Henry Wells, Francis Ngure
Trip Report: November 2015 Field Surveys in Kongwa, Dodoma, Tanzania

I. Introduction:

This report reviews the findings from a two weeks scoping field survey in Kongwa, Tanzania, conducted by Henry Wells and Francis Ngure, along with Lidia Munuo and Clara Mollay from the Nelson Mandela African Institute of Science and Technology (Arusha, TZ campus), in November of 2015. The objective of this trip was to collect samples of foods prone to mycotoxins, as well as to conduct interviews with the household members that provided the samples and with owners or operators of grain mills in villages. Furthermore, this trip presented an opportunity to combine food sampling and gathering geographic data, for later use in site selection and planning for a community based mycotoxins mitigation trial.

Owing to advances made in prior GIS analysis, and a previous short scouting trip during the summer of 2015, we decided to focus our sampling efforts in Kongwa District of Dodoma Region, central Tanzania. Kongwa seemed likely to be prone to mycotoxins contamination based on environmental drivers of high incidence of infection by mycotoxigenic fungi in crop fields, and also showed a sufficient amount of maize and groundnuts grown within and around the district.[1] Furthermore, according to projections made by the WorldPop project based on Tanzania's 2012 census, there appeared to be a sufficient number of births and pregnancies per year in Kongwa for our proposed trial sample size.[2]

The scoping survey was meant to address gaps in our own knowledge related to levels of contamination in the main staple foods (maize and groundnuts), additional *Lishe* ingredients that are prone to mycotoxins contamination, as well as complementary feeding practices among households in Kongwa. With respect to both staple foods and complementary foods, and to supplement the data on levels of contamination, our goals for this trip addressed three main areas of practical knowledge:

1. Markets for food in rural areas, and behavior patterns related to sourcing of food as well as utilization of grain mills in villages.
2. Seasonality of food production, agronomic challenges, as well as seasonal changes in market behavior and post-harvest handling of grains.
3. Contextual knowledge of food safety practices at the community level, mostly related to pre-cleaning and screening of grain and groundnuts, as well as storage practices.

Having gathered information addressing each of these areas, as well as geographic coordinates and mycotoxin contamination levels, we generated data that will be important in informing the site selection and intervention strategy for the research trial.

II. Sampling and Data Collection Methods:

We spent our first day in Kongwa conducting interviews with two key informants: The District-level Agriculture, Irrigation and Cooperatives Officer (DAICO) and the District-level

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[1] See mapping work done by Henry Wells. Data sources: WorldClim, USAID Famine Early Warning System (FEWS), Harvest Choice, GlobCover 2009, VitalSigns Tanzania

[2] Data source: WorldPop project (http://www.worldpop.org.uk/). Confirmed from records of 2012 census obtained in Kongwa during the November 2015 field trip.

Nutrition officer (DNuO) for Kongwa. These interviews provided us with records of the 2012 census as well as knowledge of other projects, both in community nutrition as well as agricultural extension, which were at work in some of Kongwa's villages.[3] Bearing this in mind, we focused our sampling on relatively small villages that were mostly away from main roads and large town centers. These villages were the most appropriate given that the sourcing of food in smaller villages was more locally-based than in larger ones, both in terms of utilization of homegrown food and local markets for food products. Furthermore, smaller and more rural villages would be easier to manage in a household-level mycotoxins intervention in a trial setting, as there would be fewer elements to control in the sourcing of a family's food supply.

In total, we conducted nine full days of village-based sampling and surveys, one day of key informant interviews at the district headquarters, and one day at a large regional market in Kibaigwa Ward, Kongwa. Excluding the visit to the Kibaigwa market, we covered 9 wards of Kongwa, for a total of 19 villages. From these 19 villages, we collected a total of 128 samples of milled maize, as well as 79 samples of whole-kernel maize, 38 of whole groundnuts, and a total of 41 other samples of complementary foods commonly used in *Lishe* (baby food) flours.

