Available online at http://www.sjbas.com.ng
Savanna Journal of Basic and Applied Sciences (June, 2025), 7(1): 71-78
P:ISSN 2705-3164 | E:ISSN 2695-2335

Assessment of Heavy Metals and Mycotoxins in Pepper (*Capsicum chinense)* and Date Fruits (*Phoenix dactylifera*) Sold Along Traffic Congested Areas in Ilorin

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ABSTRACT

Pepper (*Capsicum spp.*) and date fruit (*Phoenix dactylifera*) are widely consumed for their flavour-enhancing properties and nutritional benefits. However, their potential contamination with heavy metals and mycotoxins due to environmental exposure raises safety concerns. This study evaluated the concentrations of lead (Pb), cadmium (Cd), chromium (Cr), and arsenic (As) in eight pepper and date fruit samples collected from traffic-congested areas in Ilorin, North Central Nigeria. The samples were digested using wet acid digestion and analysed using Atomic Absorption Spectrophotometry (AAS). Aflatoxin contamination in exposed pepper (EP), exposed date (ED) and refrigerated pepper (RP), and refrigerated date (RD) samples was extracted using standard methods and determined by High-Performance Liquid Chromatography (HPLC). Results showed that Pb concentrations in pepper and date fruits ranged from 13.4–36.4 mg/kg and 8.5–42.8 mg/kg, respectively, while Cd levels varied from 2.3–21.0 mg/kg in pepper and 3.2–35.8 mg/kg in date fruits. As concentrations ranged from 0.5–22.6 mg/kg in pepper and 1.1–18.9 mg/kg in date fruits, whereas Cr levels were between 9.1–53.5 mg/kg and 16.9–45.9 mg/kg, respectively. While some values exceeded FAO/WHO limits, others remained within permissible levels. Aflatoxin analysis detected no aflatoxins in date samples, while aflatoxin G2 (1.093 ng/g) was found in EP within EU regulatory limits (10.0 ng/g). Fungal analysis identified *Fusarium oxysporum* as the dominant contaminant of 5 %. These findings highlight potential health risks associated with metal contamination and the presence of fungi in the samples, underscoring the need for regular monitoring and stringent food safety regulations to protect consumer health and ensure compliance with international standards.

Keywords: Mycotoxins, Heavy metals, Spices, Fruits, Congested Areas

INTRODUCTION

Mycotoxins and heavy metals are major contaminants in foods, presenting significant public health concerns. Mycotoxins, produced by certain molds, contaminate crops and food products, leading to toxic effects in humans and animals (Smith *et al.*, 1985). Common mycotoxins include *aflatoxins, ochratoxins, fumonisins, and trichothecenes,* which are known for their carcinogenic, hepatotoxic, nephrotoxic, and immunosuppressive effects. Mycotoxins are toxic secondary metabolites that fungi such as Aspergillus, Fusarium, and Penicillium species produce. These toxins contaminate many food products, including cereals, nuts, fruits, and processed foods. Aflatoxins, particularly aflatoxin B1, are among the most potent carcinogens known (Williams *et al.,* 2004). Aflatoxins are toxic metabolites produced by certain fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*. These compounds are known for their carcinogenic potential, especially aflatoxin B1, which is the most potent. The presence of aflatoxins in foods, spices, and fruits poses significant health risks, including liver cancer. Consequently, regulatory agenciesthe presence of aflatoxin in various food commodities to protect public health. The presence of these metabolites in food, such as in pepper and dates, can affect international trade and cause significant economic burdens.

Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are environmental pollutants that can enter the food chain through soil, water, and air contamination (Patrick, 2006). These metals accumulate in the body over time, causing various health issues, including neurological damage, kidney dysfunction, and increased cancer risk.

