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Evaluation of aflatoxin B1 (AFB1) contamination levels in corn and corn-growing soils from the leading corn-producing districts of Sri Lanka

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Abstract

Aim: Mycotoxins are the third most dangerous food contaminants, with one billion metric tons of food being contaminated annually. This study was conducted as a comprehensive assessment of aflatoxin B1 (AFB1) contamination in corn kernels and corn-growing soils across the six main corn-producing districts of Sri Lanka.

Methods: A total of 12 soil samples were collected from the front, middle, and rear regions of each field from the subsurface and at various depths. In addition, six healthy corn kernel samples were harvested from the same locations. AFB1 was detected using enzyme-linked immunosorbent assay (ELISA). To verify the accuracy and precision of the assay, a recovery evaluation was conducted. To assess the distribution and correlations of AFB1 concentration in maize, its growing soil, and other environmental parameters, a comprehensive statistical study was conducted.

Results: AFB1 level patterns implied that environmental factors influence the variability across the six districts. The temperature significantly affected AFB1 contamination in corn kernels with a p-value of 0.00014 ($p < 0.05$). Corn AFB1 levels showed a significant correlation with AFB1 levels in corn growing soils, with a p-value of 0.0261 ($p < 0.05$). Moreover, maximum AFB1 contamination was recorded at temperatures ranging from 26°C to 30°C.

Conclusions: This study reveals a concerning trend; most of the corn samples from these districts exceeded the regulatory AFB1 levels set by the United States Food and Drug Administration (US FDA), and a significant positive correlation of corn AFB1 with soil AFB1 highlights soil as a potential reservoir for AFB1-producing fungi. Moreover, linking environmental elements to AFB1 data might encourage adaptive management strategies, which may help reduce contamination.

Keywords

aflatoxin (AFB1), maize (corn), soil contamination, Sri Lanka

Introduction

Mycotoxins represent a significant risk to global food safety and security. Each year, one billion metric tons of food products are contaminated, ranking mycotoxins as the third-highest hazardous category reported in the Rapid Alert System for Food and Feed of the European Union [1, 2]. The United Nations Food and Agriculture Organization states that 25% of crops produced worldwide are contaminated and wasted annually due to mycotoxins [3]. These mycotoxins include trichothecenes, fumonisins, ochratoxins, and aflatoxins [4], which are widely recognized for their harmful effects on humans and animals [5, 6], posing a serious threat to public health, agricultural productivity, and food safety worldwide.

Corn is recognized as one of the most susceptible crops to mycotoxins and contamination from mycotoxigenic fungi [7, 8]. Additionally, *Aspergillus* spp. prefer polysaccharides as its main substrate [9]. The high carbohydrate content in corn provides a secure environment for the fungus to thrive. Furthermore, its open husk structure and mechanical injuries may provide direct access to fungal spores. Among all mycotoxins, aflatoxins pose the greatest health risks due to their hepatotoxic and carcinogenic properties [10, 11]. Primarily, aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* found on cash crops, maize, and peanuts, particularly in warm and humid climates [12]. The principal aflatoxin types include B1, B2, G1, and G2, commonly designated as aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2. Among these, AFB1 is regarded as the most toxic and highly carcinogenic [13]. In addition to corn, aflatoxin contamination is also frequently reported in cereals [14]. Aflatoxin production in corn begins when the corn kernels in the field are infected, particularly by *Aspergillus* spp. [15, 16]. *Aspergillus* spp. thrives in warm and humid environments, and Sri Lanka is an ideal location with warm, tropical, humid weather, which is favorable for growing and spreading aflatoxin contamination rapidly [17]. Moreover, Molnár et al. [18] demonstrated that elevated temperatures and drought conditions promote *Aspergillus* infection in maize kernels, particularly when exposure occurs during the silking stage.

The United States Food and Drug Administration (US FDA) has established a maximum limit of 20 parts per billion (ppb) for total aflatoxins in food [1]. Therefore, the accurate detection and quantification of AFB1 are essential for ensuring food safety and mitigating its impact on communities, including its monitoring in maize and associated soil samples. A range of analytical techniques has been employed to detect aflatoxin contamination, each with distinct advantages and limitations, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay (ELISA) [15]. Among these, ELISA is particularly well-suited due to its rapid and routine application in aflatoxin detection [19].

Corn is one of the major staple foods worldwide, especially in Sri Lanka, where it is regarded as the second most produced crop and is utilized for both human consumption and animal feed. Since 2007, the annual corn production in Sri Lanka has rapidly increased due to private sector involvement in cultivation [16]. According to the US Department of Agriculture, Sri Lanka comprises five major corn-growing districts: Ampara, Anuradhapura, Badulla, Monaragala, and Kurunegala (Figure 1). In 2022, these districts accounted for approximately 80% of the total corn production in Sri Lanka, with Anuradhapura contributing 33%, followed by Ampara (17%), Monaragala (16%), Badulla (11%), and Kurunegala (4%) [8]. Additionally, these districts belong to different agro-ecological regions due to variations in rainfall, temperature, and altitude [20]. A single study focused on corn in Sri Lanka, conducted in one district, found that corn kernel samples exhibited lower AFB1 levels compared to the soils [14]. However, as the study was confined to a single district, it provides valuable localized insights while highlighting the need for broader investigations to better understand AFB1 contamination in maize and associated soils across Sri Lanka. Most studies indicate that AFB1 contamination in maize is a multifaceted issue influenced by both environmental and agronomic factors. Building on identified gaps in the literature, the present study undertook a comprehensive evaluation of AFB1 contamination in maize kernels and their associated soils across Sri Lanka, with a focus on the country's principal maize-producing districts. Accordingly, the study was designed to test the overarching hypothesis that AFB1 contamination in maize and its growing soils in Sri Lanka is not random but is significantly shaped by the interaction of environmental conditions (e.g., temperature and soil pH) and the role of soil as a reservoir for aflatoxigenic fungi. To address this, a multi-district survey was conducted across major maize-producing regions, incorporating systematic sampling of both soil and kernel samples, alongside robust nonparametric statistical analyses to evaluate the relationships between these factors and AFB1 contamination.

