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Original Research

Mycobiota and aflatoxins in fresh and dried fig fruits in Palestine

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Sahar M. Huntoli, Rana M. Jamous, Salam Y Abuzaitoun, Nidal Za'tar, Mohammed S. Ali-Shtayeh

Mycobiota and incidence of aflatoxigenic fungi in fig fruits from Palestine

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ABSTRACT

Huntoli S. N., Jamous, R. M., Abuzaitoun, S. Y., Za'tar, N., & Ali-Shtayeh, M.S. (2014). Mycobiota and incidence of aflatoxigenic fungi in fig fruits from Palestine. *Biodiversity & Environmental Sciences Studies Series*, 9 (1),1-16.

Fifty-one samples of fig fruits (22, fresh; 4, shriveled-ripe; 2, from drying plot (*mustah*); 3, shriveled-ripe ten days post sun-drying; and 20, stored dried figs) were collected from 7 locations from the West Bank over a 12-month period. The samples were assayed for mycobiota including *Aspergillus flavus* from the surface and internal of the fruits. Twenty-one species belonging to 14 genera were isolated from fruits surface; the most frequent fungi were species of *Aspergillus* and *Cladosporium* which were encountered in 86.3, and 74.5% of the samples, respectively with *A. flavus* being found in 41.2% of the samples. Twenty-seven species belonging to 17 genera were isolated from the internal of fruits. The most frequent fungi were *Aspergillus* species and *Alternaria alternata*, which were encountered in 92.2 and 58.8% of the samples, respectively, with *A. flavus* being found in 58.8% of the samples. The highest total means mycobiota level was recorded in fig fruits collected from shriveled-ripe figs (under the tree), and the lowest level was recorded in stored dried figs (quttain). Total mean mycobiota level was significantly affected by the geographical location of the fruit production site; the highest level was recorded in Selwad (Ramallah District). The highest population levels of *A. flavus* were found in the dried fruits. A total of 73 isolates of *A. flavus* were isolated from fig fruits. Fifty (68.5%) of these isolates were found to be aflatoxigenic. VICAM analysis using monoclonal antibodies revealed that 9 samples out of 30 samples contained aflatoxin. The level of aflatoxins was (≤ 0.4 µg/kg), which did not exceed the FDA permitted levels (< 20 µg/kg), with the highest level of contamination being found in the dried fruits.

Introduction

Fig fruit (*Ficus carica* L.) is one of the most important agricultural products of the tropic and subtropics areas. In Palestine, as well as in other Mediterranean countries, the fig is included in the diet since the ancient years and it is considered as the symbol of immortality (Shtayeh et al., 1991; Nozedar, 2008). The leaves, roots, fruit, and latex of the plant are known for their health-promoting properties including acetylcholinesterase inhibition, antifungal, anti-helminthic and anticarcinogenic activities (Ali-Shtayeh & Jamous, 2008).

Mold contamination of figs occurs during ripening, harvest, post-harvest, storage, and processing of raw materials (Heperkan et al., 2012). During these periods, favorable conditions, e.g., high temperatures, high moisture content, and high level of sugar content and other nutrients play an important

role in the growth of fungi and mycotoxin production (Battilani et al., 2011; Embaby et al., 2012). The most important filamentous molds that grow and produce mycotoxins (secondary metabolites) in food and dried fruits are *Aspergillus, Fusarium*, and *Penicillium* species (Ozer et al., 2012; Saadullah, & Abdullah, 2015; Campos et al. 2008). These fungi can cause food spoilage, biodeterioration and are capable of producing different mycotoxins. *Aspergillus* species are the most common toxigenic species in various foods and feeds (Kumar et al., 2008). These species produce the most carcinogenic mycotoxins, the aflatoxins (AFs) (Ozer et al., 2012; Creppy, 2002).

Aflatoxins are highly toxic, carcinogenic and mutagenic polyketide secondary metabolites and are known to contaminate a wide cultivar of foods and agricultural products such as dried fruits (Perrone et al., 2014; Hedayati et al., 2007). AFs are produced by *Aspergillus* species, mainly *A. flavus, A. nomius, A. parasiticus*, and *A. astellatus* (Payne & Brown, 1998; Reiter et al., 2009; Peterson et al., 2000). AFs are a worldwide significant problem in terms of public health, agriculture, and economics (Moss, 1998). The main problem for fig consumption is the contamination of the dried fig by AFs. Food processing techniques are not sufficient to eliminate AFs from contaminated food due to their heat resistant nature (Betina, 1989).

Preservation of fig fruits by sun-drying has been practiced for centuries (Cagan & Svercel 2001). It is limited to climates with a hot sun and a dry atmosphere and to certain fruits, such as figs. In Palestine, as in some Mediterranean countries (Zinedine et al., 2007; Gilbert & Senyuva, 2008), when fig fruits have reached an appropriate degree of maturity, they are gathered and transported to the drying place (mustah) which is a piece of ground fenced off to prevent access of animals. The ground in the mustah is sometimes covered in herbs to avoid contact with the soil. Figs are spread out over the surface of mustah without preliminary treatment, and after a few days of drying (about 5 days), fruits are collected and stored.

Traditional sun-drying as described above has a high risk of fungal infection when the semi-dry fig (31-36 % moisture content; aw = 0.8-0.87) has been in contact with the soil (Özay & Alperden, 1991). There is a subsequent risk of fungal growth and toxin production, during the drying period when the figs are within a critical range of water activity. Under favorable conditions for fungal growth (optimum water activity of around 0.8, and temperatures from 25-30 ºC), fungal invasion can occur either through spore-contaminated dust or insect transmission to the fruit on the tree or directly from the soil or during the course of subsequent sun-drying.

Natural occurrence of AFs and fungal contamination of many dried fruits were investigated in many parts of the world (Karaca & Nas, 2008; Karaca et al., 2010 and references therein). The presence of fungal contamination in raw and dried figs usually leads to nutrient losses, alteration of organoleptic characteristics and decline of product shelf-life in the market. The mycological quality of raw materials utilized to elaborate the final product needs to be controlled; it allows to predict the toxicological potential present in the dried figs.

