FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

FEED THE FUTURE INNOVATION LAB FOR THE REDUCTION OF POST-HARVEST LOSS

PHLIL NEPAL BUY-IN FINAL REPORT

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FEED THE FUTURE INNOVATION LAB
Post-Harvest Loss Reduction

KANSAS STATE UNIVERSITY

Where PHLIL works:

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Kansas State University: lead (Dr. Jagger Harvey); mycology (Prof. John Leslie), mycotoxin, agricultural economics (Dr. Jisang Yu); planning and support (Dena Bunnel, Caroline Kolins, Catherine Hickman)

University of Nebraska-Lincoln (Dr. Andreia Bianchini and team): mycotoxin/mycology analysis, capacity building

Nepal Development Research Institute (Dr. Jaya Gurung and team): survey design and implementation, lab establishment, sample analysis, policy linkages, in-country lead

Helen Keller International (Dale Davis and team): survey implementation lead

Nepal Academy of Science and Technology (Jaishree Sijapati and team): mycotoxin laboratory establishment and hosting, shared research capacity

CSIRO Australia (Ross Darnell): modelling, sampling, stats

Nutrition Innovation Lab (Prof. Patrick Webb, Drs. Shibani Ghosh, Robin Shrestha, Joanna Andrews-Trevino, and team): Diet-exposure/stunting association (Banke), Tufts University and partners

Mars Global Food Safety Center (Drs. Bob Baker, Guangtao Zhang, Abigail Stevenson and team): collaborations on lab design, capacity building, research, intervention identification, and funding

ISPA (Dr. Antonio Logrieco and team): multi-mycotoxin/mycology analysis: pilot snapshot

Romer China: mycotoxin kit and equipment donation, technical information

Tribhuvan University (Prof. KC Gopal, Ram Kumar Shrestha): research and capacity building

Broader stakeholder group: initial and ongoing consultation, including August priority setting workshop. Includes key input from Dr. Sabnam Shivakoti, Dr. Matina Baidya and Krishna Rai, collegues at NARC, Plant Quarantine, Ministry of Agriculture, Ministry of Health and others.

Report Outline:

I. Background and project summary

Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss Buy-In Project

Nepal ranks among the poorest and least developed countries in the world. High rates of chronic malnutrition and stunting among children are of significant concern. Every person's Right to Food is an unalienable and fundamental provision in the newly ratified Constitution of Nepal. It is reinforced by a policy of supporting sustainable agricultural production and effective distribution of food. This is evident through the formulation and implementation of the Agriculture Development Strategy (ADS) and the Multi-Sectoral Nutrition Plan (MSNP I and II) both of which aim to reduce poverty and enhance food security and nutrition through multi-sectoral means.

Approximately one third of food produced globally is lost or wasted. Developing countries, including Nepal, suffer from high levels of post-harvest loss. Proper drying and storage practices can effectively address mycotoxins as one category of these losses, as well as post-harvest losses more broadly. Given the investments and hard-won increases in crop production, reducing post-harvest losses is a low hanging fruit with high, short-term potential to enhance the nutritional status and resilience of households.

A key area that cuts across nutrition, food security and economic development is food safety. Challenges to food safety impact the health and economic vitality of millions of people in low-and-middle income countries around the world. Problems relating to food safety contribute significantly to ill health, food-borne disease outbreaks, and even mortality. These are dangers of particular concern in relation to poor households, especially for pregnant women, newborns and infants under the age of two.

Contamination of food and feed by fungi and harmful chemicals they produce (mycotoxins) is detrimental to health, agriculture, trade and the environment. It has been estimated that one of these toxins, aflatoxin (produced by *Aspergillus* fungi) contaminates up to a quarter of the global food supply, threatening the health of more than 4.5 billion people globally. Recent evidence suggests that mycotoxin contamination in the food supply may play a significant role in stunting children's development. In humans, mycotoxins cause cancer and are associated with child stunting, immune system suppression and nutrient uptake inhibition.