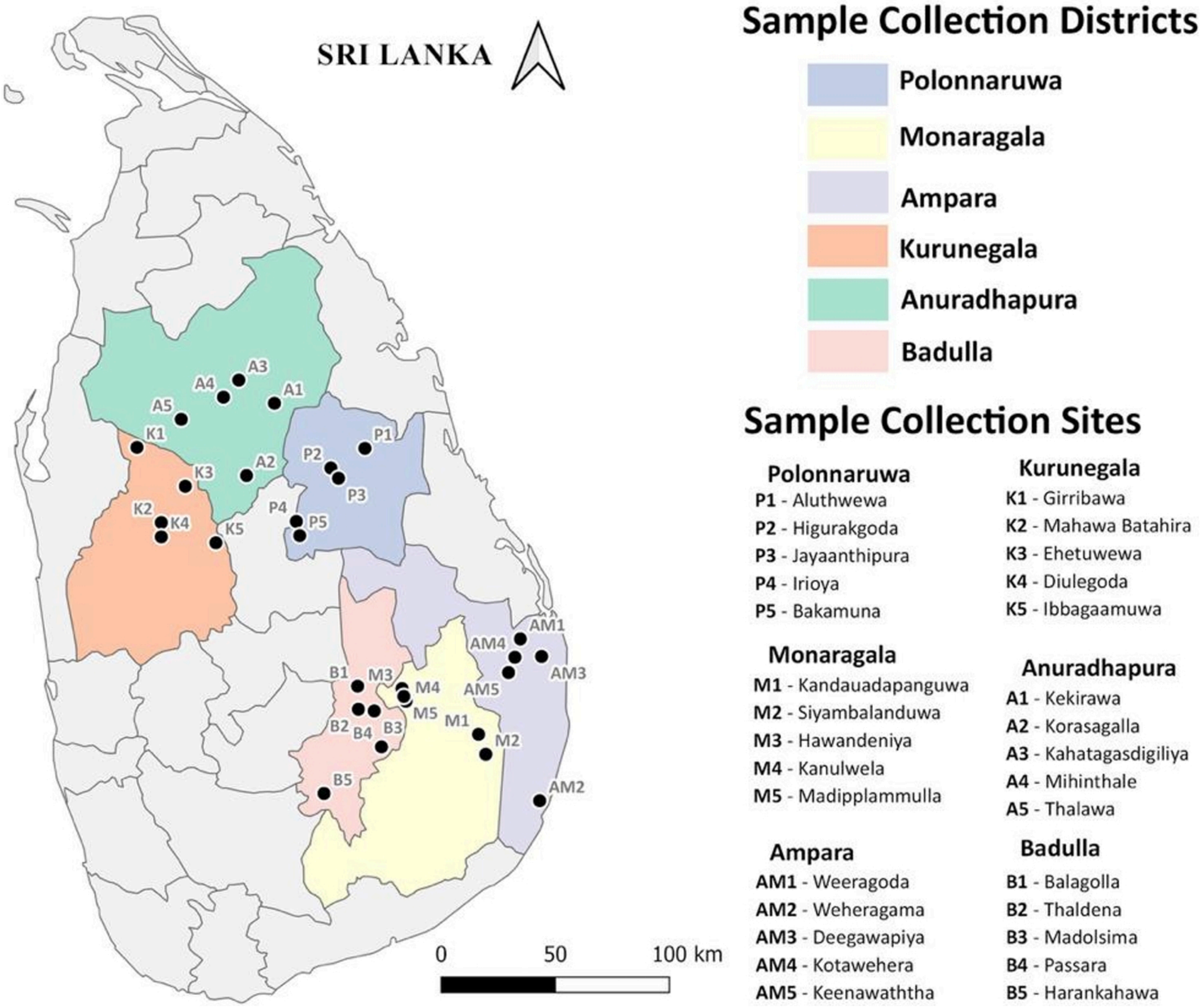

Figure 1. Sampling locations of major corn-producing districts in Sri Lanka. In addition to the five officially recognized high-production districts, Polonnaruwa was included based on its considerable maize production and its representation of agro-ecological conditions within the North Central Province. Map created using QGIS version 3.34.15.

Materials and methods

Sample collection

Six principal maize-producing districts in Sri Lanka were selected as study sites—Polonnaruwa together with five other major maize-growing districts (Figure 1)—with Anuradhapura and Polonnaruwa representing the North Central Province. Polonnaruwa was included due to its substantial maize yield and its location within the North Central Province alongside Anuradhapura, allowing for broader regional representation of agro-ecological conditions. In each district, five corn-growing fields (each over one hectare) were selected. At each sampling field, three corn samples were collected, with two biological replications per sample from the front, middle, and rear of the field. For soil, six samples were collected from three locations (front, middle, and rear) within each field, comprising three subsurface soil (5–10 cm) and three surface soil samples (0–5 cm), each with two biological replications. Total soil samples (n = 360) and corn kernel samples (n = 180) were collected during harvest from the same sites as the soil samples to maintain data consistency across soil and crop evaluations. Only healthy kernels, without visible signs of mold, were chosen for analysis. They were obtained during harvest, when the kernels were already dry, albeit fresh from the field. All sampling activities occurred during harvest periods to ensure consistency across different sites (approximately 500 g per soil sample and 300 g per corn sample). The samples were stored in sterile zip-lock bags, sealed, and kept at 4°C during transport, then maintained at –20°C for 3 to 4 days prior to AFB1 analysis, until extraction methods were established. The air temperature at each sampling location, with three replicate readings within each sampling site, was recorded.