The ingestion of AFs from contaminated food has led to serious health complications in humans (Sherif et al., 2009). Different countries have, therefore, applied stringent rules for AFs in food to maintain the health of people (Juan et al., 2012). The safe limit of AFs lies in the range of 4–30 µg/kg for human consumption. The EU has the strictest standard level with AFB1 and total AFs not beyond 2µg/kg and 4µg/kg, respectively, in any product meant for direct consumption (EC, 2010). Similarly, the maximum acceptable limit set for AFs in the USA is 20 µg/kg (Wu, 2006).

Many studies have been carried out on aflatoxins contaminating figs especially dried figs, in several countries (Karaca et al., 2010; Ozer et al., 2012; Saadullah, and Abdullah, 2015; Campos et al. 2008; Zohri & Abdel-Gawad, 1993; Saadullah and Abdullah, 2015; Demir et al. , 1990). The aims of the present study were to isolate and identify the mycobiota and determine the AFs natural occurrence in raw and dried fig fruits over a period of twelve months. Therefore, the aflatoxigenic capacity of *A. flavus* was investigated.

Materials and Methods

Sample Collection

Fifty-one samples of fig fruits (22, fresh; 4, shriveled-ripe; 2, from drying plot (*mustah*); 3, shriveled-ripe ten days post sun-drying; and 20, stored dried figs) were collected from 7 locations from the West Bank over a 12-month period.

Fresh fruits were picked up as described by Tous & Ferguson (1996). Each collected sample was placed in a sterile labeled polyethylene sealed bags to minimize loss of water content and provide sufficient aeration (Zohri & Abdel-Gawad, 1993). The samples were kept in a cooled container and transferred immediately to the laboratory for processing either on the same day or kept in the refrigerator at 8 ^{o}C for the second day. About one third of each sample was stored in the freezer for the detection and quantitation of aflatoxin from samples with aflatoxigenic *A. flavus* (Doster & Michailides, 1998). Moisture content of fig fruits was determined using oven drying method (AOAC International, 2002). Samples were taken and dried at 105 ^{o}C for 24 hours**.**

Mycological studies

***Determination of fig fruit external mycobiota*.** External mycoflora was determined according to French Standard AFNOR V08-301. A 40 g subsample was placed with glass balls in 360 ml of dilution liquid (Tryptone, 1.6gm; Sodium chloride, 8.5gm; Tween 80, 2 drops; 1000 ml distilled water), and mixed thoroughly on an orbital shaker (400 r.p.m.) for 20 min (Bauduret, 1990, Dalcero et al., 1998). Ten-fold dilutions were prepared in 1% peptone-water solution to give 10^{-2}, 10^{-3} and 10^{-4} dilution. For each dilution, 1 ml was pipetted into 4 sterile disposable petri dishes. M2 agar (2% malt extract; 0.2% yeast extract and 1.8 % agar) supplemented with 60 µg/ml chloramphenicol (Sigma, St.-Louis, USA) and 50 µg/ml gentamycin sulfate (Sigma) was added to 4 plates. M5 agar (5% malt extract; 0.2% yeast extract; 5% sodium chloride and 1.8% agar), also containing antibiotics, was poured in the other 4 plates. Two plates of each medium were incubated at 25 ^{O}C, and the others at 35 ^{O}C. (Bauduret, 1990; Diener & Davis, 1966), as the optimum temperature for *A. flavus* growth occurs at 35 ^{o}C and the maximum aflatoxin biosynthesis occurs at 25-30 ^{o}C on both synthetic and natural media (Smith & Moss, 1985; Van Egmond, 1989). After 4 to 6 days of incubation, molds were identified and counts were made of individual species and genera as colony forming units (CFU) gm^{-1} dry weight (DWt.) of sample. Representative isolates of recovered fungi were grown on PDA and incubated at 25 ^{o}C for identification. *A. flavus* colonies were recorded as CFU gm^{-1}, and stored for aflatoxin detection.

***Determination of fig fruit internal mycobiota*.** Internal mycobiota was determined for all samples. From each sample, 24 whole fig fruits were surface-sterilized for one minute in a commercial 5% aqueous solution of sodium hypochlorite, then washed twice with sterile distilled water (Gonzalez, Pacin, Resuik & Martinez, 1996) and dried by placing them on sterile toilet paper under aseptic condition in a laminar flow desk. Each fig fruit was cut to 4 pieces, 12 fruits were plated on M2 agar medium, and another 12 fruits on M5 agar medium (one fruit = 4 pieces per plate). Of each medium, 6 of the inoculated plates were incubated at 25 ^{o}C and the other 6 at 35 ^{o}C for 4-6 days. The plates were observed daily and colonies were recorded as colonies per 10 fig fruits. Representative fungal isolates were grown on PDA and incubated at 25 ^{o}C for identification and aflatoxin detection for *A. flavus* isolates.

***Identification of fungi*.** Fungal isolates recovered from M2 and M5 agar plates were first grown on different media (Sabouraud's dextrose agar (SDA) (Oxoid), PDA and CzA medium), and incubated for 10 days at 25 ^{o}C. The isolates were grouped on the basis of their colonial morphology (color, texture, and growth characteristics). Microscopic examination was carried out using fresh direct mounts in lactophenol cotton blue under high powers (X40, X100). Cultures, which did not produce any reproductive structures, were incubated in the light for two days and then examined under the microscope. Identification of isolates was made with the aid of several taxonomic references including (Campell et al., 1980; Domsch et al., 1980; Kozakiewicz, 1989; Onions et al., 1981; Klich, 2002; Samson et al., 2007; Noonim et al., 2008; Pitt & Hocking, 2009). Fungal isolates including *A. flavus* were maintained on SDA slants supplemented

with 60 µg/ml chloramphenicol and 50 µg/ml gentamycin sulfate in 30-ml sterile screw-capped culture tubes and then stored in a refrigerator at 4 °C, and sub-cultured monthly.

Detection of Aflatoxin

Qualitative analysis

Production of blue fluorescence on coconut-agar medium. A qualitative test for aflatoxin-producing strains of *A. flavu*s was carried out by culturing *A. flavus* isolates on coconut agar medium (CAM) (Davis *et al*., 1987) (per liter: agar, 4.0gm; shredded coconut, 100 gm). A mycelial plug from PDA was placed onto the center of CAM plate and the plates were incubated at 25 °C for a period of up to one-week (Lin & Dianese, 1976). The reverse side of each plate was examined daily under UV Lamp (under long-wave, 365 nm) for blue fluorescence. Uninoculated plate was observed as a reference (Davis, Iyer & Diener, 1987; Lin & Dianese, 1976). The intensity of fluorescence was expressed by number of "+" signs (Lin & Dianese, 1976).