We traveled to each village with a guide from the DAICO's office, who helped introduced us to the village leaders and obtained verbal consent for our visit and survey. Upon arrival to each village, we paid a courtesy visit to the office of the Village Executive Officer (VEO), where we signed a guestbook and introduce our field visit activities. After obtaining verbal consent the VEO gave us at least two village leaders to guide our two teams for sampling at grain mills and nearby households.[4] We paid all of our respondents for the samples and time they donated to us. We paid mill operators TZS 1,000 for their time during interviews, and the mill customers TZS 1,000 for about 50-100 g of milled maize, TZS 2,000 for 0.5 kg of whole-kernel maize sample, and TZS 2,500 for a customer who donated both a milled and a whole-kernel sample. At the household level we collected a wider range of food samples, and paid 1,000-6,000 TZS depending on the number and type of samples he collected. A sample of about 250 g of groundnuts was compensated at TZS 2,000 whereas *Lishe* was compensated at about TZS 3,000.

Short survey questionnaires and collection of geographic coordinates for each mill and household was done using the Open Data Kit software (https://opendatakit.org/). Open Data Kit is an open-source, web-based data management platform that allows users to build and customize survey forms, link their forms to a mobile device or tablet for portable use, and sync the responses to the forms collected via those devices to a cloud database. This software was useful in matching the location data and sample ID's with the survey respondents, and recording qualitative information about all samples collected and locations visited. Qualitative data gathered include information about the sources of the food samples and respondents' food production or utilization habits, mills and their operation, seasonality of food and village descriptive characteristics.

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[3] See H. Wells and F. Ngure's field notes document from the trip for more specific information on names of other projects and villages where they are involved.

[4] These were important steps to take for us to be welcomed into the villages for any sampling activities—if we had not done so, we likely would not have been able to conduct any sampling at all. Furthermore, a small courtesy payment of 10,000-20,000 TZS was made to each village level guide, as well as a half per-diem payment of 40,000 TZS to our guide from the DAICO's office, and per-diem payments of 80,000 TZS to our driver and 100,000 TZS each to Clara and Lidia, respectively.

To analyze samples, we utilized mReader technology from MobileAssay (mobileassay.com), which analyzes images of Neogen Reveal Q+ test strips for various mycotoxins using an android app. The samples were extracted and the test strips prepared following the Neogen (http://www.neogen.com/) Reveal Q+ protocol for both Aflatoxins and Fumonisin. These tests were done for milled maize while sampling was still ongoing in Dodoma. Other types of food samples (groundnuts, millet, sorghum, whole-grain maize, etc.) were analyzed at NM-AIST mycotoxins lab after our return to Arusha. Since the Neogen Q plus is not validated for most complex food matrices, repeated analyses of groundnuts, millet, sorghum, finger millet, sesame and Lishe flours was done at Beca-ILRI, in Nairobi, using the Helica Aflatoxin B1 low matrix ELISA kits. The results from these assays will further be cross-validated using internal standards and testing of subsets of samples on alternate protocols such as HPLC and LCMS.

R software was used for data cleaning, analysis and visualization, both for geographic and non-geographic variables. Values for geographic variables such as rainfall and elevation were obtained by extracting values from raster layers representing each variable within a 5-km radius buffer area around each mill point. Raster data layers for rainfall, elevation, and population demographics came from various publicly available sources, all of which use a mixture of field-based sampling techniques and remote sensing using satellite imagery. More information on the sources of remotely sensed data methods used for geographic data analysis and mapping can be found in an appendix to this report.

III. Results

Overall, we observed a range of 0.2 – 141.5 ppb Aflatoxin in samples of milled maize collected during our surveys. While the distribution of values for Aflatoxin in milled maize was substantially right-skewed, 48% of those samples registered over the legal limit of 10 ppb AF, and 17% over 50 ppb AF. These are comparable to the levels observed in other studies in central Tanzania. These results were also in agreement with the experience of our colleagues from NM-AIST in terms of levels of contamination that were expected in similar contexts in Tanzania[5].