Sample preparation and quantification of AFB1

Soil samples were individually sieved through a 2 mm mesh and air-dried under ambient conditions until a constant weight was achieved. Separately, both the sieved soil and maize kernels were homogenized into a fine powder using a kitchen blender. The corn seed powder and soil samples were measured precisely to 50.00 ± 0.05 g each and transferred into conical flasks. Each sample was mixed with 250 mL of 70% methanol and 10 g of NaCl, thoroughly shaken for 3 minutes, and allowed to settle for 15 minutes. The resulting supernatants were filtered with Whatman No. 1 filter paper, and the extracts were analyzed within 24 hours according to the manufacturer's protocol (Celer AFLA B1) (Cat. nr HU0040004) ELISA kit.

The prepared extracts were utilized to detect AFB1 using ELISA, following the protocol provided in the AFB1 ELISA kit from Gold Standard Diagnostics, Celer AFLA B1. Before analysis, all samples and assay reagents were equilibrated to room temperature (25°C). For ELISA analysis, each biological replicate was further measured in two technical replicates. Absorbance readings were taken at a wavelength of 450 nm, as specified in the manufacturer's instructions, using a Thermo-Scientific Multiskan™ GO microplate reader (Ratastie 2, FI-01620 Vantaa, Finland), with each sample measured in duplicate. The calculated B/B_0 values for each standard and sample facilitated the generation of the standard curve, from which the concentrations of the samples were interpolated.

$$Absorbance\ (\%) = B\,/\,B_0 \times 100\%$$

Where, B: absorbance of standards or samples, B_0: absorbance of zero standard.

AFB1 detection was calculated using the Excel template provided by the manufacturer, available on the Gold Standard Diagnostics website (www.goldstandarddiagnostics.com). The whole experiment was conducted in a completely randomized design with duplicates.

Accuracy and repeatability

In order to verify the accuracy and precision of the assay, a recovery evaluation was conducted in accordance with the specifications outlined in the ELISA kit. These specifications define the limit of quantification (LOQ) as 2 ppb and specify acceptable recovery ranges of 134 ± 19 and 77 ± 7, respectively. For the recovery assessment, representative maize and soil samples previously determined to be 'not detected' for AFB1 by ELISA were selected for spiking experiments. Each sample matrix (5 g) was fortified with AFB1 at three concentration levels (10, 15, and 20 ppb), with two independent replicates analyzed at each level. The resulting data, including mean recovery concentrations, standard deviations, and percentage recoveries, are presented in Table 1.

Table 1. AFB1 recovery results in spiked corn and soil samples (n = 2).

Sample type	Spiked level (ppb)	Mean recovered AFB1 ± SD (ppb)	Recovery (%) ± SD (ppb)
Corn	10	7.394 ± 0.401	73.938 ± 0.401
	15	12.302 ± 2.519	82.016 ± 2.519

Table 1. AFB1 recovery results in spiked corn and soil samples (n = 2). (*continued*)

Sample type	Spiked level (ppb)	Mean recovered AFB1 ± SD (ppb)	Recovery (%) ± SD (ppb)
	20	16.454 ± 1.067	82.268 ± 1.067
Soil	10	8.285 ± 1.172	82.846 ± 1.172
	15	14.858 ± 1.375	99.053 ± 1.375
	20	18.033 ± 1.019	90.167 ± 1.019

AFB1: aflatoxin B1; ppb: parts per billion.

Environmental factor analysis

In this study, key environmental factors were evaluated by measuring daytime air temperature and the pH of soil samples at each sampling site. Three replicates were taken at each sampling site for both temperature and pH. The parameters were obtained in the field using a ventilated thermometer and Thermo-Fisher^{TM} Scientific Eutech^{TM} pH 450 Portable pH Meter (pH 450 Portable Meter Kit, Singapore), following the manufacturer's instructions to ensure accuracy and reliability.

Statistical analysis

A comprehensive statistical analysis was conducted to evaluate the distribution and relationships of AFB1 concentrations in maize, its growing soils, and associated environmental factors. Initial exploratory data analysis employed box plots, control charts, and summary statistics to visualize data patterns. The Shapiro-Wilk test was first performed to assess the normality of the distribution of mean location-based AFB1 concentrations in maize and soil; as the data did not meet normality assumptions, a $Log_{10}(X + 1)$ transformation was applied to both maize and soil mean location AFB1 concentrations to reduce right skewness. Consequently, nonparametric statistical tests were used for subsequent analyses.

Spearman's rank correlation coefficients were computed for $Log_{10}(X + 1)$-transformed AFB1 data, and graphical analyses were performed to examine relationships between transformed mean location AFB1 values in maize kernels and maize-growing soils, as well as environmental parameters such as mean pH and average location temperature recorded in this study. For the analysis of the relationship between $Log_{10}(X + 1)$-transformed mean location maize and soil AFB1, mean soil AFB1 values were obtained by averaging surface and subsurface soil AFB1 concentrations, which were then $Log_{10}(X + 1)$-transformed. All statistical analyses were performed using RStudio (version 2025.09.2+418). All statistical significance was determined at $p < 0.05$.

Results

AFB1 concentrations across all selected districts, encompassing both maize kernel and soil samples, exhibited a range of minimum and maximum values that reflect the overall contamination levels in Sri Lanka (Table 2). The mean AFB1 concentrations in maize samples collected from six districts (n = 30) are presented in Figure 2. Numerous locations showed average AFB1 levels exceeding the safe limit established by the US FDA for corn consumption, which is 20 ppb. Additionally, 13 locations showed corn concentrations below the detection limit, with three for soil (Figure 3).

Table 2. Distribution of aflatoxin B1 (AFB1) concentrations in corn and corn-growing soil samples from six major corn-producing districts in Sri Lanka (ND indicates values below the limit of detection).