Production of bright greenish yellow fluorescence (BGYF). BGYF results when *A. flavus* produces kojic acid, which is then converted to the fluorescent compound by peroxidase in the plant (Marsh et al., 1969). Fungi can provide peroxidases needed to convert the kojic acid to the fluorescent compound (Wicklow & Hessetline, 1979). Twenty samples of dried figs "Quttain" were incubated at 25 °C for 40 days to detect the presence of (BGYF) under long-wave UV lamp (365 nm) and to study the effect of incubation time on aflatoxin production. The results were recorded as number of "+" signs.

Quantitative analysis by immuno affinity column. Since not all isolates of *A. flavus* can produce AFs, the determination of aflatoxin-producing ability of a particular isolate is important in biochemical, genetical, toxicological, and epidemiological studies. Aflatoxin was determined in all samples containing at least one aflatoxigenic strain by using AflaTest® column bound with specific antibodies to aflatoxin (VICAM, 1999). Aflatoxin concentration was measured in µg/kg using a calibrated fluorometer.

Statistical analysis

Data on the distribution of external and internal mycobiota in fig fruits were analyzed using the ANOVA test to detect the significant differences in relation to the state, cultivar, and site of production. Mean separation was carried out using Scheffe's test. Also, a t-test was used to detect the significant difference between pairs of variables ($P< 0.05$). All analysis was conducted using the SPSS database for Windows, version 17.

Results and Discussion

Frequency of occurrence of external and internal mycobiota

The biodiversity of the external and internal mycobiota recovered from fig fruits at different stages for making "Quttain" is evident. A total of 21 species of fungi belonging to 14 genera were recovered from fig fruits surface, and 27 species of fungi belonging to 17 genera were recovered from the internal of fig fruits (Table1). The most frequent fungi encountered on external and internal fig fruits were *Aspergillus* spp. Seven species of *Aspergillus* were identified, of which A. niger (82.4 and 92.2) was the most frequent (frequency; 82.4 in the external and 88.2 in the internal fruits) , followed by *A. flavus* (41.2% and 58.8), *A. terreus* (9.8% and 23.5%), and *A. candidus, A. fumigatus, A. versicolor* and *A. glaucus* (Range: 2-11.8%). *Cladosporium* was the second most frequent genus represented by 3 species, of which *C. herbarum* (frequency; 54.9% in the external fruits and 19.6 in the eternal). *Penicillium* species ranked third recovered from 68.6% of the external fruit samples and 29.4% of the internal fruit samples, and *Alternaria alternata* was isolated from 49% of the collected external fruit samples and 58.8% internal fruits, *R. stolonifer* was represented in 51% of the internal fig fruit samples and 15.7% of the external fig fruit samples (Table 1). The highest number of fungal species were recovered from the surface fresh fig frutes (21 fungal species) followed by 'shriveled-ripe ten days post sun-drying ' (18 fungal species), out of the twenty-two fungal species there were six species present in all fig fruit states and the internal fresh stored dried figs (17 fungal species).

Table 1. External and internal mycobiota recovered from fig fruit surface (number of positive samples (frequency %)).