In Nepal, Department of Food Technology and Quality Control (DFTQC) regulates mycotoxins under its remit, and has set limits of 20ppb and 50ppb for aflatoxin contamination of food and feed, respectively. Previous studies published by the Nepal Agricultural Research Center, Tribhuvan University and others, revealing that mycotoxins including aflatoxin are an issue in the Nepali food supply. While these handful of studies establish that mycotoxins are present, the complex, dynamic and pervasive nature of mycotoxin contamination requires a broader and more intensive study to adequately characterize and chart the way forward to reduce mycotoxin contamination in the Nepali food system.

Recent findings published by the Nutrition Innovation Lab (NIL) report widespread aflatoxin (a particularly damaging mycotoxin) exposure among pregnant women in Banke, a mid-western district (of Province 5) in Nepal with some of the highest rates of stunting. Digging deeper into the sources of aflatoxin exposure, the Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss (PHLIL) led this USAID-funded project, with NIL as a key collaborator, to find the sources, causes and innovative solutions to mycotoxin contamination in the Nepali food and feed supply.

The PHLIL Mycotoxin Assessment Buy-In Scope of Work laid out the following objectives:

> ***Specific Objective 1:***
> *To screen for mycotoxin content in maize, groundnuts, rice, chilies, wheat, selected spices, and animal feed products (poultry and livestock); and assess mycotoxin content in greater detail in the subset of identified higher-risk commodities in follow-up surveying.*
>
> ***Specific Objective 2:***
> *To determine the types of toxigenic fungus species and subsequent mycotoxins present in selected commodities, and at a modest pilot scale in associated soil that could contaminate food supplies.*
>
> ***Specific Objective 3:***
> *To determine ecological distribution of toxic fungi in relation to mycotoxin production.*
>
> ***Specific Objective 4:***
> *To investigate mycotoxin prevalence in various crop storage systems in Kathmandu Valley and Nepalgunj, and if present, to make recommendations for how to reduce the prevalence of mycotoxins in storage systems. More specifically, to explore the applicability of systems designed to reduce the prevalence of mycotoxins in other regions of the world, and to gauge whether or not these existing tools or approaches would potentially be feasible and effective in Nepal.*
>
> ***Specific Objective 5:***
> *To build the capacity of, but not limited to, NAST, Nepal Development Research Institute, Tribhuvan University, the Agriculture and Forestry University, DFTQC, and the relevant research division in NARC. Primary partners will have intensive capacity building, given that they will execute the processing and laboratory analysis of survey samples; and others will receive more targeted information and training.*

By design, the PHLIL Buy-In project approach simultaneously empowered and equipped the National Agricultural Research System, enhancing their human and institutional capacity to address this complex challenge within and beyond the project lifespan. This included:

- establishment of a context-appropriate mycotoxin laboratory at the Nepal Academy of Science and Technology's (NAST) Biotechnology Research Center in Kathmandu, in collaboration with the Mars Global Food Safety Center;

- a leading technical role for the Nepal Research Development Institute, established as a primary means of mainstreaming research findings into Government policy;
- immersive training of six members of the Nepali technical team at the University of Nebraska-Lincoln (UNL), Kansas State University and at Mars facilities in India;
- hosting a Nepali researcher at UNL to validate testing methods and analyze samples in parallel with NAST;
- integration of mycotoxins into the Tribhuvan University curriculum and research capacity;
- collaboration with NIL to characterize the associations between dietary exposure to aflatoxins and health outcomes, in Banke;
- and an opportunity for downstream integration of survey efforts and interventions into Helen Keller International's broader efforts to improve nutrition in Nepal, currently including several million beneficiaries.