Sample type	District	Location	AFB1 min ppb	AFB1 max ppb	AFB1 mean ± SD ppb	Air temperature mean ± SD (°C)	Soil pH mean ± SD
Corn	Ampara	Deegawapiya	ND	ND	ND	30.233 ± 0.115	
		Keenawaththa	1.656	2.205	1.970 ± 0.100	29.866 ± 1.040	
		Kotawehera	ND	ND	ND	36.066 ± 0.737	
		Weeragoda	13.13	26.992	18.850 ± 4.288	32.133 ± 0.757	
		Weheragama	ND	ND	ND	30.966 ± 0.321	

Table 2. Distribution of aflatoxin B1 (AFB1) concentrations in corn and corn-growing soil samples from six major corn-producing districts in Sri Lanka (ND indicates values below the limit of detection). (*continued*)

Sample type	District	Location	AFB1 min ppb	AFB1 max ppb	AFB1 mean ± SD ppb	Air temperature mean ± SD (°C)	Soil pH mean ± SD
	Anuradhapura	Kahatagasdigiliya	2.428	37.348	21.403 ± 11.619	32.900 ± 0.100	
		Kekirawa	10.626	39.811	28.467 ± 5.714	32.666 ± 0.404	
		Korasagalla	ND	ND	ND	33.333 ± 0.416	
		Mihintale	9.719	39.000	21.997 ± 9.38	33.000 ± 0.871	
		Thalawa	ND	6.517	1.560 ± 2.702	32.666 ± 0.152	
	Badulla	Balagolla	ND	ND	ND	31.966 ± 0.057	
		Haldummulla	ND	ND	ND	32.100 ± 0.264	
		Madulsima	ND	ND	ND	31.933 ± 0.115	
		Passara	ND	ND	ND	32.166 ± 0.152	
		Thaldena	ND	ND	ND	31.933 ± 0.208	
	Kurunegala	Diulegoda	7.895	39.000	20.385 ± 10.100	28.633 ± 1.556	
		Ehetuwewa	ND	ND	ND	33.566 ± 0.351	
		Giribawa	13.255	39.000	28.744 ± 13.566	33.100 ± 0.300	
		Ibbagamuwa	ND	ND	ND	23.033 ± 1.556	
		Mahawa batahira	ND	ND	ND	30.200 ± 0.100	
	Monaragala	Hewandeniya	1.656	2.205	1.970 ± 0.100	33.500 ± 1.562	
		Kandaudapanguwa	1.64	3.492	2.373 ± 0.477	32.800 ± 2.081	
		Kanulwela	ND	2.255	1.373 ± 1.204	26.400 ± 0.556	
		Madippalanmulla	1.839	11.141	4.334 ± 2.198	33.066 ± 2.081	
		Siyambalanduwa	1.763	2.618	2.210 ± 0.266	31.800 ± 0.435	
	Polonnaruwa	Aluthwewa	11.636	18.453	13.017 ± 1.808	20.933 ± 0.404	
		Bakamuna	5.081	39.000	19.141 ± 17.222	19.766 ± 0.351	
		Higurakgoda	3.719	39.000	25.737 ± 12.63	22.933 ± 0.550	
		Iriwewa	ND	ND	ND	21.233 ± 0.321	
		Jayanthipura	ND	1.571	0.262 ± 0.454	24.100 ± 0.600	
Soil	Ampara	Deegawapiya	2.828	15.026	5.250 ± 1.584	30.233 ± 0.115	7.533 ± 0.990
		Keenawaththa	4.719	108.006	18.403 ± 17.858	29.866 ± 1.040	10.116 ± 0.640
		Kotawehera	ND	151.349	26.124 ± 35.897	36.066 ± 0.737	9.566 ± 0.840
		Weeragoda	3.770	18.158	8.772 ± 0.749	32.133 ± 0.757	8.883 ± 0.190
		Weheragama	1.258	10.599	3.884 ± 1.181	30.966 ± 0.321	8.250 ± 0.936
	Anuradhapura	Kahatagasdigiliya	2.699	10.650	4.628 ± 1.265	32.900 ± 0.100	6.556 ± 0.051
		Kekirawa	3.284	14.394	5.366 ± 2.443	32.666 ± 0.404	6.583 ± 0.225
		Korasagalla	ND	5.564	4.191 ± 0.675	33.333 ± 0.416	7.363 ± 0.570
		Mihintale	2.749	6.406	4.225 ± 0.788	33.000 ± 0.871	6.373 ± 0.215
		Thalawa	1.667	7.583	4.018 ± 0.481	32.666 ± 0.152	6.216 ± 0.080
	Badulla	Balagolla	ND	3.401	0.859 ± 0.504	31.966 ± 0.057	6.176 ± 0.362

Table 2. Distribution of aflatoxin B1 (AFB1) concentrations in corn and corn-growing soil samples from six major corn-producing districts in Sri Lanka (ND indicates values below the limit of detection). (*continued*)

Sample type	District	Location	AFB1 min ppb	AFB1 max ppb	AFB1 mean ± SD ppb	Air temperature mean ± SD (°C)	Soil pH mean ± SD
		Haldummulla	ND	5.415	1.498 ± 0.864	32.100 ± 0.264	6.720 ± 0.115
		Madulsima	ND	ND	ND	31.933 ± 0.115	5.726 ± 0.140
		Passara	ND	2.745	0.460 ± 0.555	32.166 ± 0.152	7.210 ± 0.565
		Thaldena	ND	1.777	0.341 ± 0.395	31.933 ± 0.208	5.72 ± 0.020
	Kurunegala	Diulegoda	10.125	20.773	13.981 ± 2.062	28.633 ± 1.556	7.226 ± 0.110
		Ehetuwewa	ND	21.209	7.379 ± 5.656	33.566 ± 0.351	7.056 ± 0.430
		Giribawa	6.112	181.810	37.095 ± 43.693	33.100 ± 0.300	6.365 ± 0.289
		Ibbagamuwa	7.927	32.767	14.426 ± 3.914	23.033 ± 1.556	7.270 ± 0.135
		Mahawa batahira	13.670	133.606	40.168 ± 30.912	30.200 ± 0.100	6.493 ± 0.184
	Monaragala	Hewandeniya	2.366	22.974	13.158 ± 3.745	33.500 ± 1.562	5.986 ± 0.277
		Kandaudapanguwa	1.562	28.627	7.506 ± 7.179	32.800 ± 2.081	6.696 ± 0.890
		Kanulwela	1.161	6.020	3.977 ± 1.047	26.400 ± 0.556	7.543 ± 0.320
		Madippalanmulla	2.728	17.804	8.111 ± 7.567	33.066 ± 2.081	6.273 ± 0.279
		Siyambalanduwa	2.695	17.804	6.941 ± 2.535	31.800 ± 0.435	7.596 ± 0.453
	Polonnaruwa	Aluthwewa	11.487	18.142	13.563 ± 0.896	20.933 ± 0.404	6.413 ± 0.175
		Bakamuna	1.429	13.723	9.326 ± 4.178	19.766 ± 0.351	6.440 ± 0.087
		Higurakgoda	7.428	18.492	11.368 ± 0.761	22.933 ± 0.550	6.656 ± 0.297
		Iriwewa	ND	ND	ND	21.233 ± 0.321	7.290 ± 0.272
		Jayanthipura	ND	ND	ND	24.100 ± 0.600	6.483 ± 0.283