Mycobiota	External					Internal					Total (51)	
	Fruit state*(No. of samples)					Fruit state*(No. of samples)						
	a (22)	b(4)	c (2)	d(3)	e(20)	a (22)	b (4)	c (2)	d (3)	e (20)	External	Internal
Acremonium kiliense (Grütz)	1(4.6)	0(0)	0(0)	0(0)	0(0)	0 (0)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)
Alternaria alternata (Fr.) Keissler	19(86.4)	4(100)	2(100)	3(100)	2(10)	13(59.1)	4(100)	2(100)	3(100)	3(15)	30(58.8)	25(49)
Aspergillus candidus (Link ex Link)	3(13.60	2(50)	0(0)	0(0)	1(5)	0(0)	1(25)	0(0)	0(0)	1(5)	6(11.8)	2(3.9)
A. flavus (Link ex Gray)	9(40.9)	1(25)	1(50)	3(100)	16(80)	7(31.8)	1(25)	1(50)	2(66.7)	10(50)	30(58.8)	21(41.2)
A. fumigatus (Fresenius)	0(0)	0(0)	0(0)	0(0)	1(5)	0(0)	0(0)	0(0)	0(0)	2(10)	1(2)	2(3.9)
A. glaucus (Link ex Fries)	1(4.6)	0(0)	0(0)	0(0)	0(0)	1(4.5)	0(0)	1(50)	0(0)	0(0)	1(2)	2(3.9)
A.niger (Van Tieghem)	18(81.8)	4(100)	2(100)	3(100)	18(90)	14(63.6)	4(100)	2(100)	3(100)	19(95)	45(88.2)	42(82.4)
A. terreus (Thom)	0(0)	0(0)	0(0)	1(33.3)	11(55)	0(0)	0(0)	1(50)	0(0)	4(20)	12(23.5)	5(9.8)
A. versicolor (Vuill.) Tiraboschi	0(0)	0(0)	0(0)	0(0)	2(10)	0 (0)	0(0)	0(0)	0(0)	0(0)	2(3.9)	0(0)
Auerobasidium pullulans (de Bary) Arnaud	5(22.7)	0(0)	1(50)	0(0)	0(0)	2(9.1)	0(0)	1(50)	0(0)	0(0)	6(11.8)	3(5.9)
Cladosporium herbarum (Pres.) Link ex S. F.Gray	4(18.2)	0(0)	2(100)	2(66.7)	2(10)	11(50)	2(50)	2(100)	3(100)	10(50)	10(19.6)	28(54.9)
C. resinae (Lindau) de Vries	3(13.6)	1(25)	0(0)	0(0)	1(5)	6(27.3)	1(25)	0(0)	0(0)	0(0)	5(9.8)	7(13.7)
C. sphaerospermnm (Penz.)	2(9.1)	1(25)	0(0)	0(0)	0(0)	4(18.2)	4(100)	1(50)	2(66.7)	1(5)	3(5.9)	12(23.5)
Fusarium oxysorum (Schlecht)	3(13.6)	0(0)	0(0)	0(0)	0(0)	0 (0)	0(0)	0(0)	0(0)	0(0)	3(5.9)	0(0)
Helminthosporum cynodonties (Link ex Fries)	1(4.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	0(0)	1(2)	1(2)
Humicola grisea (Traaen)	1(4.6)	0(0)	2(100)	2(66.7)	5(25)	0(0)	0(0)	1(50)	3(100)	1(5)	10(19.6)	5(9.8)
Moniliella acetobutans (Stolk & Dakin)	0(0)	0(0)	0(0)	0(0)	1(5)	0(0)	0(0)	0(0)	0(0)	1(5)	1(2)	1(2)
Mortierella ramanniana (Möller) Linnem	1(4.6)	0(0)	0(0)	0(0)	1(5)	0 (0)	0(0)	0(0)	0(0)	0(0)	2(3.9)	0(0)
Mortierella spp.	0(0)	0(0)	0(0)	0(0)	3(15)	0(0)	1(25)	0(0)	0(0)	0(0)	3(5.9)	1(2)
Paecilomyces lilacinus (Thom) Samson	1(4.6)	0(0)	0(0)	0(0)	0(0)	0 (0)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)
Papulaspora corprophila (Preuss)	1(4.6)	0(0)	0(0)	0(0)	0(0)	0 (0)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)
Penicillium chrysogenum (Thom)	4(18.2)	1(25)	1(50)	0(0)	9(45)	12(54.5)	4(100)	1(50)	2(66.7)	14(70)	15(29.4)	33(64.7)
P. citrinum (Thom)	0 (0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(5)	0(0)	1(2)
P. oxalicum (Currie & Thom)	0 (0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(5)	0(0)	1(2)
Pseudobotrytis terrestris (Timonin) Subramanian	1(4.6)	0(0)	0(0)	2(66.7)	0(0)	1(4.5)	0(0)	0(0)	0(0)	0(0)	3(5.9)	1(2)
Rhizopus stolonifer (Ehrenb. ex Link) Lind	8(36.4)	1(25)	2(100)	3(100)	12(60)	2(9.1)	0(0)	2(100)	0(0)	4(20)	26(51)	8(15.7)
Ulocladium chartarum (Preuss) Simmons	2(9.1)	1(25)	0(0)	1(33.3)	4(20)	4(18.2)	0(0)	0(0)	0(0)	6(30)	8(15.7)	10(19.6)
Verticillinm albo-atrum (Reinke & Berthold)	3(13.6)	0(0)	0(0)	0(0)	1(5)	0(0)	1(25)	0(0)	0(0)	3(15)	4(7.8)	4(7.8)
V. lateritium (Ehrenbery) Rabenhost	0(0)	0(0)	0(0)	0(0)	1(5)	0 (0)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)
Unidentified hyphomycetes	0 (0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(5)	0(0)	1(2)
Total number of fungal species	**21**	**9**	**8**	**18**	**12**	**11**	**10**	**11**	**8**	**17**	**27**	**22**

*a fresh-fruits; b, shriveled-ripe; c, from drying plot (*mustah*); d, shriveled-ripe ten days post sun-drying e, stored dried figs (quttain) (> two months in storage).

This study shows that species of *Aspergillus*, *Cladosporium*, *Penicillium* and *Alternaria alternata* were present in high frequency.This result coincides with those of previous studies carried out on dried figs in Egypt (Zohri & Abdel-Gawad, 1993) and Iraq (Saadullah and Abdullah, 2015). However, higher incidence of *A. alternata* and *Cladosporium* species, and lower incidence of *Aspergillus* species and *Penicillium* species, were encountered in this study. The high frequency of these fungi may be due to their ability to produce dry spores readily distributed into the atmosphere by the slightest physical disturbance (Smith & Moss, 1985). All of these fungi were isolated previously from seeds, grains, fruits, and vegetables by several researchers in many laboratories (Zohri & Abdel-Gawad, 1993).

Population levels of external and internal mycobiota in different fruit state

The population levels of of External and internal Mycobiota in different Fruit State was recovered at 25 °C on M2 Medium, Abundance or contamination level (CFU gm-1 DWt.) of external mycobiota of 36 fig fruit samples are presented in Table 2. The external total mycobiota population levels were ranged between 600.1-7197 CFU gm-1 DWt, the highest levels were achieved in shriveled-ripe and fresh fruits. The internal total mycobiota population levels were ranged between 36.6-144.1 CFU gm-1 DWt, the highest levels were acheived in drying plot fruites. Total mean mycobiota population levels were not significantly affected by fig state ($p \geq 0.05$).

In the external fruits, three fungal species (*A. alternate, A. niger* and *C. herbarum*) were detected in all fruit states, however the population level of these species were significantly affected by the fig fruit state ($p \leq 0.05$) with the highest population level of *Alternaria alternate* (2,435.3 CFU gm-1 DWt.) in fig fruits during the drying stage in the mustah and the highest population levels of *C. herbarum* (6,048.2) was recorded in fresh fig fruits, while, *A.niger* highest mean level (353.6) was recorded was recorded in stored dried fig fruits

In the internal fruits, two fungal species (*A. alternate, A. niger*) were detected in all fruit states, however the population level of these species were significantly affected by the fig fruit state ($p \leq 0.05$) with the highest mean population level of *Alternaria alternate* (100CFU gm-1 DWt.) in the shreveild-ripe fruits and the highest population levels of *A. niger* (21.1) was recorded in stored dried fig fruits.

The Higher population levels of Alternaria alternata (2435.3) recovered from fruits surface during drying state in the mustah in this study may be attributed to the fact that this fungus is a cosmopolitan saprophyte especially on senescent plant material, foodstuffs, and textiles and is frequently isolated from soil (Smith & Moss, 1985). Also, over-ripening and high moisture content of fruits may increase the probability of contamination.

Table 2. Distribution and population levels (CFU gm-1DWt.) of fig fruits external and internal mycobiota on M2 medium according to the fruit state.