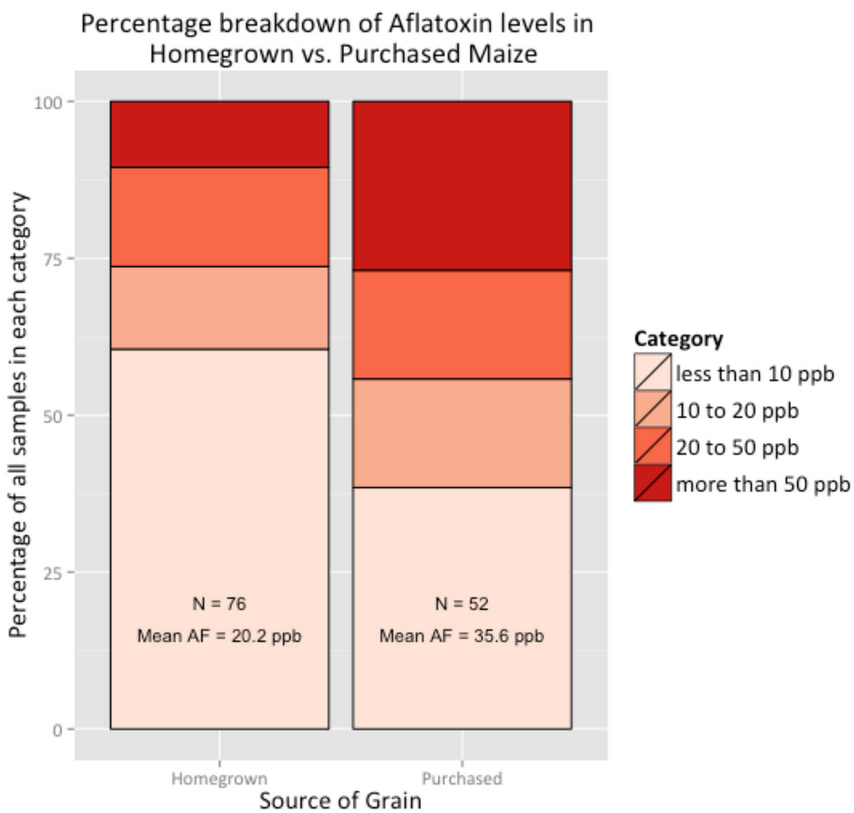
None of the 128 samples of milled maize tested registered a Fumonisin value over the legal limit of 1 ppm, which our colleagues also identified as not entirely unpredictable. However, as other studies in similar regions have shown greater prevalence of FUM contamination in maize over 1 ppm, further testing will likely be needed. Furthermore, it is worth restating that the assay methods used on the food samples are still in the process of being validated, and that less work has gone toward development of standards for FUM than for AF, indicating that the FUM data are likely less reliable.

A summary of the sample types collected by village and ward visited is included below in Table 1. Note that the toxin values in table 1 represent ONLY for the milled maize samples, as the sampling for those was the most consistent. Lab assays of the whole maize samples are yet to be done. For non-maize samples, groundnuts registered the highest average contamination with AF, with a mean of 20 ppb and standard error of 5.8, a max of 120 ppb, and 27% of samples registering over the 10 ppb legal limit for AF. In addition, levels of contamination in *Lishe* flours were worryingly high – groundnuts and lishe had geometric mean AF levels of 18.1 and 110.8, respectively (discussed in more detail below). Otherwise, fairly little contamination was

[5] Need citations here for specific comparisons with published papers!

observed in the remainder of non-maize samples (Millet, sorghum and finger millet). Four of these registered over 10 ppb AF and none over 20. These values need to be validated against results from other assay protocols. Validation using ELISA, HPLC and LCMS protocols will be underway soon at our collaborating institutions; BecA (Nairobi), NM-AIST and Cornell.

