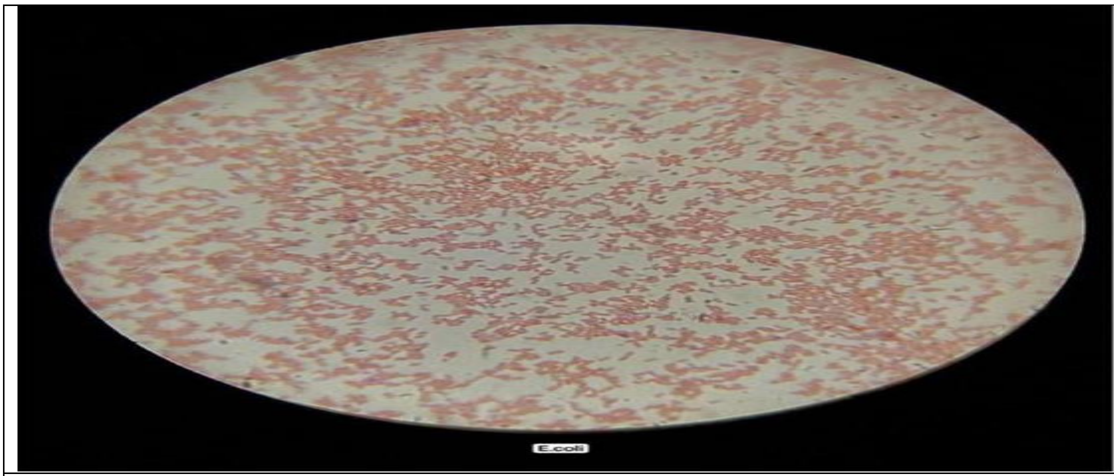

Figure (2): Gram-negative cells of E. coli bacteria under a microscope at a magnification of 40x

Table 2: Results of biochemical tests for *E.coli* bacteria

Motility	MR	Urease	Indole	Citrate	Oxidase	Catalase
+	+	-	+	-	-	+

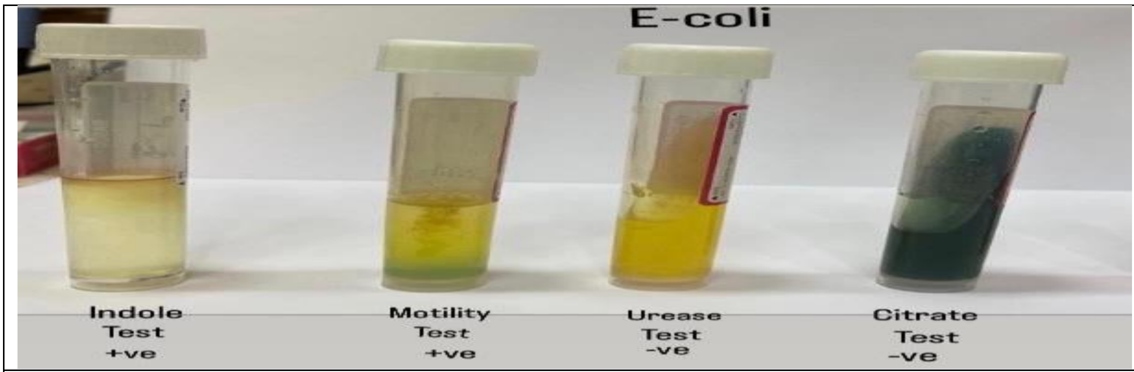

Figure (3): Results of biochemical tests for E. coli

***Staphylococcus* spp.**

Microscopic examinations showed that the bacterial smears stained with Gram stain are spherical Gram-positive cells, as the staphylococci appeared in irregular clusters under the microscope, as in Figure 4.

Staphylococci appeared on the Blood agar medium in the form of small, somewhat convex white colonies with complete decomposition around the bacterial colonies. Staphylococci isolates were grown on the mannitol salt medium and gave a positive test for mannitol sugar fermentation with the appearance of colonies and the surrounding medium being yellow indicating the formation of acid as a result of mannitol sugar fermentation and a change in the pH of the medium from neutral to acidic due to the activity of these bacteria as shown in Figure (5)

All isolates were positive for catalase, negative for the oxidase test, and positive for urease. As for the results of the IMVC tests, the bacteria were negative for the catalase test, the motility test was negative, and the staphylococci were positive for the citrate test, as shown in Figure 6 and Table 3.