Mycobiota	Fruit state*(No. of samples)									
	External					Internal				
	a(7)	b(4)	c(2)	d(3)	e(20)	a(7)	b (4)	c (2)	d(3)	e (20)
Alternaria alternate	69	269.5	2435.3	211.4	6	99.8	100	78.3	58.3	0.5
Aspergillus flavus	0	0	0	0	65.7	0.2	0	0	5	2.5
A. niger	34.5	68.5	32.9	314.2	353.6	2.4	3.3	2.5	18.3	21.1
A. terreus	0	0	0	0	0	0	0	0	0	1.3
Cladosporium herbarum	6048.2	1604	722.7	380.2	50.2	0	0	48.3	21.1	0.9
C. sphaerospermum	0	4303.3	1409.4	695.4	0	0	0	0	0	0
Penicillium chrysogenum	713.9	714.5	164.3	0	53.4	6.4	0	0	0	5.9
Other species**	31.1	237.2	66.5	0	71.2	30.7	1.7	15.0	19.5	4.4
Total	**6896.7**	**7197**	**4831.1**	**1601.2**	**600.1**	**139.5**	**105**	**144.1**	**122.2**	**36.6**

* As in Table 1; ***Aspergillus candidus, A. fumigatus, A. glucaus, A. terreus, Auerobasidium pullulans, Acremonium kiliense,A. versicolor,Cladosporium resinae,C. sphaerospermum, Fusarium oxysporium, Helminthosporum cynodontis, Humicola grisea, Rhizopus stolonifer, Ulocladium chartarum, Moniliella acetobutans, Mortierella ramanniana, Mortierella spp, Paecilomyces lilacinus, Papulaspora corpophila, Penicillium citrinum, P. oxalicum, Pseudobotrytis terrestris, Vertcillium albo-atrum,* unidentified hyphomycetes, *V. lateritium.*

Population Levels of External Mycobiota According to Fruit Cultivar

Distribution of fig fruit mycobiota in defferent fruit cultivar is presented in Figure 1. In the external mycobiota, Population levels of *A. alternata* were significantly different by fig fruit cultivar (P=0.02), with the highest mean level (1,656.9) found in *Biadi* and *Kharroubi* mixture fruit cultivars. Total mean mycobiota population levels were not significantly (P=0.09) affected by fig cultivars, with the highest mean level (5474.9) was recorded in *Biadi* fig fruits. In the internal mycobiota, Population levels of *C. herbarum*, *H. grisea*, and *R. stolonifer* were significantly (P<0.05) affected by fig fruit cultivar, with the highest mean level (36.7, 6.7 and 11.7, respectively) found in the *Biadi* and *Kharroubi* or *Swadi* mixture cultivars.

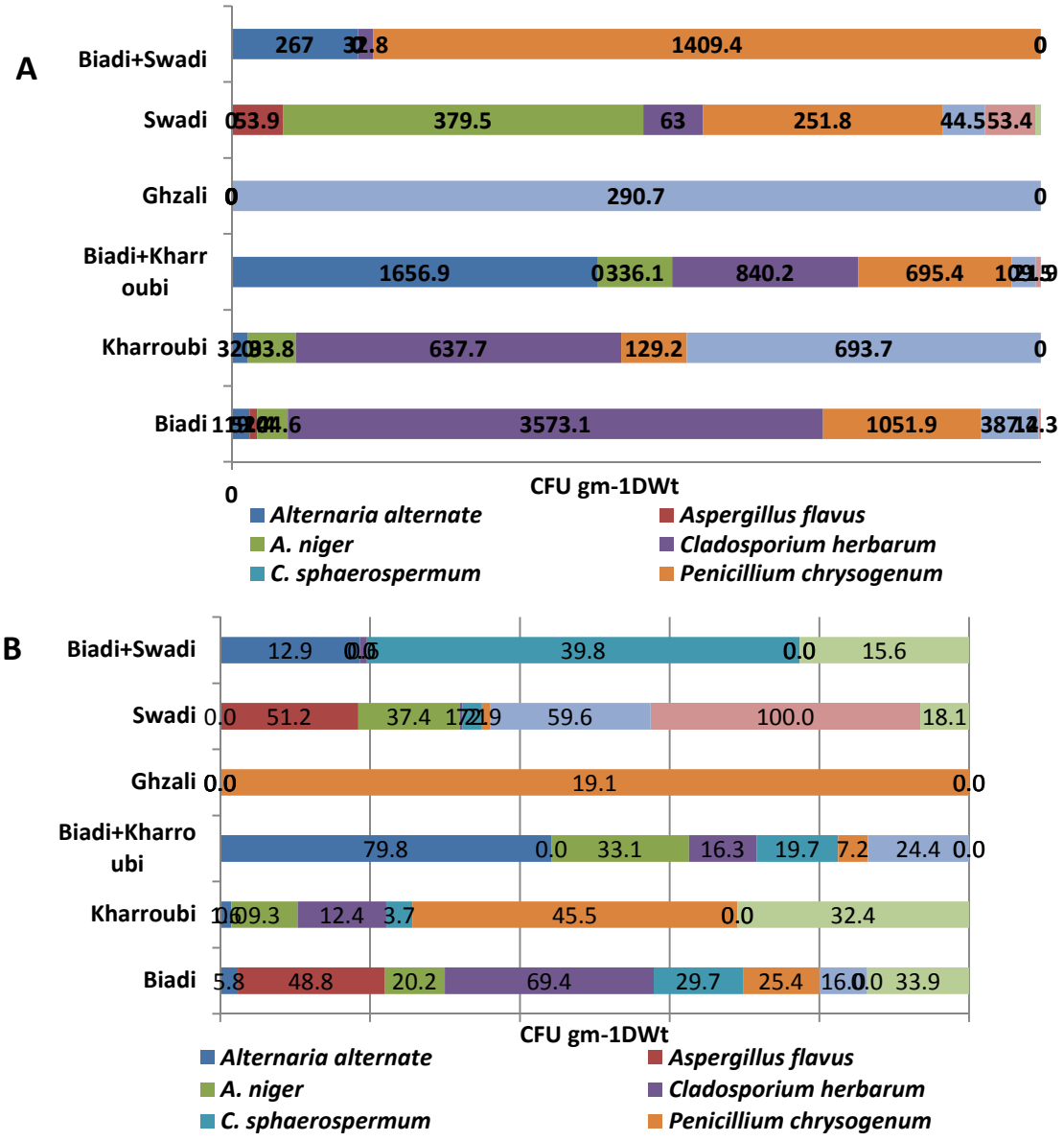

Figure 1. Distribution and population levels (CFU gm-1DWt.) of fig fruits external mycobiota according to fruit cultivar.(A) internal and (B) external.