A statistically significant difference was observed between homegrown (N=76) and market-purchased (N=52) maize samples (an unequal-variance t-test, p value =0.027). This difference is clearly visible in the distributions of the two categories of milled maize samples.[6] Some survey respondents themselves reported they often begin buying maize from local traders about six months after harvest, when homegrown stores begin to run low. It is likely that market sources maize is stores store longer, often in sub-optimal conditions that favour AF accumulation. Our visit to the Kibaigwa market also enlightened us to the fact that many traders will mix ‘good’ and ‘bad’ quality batches together to help improve and stabilize sale prices. In addition some larger traders sourced grain from other regions.[7]



[6] Insert citation here relating this finding to those of other studies. Make note of any differences observed in other regions (ex. Eastern Kenya, owing to different market & ecological dynamics)

[7] Cite C. Barrett and M. Burke papers on seasonal price arbitrage in East African grain mkts.

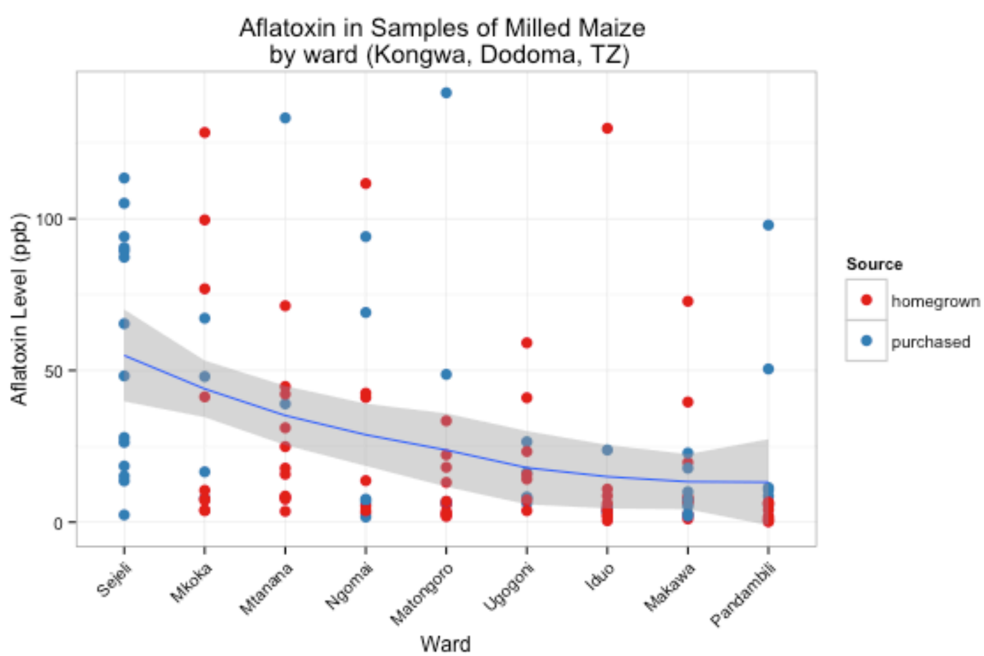
Table 1: Kongwa Village-level Sampling Summaries ***(Note: toxin levels reflect tests of ONLY milled maize samples)***