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Pepper (*Capsicum chinense spp*.) is a widely consumed spice known for its flavour-enhancing properties and health benefits. It is rich in vitamins A and C, antioxidants, and capsaicin, which is linked to various therapeutic effects (Bhandari *et al.,* 2020). Spices, including pepper, play a crucial role in culinary traditions and have significant economic importance globally (Khan *et al.,* 2021). Pepper, as an aromatic substance derived from plants, is used to enhance flavour, aroma, and colour in food (Khan *et al.,* 2021). The consumption of spices, including pepper, is associated with numerous health benefits, such as antioxidant and anti-inflammatory effects (Bhandari *et al.,* 2020). However, the cultivation and sale of these spices can be impacted by environmental factors, particularly in urban areas where pollution is prevalent.

Traffic congestion in cities like Ilorin contributes to the accumulation of heavy metals and mycotoxins in food products due to vehicular emissions and improper waste disposal (Olayemi *et al.,* 2022). The assessment of heavy metals and mycotoxins in pepper sold in these areas is essential, as these contaminants pose serious health risks, including neurotoxicity and carcinogenic effects (Mokhtar *et al.,* 2021). Understanding the levels of these harmful substances in pepper can inform public health policies and promote safer consumption practices. Thus, the objective of this study was to assess the level of AFs contamination in pepper and data samples collected randomly from different locations in selected markets in the Ilorin metropolis.

MATERIALS AND METHODS

Sample Collection and Preparation

Pepper and date fruit samples were collected randomly from eight different traffic-congested areas in Ilorin (Table 1), into polyethylene bags. The locations (Table 1) include Amilegbe, Ipata, Maraba, Ojagboro, Post Office, A Division, Gambari, and Oja Oba accordingly. The samples were sun-dried for four weeks to reduce moisture content and ground using an industrial blender into a powdered form.

Table 1: Sample Location coordinates

Location	Longitude	Latitude
Gambari	8.5333^0N	4.5833^0 E
Amilegbe	8.5167^0N	4.5333^0 E
A-Division	8.4833^0N	4.5167^0 E
Post-Office	8.4667^0N	4.4833^0 E
Ojaoba	8.5167^0N	4.5667^0 E
Ipata	8.4833^0N	4.5333^0 E
Maraba	8.45^0N	4.5167^0 E
Ojagboro	8.4667^0N	8.4667^0N

Heavy Metals Analysis

The samples were processed for elemental composition analysis using Atomic Absorption Spectroscopy after an acid digestion preparation procedure. The digestion procedure- 10 mL HCl, HNO_3, and $HCLO_4$ sequentially added to the samples, heated to dryness on a digestion block, was adapted from Anton Pear's multiwave 3000 microwave digestion method. The digestates were analysed for As, Cd, Cr, and Pb by atomic absorption spectroscopy.

Microbial Analysis

The exposed and refrigerated pepper and date fruits were transferred to the laboratory for microbiological investigations. A comprehensive microbiological analysis was conducted to assess the presence of various microorganisms. The total bacterial count was determined using the pour-plate method on plate count agar, as recommended by the Bacteriological Analytical Manual. Coliform bacteria and *Escherichia coli* (E. coli) detection was performed using the three-tube most probable number (MPN) method (BAM, 2001). Positive results were confirmed through additional testing on eosin methylene blue (EMB) agar. Yeast and mold growth were assessed using the spread plate technique on potato dextrose agar. *Staphylococcus aureus* detection was accomplished through the use of a Baird-Parker agar medium. Characteristic colonies were then subjected to coagulase and mannitol fermentation tests for confirmation (BAM, 2002).

Aflatoxins Quantification Procedure

Dates and pepper samples (refrigerated and exposed) randomly pulverized from different locations in the open markets in Ilorin were extracted for Aflatoxin (Afs)-producing fungi using methanol and water (80:20, v/v). These extract samples were then

cleaned up using an immunoaffinity column (IAC) and determined with a high-performance liquid chromatography-fluorescence detector (HPLC-FLD).