ppb: parts per billion.

The lowest mean AFB1 concentration in maize was recorded at Kandaudapanguwa in the Monaragala District (1.64 ppb), while the highest was observed at Kekirawa in the Anuradhapura District (39.811 ppb). For soil samples, the lowest mean concentration occurred at Kanulwewa in the Monaragala District (1.161 ppb), whereas the highest was recorded at Giribawa in the Kurunegala District (181.81 ppb). In comparison, Jayaratne et al. [14] previously reported substantially higher AFB1 levels, with maize kernels from the Anuradhapura District ranging from 60 ppb to 70 ppb and corresponding soils between 350–400 ppb. In contrast, the present study recorded lower levels at Kekirawa, with 7.27 ppb in maize and 80.95 ppb in soil.

The Shapiro-Wilk normality test showed that the data were not normally distributed for both the corn kernel and the corn growing soil. The test indicated non-normality for the corn kernel samples [df = 29, w = 0.69345, $p = 9.315 \times 10^{-7}$ ($p < 0.05$)] and for the soil samples [df = 29, w = 0.78348, $p = 3.326 \times 10^{-5}$ ($p < 0.05$)].

Statistical analysis using Spearman's correlation demonstrated a significant positive association between recorded average air temperature and $Log_{10}(X + 1)$-transformed mean AFB1 concentrations in corn [p-value = 0.00014 ($p < 0.05$); correlation coefficient (r) = 0.5578; degrees of freedom (df) = 28]. In

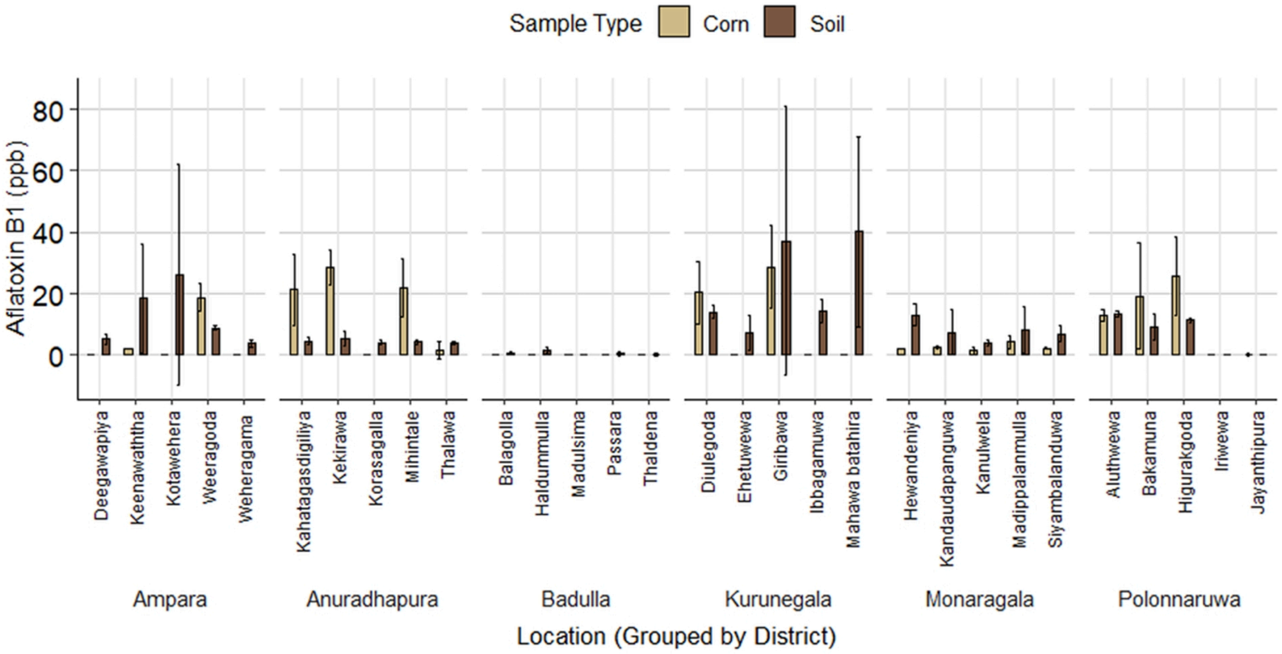

Figure 2. Distribution of AFB1 in corn and soil across the top corn-growing districts (including Polonnaruwa) in Sri Lanka. AFB1: aflatoxin B1.