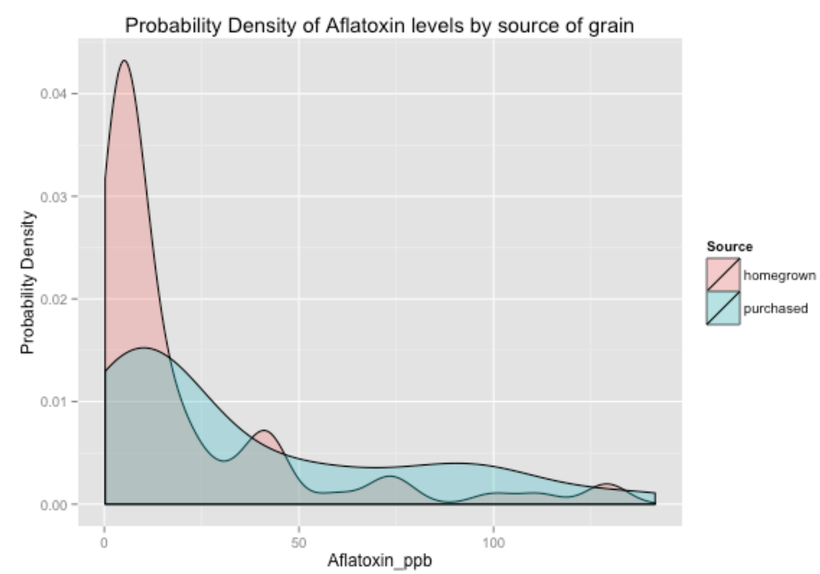
Village	Ward	# Mills Visited	# HH's visited	Milled Maize Samples	Whole grain maize samples	Groundnut samples	All other samples	Mean [std error] Aflatoxin (ppb)	Mean [std error] Fumonisin (ppm)
Mkoka	Mkoka	2	3	5	4	3	3	7 [1.1]	0.02 [0.008]
Mvungurumo	Mkoka	2	3	6	6	2	0	61 [17.0]	0.25 [0.131]
Mnuku	Mkoka	1	1	2	2	0	0	58 [41.5]	0.85 [0.100]
Ibwaga	Ugogoni	2	3	6	4	4	6	13 [3.3]	0.08 [0.032]
Mautya	Ugogoni	2	3	6	5	2	3	26 [8.5]	0.11 [0.066]
Mtanana 'A'	Mtanana	1	2	5	4	1	0	55 [20.2]	0.19 [0.135]
Ndalibo	Mtanana	3	4	8	6	3	1	22 [7.9]	0.09 [0.069]
Matongoro	Matongoro	3	4	8	5	3	2	34 [16.3]	0.11 [0.057]
Mlanje	Matongoro	1	3	6	5	3	4	7 [3.1]	0.33 [0.117]
Pandambili	Pandambili	2	3	9	3	1	0	5 [1.0]	0.06 [0.113]
Silwa	Pandambili	2	4	8	3	2	1	21 [12.4]	0.23 [0.099]
Iduo	Iduo	2	3	9	5	2	4	19 [13.9]	0.13 [0.060]
Suguta	Iduo	1	3	6	6	3	5	7 [3.5]	0.58 [0.262]
Sejeli	Sejeli	2	4	7	5	0	1	56 [16.2]	0.42 [0.213]
Msunjilile	Sejeli	1	4	7	3	0	0	58 [14.6]	0.52 [0.131]
Manyata	Ngomai	1	2	4	0	2	1	14 [9.5]	0.26 [0.152]
Chilanjilizi	Ngomai	2	4	9	4	3	3	39 [14.2]	0.29 [0.106]
Makawa	Makawa	2	4	9	6	3	0	8 [2.1]	0.29 [0.112]
Muungano	Makawa	2	3	8	3	1	0	21 [8.5]	0.45 [0.119]
OVERALL	19 villages, 9 wards	34	60	128	79	38	41	26.5 [3.05]	0.254 [0.029]

Not listed in Table 1: Also collected were 7 samples of commercial 'lishe' (mixed baby food flours) for lab evaluation.