Fruit cultivar had a considerable effect on mycobiota levels with Biadi fruits yielding the highest levels. This cultivar is used to make "Quttain" by drying in the mustah during which the fruits are exposed to higher contamination levels. On the other hand, the lower levels yielded by Ghzali fruits may be partly due to the fact that these fruits are consumed fresh, and thus exposed to fewer levels and duration of contamination . Fig cultivars yielded comparable total mean mycobiota population levels of internal mycobiota.

Table 3. Distribution and population levels (CFU gm^{-1}DWt.) of fig fruits mycobiota according to fruit production site

Mycobiota	Production site* (No.of Samples)								
	1(8)	2 (10)	3 (7)	4 (1)	5 (2)	6 (1)	7 (1)	8 (4)	9 (2)
	Population levels (CFU gm^{-1}DWt.) of fig fruits external mycobiota								
Alternaria alternata	729.9	122.6	0	0.0	60	0.0	0.0	0	0
Aspergillus flavus	0	0	95.4	0.0	0	0.0	0.0	161.7	0
A. niger	172.4	39.1	252.4	61.1	238.5	607.5	142.1	907.4	71.2
Cladosporium herbarum	5070.2	1090.3	108.5	0.0	60	0.0	0.0	0	0
C. sphaerospermum	2034.8	584	0	0.0	0	0	0.0	0	0
Penicillium chrysogenum	932.2	78.8	49.5	0.0	89	303.8	0.0	44.5	0
Other species**	40.3	103.9	130.8	61.1	59	0	0	66.8	0
Total	8979.8	2018.7	636.6	61.1	506.5	911.3	142.1	1180.4	71.2
	Population levels (CFU gm^{-1}DWt.) of fig fruits internal mycobiota								
Alternaria alternata	92.1	69.3	2.5	0	0	0	0	0	0
Aspergillus flavus	0.2	1.5	5	2.4	0	10	0	0	1.7
A.niger	6.9	7.5	37.1	13.1	23.3	23.3	11.7	35.8	5.8
A. terreus	0	0	2.1	0.7	1.7	0	0	0	5.8
Cladosporium herbarum	13.8	5	4.6	0	0	0	0	0	0
Penicillium chrysogenum	0.6	4.7	9.2	2.6	0	55	0	0.8	0
Other species**	11.8	24.2	2.9	0.7	10	0	15	8.3	0
Total	125.4	112.2	63.4	19.5	35	88.3	26.7	44.9	13.3

1, Silwad; 2, Al-Mazra'h Al-Qibliyah; 3, Al-Mazra'h Al-Sharqiyah; 4, Al-Sawiah; 5,Til; 6, Kubar; 6, Al-Mikhmas; 7, Syria; 8, Turkey.

Population levels of fig fruits mycobiota from different production site

Distribution of fig fruits mycobiota according to the production site is shown in Table 3. Population levels of external and external mycobiota and total mean population levels were significantly ($P \leq 0.05$) affected by the fruits production site.The highest total population level (8979.8 in the external nd125.4 in the internal) were detected in samples collected from silwad, C.herbarum was the most abundant fungi of the external mycobiota with population level of 5070 and the most abundant fungi of internal mycobiota was A. alternata with population level of 92.1.

Differences in total mean mycobiota levels (external & internal) in samples from different geographical locations may be contributed to differences in climate, fruit uses, and cultivar.

Effect of Different Variables on the Recovery of External Mycobiota and *Aspergillus flavus* Population Levels

The effect of fruits moisture content, incubation temprature and medium on the recovery of mycobiota and *Aspergillus flavus* were tested, The moisture content of fig fruits did not have a significant effect on the total mean external mycobiota level including A. flavus levels. On the other hand, a significant correlation ($P<0.05$) was detected between total mean internal mycobiota level and moisture content. This

may explain the low total mean mycobiota levels at the dried state with low moisture content, which could have prevented the growth and development of fungi (Bauduret, 1990).

The Effect of Incubation Temperature and Media on Total Mycobiota and Aspegillus flavus Population Levels is presented in Table 4, the total mean external mycobiota levels on M5 medium were higher than that on the M2 medium. This may be due to different cultural compositions of both media, e.g. M5 media contain sodium chloride (NaCl) and this is very effective in the inhibition of fast-growing "speeder" fungi such as Mucor and Trichoderma. Yet, it does not inhibit the growth of other fungi species, including mycotoxin-producers (Bars & Bars, 1998). Hence most of the mycobiota recovered on M5 medium were not fast-growing.

Total mean mycobiota levels (external and internal) were significantly affected (P = 0.01) by incubation temperature with the higher levels for all samples being recorded at 25 oC than that at 35 oC for different media used in this study. On the other hand, higher population levels of A. flavus were recorded at 35 oC more than at 25 oC, as the optimum temperature for A. flavus, is 37 oC with maximum aflatoxin biosynthesis at 27.2-30 oC (Jacobson et al., 1993).

Table 4. Effect of incubation temperature and media on: (A) Total mycobiota, (B) *Aspergillus flavus* population levels (CFU gm-1 DWt.).

	External		Internal	
Media (Temp)	**Total mean mycobiota**	**A. flavus**	**Total mean mycobiota**	**A. flavus**
	CFU gm-1 DWt.		**CFU gm-1 DWt.**	
M2 (25 °C)	1164.9	36.5	77.3	1.9
M2 (35 °C)	786.2	37.8	49.7	4.2
M5 (25 oC)	2804.2	9.5	60.6	0.7
M5 (35 °C)	1256.6	114.2	39.9	2.1

Aflatoxigenic A*spergillus flavus* Group

External A. flavus was encountered in 41.2% of fig fruits studied, with an average population level (CFU gm-1 DWt.) of 36.5, comprising 3.1% of total external mycobiota populations (1164.9). Internal A. flavus was found in 58.8% of fig fruits, with an average population level (CFU/10 fruits) of 1.9 comprising 2.5% of total internal mycobiota populations (77.3). Seventy-three A. flavus isolates recovered from 51 samples were analyzed for aflatoxin production. Samples that contained at least one aflatoxigenic isolate of A. flavus were also examined for the presence of aflatoxin.

