Investigation of the potential to reduce waste through sampling and spatial analysis of grain bulks

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Abstract
Batches of grain are accepted or rejected based on average mycotoxin concentrations in a composite grain sample. Spatial analysis of mycotoxins in two grain bulks was performed to determine the spatial distribution of toxins, whether they were co-located and the proportions of grain over legislative limits. The 2D distribution of deoxynivalenol (DON) and ochratoxin A (OTA) in a truck load of wheat grain was analysed, as was the distribution of fumonisins (FB1 and FB2) in a 3D maize grain pile. The data had been previously analysed, but results here show that highly skewed data would need to be transformed to investigate spatial autocorrelation properly. In the truck of wheat grain, DON and OTA showed co-variation and, in contrast to previous studies, OTA showed spatial structure when converted to normal scores. Spatial analysis of the maize pile showed that FB1 and FB2 contamination levels were each highest near the outer face and base of the grain pile. Simulations for both grain bulks showed that, for average toxin concentrations close to legislative limits, the proportion of grain over the legislative limits can vary greatly and could be very small when toxin contamination is highly positively skewed. The implications of the results for management were considered. Post-harvest, strategically placed sensors could be used to monitor environmental conditions within the stored grain in real time and detect the first signs of spoilage allowing swift remediative action so less grain is wasted. Pre-harvest approaches for mycotoxin management are suggested as additional food waste reduction strategies.

1. Introduction

The FAO (2017) estimates the world will need to produce 56% more food to feed a world population over 9 billion by the year 2050 (Ranganathan et al., 2018). Searchinger et al. (2019) noted that key elements in producing a sustainable food future are reducing waste, increasing food production while maintaining (or reducing) the land used in agriculture, and cutting greenhouse gas (GHG) emissions.

The pursuits of precision and digital agriculture have the potential to address the problems associated with feeding the future global population, whilst not extending the cultivated area and reducing waste could contribute to reductions in GHGs. Mycotoxins are naturally-occurring toxic secondary metabolites produced by fungi in a range of commodities both in the field before harvest and post-harvest during storage (Kushiro, 2008; Wang et al., 2016). Food standard regulations around the world cover a range of mycotoxins that are harmful to human and animal health (Omotayo, Omotayo, Mwanza, and Babalola, 2019). The legislated maximum permissible concentrations of mycotoxins vary by country, toxin and food commodity (Mazumder & Sasmal, 2001). It has been suggested that lack of legislation for aflatoxin, a mycotoxin that is particularly carcinogenic, may be

linked, at least in part, to between 2 and 10 times (Henry et al., 1999) and 16-31 times more deaths from liver cancer in some less developed countries (Liu and Wu, 2010). This illustrates the great importance of food safety regulation. However, testing for mycotoxin contamination is expensive (Papadoyannis, 1990, p. 504) so it does not take account of spatial variability in the distribution of these toxins within batches of grain and other foodstuffs. The standard protocol is to collect numerous samples at regular intervals throughout a batch of grain. These are then mixed together to make one composite sample, in which the mycotoxin levels are measured to determine an average concentration of contamination for the whole batch of grain (EU, 2006a; Maestroni & Cannavan, 2011). This approach could potentially result in large quantities of uncontaminated grain being wasted or highly contaminated grain being missed if the toxin levels are very high in small, localised pockets within the batch of grain.

Due to the sampling approach used, little is known about the spatial variation of mycotoxins in storage settings. Spatial studies of mycotoxins have been minimal due to the costs of testing for them. For such studies of representative sampling, validated accredited methods are required to ensure the accuracy of the results. This is expensive, and because of the small margins in the grain industry, it is often not carried out. Portell et al. (2020) visually investigated the three dimensional (3D) spatial extent of *Fusarium graminearum* colonisation of wheat grain at different moisture contents and following inoculation at different positions within clear storage jars. Colonisation dynamics and respiration were affected by the inoculation position, but dry matter loss and fungal biomass were mainly affected by the moisture content. Biselli et al. (2008) measured deoxynivalenol (DON) and ochratoxin A (OTA) contamination of 100 samples from a truck load of grain. They compared the average concentration for a bulked sample with the average concentrations of the 100 individual samples, and found that the former was representative of the latter for DON, but not for OTA, which had a far greater coefficient of variation. Using the same dataset, Rivas Casado et al. (2009a) investigated the spatial structure of DON and OTA variation within the truck-load of grain using variograms. They concluded that DON showed spatial structure, but that OTA did not. Rivas Casado et al. (2009b) then used this information to simulate data sets that could be repeatedly sampled to help determine suitable sampling protocols for the future. The dataset generated by Biselli et al. (2008) showed spatial variation in 2 dimensions (2D), but variation within grain bulks needs to be examined in 3D. Rivas Casado et al. (2010) sampled stored maize in a silo in 3D with the aim of characterising the spatial structure and determining a suitable grain sampling and bulking strategy. However, they found no spatial structure in the data, so the latter was not possible.

The mycotoxin levels in the truck load of wheat grain and the maize pile investigated by Rivas Casado et al. (2009a) and (2010), respectively were highly skewed. As variograms are based on variances, they can be sensitive to a few large outliers or asymmetry in the data distribution (Kerry & Oliver, 2007a,b). Rivas Casado et al. (2009a and 2010) did not employ any method of dealing with the asymmetry in OTA or FB1 and FB2 concentrations, respectively. Typical methods of dealing with asymmetry for variogram computation involve transforming the data to logarithms or indicators, removing outliers or computing normal scores (Kerry & Oliver, 2007a,b). The aim in the present study was to re-analyse the data investigated by Rivas Casado et al. (2009a) and (2010) to determine if dealing with the non-normality in OTA and FB1 and FB2 data could reveal spatial structure in OTA and any spatial association with DON data, and whether there was spatial structure in the FB1 and FB2 concentrations in 3D.

These aims were partially fulfilled by Kerry, Ingram, Garcia-Cela, and Magan (2019) in an initial examination of the spatial variation in DON and OTA in the truck load of grain and FB1 and FB2 in the stored maize grain. However, they did not interpolate (krige) the data to visualise and investigate the

proportions of the two grain bulks that exceeded legislative limits in order to quantify the amount of waste that would occur. This has been done in the current paper and geostatistical simulation has also been employed to determine the range and degree of uncertainty in the proportions of grain that were above the legislative limits. To do this, concentrations were simulated for batches of grain with the same variograms as the sample data, but a mean concentration that was equal to the legislative limit. This was done to determine what range of proportions of uncontaminated grain would be wasted in such situations and to suggest future management strategies that could reduce some or all of this waste.

2. Materials and methods

2.1 Data collection

The data for this paper come from two previous studies. Data on different mycotoxins were collected from two grain bulks, one in a truck and one in a grain pile. The variations in DON and OTA in the truckload of previously stored wheat were biosystems engineering 207 (2021) 92 e105 93 examined in two dimensions. The truckload of wheat, with dimensions of 2.5 m x 10 m (Fig. 1a), was sampled from above in the horizontal plane at a spacing of 0.5 m producing a grid of 100 points (Biselli et al., 2005, Fig. 1a). A five-aperture probe sampler was vertically inserted into the grain at each grid point to take a single incremental sample containing grain from five depths. Each sample was mixed and subsampled before the DON and OTA concentrations were measured. Full details of the laboratory procedures used have been given by Biselli et al. (2005). The final sets of values describe the spatial variation of DON and OTA concentrations (mg kg^{1}) in a two dimensional horizontal plane but as values were averaged with depth, there is no insight into how DON or OTA changed with depth.