Table 2: Ward-level characteristics ***(Note: toxin levels reflect tests of ONLY milled maize samples)***

Ward	Area (km^2)	Elevation Mean (masl)	Pre-Harvest Rainfall Mean (mm)	Peri-Harvest Rainfall Mean (mm)	Months of Harvest	Geometric Mean AF (ppb) in Milled Maize	Range AF (ppb) in milled maize	Number of milled maize samples collected
Mkoka	1068	1199	678	37	April-June	21.0	3.8 – 128.4	13
Ugogoni	1682	1122	615	26	May-July	19	1.1 – 72.8	12
Mtanana	5089	1135	656	39	June-July	22.1	3.6 – 133.2	13
Matongoro	1063	1242	657	40	June-July	10.0	1.9 – 141.5	14
Pandambili	890	1295	644	50	May-July	4.1	<1 – 97.9	17
Iduo	829	1187	634	40	June-Sept	5.5	<1 – 129.8	15
Sejeli	2834	981	672	39	May-July	38.7	2.4 – 113.4	14
Ngomai	2014	1248	646	45	May-July	13.7	1.7 – 111.6	13
Makawa	1674	1343	632	39	June-July	8.1	1.1 – 72.8	17





High levels of AF were found in groundnuts and *Lishe*. Overall, groundnuts showed a range of <1 – 1176.9 ppb AF, with a geometric mean of 18.2 ppb. Thirty two percent of all groundnut samples had over 50 ppb AF. Ranges of AF observed in groundnuts varied greatly by ward, as shown in table 5a below.

Levels of AF observed in *Lishe* (baby food) flours, both home-produced and from commercial sources, were also alarmingly high. Overall, the 13 samples of lishe collected showed a geometric mean of 110.8 ppb AF with a minimum of 12.2 ppb and a maximum of 411.5 ppb. As detailed in table 5b below, the geometric means of the two subsets of samples, home-produced and commercially sourced, were roughly equal, but the range of values observed in home-produced *Lishe* flours was much wider.

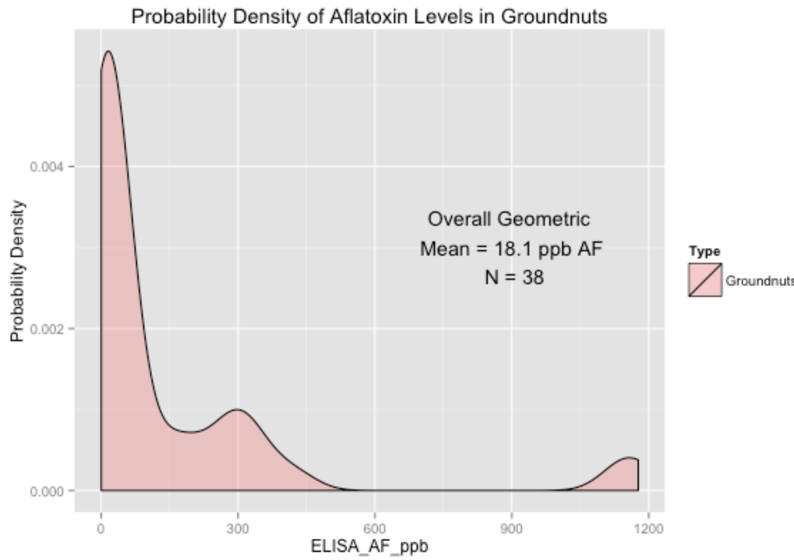


Table 5a: Groundnuts

Ward	Geometric Mean AF (ppb)	Min AF	Max AF	Median AF	N samples
Iduo	48.6	8.1	421.7	17.4	5
Makawa	10.5	5.5	15.9	11.9	4
Matongoro	48.1	9.6	269.7	49.3	6
Mkoka	7.9	0.3	155	8.1	5
Mtanana	234.7	51.8	629.3	306.1	4
Ngomai	123.1	8	1176.9	160.6	5
Pandambili	15.6	10.7	28.4	12.5	3
Ugogoni	18.4	4.3	1137.6	10.5	6

Table 5b: Lishe (Baby Food Flours)

Source	Geometric Mean AF (ppb)	Min AF	Max AF	Median AF	N samples
Commercial	109.6	68.3	255.2	87.7	5
Home-produced	111.6	12.2	411.5	198.6	8

*** Note: No gnut collected in Sejeli ward (none available due to severe food insecurity)**

Note: All non-maize food samples were all analyzed using the HELICA AF B1 low matrix kits.