Qualitative and Quantitative Detection of Aflatoxin

Of the 73 isolates of *A. flavus* screened in this survey, 50 (68.5%) were aflatoxigenic (Table 5). Among 30 fig fruits samples containing at least one fluorescent isolate on CAM, 9 (30%) contained aflatoxin. So, the CAM screening method appears to be a good microbiological examination routine to define the risks of aflatoxin presence or absence in samples. Again, a good correlation seemed to exist between the presence of at least one toxigenic isolate and the presence of aflatoxin in the samples.

Thirty samples of fig fruits each with at least one aflatoxigenic A. flavus were analyzed for aflatoxin production using polyclonal antibodies by VICAM. Only 9 samples (30%) were shown to contain AFs (Table 5). Two samples of fresh fig fruits, which showed the only pigmentation without fluorescence on CAM, were found to be devoid of aflatoxin.

The Effect of Storage Period on Aflatoxin Formation was presented in table 5 Out of 20 samples of dried fig fruit incubated at 25° C for 40 days, 17(85.0%) contained fruits which produced external bright–greenish-yellow fluorescence (BGYF) under (365 nm) UV lamp (Table 5), and 7(41.2%) contained

aflatoxin. BGYF is used commercially in Turkey to remove aflatoxin-contaminated figs (Ozay & Alperden, 1991; Sharman et al., 1991). However, in situations where high percentage of contaminated figs only having internal fluorescence, BGYF will probably not be used for the removal of contaminated fig products or as a presumptive test for aflatoxin in figs (Doster & Michailides, 1998). Nonetheless, BGYF will be probably advantageous for reducing aflatoxin contamination during the manufacturing of fig paste (since the fruits are cut in quarters during processing) or other specific conditions.

An orange-yellow pigmentation on CAM medium was seen before the appearance of fluorescence (Lin & Dianese, 1976). But the production of yellow pigments was not a reliable indicator of aflatoxin producing ability (Davis *et al.*, 1987). The results confirm this observation. In fact, two of the tested samples of fresh fruits showed pigmentation only without fluorescent on CAM were found to be devoid of aflatoxin.

Table 5. The occurrence of aflatoxigenic *Aspergillus flavus* isolates and aflatoxins in fig fruits at different states.

	Fruit state*					Total no. of isolates
	a	**b**	**c**	**d**	**e**	
No. of isolates screened/ No. of samples examined	(20/22)	(2/4)	(2/2)	(6/3)	(43/20)	73
No. of isolates showing fluorescence on CAM	14	1	2	4	29	50
No. of samples showing at least one aflatoxigenic isolate	10	1	1	3	15	30
No. of AFs –positive samples	1	1	0	0	7	9
Aflatoxin concentration (µg/kg)	0.41	0.23	0	0	0.0059-0.41	0.0059-0.41

* As in Table 1; Note: 2 samples of fresh figs showing the only pigmentation without fluorescence on CAM were found to be devoid of aflatoxin.

The formation of AFs depends on the food on which the fungi grow and the conditions of heat and humidity during crop growth and storage. Aflatoxins have been found in a wide cultivar of foodstuffs around the world, particularly in countries with climates of high temperature and humidity or where poor storage conditions, which favor the growth of fungi, were used (MAFF, 1996). However, a suitable substrate is required for fungal growth and subsequent toxin production, although the precise factor (s) that initiates toxin formation is not well understood (ICMSF, 1996).

Mycotoxins cannot be produced unless fungal growth occurs. However, the presence of mycotoxigenic fungi in or on a food does not automatically mean the presence of mycotoxins, but that potential for mycotoxin production exists. On the other hand, the absence of toxigenic fungi does not guarantee that the commodity is free of mycotoxins, as the toxins may persist long after fungi have lost viability (CAST, 1989), so good processing and storage of the product would prevent toxigenic fungi growth and aflatoxin biosynthesis.

In this study aflatoxin concentration in aflatoxin positive samples ranged between (0.0059 – 0.4 µg/kg), which is below the safe levels of AFs which lie in the range of 4-30 µg/kg for human consumption. The EU has the strictest permitted level with AFB1 and total AFs not exceeding 2 µg/kg and 4 µg/kg , respectively in any product for direct consumption

(EC, 2010). Likewise, the maximum permitted limit set for AFs in the USA FDA is 20 µg/kg (Wu, 2006). Low levels of aflatoxin contamination were also detected in figs by (Buchanan, Sommer & Fortlage, 1975). Akerstrand and Moller (1989) examined 103 samples of dried figs in Sweden and reported that 53 samples had aflatoxin concentration exceeding 5 µg/kg, with a maximum of 203 µg/kg being detected. Sharman *et al*. (1991) examined samples of dried figs and fig pastes during the periods from November 1988 to January 1989 and from April 1989 to July 1990. they reported that 24% of samples tested in the first period had total aflatoxin concentration above 10 µg/kg (with the highest level being 165 µg/kg) while in the second period 11% of the 112 samples of fig paste and 9% of 93 samples of whole dried figs were contaminated with total aflatoxin concentration above 10 µg/kg (with the highest level being 40 µg/kg).

Physical removal of fluorescent dried figs under UV radiation is a practical and useful way of getting rid of most of the aflatoxin-contaminated figs (Steiner et al., 1988) but this process is not useful for figs contaminated with other mycotoxins. Obstructing fungal contamination in the orchard is probably the most rationalistic method for aflatoxin prevention (Altındişli et al., 1999). It will be possible to overcome mycotoxin problems if the necessary protections are taken.

To reduce the time the figs, spend under conditions favorable for *A. flavus* growth and aflatoxin production, traditional sun-drying can be replaced by solar drying (Gilbert and Senyuva, 2008). Solar drying uses a system that forces sun-heated air at 20 ºC above ambient temperature over the figs contained in a drying chamber (Özay et al., 1995; Ali-Shtayeh and Jamous, 2010). The shorter drying time reduces risks of fungal growth, and the solar-dried product is in fact preferred by consumers in terms of both color and flavor (Gallali et al., 2000).