Data were collected from the stored maize grain pile in 3D. A bed of maize in a large grain store with an exposed surface area of ~3800 m2 and height of ~10 m was sampled in three planar layers inclined at 45°, parallel to the open face of the bed, providing a three-dimensional grid of points (Rivas Casado et al., 2010, Fig. 1b). There were 50 points in each layer on a 10 x5 point grid with 0.5 m spacing between points. There was also a spacing of 0.5 m between each of the layers (Fig. 1b). Grain samples were taken using a probe with a conical chamber at the tip that could be opened and closed using a lever at the end of the shaft (Fig. 1c), collecting about 200 g of grain. A 0.5 m square metal frame was placed on the exposed grain face to locate the sampling points where the probe would be inserted. Samples from the three different layers were taken at each point, before moving the frame, to minimise disturbance of the grain (Rivas Casado et al., 2010).

The FB1 and FB2 contents were analysed using the validated method detailed by Rivas Casado et al. (2010). Samples were ground, passed through a 1 mm sieve and homogenised. A solution of acetonitrile:methanol:water (25:25:50, v/v/v) was used to extract fumonisins with a grain to solution ratio of 1:5. Following further sample preparation, concentrations of FB1 and FB2 (mg kg^{1}) were determined using a liquid chromatography mass spectrometry system (Thermo-Fisher Scientific, San Jose, CA, USA).

2.2. Data analysis

The OTA data from the truck load of grain were transformed to normal scores (NST). Normal score ties were broken using the local mean of the eight nearest neighbour points. Normal scores were calculated in the same way for FB1 and FB2 from the grain pile. Omni-directional variograms in two dimensions with a lag separation of 0.5 m were computed and modelled for DON and OTA in the truck load of grain and FB1 and FB2 in each layer of the grain pile using SpaceStat (Jacquez et al., 2014). A

two-dimensional cross-variogram with a 0.5 m lag was computed between DON and OTA NST for the truck load of grain using SGems (Remy et al., 2009). Omnidirectional variograms in three dimensions with a lag separation of 0.5 m were computed for FB1 and FB2 concentrations in the grain pile using SGems (Remy et al., 2009). Residual maximum likelihood (REML) variograms were also computed for FB1 and FB2 in each layer of the grain pile using GeoR (Ribeiro et al., 2001).

The global Moran's I statistic investigates spatial autocorrelation. It involves standardising the data to a mean of zero and unit variance and this can be problematic when data are highly skewed. However, in using the mean and standard deviation of a local neighbourhood to standardise data, the local Moran's I (LMI) is less likely to encounter problems with skewed data. Univariate global and LMI analysis (Anselin, 1995) was done using SpaceStat for DON and OTA NST from the truckload of grain and for the FB1 and FB2 concentrations of the individual layers in the grain pile. The global Moran's I statistic determines whether there is significant positive (clustering) or negative (spatial outliers) spatial autocorrelation in the data compared to a spatially random distribution. The LMI statistic computes the statistic for a moving window to determine whether a given observation is similar to or different from the mean of neighbouring values (here within 1 m). Maps were produced to show whether points were part of significant (p ¼ 0.05) clusters of low (LL-low values surrounded by low values) or high (HH e high values surrounded by high values) values, were significant spatial outliers (LH e low values surrounded by high values or HL e high values surrounded by low values) or were not significantly (NS) different from a random distribution. Monte Carlo simulation was used to determine the p-value of the LMI statistic for each observation. The number of tests was large and each point was reused in several tests so the risk of false positives was increased, hence the Simes correction (Jacquez et al., 2014) for multiple testing was used to adjust for this. Bivariate global and LMI analysis between DON and OTA NST in the truck load of grain and between different layers for FB1 and FB2 in the grain pile was performed. This indicates whether clusters in the two variables or layers are co-located by using the standardised values of the second variable as the neighbours for each point in the first variable. This means that HH and LL clusters are significant clusters with high or low values, respectively of both variables or in both layers, and show positive spatial correlation in the variables. Any HL or LH clusters indicate significant clusters of high values in the first variable and low values in the second variable, or low values in the first and high values in the second variable. Such locations show negative spatial correlation between variables.

The 2D variograms for the truck load of grain from ordinary kriging of the raw DON and OTA NS data to a 0.1 m grid give a more complete picture of likely variation of DON and OTA in the grain. Kriged OTA NST data were back-transformed to the original scale. This analysis was performed in SpaceStat (Jacquez et al., 2014). The 3D variograms were used for ordinary kriging of the FB1, FB2 NST data in the grain pile to a 0.05 m grid. Following kriging, 3D local Moran's I analysis of FB1 and FB2 was performed following the approach of Kerry et al. (2017) and kriged NST values were back-transformed to the original scale.

Finally, the mycotoxin data from the truck of wheat grain and the maize grain pile and their associated histograms and variograms were used for conditional sequential Gaussian simulation (SGS) of the data to 0.1 m and 0.05 m grids, respectively, in SGems (Remy et al., 2009). SGS converts data to NS so that data being simulated have a normal distribution. Following simulation, the values are back-transformed to the original scale. Here 100 realisations were simulated for each dataset. The simulated values were converted to z-scores (zero mean and unit variance) by subtracting the mean from each value and then dividing by the standard deviation of the original dataset. Although data for OTA and FB1 and FB2 were highly positively skewed, the values of the z-scores reflected this. There were fewer negative z-scores and more positive and large z-scores. These z-scores from simulation

were analysed to compute summary statistics for the 100 realisations and determine what proportion of points were above the mean of zero and would be rejected in a scenario where the mean concentration was equal to a legislative limit.