Twenty-five (25 g) of the homogenized sample was weighed, followed by the addition of 5 g of sodium chloride to the sample. 150 mL of methanol-water mixture was added in a ratio of 70:30 to the sample, then the mixture was shaken for 30 minutes at 175 RPM using an orbital shaker. The mixture was filtered using pre-folded filter paper. 70 mL of phosphate-buffered saline (PBS) was added to 10 mL of the filtrate, and an immune-affinity clean-up column was used to clean up 50 mL of the mixture, after which the column was washed with 20 mL of PBS. The mixture was eluted with 1.5 mL of HPLC methanol grade into vials, then the eluate was diluted with 1.5 mL of deionized water, and then the mixture was shaken for 2 minutes on a vortex mixer. The prepared sample was then injected into the High-Performance Liquid Chromatography (HPLC) system. Figure 4 shows the flow chart of HPLC determination of total Aflatoxins using pre-column derivatization (AOAC International, 2019).

Analytical Validation

After the calibration curve was determined, aflatoxin determination was validated by evaluating its linearity, regression coefficient (R^2), LOD, LOQ, and recovery (Table 2). The values of the regression coefficient (R^2) were > 0.970 for all analytes, indicating that the data were fitted to the regression line. In this study, the LODs for AFB1, AFG1, AFB2, and AFG2 were 0.00514, 0.01934, 0.00079, and 0.00078, respectively. The recoveries of AFB1, AFG1, AFB2, and AFG2 were found to be 98.9 ± 1.50 %, 92.30 ± 1.52 %, 97.80 ± 2.31%, and 98.70 ± 1.52%, respectively. The limit of quantification is the lowest concentration of the measurand that can be determined with an acceptable level of repeatability, precision, and trueness. The limit of quantification (LOQ) is "the lowest concentration at which the performance of a method or measurement system is acceptable for a specified use."

Table 2: Method validation for the determination of AFB1, AFG1, AFB2, and AFG2 in dates and peppers (n = 4).

Aflatoxin	Aflatoxin Concentration range (ppb)	R^2	LOD	LOQ	Recovery (%)
AFB1		0.98760	0.00514		98.89+1.50
AFB2		0.98753	0.00079		92.30
AFG1		0.99884	0.01934		97.80
AFG2		0.99719	0.00078		98.70