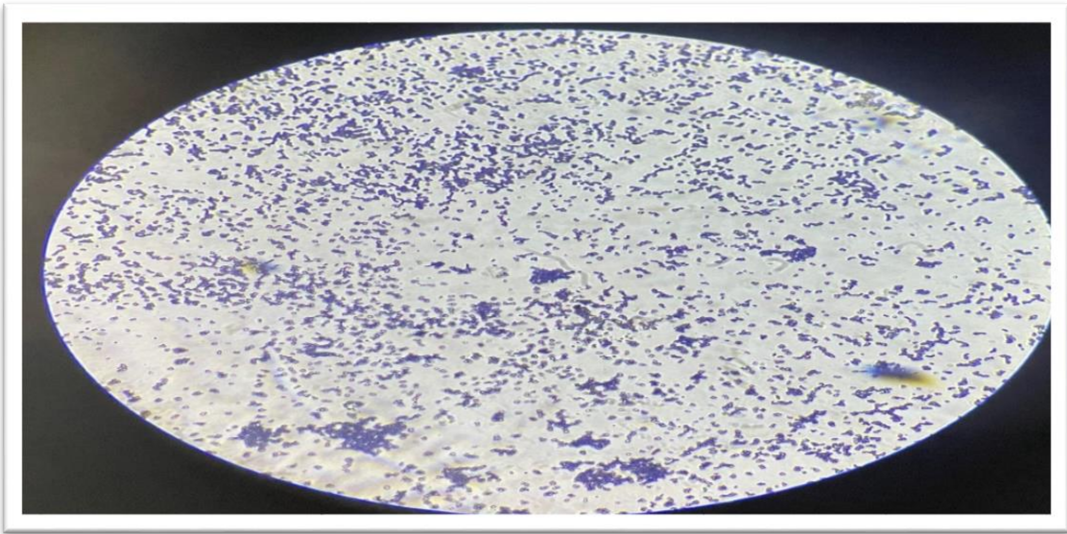

Figure 4 shows the shapes of staphylococci under the microscope.

Figure (5): Staphylococcus *aureus* growth on a blood agar medium, showing the decomposition of colonies, and also growth on a mannitol salt medium, showing the medium turning yellow as a result of the fermentation of mannitol sugar.

Table 3: Results of biochemical tests for Staphylococcus *aureus*.

Motility	Urease	Indole	Citrate	Oxidase	Catalase
-	+	-	+	-	+

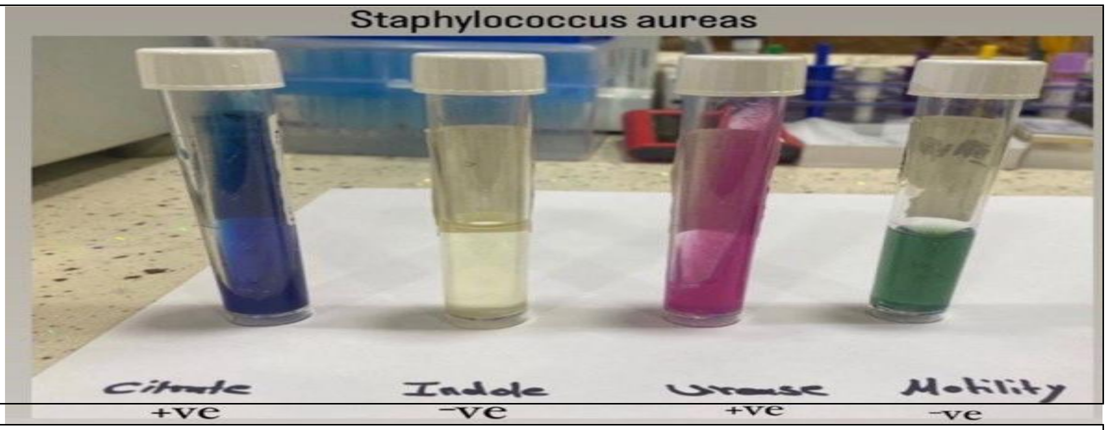

Figure (6): Results of biochemical tests, Staph. Aureus

Several fungal isolates belonging to Aspergillus, including A. nigar, were obtained according to the shape and edges of the fungal colony on Sabouraud dextrose agar and the shape of the fungus under the microscope, as shown in Figure 7.

Figure (7): A: Fungal colonies under the microscope. B: Aspergillus growth medium (Sabouraud dextrose agar).