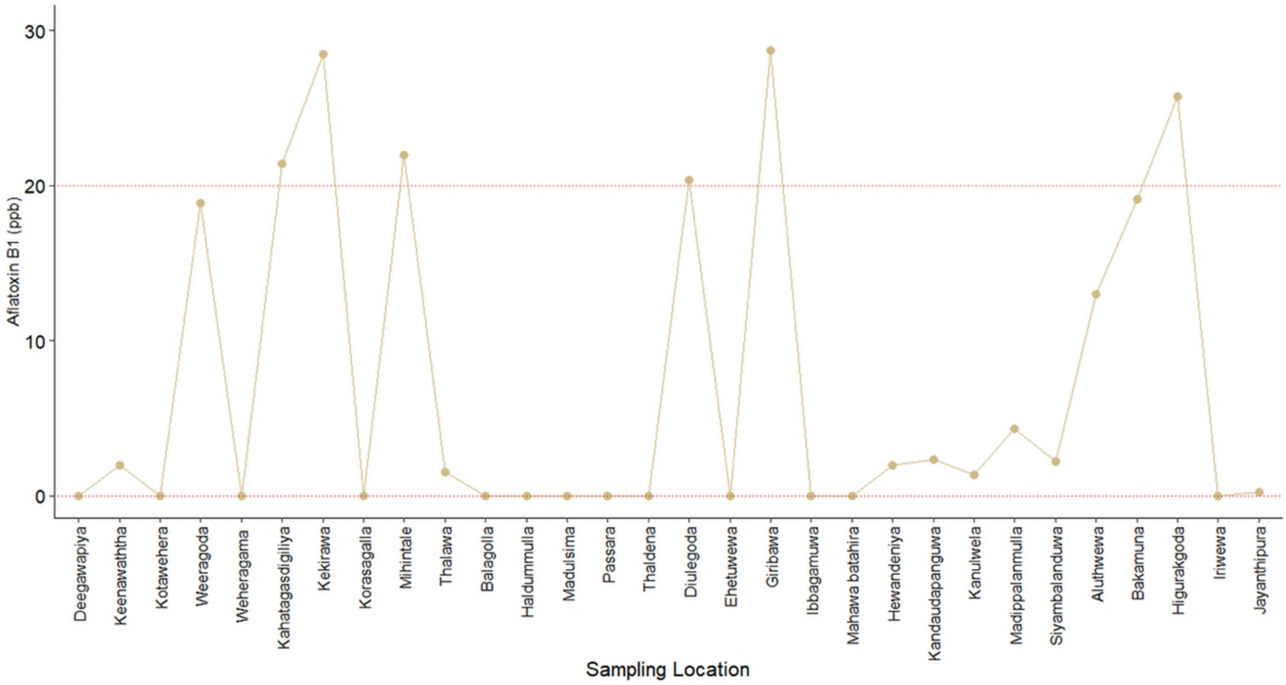

Figure 3. Control chart of AFB1 concentrations in corn samples across Sri Lanka. The upper dashed line indicates the US FDA regulatory limit (20 ppb), while the lower dashed line represents the non-detect baseline (0 ppb). AFB1: aflatoxin B1; ppb: parts per billion; US FDA: United States Food and Drug Administration.

contrast, no significant association was observed for soil samples [p-value = 0.3968 ($p > 0.05$); correlation coefficient (r) = 0.1605; degrees of freedom (df) = 28].

Mean AFB1 concentrations in maize-growing soils also showed a positive correlation with AFB1 levels in maize kernels [p-value = 0.0261 ($p < 0.05$), r = 0.4056, degrees of freedom (df) = 28] (Figure 4).

Discussion

Environmental parameters, particularly temperature and soil pH, play a significant role in AFB1 production by influencing the metabolic activity of *Aspergillus* spp. Optimal AFB1 production has been reported at a pH of 5.5–6.5 and temperatures between 20°C and 26°C, while contamination decreases between 26°C and

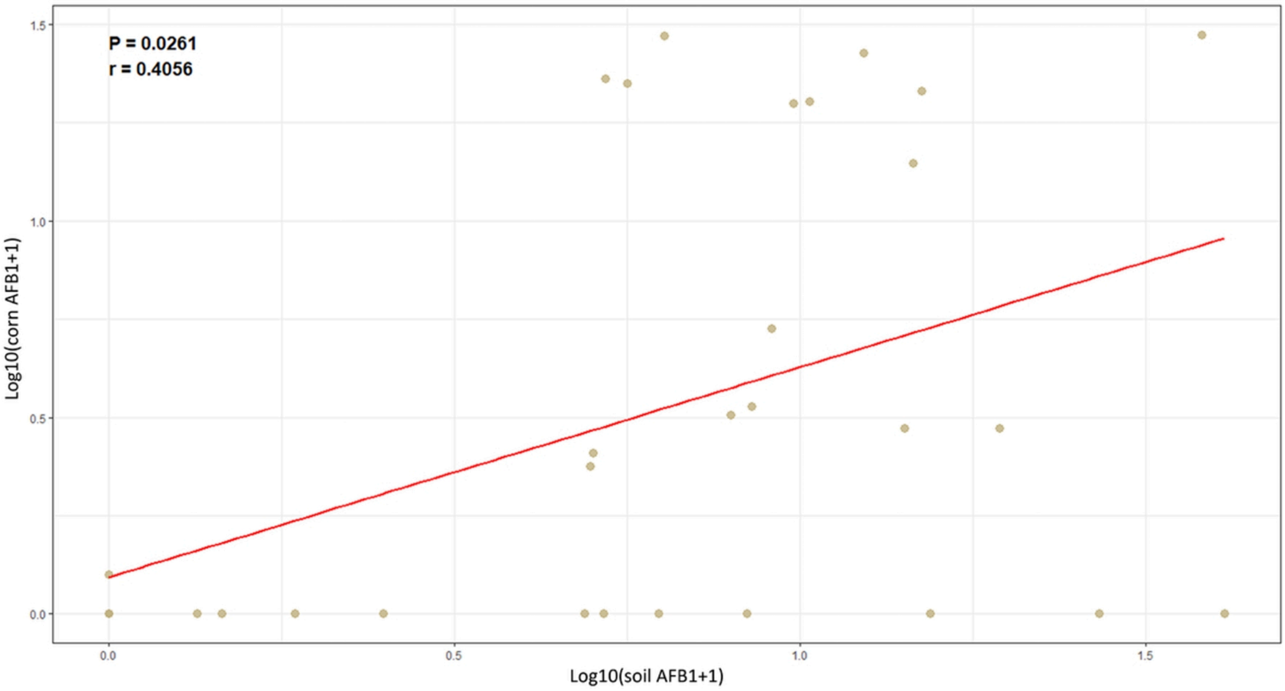

Figure 4. Spearman's correlation analysis between $Log_{10}(X + 1)$ transformed mean AFB1 concentrations in soil and corresponding corn samples. AFB1: aflatoxin B1.