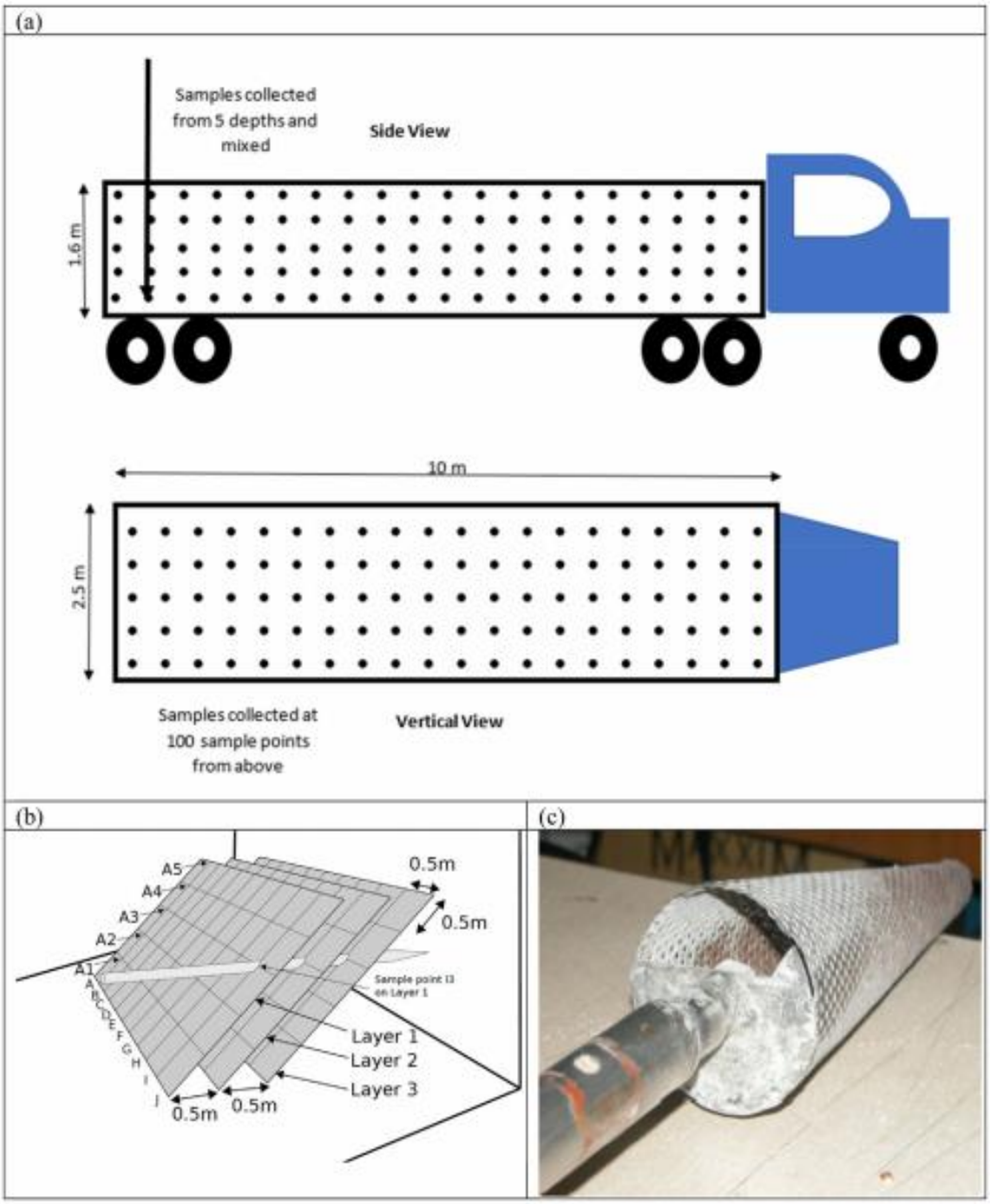

Fig. 1 e (a) Side and vertical view of truck load of wheat grain showing the sampling scheme, (b) Sampling scheme for the maize grain pile in the silo in relation to the wall and floor and (c) Probe used to sample the maize grain pile (Adapted from Rivas Casado et al., 2010).

3. Results and discussion

3.1. Frequency distribution of mycotoxins

Figure 2 shows the histograms for DON and OTA contamination in the truck load of wheat. DON had a relatively normal distribution with a slightly elevated skew (1.21) caused by a large outlier and slight asymmetry (Fig. 2a). DON had a large range of values of up to ~2700 mg kg1 and there were no points with 0 mg kg^{1}. Most points had values between 1000 and 1500 mg kg^{1}. The histogram for OTA (Fig. 2b) showed a highly skewed (4.36) distribution, approximating a Poisson distribution, with a large number of points with 0 mg kg^{1} and the highest concentration being ~9 mg kg^{1}. Most values in the distribution are below 1 mg kg^{1}. Rivas Casado et al. (2009a) noted the strongly skewed distribution of the OTA data but did not address this problem, although they log-transformed the DON data which were far less skewed. The histograms in Fig. 3a, b shows the distribution of FB1 and FB2 in the stored maize grain. Both histograms showed marked asymmetry and FB2 had a larger skew (1.91). For FB1, the values reached up to ~8000 mg kg^{1} whereas maximum values for FB2 were ~6000 mg kg^{1}. For FB1, most of the values were below 4000 mg kg^{1} whereas for FB2, most values were below 2000 mg kg^{1}. The positively skewed distributions of all of the toxins examined in the truck and the grain pile suggests that concentrations of mycotoxins typically have skewed concentrations. Indeed, it is well known that environmental contaminants often have highly skewed distributions (Goovaerts et al., 1997; Van Meirvenne & Goovaerts, 2001) and this has implications for how the contaminant is dealt with or managed.

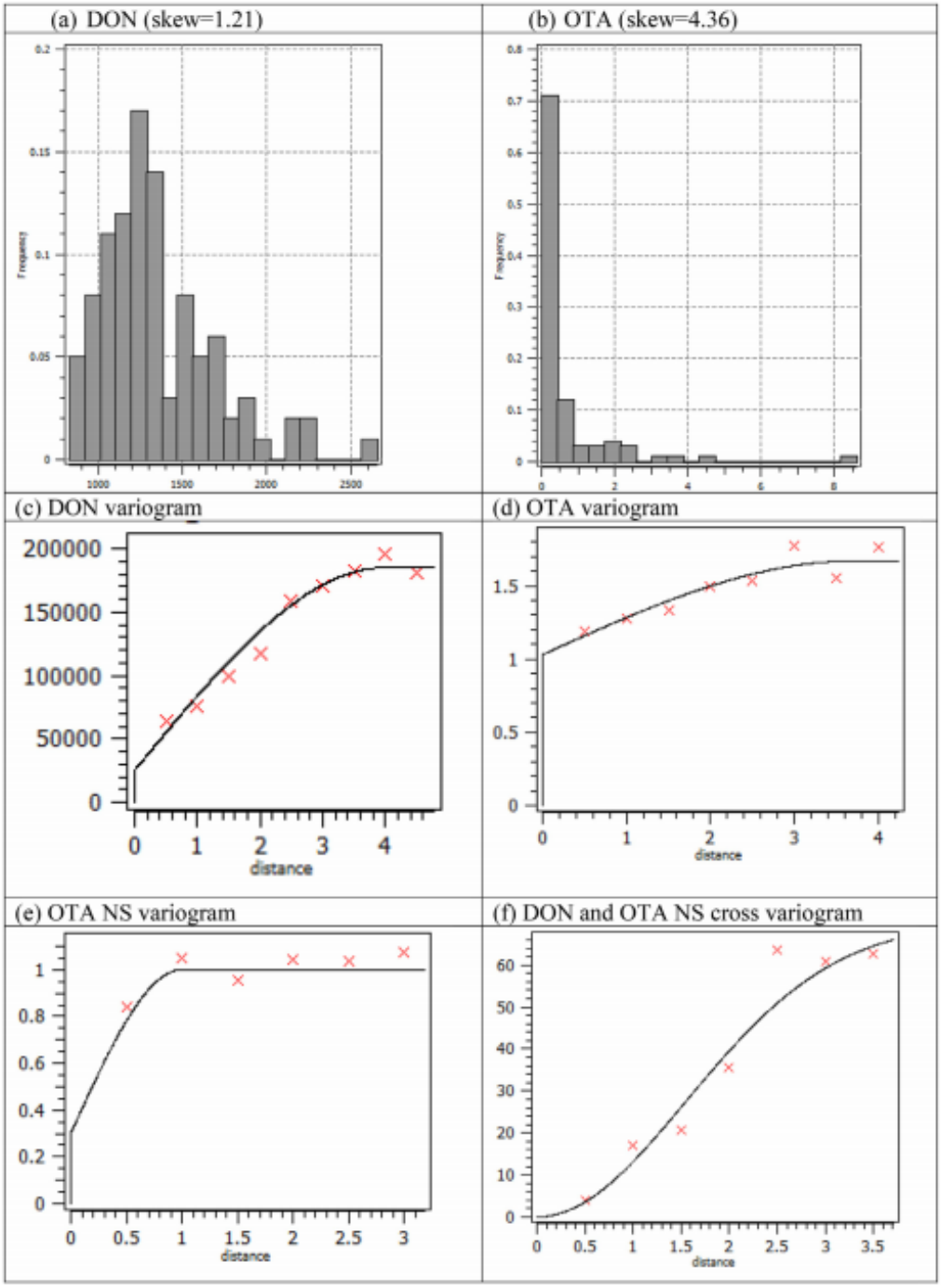

Fig. 2 e Histograms of Mycotoxins in ppb in the truck load of grain (a) DON and (b) OTA and 2D variograms of (c) DON, (d) OTA, (e) OTA NST and (f) cross variogram for DON and OTA NST.