A further suite of unique research elements was included in this project, which are broadly scalable and will accelerate the findings towards achieving improved health, nutrition and economic outcomes. Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) is providing senior research expertise and in-kind resources to develop risk maps as tools to estimate and address overall aflatoxin risk across key districts in Nepal. Since contaminated commodities may simply be dumped or shunted for consumption by the most vulnerable consumers in society, exacerbating the problem, agricultural economic questions around incentives and alternatives are a central part of the survey design. In further collaboration and with co-funding from Mars Inc., a metagenomics approach is being adapted for use in characterization of fungal populations in crops and soil, which could hold preventative and surveillance insights into tailored and responsive mycotoxin mitigation strategies.

From the surveys underpinning these analysis, PHLIL collected a total of 3,215 samples from markets (1,252) and households (1,963) across 20 districts of Feed the Future's "Zone of Influence", in addition to 211 samples analyzed in an initial market snapshot. The SOW specified 2,000, however the project team went to great lengths to expand this by 1,426 samples (surpassed by 71.3%), to enable interrogation of the full set of questions as thoroughly as possible. More broadly, co-funding from many if not all of the institutions involved exceeded that initially pledged, due to the strong commitment of the teams and their institutions. Surveys were carried out pre- and post-monsoon season. The market survey collected information related to cleaning, sorting and storage practices in the market, while the household survey characterized both pre- and post-harvest practices in each household. Samples of maize, groundnuts, dried chilies, ground chilies, rice, soy nuggets, animal feed and complementary foods for children were collected.

Project results and partnerships are already moving towards solutions. Survey results revealed unsuspected, commonly consumed chilies as a potential culprit (analysis was conducted by Italy's Institute of Sciences of Food Production, linking this project to the European Union mycotoxin network). In a PHLIL program "South-South" collaboration, PHLIL researchers at Bangladesh Agricultural University attended the 2018 Nutrition Symposium in Kathmandu to feature drying and storage solutions identified in the broader PHLIL project, in direct response to requests from the Ministry of Agriculture and others for immediate solutions. The BAU team trained twenty-eight Nepali scientists from government and private organizations on the proper use of the BAU-STR dryer. After the training, one dryer was handed over to Helen Keller International (HKI) and another one to the Nepal Agricultural Research Council (NARC). These two dryers are now in the hands of researchers and development practitioners, as a viable solution for arresting mycotoxin development due to poor drying practices, with requests for purchase by development projects in the ZOI.

The mycotoxin research laboratory at NAST and its now well-trained mycotoxin technical team represent a key component of the sustainable impact of this project. Selection of NAST was conducted in consultation with the USAID Nepal Mission and the USAID Bureau for Food Security after visiting several of the major research institutions in Kathmandu. The PHLIL project mycotoxin laboratory at NAST is an adapted version of the mycotoxin analysis laboratory installed in Mars Inc. factories around the world, coupled with aspects of other PHLIL-supported labs established in the program's six other project countries. The design represents an out-of-the-box blueprint that can be deployed at other institutions in Nepal and in other Feed the Future countries.

PHLIL team members have also provided trainings to Nepali scientists on scientific writing, mycotoxin theory and lab procedures, and risk communication. Later in the project, the Nepali technical team members independently led a short training for other Nepali institutions in mycotoxin analysis, backstopped from afar by the international team, marking the maturation of the NAST lab as a leading capacity building and research platform for Nepal. To further ensure that the Nepali mycotoxin team and broader national system are equipped to constructively communicate about mycotoxins, concurrent discussions about risk communication are underway.

The outcomes of this project include the country of Nepal using now-enhanced capacity to safeguard their food supply to improve nutrition nationally, backed by mycotoxin evidence and technical leadership and expertise within the national system. Beyond Nepal, the insights gleaned from this project can help extend similar spillover benefits to other Feed the Future countries. The project is intended to provide foundational findings on this link between agriculture and health, informing agriculture and health investments by the international community. The capacity development, research strategies and innovations will be scalable to help better address the one quarter of the global food supply at risk of contamination by aflatoxin. Moreover, research findings will better equip us to safeguard against the recently estimated $1.68 billion in potential aflatoxin-related losses to US corn production, in potential years of bad climatic conditions.