RESULTS AND DISCUSSION

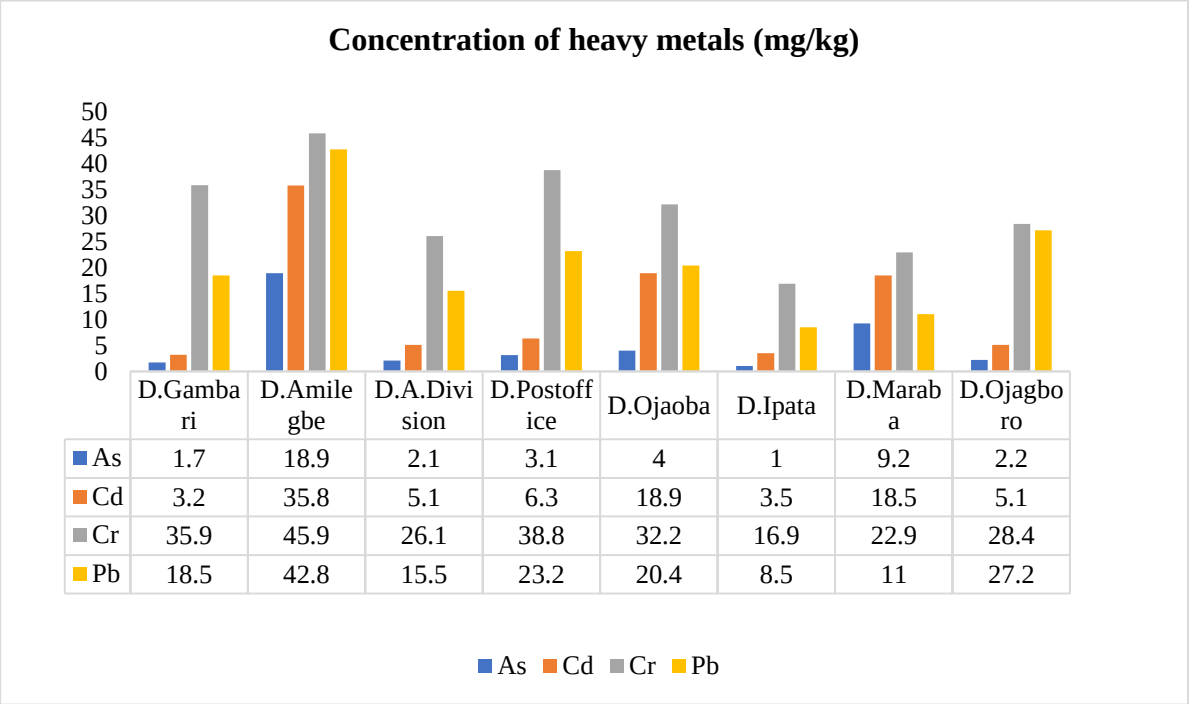
Table 3 presents the concentrations of several heavy metals (mg/Kg) found in dates sold in eight traffic-congested areas of Ilorin, Kwara State, Nigeria. All eight date samples were analysed for arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb). The highest concentrations identified were Pb (42.8 mg/kg) in sample D Amilegbe, Cd (35.8 mg/kg) in the same sample, Cr (45.9 mg/kg) also in sample D Amilegbe, and As (18.9 mg/kg) in the same sample. The concentration of Pb across all eight samples ranged from 8.5 mg/kg to 42.8 mg/kg. Lead has been shown to adversely affect various metabolic processes essential for plant growth and development, including photosynthesis, transpiration, DNA synthesis, and mitotic activity (Kimani *et al*., 2011). The levels of Pb found in the date samples were significantly higher than the permissible limit of 30 mg/kg for fruits set by the FAO/WHO (FAO/WHO, 2003). The concentration of cadmium in all samples ranged from 3.2 mg/kg to 35.8 mg/kg, which poses a potential health risk to consumers. Arsenic levels ranged from 1.0 mg/kg to 18.9 mg/kg in the samples, surpassing the WHO's maximum permissible limit of 1 mg/kg for fruits (Baig *et al.*, 2019). Additionally, chromium concentrations varied from 16.9 mg/kg to 45.9 mg/kg across the samples, with the WHO's acceptable standard being 2.30 mg/100g (Mundi *et al*., 2019). Statistical analysis revealed significant differences in the concentrations of selected heavy metals among the analysed Date samples. Heavy metals in the Date samples were recorded in the order of Pb >Cr >Cd> As.

Table 3: Concentration of heavy metals (mg/kg ± SD) in different date samples collected.

Sample Code	**As**	**Cd**	**Cr**	**Pb**
D Gambari	1.7±0.001	3.2±0.001	35.9±0.004	18.5±0.004
D Amilegbe	18.9±0.001	35.8±0.003	45.9±0.040	42.8±0.003

D A-Division	2.1±0.001	5.1±0.001	26.1±0.001	15.5±0.004
D Post Office	3.1±0.002	6.3±0.002	38.8±0.006	23.2±0.001
D Ojaoba	4.0±0.000	18.9±0.004	32.2±0.001	20.4±0.003
D Ipata	1.0±0.001	3.2±0.003	16.9±0.004	8.5±0.001
D Maraba	9.2±0.001	18.5±0.004	22.9±0.004	11.0±0.007
D Ojagboro	2.2±0.001	5.1±0.002	28.4±0.003	27.2±0.006

Where D is the date sample from different locations.