3.2. Spatial analysis

3.2.1. Variogram and Moran's I analysis of DON and OTA contamination in a truck load of grain

As the histograms for each mycotoxin showed marked asymmetry, the use of log-transformed and normal score transformed data (Webster & Oliver, 2007) was investigated for computing variograms and the Moran's I. Table 1 shows the variogram parameters for DON and OTA contamination in the truck load of grain. The raw DON values showed good spatial structure with a

variogram range of 4 m and nugget:sill ratio of about 13% (Fig. 2c). These parameters were similar to those found for the log-transformed data by Rivas Casado et al. (2009a). The variogram for OTA (Fig. 2d) had a similar range to that of DON but a large nugget effect (62%, Table 1) showing a large proportion of spatially random variation. However, the variogram of OTA NST (Fig. 2e) had a nugget:sill ratio of 30% and the range decreased to 1 m suggesting that patches with similar values were small. However, these smaller patches could appear as a greater proportion of random variation if the skewed data were not dealt with. Rivas Casado et al. (2009a) suggested that there was no spatial structure in OTA perhaps due to the high proportion of nugget variation, but after dealing with the highly skewed data using NST, new patterns were found. The global Moran's I values (Table 1) also agree with these findings. Raw DON values showed strong (0.468) positive spatial autocorrelation that was significant at p ¼ 0.001. In contrast, raw OTA values showed negative spatial autocorrelation that was not significant (p ¼ 0.494) whereas the OTA NST data showed moderate, positive and significant spatial clustering (p ¼ 0.001). These results show that highly skewed data need to be transformed to properly investigate spatial autocorrelation in their patterns.

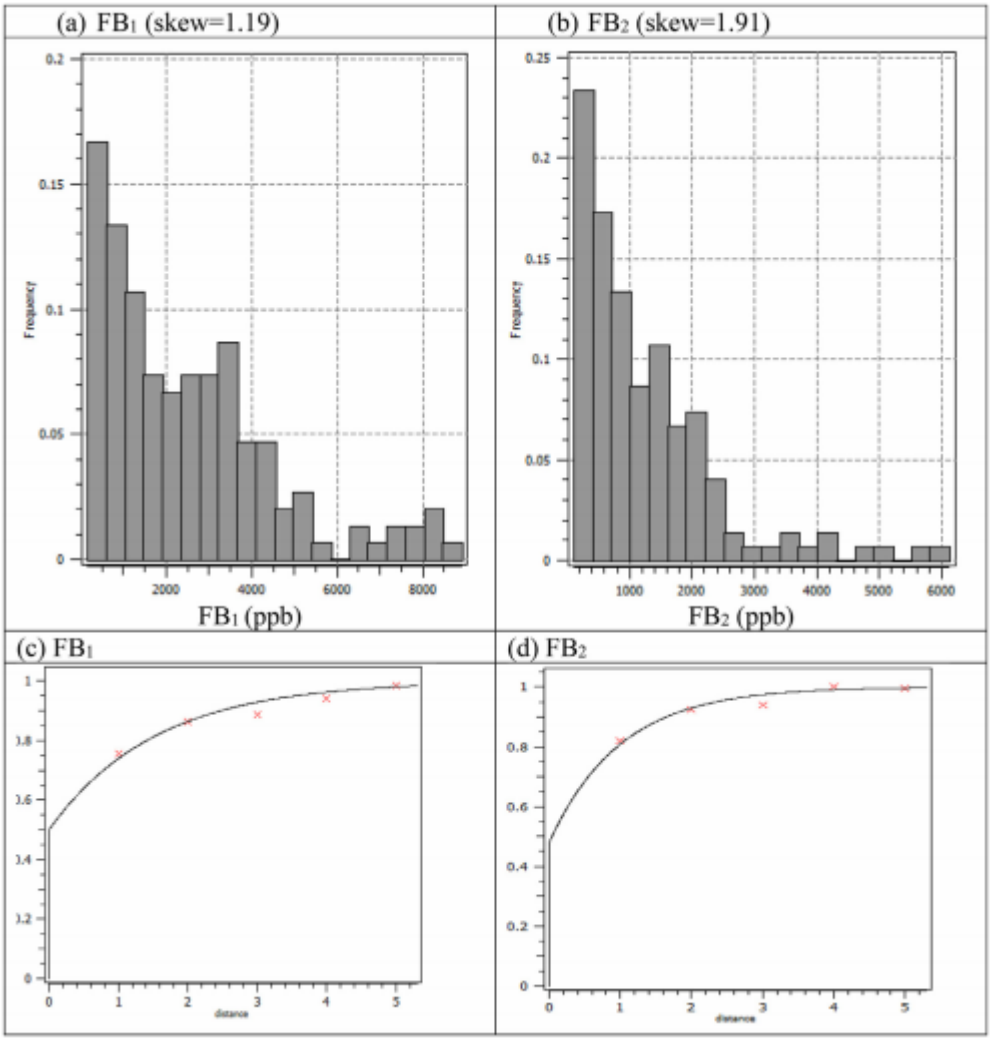

Fig. 3 e Histograms of Mycotoxin concentrations in (ppb) (a) FB1 and (b) FB2 and variograms for (c) FB1 and (d) FB2 in the grain pile.

Figure 4a and c shows the spatial patterns of the raw values of DON and OTA in the truck. These patterns show that most spatial variation occurred along the long axis of the truck, which is consistent with the method of loading the truck. Indeed, examination of directional variograms (not shown) for the long and short axis of the truck showed spatial structure for the long axis with parameters almost identical to the omni-directional variogram and predominantly random variation across the short axis of the truck. Rivas Casado et al. (2009a) also observed similar differential spatial variation in the long and short axis of the truck. However, they concluded that the seemingly random variation across the short axis was a result of it being so short and there being low numbers of comparisons at each lag. Figure 4b and d shows how DON and OTA values are clustered in space using the univariate local Moran's I. These maps are consistent with the findings of the variogram and global Moran's I analysis in that they show larger significant clusters of HH and LL points for DON than for OTA.

Table 1. Univariate and bi-variate global Moran's I values and variogram and cross-variogram parameters for DON and OTA contamination in the truck load of grain

Variable(s)	Global Moran's I	*p* value	Model	Nugget:sill ratio (%)	Variogram range (m)
DON	0.468	0.001	Spherical	13	4.00
OTA	-0.012	0.494	Spherical	62	3.57
OTA NST	0.124	0.001	Spherical	30	1.00
DON and OTA NST	0.157	0.001	Gaussian	0	3.00