30°C and is completely inhibited at 30°C [4]. In this study, temperatures recorded across maize-growing regions of Sri Lanka fell around this range. The mean air temperatures observed in each district—Ampara (32.01°C), Anuradhapura (31.51°C), Badulla (21.79°C), Kurunegala (29.70°C), Monaragala (32.91°C), and Polonnaruwa (31.85°C) underscore the relevance of these environmental conditions to the risk of aflatoxin contamination in maize. The tropical climate of Sri Lanka is particularly favorable to *Aspergillus* spp., making it important to examine the relationship between environmental parameters and AFB1 levels [7]. Importantly, maize is particularly vulnerable during silk emergence, when prevailing environmental conditions critically determine the extent of AFB1 infection [5]. However, contamination by *Aspergillus* spp. may be influenced by rainfall, agronomic practices, and post-harvest handling factors, which were not captured in the present study. These variables will be considered in future studies.

Aspergillus spp. exhibit optimal growth at temperatures ranging from 25°C to 30°C, with growth reduced to half the maximum at 20°C and five times lower at 15°C [11]. This supports the idea that mean corn AFB1 levels have a significant positive correlation [for corn, p-value = 0.00014 ($p < 0.05$)] with the recorded average air temperature (Figure 5), indicating AFB1 contamination may be significantly driven by average air temperature. Moreover, the statistical analysis showed a positive correlation between mean corn kernel AFB1 levels and mean soil AFB1 levels [p-value = 0.0261 ($p < 0.05$)]. Previous studies suggest that soil may act as a primary inoculum source for aflatoxin-producing *Aspergillus* spp. in corn kernels, with spores being transferred into the kernels via wind and rain [14]. While the positive correlation observed between mean soil AFB1 levels and maize kernel AFB1 levels supports a potential association, it does not confirm soil as the primary source of contamination, as the origin of the inoculum was not directly assessed. Additionally, this study found no significant correlation between AFB1 concentrations in maize kernels and the corresponding soil pH. However, earlier research demonstrated that *Aspergillus* spp. producing AFB1 is more prevalent in high pH soils than in low pH soils, such as those used for growing peanuts [21–27], providing clear evidence of the impact of *Aspergillus* spp. in the soil. Although ELISA assays indicate these AFB1 contaminations, these findings are reported with caution due to potential cross-reactivity and matrix effect associated with the assay; these values may reflect slight over- or underestimation, therefore these findings should be considered as preliminary evidence and future studies should use more specific analytical techniques.