This project has established a robust evidence base, cultivated national research capacity and helped inform and foster a growing multi-sectoral community to address mycotoxins into the future. Poor agricultural production, harvest and post-harvest practices, vulnerable value chains, trade barriers, and poor transportation and infrastructure increase the risk of mycotoxin contamination (including aflatoxins) and create barriers for control. To effectively and sustainably reduce the specter of mycotoxin exposure in Nepal, we are collectively working towards informing sound policy, underpinned by robust evidence and national research capacity; as well as a coordinated mycotoxin mitigation strategy involving multi-sectoral, multi-level collaborations. Specific agricultural practices and recommendations are covered below and in recommendations, findings and various annexes (I, VIII – XII). Understanding the challenge of abating mycotoxins within the food system is critical in achieving the goals laid out by the MSNP II and USAID CDCS. Indeed, it is key to Nepal's achievement of the SDGs, since good health and nutrition represent fundamental platforms on which so many other SDGs have to build.

It is important to note that addressing mycotoxins has become a pressing global issue, with many research groups and national systems fervently working towards innovative and sustainable mitigation strategies. In this sense, and through the broader collaborations of the Innovation Labs and other partners here, Nepal does not stand alone in confronting this challenge. Countries here in the region and around the world, including the USA, are actively working at reducing mycotoxins in the food and feed chains, and consequent exposure and adverse health effects. PHLIL has now worked at characterizing and addressing mycotoxins in Afghanistan, Bangladesh, Ethiopia, Ghana, Guatemala, Honduras, Nepal and beyond. PHLIL stands ready to continue supporting Feed the Future and in-country partners to secure a safe and nutritious harvest, to feed our collective future.

Overall conclusions:

1. Aflatoxin was an issue in many different food and feed commodities, with maize and groundnut being high risk, chilies and soy nuggets being medium, and rice and wheat-based infant weaning formula being low. This does not capture potential risk levels for other mycotoxins.
2. The Nepali agricultural value chain actors are generally a naïve population with respect to appropriate GAP to keep food and feed safe from aflatoxin contamination:
    i. Farmers and storekeepers felt that they are generally observing good harvesting, storage cleaning and storage practices. Practices and resulting aflatoxin contamination issues tell a different story.
    b. Farmers who report a higher awareness about fungal-related issues in their stored grains were actually storing longer, and were not observing GAP.
3. Improved drying technologies are not in use by Nepali farmers in the districts surveyed, apart from using a tarp rather than drying on uncovered ground: 99.8% of surveyed farmers used sunlight to dry (one farmer reported "other"). Even for the basic, inexpensive technology of using a tarp for drying, rather than placing maize on uncovered ground (where mycotoxin-producing fungi reside, not to mention other food safety hazards such as animal feces) is not used by the majority of farmers (57.8% reported drying maize on bare ground).

4. Improved storage technologies that prevent post-harvest losses including aflatoxin contamination are essentially absent in these farming communities.
5. An association between aflatoxin in infants' food and blood serum aflatoxin levels and/or stunting was not established. However, the wealth of information gathered from the broader PHLIL study, as well as from NIL's extensive work, nonetheless charts a path forward to mitigating aflatoxin contamination and the negative health effects in the Nepali population. We *do* know from the broader work: risk levels for aflatoxin contamination in Nepal for various foods and feeds; geographic areas, climatic features and practices that increase risk of aflatoxin contamination of maize (risk mapping and broader survey) and other commodities; current practices along the value chain, from farm to market, including those that are associated with higher aflatoxin prevalence in multiple food commodities and/or are known to increase risk of aflatoxin contamination; and stakeholder-informed and –formulated short, medium and long-term strategies to address aflatoxin contamination in food and feed in Nepal.