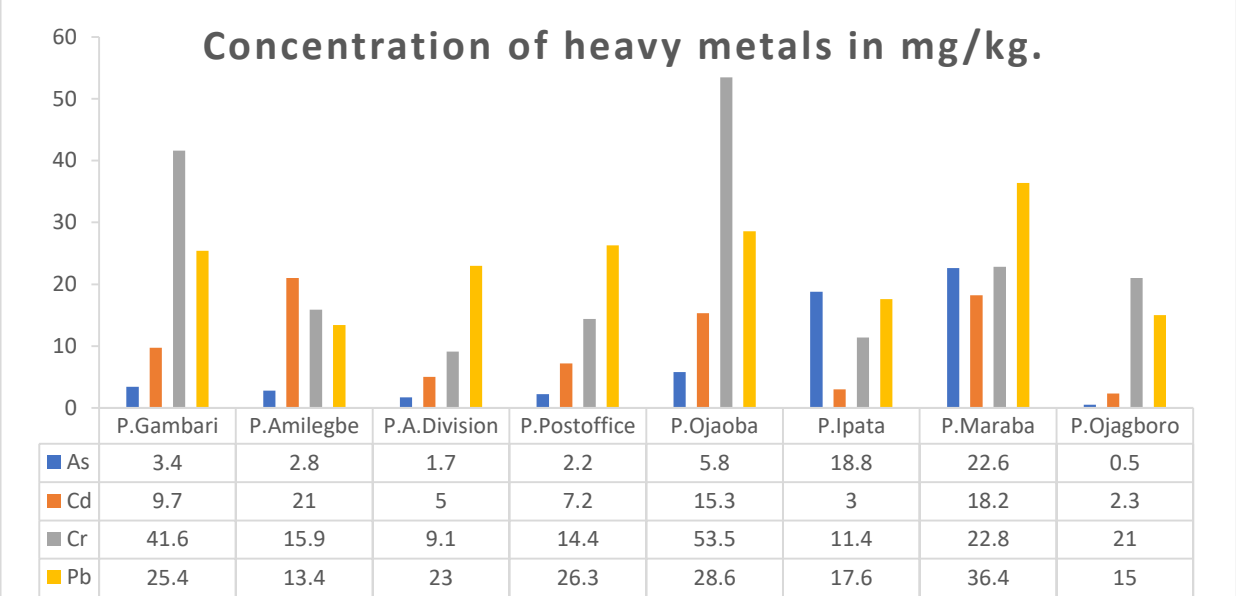
	D.Gambari	D.Amilegbe	D.A.Division	D.Postoffice	D.Ojaoba	D.Ipata	D.Maraba	D.Ojagboro
As	1.7	18.9	2.1	3.1	4	1	9.2	2.2
Cd	3.2	35.8	5.1	6.3	18.9	3.5	18.5	5.1
Cr	35.9	45.9	26.1	38.8	32.2	16.9	22.9	28.4
Pb	18.5	42.8	15.5	23.2	20.4	8.5	11	27.2

Figure 1: Concentration of heavy metals (mg/kg)

Table 4: Concentration of heavy metals (mg/kg ± SD) in different pepper samples collected.

Sample Code	As	Cd	Cr	Pb
P Gambari	3.4±0.001	9.7±0.001	41.6±0.003	25.4±0.004
P Amilegbe	2.8±0.001	21.0±0.039	15.9±0.004	13.4±0.003
P A-Division	1.7±0.001	5.0±0.007	9.1±0.004	23.0±0.003
P Post Office	2.2±0.001	7.2±0.003	14.4±0.003	26.3±0.002
P Ojaoba	5.8±0.004	15.3±0.001	53.5±0.004	28.6±0.004
P Ipata	18.8±0.006	3.0±0.001	11.4±0.004	17.6±0.003
P Maraba	22.6±0.004	18.2±0.004	22.8±0.006	36.4±0.001
P Ojagboro	0.5±0.001	2.3±0.002	21.0±0.004	15.0±0.006

P – Pepper samples from different locations.



	P.Gambari	P.Amilegbe	P.A.Division	P.Postoffice	P.Ojaoba	P.Ipata	P.Maraba	P.Ojagboro
As	3.4	2.8	1.7	2.2	5.8	18.8	22.6	0.5
Cd	9.7	21	5	7.2	15.3	3	18.2	2.3
Cr	41.6	15.9	9.1	14.4	53.5	11.4	22.8	21
Pb	25.4	13.4	23	26.3	28.6	17.6	36.4	15