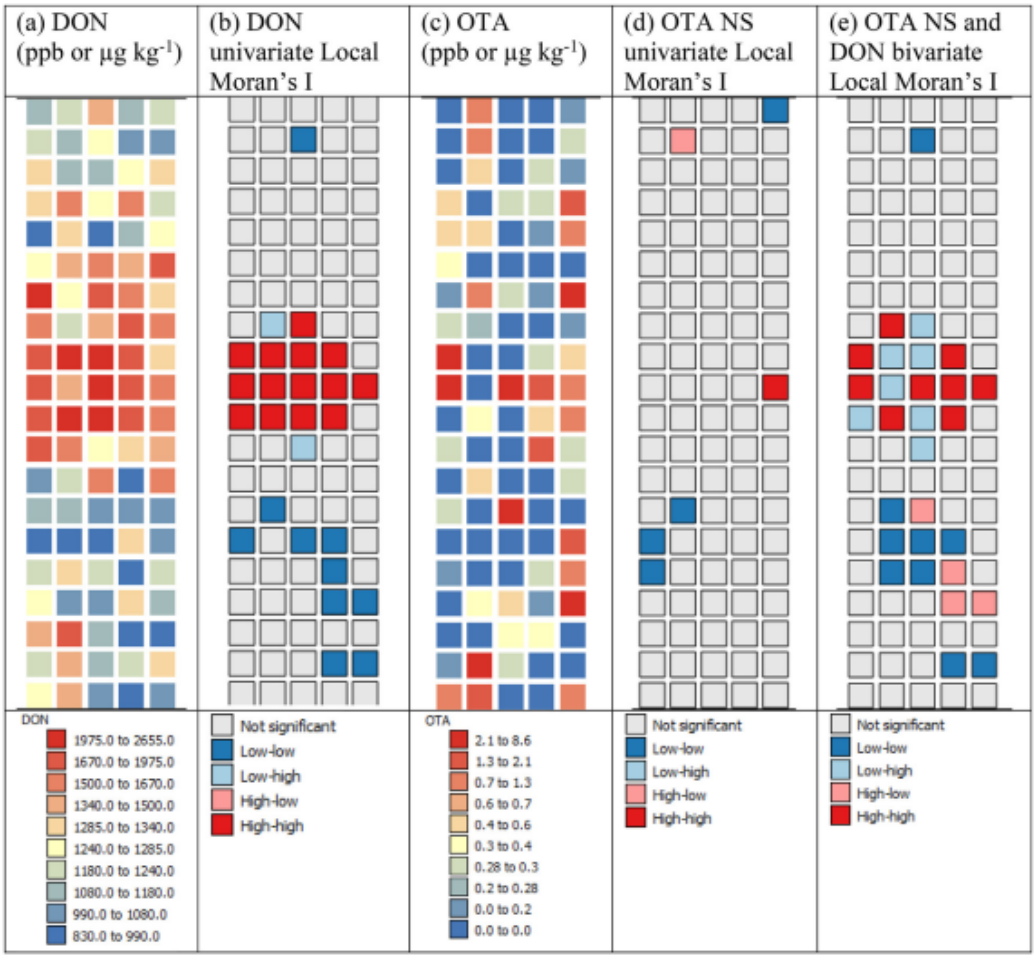

Fig. 4 e Maps of raw values of DON (a) and OTA (c) and distribution and clusters from uni- (b, d) and bi-variate (e) LMI analysis of the mycotoxin concentrations in the truck load of grain.

Figure 5a and b shows the kriged DON and OTA NST, respectively. The patterns of variation in kriged DON and OTA NST are smoother and more continuous than for the raw data. This translates into a larger patch of LL values in the univariate LMI for DON and larger LL and HH patches in the OTA NST univariate LMI than for the raw data. This also further emphasises that there are places in the truck load of grain where DON and OTA values are both positively (red and dark blue) and negatively (pink and pale blue) associated (Fig. 5e).

The spatial analyses show that clusters of DON and OTA are often co-located, but the patches of OTA are smaller. As DON is usually produced in the field (Kushiro, 2008) and OTA during storage (Wang et al., 2016), this finding is consistent with OTA developing from locations of high DON concentrations and spreading out from them. Although the two toxins are produced by different species of fungi, Hussein and Brasel (2001) noted that more than one toxin may show high concentrations in the same initiation points because mycotoxins weaken the host, which improves the environment for further fungal development. The patterns observed are also consistent with the notion that in storage settings, pockets of increased moisture may favour fungal development and toxin production by more than one fungal species. Fungal development is not possible below 0.70 water activity (aw), equivalent to about 14e15% moisture content (MC) in wheat and maize.

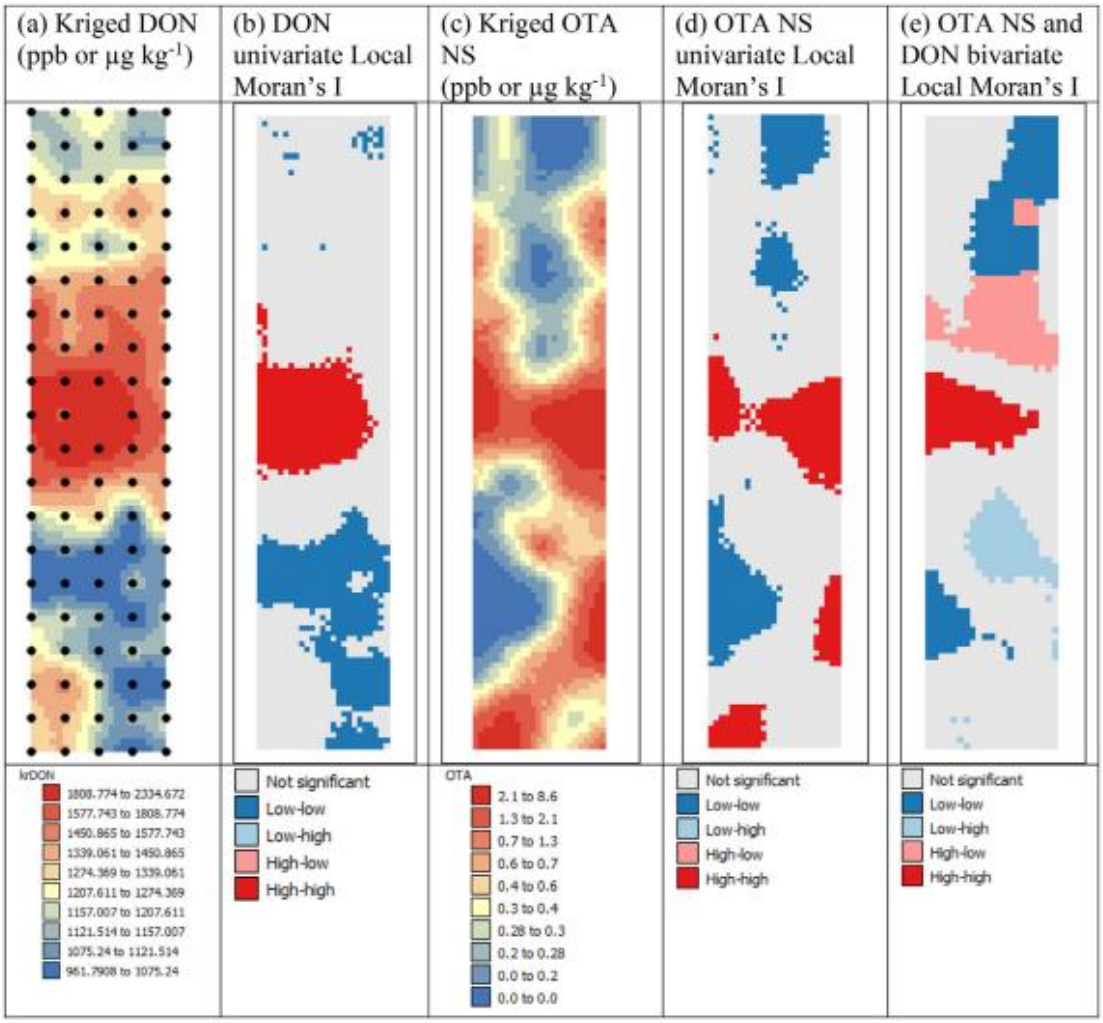

Fig. 5 e Kriged maps of DON (a) and OTA (c) distribution and clusters from uni- (b, d) and bi-variate (e) LMI analysis of the mycotoxin concentrations in the truck load of grain.