Polycyclic aromatic hydrocarbons in dried figs

The findings of the present investigation indicated variations in the amounts of PAHs across several samples of dried figs. This alteration in concentrations is ascribed to environmental circumstances and variations, as well as the characteristics of the sample sources. The analysis revealed substantial variations in PAH concentrations across different samples, with the detection of 12 PAHs: Naphthalene (NAP), Acenaphthylene (ACY), Pyrene (PYR), Chrysene (CHR), Fluorene (F), Benzo[ghi]perylene (B[ghi]P), Acenaphtene (AC), Dibenzo[a,h]anthracene (Dha), Benzo[k]fluoranthene (BkF), Benzo[b]fluoranthene (B[b]F), Anthracene (ANT), and Phenanthrene (PHE).

The following figures represent the chromatograms of polyaromatic hydrocarbons (PAHs) in random samples of the studied dried fig samples. The average Phenanthrene compound in dried figs was (11.57ppb), while the average Naphthalene (0.03ppb), Benzo[b]fluoranthene (10.57ppb), Chrysene (13.66ppb), and Benzo[ghi]perylene recorded an average of 0.39ppb.

The highest concentration of Naphthalene compound was recorded in Thai figs, where it reached 0.05ppm, while the lowest concentration (0.01 ppm) was recorded in Syrian figs. Also, Phenanthrene compound recorded the highest (13.41ppb) and the lowest value (10.5ppb) in the local origin (Erbil) and Syrian, respectively. The highest concentration of Benzo[b]fluoranthene compound (12.31ppb) was in the Thai origin, and the lowest concentration (9.41ppb) in the Iranian origin.

Benzo[ghi]perylene recorded the highest concentration (0.47ppb) and the lowest concentration (0.32ppb) from Turkish origin. The highest and lowest concentrations of Chrysene (15.31ppb) and (12.21ppb), respectively, were taken from Turkish origin, as shown in Tables 4-5. The difference in the concentration of PAHs according to their sources and locations is due to the nature of human activities and emissions from industries that are close to dried fruit sites, which release different concentrations of PAHs into the air and settle in water or soil Table 4.

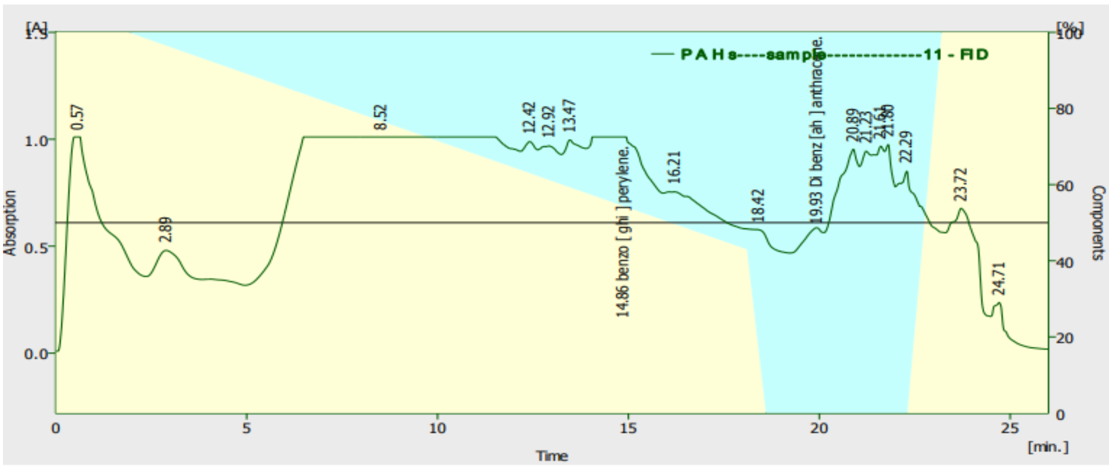

Figure (8): Chromatogram of PAHs compounds in a dried fig sample

Table 4: PAHs in dried figs

Sample	Places	NAP	PHE	B[b]F	B[ghi]P	Chr	Total PAHs (ppb)
Fig	T1	0.02	11.32	0.00	0.00	0.00	11.32
	T2	0.00	0.00	0.00	0.47	15.31	15.78
	T3	0.00	0.00	0.00	0.32	13.15	13.83
	IR1	0.04	0.00	9.41	0.00	0.00	9.45
	IR2	0.00	0.00	0.00	0.40	12.21	12.61
	I1	0.03	13.41	0.00	0.00	0.00	13.44
	I2	0.05	0.00	10.01	0.00	0.00	10.06
	TY	0.05	0.00	12.31	0.00	0.00	12.36
	S	0.01	10.52	0.00	0.00	0.00	10.53
Mean		0.03	11.75	10.57	0.39	13.66	12.19
Min		0.01	10.52	9.41	0.32	12.21	9.44
Max		0.05	13.41	12.31	0.47	15.31	15.78