Figure 2: Concentration of heavy metals detected in pepper

Table 4 shows the concentration of some heavy metals (mg/Kg) in pepper sold in 8 traffic-congested areas in Ilorin, Kwara State, Nigeria. All 8 pepper samples were analyzed for As, Cd, Cr, and Pb. The highest concentrations obtained for Pb (36.4 mg/kg) P. Maraba, Cd (21.0 mg/kg) P. Amilegbe, Cr (53.5 mg/kg) P. Ojaoba, and As (22.6 mg/kg) P. Maraba, respectively. Statistical analysis revealed significant differences in the concentrations of selected heavy metals among the analyzed pepper samples. The concentration of Pb ranged from 13.4 mg/kg - 36.4 mg/kg for the eight samples under consideration. Pb has been shown to have a toxic impact on a variety of metabolic processes essential to plant growth and development, including photosynthesis, transpiration, DNA synthesis, and mitotic activity (Kimani et al., 2011). The level of Pb in the pepper samples was very high when compared to the permissible Limit required in spices 10 mg/kg) (Baig *et al.,* 2019). The concentration of Cd in all eight samples varied from 2.3 mg/kg - 21.0 mg/kg. This level tends to pose health risks to the consumer. The concentration of As ranged from 0.5 mg/kg to 22.6 mg/kg for the samples. The MPL of As in spices proposed by WHO was 1 mg/kg (Baig et al., 2019). The concentration of Cr ranged from 9.1 mg/kg to 53.5mg/kg in all the samples. The Cr acceptable standard by WHO 2.3 mg/Kg (Mundi et al., 2019).

The WHO recommended standards for heavy metals in dried spices are Pb (10 mg/kg), As (0.2 mg/kg), Cr (0.5 mg/kg), and Cd (0.5 mg/kg).
Table 5 shows the mean of all the fungi isolated from the samples, with the highest of 2.02 ± 0.20 in sample R.D,. Although not significantly different in samples, E.D.

Table 5: Mean of Isolated Fungi from the samples

Sample	Mean± S.E
R.D	2.02±0.20
E.D	2.01±0.00

R.D = Refrigerated Date, E.D = Exposed Date.

Table 6: Mean of Isolated Fungi from the pepper samples

Sample	**Mean ± S.D**
R.P	3.50±0.05
E.P	2.50±0.01

R.P. – Refrigerated Pepper, E.P.—Exposed Pepper.

Table 6 shows that the mean of all the fungi isolated from the samples is the highest (3.5 ± 0.05) in sample R.P, although this is not significantly different in samples E.P (2.50 ± 0.01).