However, small increases in humidity can allow the growth of xerotolerant/xerophilic fungi such as *Penicillium verrucosum* (responsible for OTA contamination). The storage environment of cereals in silos presents a complex ecosystem, and the metabolic activity and damage due to insect pests or these primary species can result in an increase in moisture content in small pockets, facilitating initiation and colonisation by such mycotoxigenic spoilage fungi. Indeed, numerous studies show increased growth of a range of species when moisture availability and temperature become conducive for spore germination and colonisation (Garcia-Cela et al, 2018, 2019; Magan et al., 2020).

3.2.2. Variogram and Moran's I analysis of FB1 and FB2 contamination in the grain pile

Variograms in 3D of raw and log-transformed data were erratic or pure nugget. This is consistent with the findings of Rivas Casado et al. (2010) but 3D variograms for NST FB1 and FB2 contamination in the maize grain showed spatial structure with areas of similar values of 4 and 3 m diameter, respectively. Both toxins also showed ~50% spatially random variation (Table 2 and Fig. 3c). The effect of skew on data can be more pronounced when datasets are small (Webster and Oliver, 1992), so it is not surprising that the MoM variograms for the individual layers were predominantly erratic or pure nugget. Nevertheless, the REML variograms for layer 1 (FB1 and FB2) and layer 2 (FB1) showed some spatial structure as did the MoM variograms for layer 3 (FB1 and FB2) (Table 2). These variograms showed a decreasing range with depth of layer from the exposed grain face (see Fig. 1a). For FB2 a similar pattern to FB1 was apparent, but there was no spatial structure in layer 2 and the patches of FB2 were smaller than those of FB1 in both layers 1 and 3. This means that larger patches of both toxins were found in the outer layers of the stored grain and smaller patches or random variation were found closer to the wall of the silo (Fig. 1a). This could be a function of the degree of aeration and moisture in outer and deeper layers of the grain pile, as fungi are aerobic microorganisms that need oxygen (Semple et al., 1989). However, the spatial variation in fungal development and environmental conditions needs further investigation.

Global Moran'sI(Table 2) and variogram analysis showed that there was a lot of spatially random variation and, when there was spatial structure, it was closest to significance in layers 1 and 3, although the p values for none of the layers were significant at p ¼ 0.05. The bivariate Moran's I values between layers showed that there was more spatial association between layers 2 and 3 for both toxins and this was significant at p ¼ 0.003 and p ¼ 0.016 for FB1 and FB2, respectively. Correlations between FB1 and FB2 for each layer and all layers together were strong ($r > 0.9$). Figure 6a and b shows the front and back view of kriged FB1 NST values. These show larger values near the base of the grain pile and these larger values extend higher on the front exposed grain face than for layer 3, which is closer to the wall. Figure 6c and d further emphasise these spatial patterns. They show that 3D LMI maps had HH clusters located near rows 4 and 5 of layers 1 and 2 (Fig. 1a) and LL clusters were located in rows 1 and 2 of layers 1e3. The maps of the spatial distribution of high and low values for FB1 and FB2 were very similar so the latter are not shown.

The spatial analysis showed that FB1 and FB2 behave and are located similarly but FB2 had a shorter variogram range, indicating slightly smaller patches of contamination than for FB1. Logic might suggest that the distribution of HH and LL clusters within the stored grain reflects a case where moisture gathers under the influence of gravity towards the base of the stored grain and that toxins develop most in the outer layers at the base of the grain pile where oxygen and moisture supply are greatest. However, this could also be the result of differences in temperature within the grain. The environmental conditions of the grain where toxin levels are highest would need to be measured and monitored to confirm whether this is the case or not. Spatial analysis of mycotoxins and environmental conditions in more stored grain silos is needed to determine whether the outer layers at the base of grain piles generally tend to have higher toxin concentrations and if so, why. If these areas are typically

at higher risk of contamination then they should be monitored with temperature, moisture and CO2 sensors. This type of data could be utilised by grain silo managers to help evaluate the temporal relative risk of mycotoxin contamination and take immediate and appropriate remedial action. Garcia-Cela et al. (2020) defined three different risk levels based on the CO2 production levels in wheat naturally and artificially inoculated with *F. graminearum*. If the level of risk is increased (increased CO2), the immediate recommendation is to aerate the silo and reduce the moisture content, and thus reduce the colonisation by the pockets of mycotoxigenic moulds. If the risk is increased then batches may need to be diverted to animal feed or for biogas production to minimise waste.

Table 2. Univariate and bivariate global Moran's I for NS FB_1 and FB_2 within and between layers and parameters of 2D and 3D variograms for single layers and the whole grain pile

Variable(s)	Global Moran's I	*p* value	Model	Nugget:sill ratio (%)	Variogram range (m)
FB_1 (layer 1)	0.029	0.158	*Gaussian*	*50*	*3.86*
FB_1 (layer 2)	-0.021	0.500	*Spherical*	*67*	*2.78*
FB_1 (layer 3)	0.047	0.054	Spherical	52	1.02
FB_2 (layer 1)	-0.060	0.170	*Spherical*	*67*	*1.98*
FB_2 (layer 2)	-0.009	0.400	---	---	---
FB_2 (layer 3)	0.019	0.164	Spherical	48	0.98
FB_1 layers 1 and 2	0.069	0.115	---	---	---
FB_1 layers 2 and 3	**0.119**	**0.003**	---	---	---
FB_1 layers 1 and 3	0.022	0.326	---	---	---
FB_2 layers 1 and 2	0.035	0.204	---	---	---
FB_2 layers 2 and 3	**0.111**	**0.016**	---	---	---
FB_2 layers 1 and 3	-0.003	0.499	---	---	---
FB_1 all layers (3D)	0.048	---	Exponential	50	4.00
FB_2 all layers (3D)	0.068	---	Exponential	48	3.00

Bold values are significant at *p*=0.05, *Italics* show REML variogram parameters, --- missing/not valid

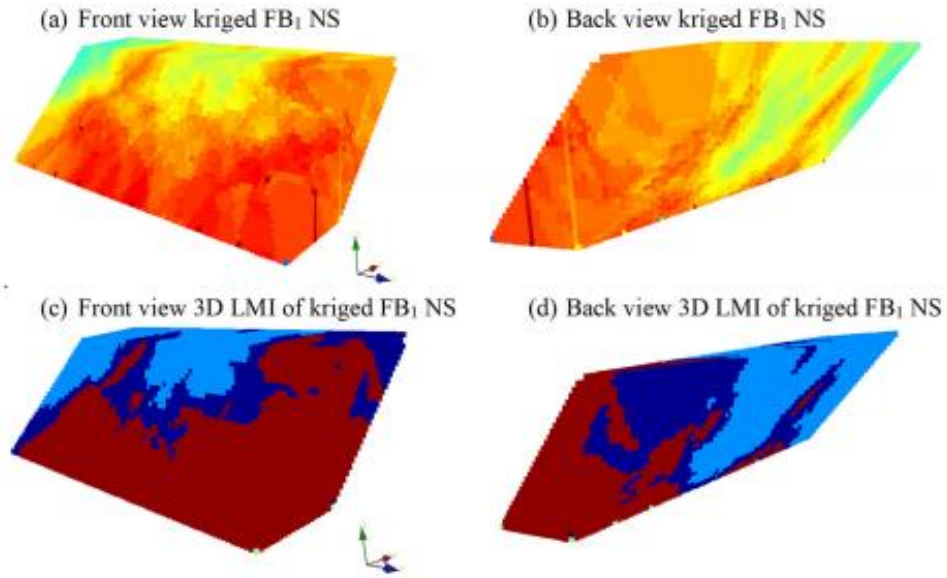

Fig. 6 e Front and back views of Kriged FB1 NST concentrations (low values yellow, green and blue and high values orange and red) and LMI for the grain pile (p ¼ 0.05 HH cluster (red), LL cluster (pale blue), NST values (dark blue)). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