There is enough evidence to demonstrate that harvesting by hand and solar drying were the most effective approaches for the reduction of mold and yeast contamination (Özay et al., 1995). After solar drying has been completed down to a moisture content of 19-22 % (aw = 0.67-0.73) (Özay and Alperden, 1991) the figs are fumigated against pests, ventilated and then sorted under UV light; any figs showing discoloration or signs of Bright Greenish Yellow (BGY) fluorescence should be removed.

This study showed that the potential hazard associated with aflatoxin in fig fruits has not been serious in Palestine. Even for fresh and dried products, the higher level was (0.4 µg/kg). However, a high incidence of aflatoxigenic *A. flavus* fungi in dried figs if processed or stored improperly could be indicating a potential mycotoxin problem in Palestine.

Conclusions

Aflatoxin contamination in dried figs is a very important problem in terms of human health and safety and can play a major role in the agricultural economy in Palestine. Fig fruits are sensitive to mold contamination and aflatoxin production during the maturation and processing stages; therefore, any kind of physical damage to fruits, and direct contact of fruits with soil must be avoided. Reducing moisture content to 40% (water activity, aw = 0.9) is the first stage of drying, and this stage decreases the risk of mold contamination and aflatoxin production. Optimization of storage conditions is effective in reducing and preventing mold contamination and aflatoxin production.

Elimination of aflatoxins without damaging the fig structure and flavor is very difficult after the toxigenic mold has sporulated and produced mycotoxins. Because mycotoxins are resistant to heat treatment; they cannot be degraded by pasteurization.

More studies on mycotoxins including aflatoxins, ochratoxin A, and patulin contamination and prevention are required in order to predict problems related to public health and safety and agricultural economy and develop effective prevention methods, since demands for figs in and from Palestine may rise.

Even though the mycotoxin problem with dried figs has been linked with aflatoxins, and lately with ochratoxin A, it has also now become obvious that figs present a good growth medium for other fungi and thus patulin and *Fusarium* toxins should also be a cause for concern in contaminated figs.

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الحياة الفطرية في موائل ثمار التين ومعدل تواجد الفطريات المنتجة للأفلاتوكسين فيها

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تم جمع إحدى وخمسين عينة من ثمار التين (22 عينة تين طازج، 4 تين جمع من تحت الشجر، 2 من المسطاح ، 3 تين مجفف حديثا، و 20 تين جاف "قطين"). جمعت العينات من 7 مواقع من منطقتي رام الله و نابلس وذلك في الفترة ما بين آب 2000 _ أيار 2001. تم عزل الفطريات الموجودة على السطح الخارجي للثمار و النامية داخلها للتعرف على توزيع الفطريات ؛ أنواعها وأعدادها باستخدام عدة طرق مرجعية للعزل و الإكثار و الزراعة مع التركيز على عزلات فطر *Aspergillus flavus* المنتجة لسموم الأفلاتوكسين. أظهرت النتائج وجود الفطريات في جميع عينات التين التي تمت دراستها. وتم في هذه الدراسة عزل 21 نوعا من الفطريات تنتمي إلى 14 جنسا من السطح الخارجي للثمار. وكانت الفطريات السائدة تابعة لجنسي *Aspergillus* و *Cladosporium* حيث عزلت من 86.3 % و74.5 % من العينات، على التوالي. أما *A. flavus* فكان يوجد في 41.2 % من العينات. وتم عزل 27 نوعا من الفطريات تنتمي إلى 17 جنسا من داخل الثمار. وكانت الفطريات السائدة هي *Aspergillus spp.* و *Alternaria alternata* حيث وجدت بنسبة 92.2% و 58.8% من العينات، على التوالي. أما فطر *Aspergillus flavus* فكان يوجد في 58.8 % من العينات. وأظهرت النتائج أن أعلى نسبة للتلوث بالفطريات كانت في المراحل الأولى للتجفيف بينما ظهرت اقل نسبة للتلوث في الثمار الجافة (القطين). و قد عزي ذلك إلى انخفاض مستوى الرطوبة الضرورية لنمو الفطر و تطوره في القطين. في حين يكون احتمال التلوث بالفطريات كبيرة في المراحل الأولى و بخاصة أن الفطريات المعزولة تستطيع إنتاج أبواغ جافة تستطيع الانتشار بالهواء و التربة. وأظهرت النتائج أيضا أن الثمار المنتجة في منطقة سلواد أكثر تلوثا بالفطريات. أما بالنسبة لفطر *A. flavus* فكانت أعلى نسبة له في الثمار الجافة (القطين). وفي دراسة تأثير الوسط الغذائي و درجة حرارة التحضين على أعداد وحدات تكاثر الفطريات المعزولة، أظهرت النتائج أن تأثير الوسط الغذائي ليس معنويا ($P > 0.05$) أما تأثير درجة الحرارة فكان معنويا ($P < 0.05$) حيث تبين بأن أفضل درجة حرارة تحضين هي 25 درجة مئوية لجميع الفطريات مقابل 35 درجة مئوية لفطر *Aspergillus flavus* وكان الوسط الغذائي (CzA) هو أفضل وسط لعزل هذا الفطر. وقد بلغ عدد عزلات *A. flavus* المعزولة 73 عزلة. وقد أظهر الفحص اختبار أل (Flourescence) للعزلات النامية على أجار جوز الهند (CAM) أن 50 من هذه العزلات منتجة للأفلاتوكسين. وظهر من دراسة العينات ال 30 التي عزل منها أل *A. flavus* المنتج للأفلاتوكسين، بوساطة الأجسام المضادة المتخصصة (Polyclonal antibodies) (باستخدام جهازفحص الافلاتوكسين أل (VICAM) أن 9 عينات كانت ملوثة بالأفلاتوكسين. وبلغ تركيز الأفلاتوكسين فيها نحو $\leq$ 0.41 جزء من بليون. مع العلم بأن هذا التركيز أقل من مستوى المعايير و المواصفات العالمية ($\leq$20 جزء من بليون). وكان 7 من هذه العينات من القطين.

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الصفحات 1–16

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