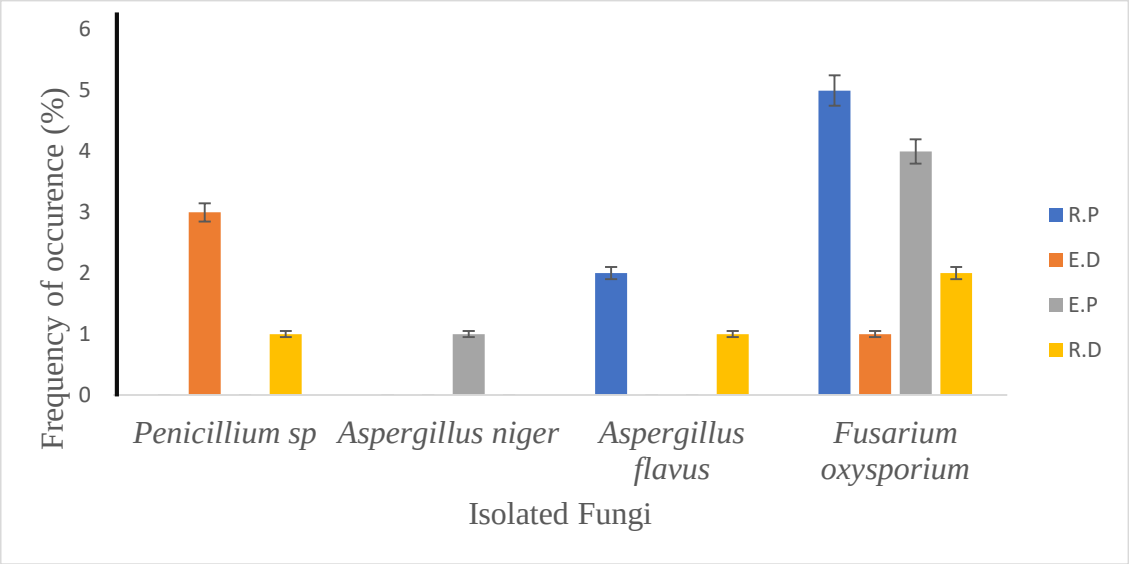
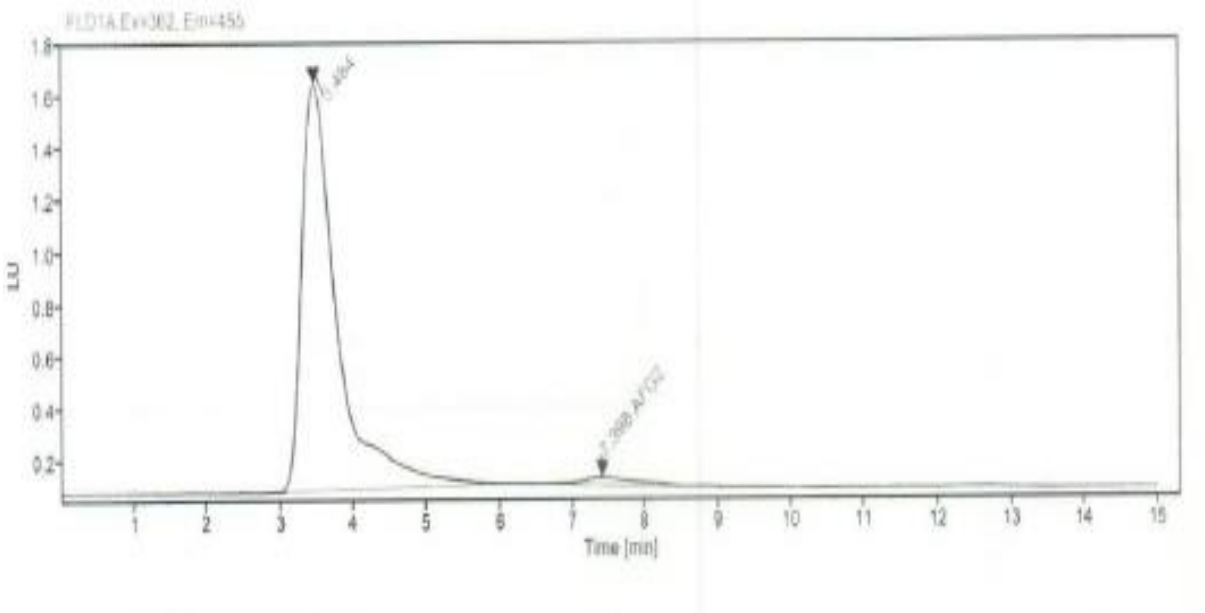


Figure 3: Frequency of Isolated Fungi from all the samples

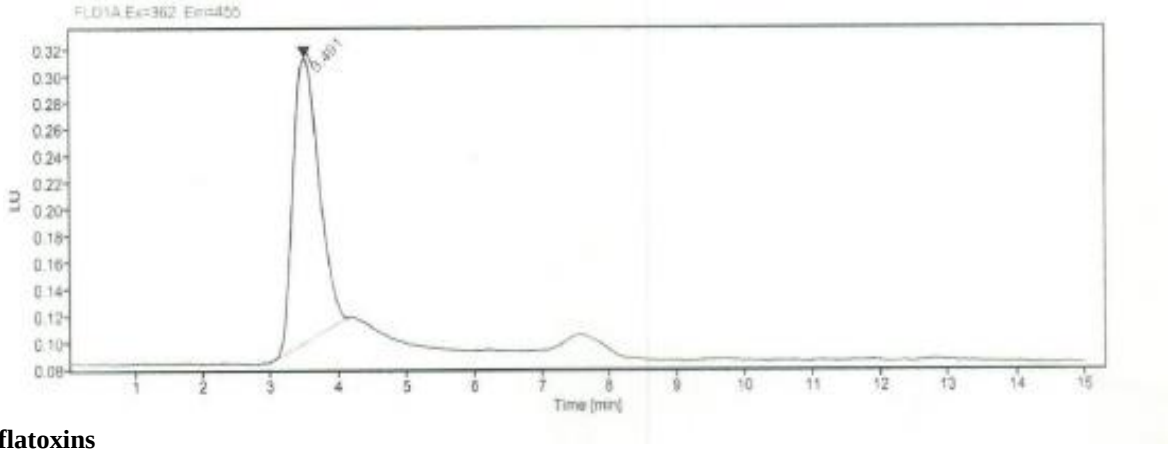
Figure 3 shows that four fungi, namely *Penicillium Sp, Aspergillus Niger, Aspergillus flavus* and *Fusarium oxysporium*, were isolated from all the samples analyzed. it was also observed that *Fusarium oxysporium* has the highest frequency of occurrence (5) in sample R.P., although not significantly different from sample E.P., but was significantly different from samples E.D. and R.D. *Penicillium Sp.* was also high in sample E.D. compare to other samples; however, *Aspergillus niger* and *Aspergillus flavus*, which were likely to secrete aflatoxin, are low in most of the samples or not even present at all.