3.3. Estimated percentages of grain discarded and their uncertainty

To determine the percentages of each batch of grain that were above the legislated limits, ordinary kriging was used. This interpolated values of each mycotoxin in the truck load of wheat grain and in the maize grain in between sampling points. To determine the uncertainty associated with kriged estimates, conditional simulations using the histograms and variograms for each toxin were performed. The simulations provide 100 possible spatial distributions of the mycotoxin levels that have those specific histograms and variograms. The SGS data were converted to z-scores with zero mean and unit variance following simulation. This means that a value greater than zero (the mean) would be above the legislative limits and any value less than zero would be below the limit.

Tables 3 and 4 show the percentages of grain that had concentrations greater than the legislative limits for the raw data values and kriged data, respectively. For DON contamination of the truck load of wheat grain, the mean batch concentrations for the raw and kriged data (1342, 1354 mg kg^1 were greater than EU (2006b) and FDA (2010) limits of 1250 and 1000 mg kg^1, respectively. This means that, in both locations, the whole truck of grain would be discarded or would have to be re-purposed for animal feed use rather than for human food use. The FDA (2010) limits for DON used for specific animals, e.g. swine, are as high as 5000 mg kg^1. Re-purposing of the grain for animal feed would result in reduced income for these batches of grain. Given that the mean batch concentration was close to the EU limit, only 55% (53% for kriged data) of the grain had concentrations greater than the EU limit. This would mean that almost half the batch of grain, which had concentrations below the EU limit, would be wastefully discarded or re-purposed for a use that would result in a lower price for the farmer. Tables 3 and 4 show that the batch of grain would not be discarded due specifically to OTA contamination as the mean concentrations of 0.57 mg kg^1 (and 2.68 mg kg^1 for kriged data) are well below the 5 mg kg^1 EU (2006b) legislative limit.

Table 3. Percentages of points in the sample data points exceeding EU and FDA toxin limits

Toxin	EU limit (µg kg^{-1})	FDA limit (µg kg^{-1})	Mean batch concentration (µg kg^{-1})	% of batch above EU limit	% of Batch above FDA limit
DON	1250	1000	1342	55 %	88 %
OTA	5	No limit	0.57	1 %	---
FB_1+FB_2	1000, 4000	4000	2495+1225=3720	83 %, 38%	38 %

Table 4. Percentages of points in the kriged data exceeding EU and FDA toxin limits

Toxin	EU limit (µg kg^{-1})	FDA limit (µg kg^{-1})	Mean batch concentration (µg kg^{-1})	% of batch above EU limit	% of Batch above FDA limit
DON	1250	1000	1354	53 %	99%
OTA	5	No limit	2.68	0.1 %	---
FB_1+FB_2	1000, 4000	4000	2061+943=3004	99 %, 16%	16 %

The SGS results (Table 5) were used to investigate the uncertainty associated with estimates of the proportion of grain that would be wasted due to DON contamination when the mean DON concentration was equal to the legislative limit. The mean percentage of grain with DON levels above the mean was 53% with a large range from 9 to 88% and an interquartile range of 37e73%. This suggests that, on occasion, data with similar characteristics to this could be produced by grain where only 9% of grain was contaminated at levels above the legislative limits. However, it may be equally

likely that 88% of the grain had toxin levels above the legislative limits. This suggests quite a wide range of uncertainty in the proportions of values that are above the legislative limits. However, DON concentrations in the truck showed spatially structured variation and little random variation (Fig. 2f and Table 1), and a distribution that was not too strongly skewed (Fig. 2a). Therefore, if the average DON concentration was at the legislative limit, on average one can expect slightly over half of the grain to be contaminated above legislative limits. For OTA, when the mean concentration was at the legislative limits, the mean proportion of grain with concentrations above the legislative limits was 26% with a range of 13e36% and interquartile range of 22e29%. This means that given data with such a high level of skewness, the mean concentration could be at the legislative limit but on average only 26% of the grain would have concentrations above the legislative limits. This would result in a significant proportion of wasted grain. There was also less uncertainty in the amount of grain with toxin concentrations above the limits as the range was narrower (13e36%) than for DON (9e88%).

The EU (2006b) has legislative limits of 4000 mg kg^1 for the sum of FB1 and FB2 in unprocessed maize and 1000 mg kg^1 in maize products intended for direct human consumption and the USA has a limit of 4000 mg kg^1 for total Fumonisins (FB1, FB2 and FB3) in whole or partially degermed dry milled corn products for human consumption (FDA, 2001). For FB1 and FB2 contamination in the maize grain pile, the mean concentration of 3720 mg kg^1 (3004 mg kg^1 for kriged data) was above the EU (2006b) limit of 1000 mg kg1 but below the EU (2006b) and FDA (2001) limits of 4000 mg kg^1. Therefore, the whole batch of maize would be rejected at the 1000 mg kg^1 limit but it would be accepted at the 4000 mg kg^1 limit. The distribution of the data was very skewed with many low values and a few very high values (Fig. 3 a and b). This is probably responsible for the fact that although only 38% (16% for kriged data) of the grain had concentrations higher than the legislative limit, the mean concentration of the batch, 3720 mg kg^1 (3004 mg kg^1 for kriged data), was close to the 4000 mg kg^1 limit. In each case, the kriged data showed a smaller proportion of points that were above the legislative limits than the raw data values. Kriging has a smoothing effect on spatial distributions. This is fine when trying to get an accurate view of the overall distribution, but if the interest is in identifying highly contaminated points that are distributional and spatial outliers, the kriging process tends to smooth and mask this aspect of variation. Nevertheless, the LMI maps for both grain bulks (see Figs. 4e6) and the global Moran's I values (see Tables 1 and 2) show that there were very few points that were significant spatial outliers and that clustering of high and low values dominated the grain bulks.

Table 5 shows the summary of the simulated values, for FB1 and FB2. When the mean concentration of the grain is at the legislative limits, the mean percentages of grain above the legislative limits for the 100 realisations were 43% and 51% for FB1 and FB2, respectively. The range of values was 38e48% for FB1 and 27e72% for FB2 and the inter-quartile range was 42e44% for FB1 and 44e57% for FB2. These values indicate, for both FB1 and FB2, that when the mean toxin concentration in the grain is at the legislative limit, on average there was about 50% or less of the grain that had toxin concentrations above the legislative limits. The range of values indicates that there is more uncertainty in the amount of FB2 than FB1 locations that were higher than the legislative limits, probably due to the more highly skewed distribution of the data (Fig. 3a and b).