Discussion

A recent study found that *Escherichia coli* was the most isolated dried fig bacteria. Turkish researchers recovered *Escherichia coli*, yeast, and mould from dried figs [21]. From dried figs, *Escherichia coli*, *Pseudomonas aeruginosa*, and Staphylococcus were also isolated [22]. A recent study found that dried vegetable and fruit outbreaks are caused by *Escherichia coli*. Fruits and vegetables are *E. coli* contaminated [23]. According to [22], Aspergillus sp. and Penicillium are the most common dried fruit fungus genera. According to [24], dried fruits yielded A. niger (25.8%), flavus (19.3%), and fumigatus (11.8%). In Duhok Governorate/Iraq, fungi from dried figs were isolated at different rates. Aspergillus has 12 species and the greatest diversity of all genera. Penicillium placed second with five species [25].

Local market dried figs are contaminated by industrial and home wastes, generator wastes, car exhausts, and excessive water discharge, according to the study. Continuous PAH exposure from the environment and diet can cause cancer. Mutations in DNA replication caused by metabolic activation of PAHs in mammalian cells to diol epoxides appear to be the main cause of cancer. Non-genotoxic PAH consequences include high blood pressure, lipid/lipoprotein problems, and insulin resistance [26]. The high molecular weight PAH benzo(a)pyrene is one of the most prominent environmental contaminants and has garnered attention for its carcinogenicity [27]. In cold seasons, PAH concentrations in fruits are increased due to emissions from many sources, especially heating sources. This increases fruit PAH deposition. According to [28], cold weather may reduce decomposition bacteria, increasing PAH concentrations. They said microbes break down PAHs. These results match [29].

The chemicals were studied at five Erbil locations. The Environmental Protection Agency (USEPA) monitored 16 major PAH chemicals using plants. Naphthalene and Indeno(cd-1,2,3) pyrene in the plant had the lowest and highest values [7].

Each plant can amass chemicals in its tissues by absorbing polycyclic aromatic hydrocarbons from the soil through the root and transferring them to the aerial section [30]. Products grown near roadways or in cities have more PAHs than those grown in rural regions. All raw rural fruits and vegetables included a minor amount of phenanthrene, fluoranthene, and pyrene. Lighter PAHs as naphthalene, acenaphthylene, and acenaphthene, were abundant [31]. Another study [32] on vegetables in Baghdad Governorate found that PAH concentrations in some samples increased due to their cultivation near public roads and car exhausts, power plants, or fuel processing stations. We quantified dietary exposure and health issues related to four EU priority polycyclic aromatic hydrocarbons (PAHs) in several Turkish vegetable cultivars. HPLC analysed PAH congeners. All the veggies tested had low PAH levels. Of all vegetable species, leafy vegetables were most contaminated. Total PAHs (benzo[a]pyrene, benzo[a]anthracene, chrysene, and benzo[b]fluoranthene) in broccoli and cauliflower were 0.77 and 0.59 ppm, respectively. Peels were more contaminated than root vegetables and fruit cores. Potato skin has the most PAHs (0.73 ppm) of root vegetables. The highest content of benz[a]anthracene (BaA) in all vegetables was 0.38 ± 0.16 in cauliflower. All samples contained benzo[a]pyrene (BaP), the most carcinogenic PAH. Benzo(b)fluoranthene (BbF) and chrysene (CHR) were less abundant in all samples. The estimated daily intake of fruits and vegetables was used to determine human PAH exposure (33,34).

Conclusion

Widespread spread of bacteria and fungi in dried fruits, where *Escherichia coli* was the dominant, followed by Staphylococcus, while *Aspergillus nigar* mold showed the largest percentage of fungal contaminants, followed by Candida mold.

PAHs in dried fruits may cause negative effects on exposed receptors, according to the environmental risk assessment. Therefore, the results of this study should help in understanding the levels, sources, and environmental risks of polycyclic aromatic hydrocarbons in dried fruits. In this study, 12 priority PAHs were studied in dried fruits collected from local markets. High levels of some PAHs were observed in dried fruits.

Funding

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Ethics:

The study protocol was reviewed by the Human Ethics Committee of the General Directorate of Education, Salahuddin, Iraq.

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Conflict of interest:

There is no conflict of interest.

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