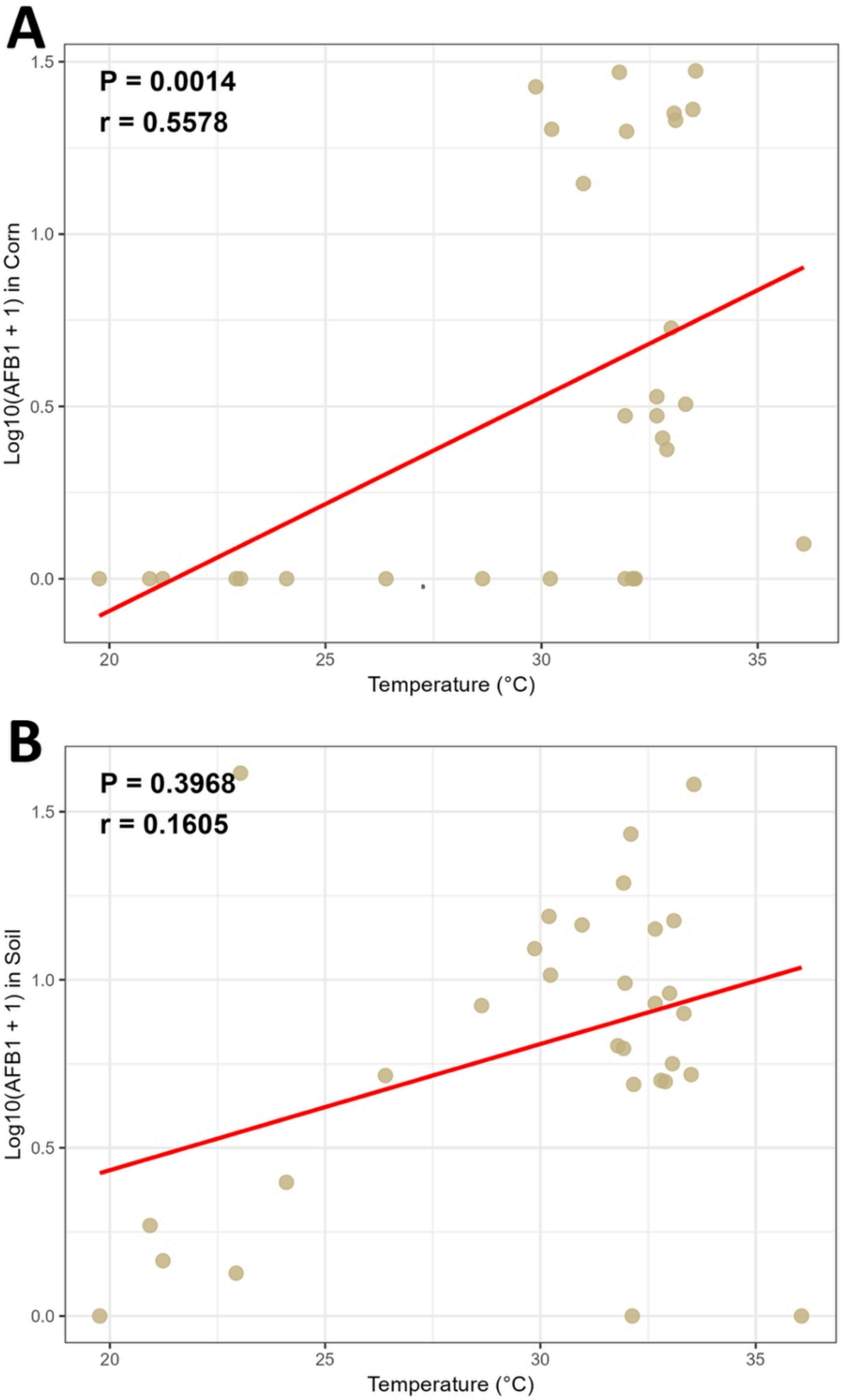

Figure 5. Spearman's correlation analysis shows (A) the association between soil and corn $Log_{10}(X + 1)$ transformed mean concentrations, and (B) the association between $Log_{10}(X + 1)$ transformed mean AFB1 concentrations and mean air temperature. AFB1: aflatoxin B1.

The recovery results ranged from 73.9% to 82.3% for maize samples and 82.8% to 99.1% for soil samples, which largely fall within the acceptable range specified by the assay kit. However, the use of only duplicate measurements represents a methodological limitation, and the reported recovery values should therefore be regarded as preliminary. Furthermore, key analytical performance parameters—including the limit of detection (LOD), linear range, intra- and inter-assay precision, and validation using certified reference materials—were not independently established in this study and instead relied on the manufacturer's specifications. As a result, the analytical output should be interpreted as semi-quantitative. Future studies should therefore incorporate full method validation with increased replication and independent determination of performance characteristics.

Despite its broad scope, this study was subject to several limitations. Seasonal rainfall patterns driven by the monsoon system in Sri Lanka posed challenges in synchronizing sampling across the study period. In addition, marked variations in ambient temperature among the six maize-growing districts complicated the formulation of unified recommendations for AFB1 management, given the strong influence of temperature on fungal proliferation. Heterogeneity in land use and land preparation practices further limited the implementation of a fully standardized sampling strategy.

Post-harvest handling practices also varied considerably across study areas, and the generally limited infrastructure and minimal adoption of value-adding processes may contribute to increased AFB1 risk, although these factors could not be quantitatively assessed in the present study. Moreover, reliable field humidity data were not available for the sampling sites during the study period. Potential influences of UV-related AFB1 degradation, as well as detailed soil physicochemical properties and microbial activity, were also not assessed.

From an analytical standpoint, future studies should incorporate confirmatory techniques such as LC-MS/MS to ensure specificity and to rule out potential interference from degradation products or co-occurring mycotoxins. Although no significant association was observed between soil pH and AFB1 levels in the present study, previous reports have indicated a possible relationship; thus, this interaction should be re-examined using improved environmental datasets and refined analytical approaches.

To address current limitations, multi-seasonal sampling is recommended to account for monsoon-driven rainfall variability, alongside agroecological analyses that better capture the combined influence of rainfall patterns and temperature regimes. Future research should also consider key agronomic and environmental variables, including land preparation practices, fertilizer application, crop management, humidity, and soil organic matter, as these are critical for developing district-specific AFB1 mitigation strategies.

Continuous monitoring of field humidity, air temperature, and soil temperature would further strengthen the understanding of environmental drivers of contamination. In addition, the potential effects of UV-related AFB1 degradation at sampling sites and detailed soil properties, particularly organic matter content, should be included to better interpret soil microbial activity and toxin degradation dynamics.

From a methodological perspective, the use of larger, more balanced datasets across districts is needed to better disentangle geographic and agronomic effects. Incorporating agroecological stratification and applying multivariate or mixed-effects models would further improve the ability to separate interacting environmental influences on AFB1 levels. Moreover, future studies should explicitly consider district-level interactions with key variables such as temperature and soil pH.

Finally, strengthening post-harvest systems through farmer education on best handling practices and promoting value addition of surplus produce could significantly reduce aflatoxin risks in the food chain. Collectively, these improvements are essential for developing sustainable, regionally tailored strategies for AFB1 mitigation.

Conclusion

This study provides important insights into the levels and determinants of AFB1 contamination in maize and its associated soils across six major maize-producing districts of Sri Lanka. The results indicate a concerning trend, with maize samples from most districts exceeding the maximum permissible AFB1 limits set by the US FDA. In contrast, samples from the Badulla District were below the detectable limit, a finding that may be associated with its comparatively lower ambient temperature range. The study further demonstrates a positive correlation between $Log_{10}(X + 1)$-transformed mean AFB1 concentrations in maize and soil, suggesting that soil may act as a reservoir for AFB1-producing fungi. These findings have important implications for maize production and aflatoxin management in tropical environments. By identifying geographic hotspots of contamination, the study supports more targeted risk assessment and mitigation strategies. Ultimately, these results can be integrated into farmer extension programmes and policy frameworks to strengthen aflatoxin control measures, thereby contributing to improved food safety, public health protection, and food security in Sri Lanka.

Abbreviations

AFB1: aflatoxin B1

ELISA: enzyme-linked immunosorbent assay

ppb: parts per billion

US FDA: United States Food and Drug Administration

Declarations

Author contributions

IMR: Conceptualization, Methodology, Investigation, Data curation, Writing—original draft, Formal analysis. KMLBK: Conceptualization, Writing—original draft, Writing—review & editing. MWARD: Methodology, Investigation, Data curation. DMIPD: Methodology, Investigation, Data curation. VYW: Writing—review & editing, Supervision. TCB: Funding acquisition, Supervision. HKSdZ: Writing—review & editing, Funding acquisition, Supervision, Visualization. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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