Finally, preliminary regression models using the natural log of Aflatoxin values from milled maize samples as a dependent variable, and various survey and remotely-sensed environmental covariates as explanatory variables, were able to explain roughly one-quarter to one-third of the variance in the observed levels of Aflatoxin across Kongwa. The inputs to the various regression models tested are summarized in tables 2 and 3 on the next page. Essentially, remotely-sensed environmental values that were used as covariates were obtained as follows: The gps waypoints representing the mills where sampling occurred were plotted on a map, and a buffer area of 8km radius was drawn around each. The buffer areas were then used to extract rainfall, elevation, slope, and aspect values from raster layers of each variable (1km^2 pixel size), which were obtained through various publicly available online data sources.[8] The most realistic model seems to be using sample-level data (i.e. all 128 data points, each plotted at the gps waypoint representing the mill closest to where it was collected) with the explanatory variables: Pre-Harvest Historical Rainfall estimate, Peri-Harvest Historical Rainfall estimate, Aspect (a categorical variable with each category representing the direction in which the slope of the landscape is facing at the mill point), and the source of the grain sample (purchased or homegrown). This model yielded an R^2 value of 0.296, 6 predictors significant at alpha=0.05 and a regression F test significant at alpha=0.001.

To obtain this model, a stepwise regression test that minimized the Akaike information criterion was performed in R using the step() function from the “stats” package. The step() function allows the user to specify criteria to select a model in a stepwise regression, while cycling through all possible ordered subsets of a specified list of predictors. Table 3 shows the results of several stepwise tests performed in this manner, using both min(AIC) and min(BIC) (Bayesian Information Criterion) as selection criteria. It is perhaps worth noting that models using mill-level averages of AF observed in milled maize yielded similarly significant model outputs, but with higher R^2; however, using the sample-level model is the more appropriate choice, due to unequal representation of mills in terms of sampling coverage, and a ‘false flattening’ of the Aflatoxin values’ distribution when averaging at the mill-level was performed.

Still, these models are under development and more detailed stepwise regression output, with the ability to see the marginal effects of each additional predictor being considered, will be helpful in understanding their broader meaning. Furthermore, these models may introduce false significance through both the representation of aspect as a simple categorical covariate, and through a failure to consider the mills’ location as its own random effect. Making these changes will allow us to better understand the potential for environmental covariates to be used in geographic modeling of Aflatoxin in the Kongwa area, and more broadly in various landscapes of East Africa where mycotoxin contamination is prevalent.[9]

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[8] Data sources listed in table 2. Insert full citation later.

[9] Citation and footnote here for IGERT team’s 2015 paper as the basis for this work in GIS analysis of mycotoxins and environmental factors.

Table 3: Metadata on variables used in linear models for Aflatoxin:

Variable	Value	Data Type	Data Source
Historical Pre-Harvest Rainfall (December-April)	8km Buffer Mean	~1km raster	WorldClim
Historical Peri-Harvest Rainfall (May-July)	8km Buffer Mean	~1km raster	WorldClim
Elevation	8km Buffer Mean	~1km raster	CGIAR-SRTM
Slope	8km Buffer Mean	~1km raster	CGIAR-SRTM
Aspect (categorical)	Dominant category in 8km buffer	~1km raster	CGIAR-SRTM
Source of Sample	Homegrown or Purchased? (Mills: p(Homegrown))	Survey Data	Nov. 2015 Kongwa Survey
Mycotoxin data	ppb value for AF	Survey Data	Nov. 2015 Kongwa Survey

Table 4: Summarizing output of various candidate models for AF in milled maize:

Data	Model Selection Method	Predictand	Predictors	Significant Predictors	R^2 Value	Model Significant? (p-value)	F test (comparing AIC vs. BIC) p-value
Mill-level	min(AIC)	AF	Pre-Harvest Rainfall, Peri-Harvest Rainfall, 5-category Aspect	6 of 7	0.498	Yes ; 0.0039	
	min(BIC)	AF	Pre-Harvest Rainfall	1 of 1	0.274	Yes ; 0.0018	0.0725
Sample-level	min(AIC)	Ln(AF)	Pre-Harvest Rainfall, Peri-Harvest Rainfall, 5-category Aspect, Source (homegrown or not)	6 of 8	0.296	Yes ; $3.5e^{-7}$	
	min(BIC)	Ln(AF)	Pre-Harvest Rainfall, Peri-Harvest Rainfall, Avg. Slope, Source (homegrown or not)	4 of 4	0.254	Yes ; $2.5e^{-7}$	0.0740

IV. Discussion and Conclusions:

Our results from mill and household surveys in Kongwa in November 2015 give us significant reason to believe that Kongwa's mycotoxin contamination in staple foods is of concern, and make Kongwa a suitable site for a larger study of these issues. The data reviewed above make it clear that there is a substantial mycotoxin contamination problem in Kongwa's food system and that the contamination is widespread, across sources of food and across villages with different characteristics. It is important to note that the difference in mean aflatoxin levels across homegrown vs. purchased maize has been commonly recorded in various regions of eastern Africa. Furthermore, our discussions with villagers, millers and traders in Kongwa suggest similar reasons why market-purchased maize may be of lower quality; namely, farmers with larger harvests may be able to be more selective in deciding which grain they store for their own consumption, and which grain gets sold on the market. Furthermore, inadequate storage of already lower-quality grain could further contribute to the market maize's mycotoxin issues, as grain could be increasingly contaminated as time progresses between harvests. Further investigation of these market and seasonal dynamics could therefore be a valuable part of a food system surveillance program in Kongwa, a district which exhibits a variety of market and village types as well as a variety of behavior patterns related to how village dwellers obtain food.

From a trial standpoint, Kongwa also benefits from being of sufficiently large population for an entire study to take place within its borders and yield adequate size cohorts of mothers and babies. Of the 87 villages in Kongwa, 74 have a population of between 1,000 and 6,000 people, with most of these villages being fairly removed from main roads and larger, peri-urban town centers. Likewise, it is appropriate to plan for coverage of roughly 60 of these villages during a trial, and during district-wide food systems mycotoxins surveillance exercises. This is beneficial for the trial as these characteristics likely result in a food supply that is comparatively more at risk of mycotoxin contamination, according to our notes with key informants from the Agriculture and Nutrition offices in Kongwa's district government, and from first-hand observations of community dynamics we were able to make while conducting our sampling. In gathering samples and data from respondents, we pointedly avoided villages that were either too urban or too close to a main road, in order to collect samples we thought best represented our target, 'at-risk' population.

Furthermore, the high levels of aflatoxin observed in groundnuts and lishe flours are important for several reasons. First, visual groundnut sorting was a commonly recorded household practice among the villagers our team spoke with in collecting samples, but the high levels of AF in the samples we collected suggest that this practice is, at least, not effective enough. In addition, the fact that both lishe and groundnuts showed a higher average level of AF contamination than maize suggests that for an intervention to be truly effective, it will have to involve a component of surveillance and mitigation of AF in non-maize samples. As discussed above, some of the key next objectives for further study and innovation will be related to assay protocol validation and development, such that a longer-term study can operate on a reliable protocol for AF in milled maize as well as comparable protocols with adequate internal standards and controls for testing for FUM, and for testing for both toxins across a range of non-maize sample types. Additionally, further experimentation with geographic analysis and modeling of the environmental, agronomic, and socio-economic drivers of mycotoxins exposure risk deserves extra attention as this study and others work toward systematizing control strategies for mycotoxins in staple food value chains of Tanzania and other sub-saharan African countries.

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