Figure 4: Chromatogram for Exposed (a) Pepper and (b) Date Fruits

(a)



b)



Aflatoxins

The presence of aflatoxin G2 in EP at a level of 1.093 ng/g is significantly below the EU's maximum allowable limit for total aflatoxins in spices (10.0 ng/g). This finding suggests that the pepper sample is well within the safety threshold set by the EU, implying it is safe for consumption based on the EU standards. The results further revealed that the detected level of aflatoxin G2 in the pepper sample is compliant with the EU regulations, indicating that the sample is safe for consumer use regarding aflatoxin contamination. The finding showed that in all the data samples (RD and ED) analyzed, AFB_1, AFB_2, AFG_1, and AFG_2 were not detected at all. Based on the EU regulatory strict limits set on aflatoxin levels in food products, Figure 4 Chromatogram for Refrigerated Pepper and date fruits maximum limits are (Aflatoxin B1: 5.0 ng/g) and (Total aflatoxins (B1 + B2 + G1 + G2): 10.0 ng/g). The non-detection of aflatoxins B1, B2, and G1, G2 in the date sample is a positive outcome, particularly the absence of aflatoxin B1 in all the samples, which is the most toxic and carcinogenic of the aflatoxins, which indicates that the date sample is free from the most harmful aflatoxins, potentially reducing the health risks associated with its consumption.

This compliance is crucial for both consumer safety and for meeting international trade standards, particularly for exports to countries with stringent aflatoxin regulations like those in the EU. Based on the quality control and monitoring perspective, although the levels of aflatoxin G2 are low, the detection still warrants continued monitoring and quality control measures. This is because the presence of any aflatoxin can indicate potential contamination sources, such as improper storage or handling, which may need to be addressed to prevent future contamination. While aflatoxin G2 levels are below the EU threshold, it is important to consider the holistic approach to food safety. Other contaminants or toxins might be present, and therefore, comprehensive testing and adherence to good agricultural and manufacturing practices are essential to ensure overall food safety. Based on this result, a consumer awareness strategy involving informing consumers about the results and the safety of the pepper sample based on international standards can help build trust and ensure transparency in food safety measures.

Conclusion

The results generally revealed the level of contamination by heavy metals in pepper and date fruit samples as very high in some areas when compared to the permissible limits stipulated by FAO/WHO. (2003), and can cause health risks, while some are still under the permissible limit. However, the high level of heavy metals discovered can be attributed to vehicular emissions, as samples are displayed along major routes. Pepper samples show no appreciable levels of aflatoxins B1, B2, and G1, but a level of aflatoxin G2 (1.093 ng/g), while date fruits show no detectable level of aflatoxins. This demonstrates compliance with the EU's recommended values for aflatoxins in spices and fruits.

Conflict of Interest: The authors declared that there is no conflict of interest.

Authors' Contributions: ODT conceptualization, investigation, methodology, IDT and OBT data curator, draft preparation, and editing. ABL

experimental, writing review. All authors approved the manuscript in the present state and approved it for submission.

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