Mycotoxins, like any contaminant in the environment (Goovaerts et al., 1997; Van Meirvenne & Goovaerts, 2001) can have a tendency towards a skewed distribution with a normal distribution contaminated by outliers or lots of low values and a few very high values forming a long positive tail in the distribution. Spatial analysis of the data in general suggests that the distribution of the data should not be ignored when investigating spatial patterns. Also, the simulation analysis suggests that the more skewed the data, the more likely that a limited number of locations with very high toxin concentrations could cause the average toxin level of a batch of grain to be above the legislative limits

while more than half of the grain had concentrations well below the limits. For DON and FB1 which showed lower amounts of skew, the amount of grain exceeding legislative limits was around 50% when the mean concentration was equal to the legislative limit, but for the OTA data, which were very skewed, only about a quarter of the grain exceeded limits when the mean concentration was at the legislative limit; and for FB2, as little as 27% of the grain was contaminated at levels that exceeded the legislative limits.

Spatial identification of the potential contaminant "hot spots" is vital information to prevent and mitigate mycotoxin accumulation in cereals, and reduce food waste. Since mycotoxin variability in grain bulks is not well understood, the true mycotoxin concentration in a bulk lot cannot be determined with 100% of certainty. Consequently, this can result in contaminated batches being processed while uncontaminated batches are rejected. As an example, European legislation requires sampling of 1 kg of cereals by taking 100 incremental samples for cereal lots 50 t (EU, 2006a). Understanding the spatial variability of mycotoxins could guide the design of accurate and less expensive sampling plans to identify accurately the mycotoxin contamination of each batch. Correct quantification of mycotoxin contamination would reduce the human exposure to misclassified batches. It also allows for bespoke remediation actions to be applied to specific lots for food or feed, or could result in highly contaminated batches being diverted for biogas production.

Strategies such as aeration (Akdogan et al., 2006), drying (Magan et al., 2020) or separating the grain at harvest based on contamination risk into micro batches (Lamb, 2020) could be applied. The variograms for OTA, FB1 and FB2 all had considerable nugget components, which suggests that there is unresolved spatial variation when sampling at an interval of 0.5 m. Sampling at a smaller interval could resolve some of this (Webster & Oliver, 2007) and reduce uncertainty from the spatially random component of variation. However, physical sampling destabilises the grain bulk, making it difficult to conduct denser spatial analysis using traditional sampling methods. A potentially more useful approach that could be employed to reduce uncertainty about the spatial distribution of mycotoxins is to use sensors that provide data on environmental variables such as temperature, CO2 and relative humidity in different regions of the grain bulk, so that a detailed spatial map can be obtained more effectively and links to detailed mycotoxin contamination levels could be developed on this basis.

Table 5. Summary Statistics of Percentage of Kriged Points above the Mean Concentrations (assumed equal to legislative limit) for 100 Simulated Realizations

Toxin	Mean % above mean concentration	Range of % above mean concentration	Interquartile range of % above mean concentration
DON	53	9-88	37-73
OTA	26	13-36	22-29
FB_1	43	38-48	42-44
FB_2	51	27-72	44-57

4. Implications of this work

As some co-location of patches of DON and OTA contamination was observed and the former develops in the field and the latter in storage, grain could easily be separated at harvest based on moisture content. Yield monitors, one of the most used precision agriculture technologies, have integrated moisture sensors which measure grain moisture via electrical capacitance (Chung et al.,

2016). The moisture sensors are used to correct the measured wet rate of grain flow to dry yield (Reyns et al., 2002). Grain could then be separated at harvest and the wetter grain dried to discourage further fungal development and potential toxin contamination by the same or other fungal species.

The co-location of DON and OTA shows that toxin levels in stored grain can be linked back to environmental conditions and management practices that the crop experienced in the field, as well as how the grain is dried and stored. Numerous studies exist in the precision agriculture literature documenting the identification of fungal infections within fields and the practice of targeted fungicide applications (De Castro, Ehsani, Ploetz, Crane, & Abdulridha, 2015a, De Castro, Ehsani, Ploetz, Crane, & Buchanon, 2015b, Di Marco et al., 2011; Fischer, 2002; Isakeit et al., 2009). As a logical extension of such studies, Kerry, Ingram, Ortiz, et al. (2019) investigated the spatial variation of aflatoxin levels within two maize (corn) fields in Alabama, USA and were able to establish management zones with different contamination risk. Such zones could be differentially managed by (1) pre-season differential planting rates and planting resistant varieties, (2) within season by differential irrigation and fungicide application rates and (3) at harvest by separating grain with different risks of contamination into micro-batches for storage. Although farmers strive to control fungal infection in the field, the risk of DON contamination pre-harvest, for example, is based on weather conditions which implicitly assumes that contamination levels would not vary within fields. Also, mycotoxin levels are usually only measured at harvest and often ignored in subsequent post-harvest storage settings.

Several studies have used hyperspectral imaging to identify various fungal infections in the field (Del Fiore, 2010; Mahlein et al., 2012). If F. graminearum infections that cause DON contamination could be identified in the field using this approach, fungicides could be applied to only the areas identified as infected. Another possibility is that fertiliser applications could be reduced in areas with fungal infection. Paungfoo-Lonhienne et al. (2015) showed that larger nitrogen fertiliser concentrations can alter the composition of the soil fungal community in favour of species with more pathogenic traits.

The 3D spatial analysis of the grain pile suggests the need for a post-harvest strategy to reduce waste. Such an approach could be to install a network of CO2 and relative humidity sensors throughout stored grain in silos to detect initiation of spoilage, allowing effective aeration regimes to be instituted or subsequent separation of batches from such a silo that should be discarded and used for biogas production or undergo remedial drying. Currently, such sensors are quite expensive to install. However, with technological developments and the increased use of digital platforms, the economic justification of such approaches could improve. This would allow effective sampling systems to be developed for ensuring that a true concentration of different mycotoxins could be quantified in cereals destined for either food or feed use.

5. Conclusions

Variogram and Moran's I analysis of mycotoxin levels in a truck load of wheat and a large maize bulk showed that adjusting for highly skewed data using the NST revealed spatial patterns in the data. In the truck load of grain, spatial structure in OTA was identified with smaller clusters than DON but with some co-location between DON and OTA clusters. As DON usually develops in the field and OTA during storage, we hypothesise that OTA may be developing from locations of high DON concentrations where the grain is more susceptible to invasion by other fungi or the environmental conditions in the grain are generally favourable for fungal development. In the maize grain pile, spatial analysis showed larger clusters and stronger spatial autocorrelation in the outer grain layers and clusters of high values at the base and low values at the top of the stored grain pile. We hypothesise that this is consistent with humidity diffusing through the grain over time under the influence of gravity and FB1 and FB2

tending to develop in wetter grain, with more oxygen rich locations at the base and in the outer layers of the stored grain. Future studies need to investigate the spatial distribution, within grain masses, of environmental variables, such as moisture, CO2 and O2 concentrations, that are likely to co-vary with mycotoxins, using robust integrated sensors.

Kriging and simulation showed that, in some cases where average concentrations were above legislative limits that would lead to a whole batch of grain being discarded, less than 50% of the grain was contaminated at levels above the actual legislative maximum allowable levels. This was particularly the case when the data were very highly skewed. This suggests that current approaches to sampling stored grain for mycotoxins are resulting in a lot of wasted grain. Simulation results showed that there was a greater degree of uncertainty about the spatial distribution of some mycotoxins than others and this needs further study. Given the quantification of uncertainty, risk can be assessed and managed by specifying the probability of threshold exceedance to give more control to decision makers. Several strategies for the management of food products earlier in the agricultural food production chain have been suggested, in addition to the best practice storage procedures. We believe that management approaches in the field as well as spatial analysis and monitoring of environmental conditions in grain masses post-harvest have the potential to significantly reduce such agricultural waste streams.

Declaration of competing 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.

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