Policy suggestions (NDRI/NAST/Stakeholder Workshop) for increasing the future provision of safe food to the Nepalese people (see also annex I):

1. Mycotoxin issues should be incorporated in Nepal government policy, so that they are recognized as an important issue by the people and are included in the mainstream of regular programming.
2. A mycotoxin testing lab should be established in each province. These labs would be managed by local provincial governments, can focus on toxins of local importance, and collectively provide first line coverage for the national domestic food supply. Mycotoxin tests should be conducted regularly on major products and suspected commodities regularly.
3. Education on food safety issues posed by mycotoxins should be incorporated into secondary school and university curricula.
4. Agricultural technicians, farmers, consumers and agricultural traders should be trained to provide awareness about mycotoxin hazards and mitigation.

   The government should provide a subsidy for recommended/improved drying and storage structures to enable adoption by farmers and/or farmers' cooperatives.
5. Importers and exporters should be made aware of existing mycotoxin regulations and asked to help enforce them to ensure the safety of food being imported into Nepal.
6. A key recommendation from the stakeholder workshop was the call for formation of a national mycotoxin steering committee (potentially an adaptation of the Partnership for Aflatoxin Control in Africa).

Recommended priority areas for agricultural interventions, from research into use, with designations as short term (ST), medium term (MT), long term LT:

1. A comprehensive and targeted awareness creation, extension and training program should be established, following a stakeholder/government/USAID Mission-informed risk communication strategy.
    a. ST aspects include:
        i. experts in the national system already being subject-matter experts, empowered with knowledge generated by this and other projects
        ii. communications/extension materials already available, including the Nepali language SAWBO aflatoxin video, which was very positively received at the national stakeholder workshop
        iii. formation of a national mycotoxin steering committee, or adoption of mycotoxin as a key issue to be addressed by an existing committee
    b. MT aspects include:
        i. national mycotoxin steering committee to synthesize, articulate and seek funding for a national mycotoxin reduction effort
        ii. Continued research to further understand interventions to reduce mycotoxin contamination and exposure at critical points of the food system
        iii. Deploy postharvest mitigation packages along the value chain and across the food system to reduce aflatoxin/mycotoxin contamination, as validated by further ST/MT research, integrated into nutrition and health intervention programs
        iv. Expand risk mapping work to be more predictive of hotspot emergence, and as a decision-making support tool to help stakeholders and the national steering committee identify the best intervention strategies for different target beneficiaries/food system actors
        v. Establishment of satellite mycotoxin laboratories in each of the provinces, with affiliated rapid response teams to measure and mitigate mycotoxin contamination at farms and markets, as issues and hotspots emerge.
    c. LT aspects include:
        i. Deployment of a model agriculture-nutrition program into the Nepali national system, reducing the risk of mycotoxin exposure and responding to emerging outbreaks

2. Research and capacity should be further extended to further characterize multiple mycotoxin threats in the food system, and extend monitoring and interventions for aflatoxin and other priority mycotoxins across the food system
    a. ST Aspects include:
        i. continued research to expand our understanding of multiple mycotoxins in various foods and feeds across the food system. IN PARTICULAR, *Fusarium* toxins (including fumonisin) were found to be a risk in the subset of samples analyzed for multi-mycotoxins and where fungal isolations were performed; there is evidence that these toxins are also associated with stunting, cancer and other negative health effects.
        ii. pilot interventions into use (as the PHLIL program does successfully in its core countries, which could provide a set interventions already validated for use in other Feed the Future countries, providing high-likelihood quick wins)
        iii. piloting the BAU-STR dryer into scaling for rice and maize in Nepal (note that the PHLIL program already used non-Nepal funds to bring the BAU-STR dryer from PHLIL Bangladesh for successful piloting)
        iv. Already identified good agricultural practices (pre- and post-harvest) promoted; the PHLIL survey revealed that almost no improved best post-harvest practices are being practiced, and many have already been validated based on broader research studies (see report and annexes I, VIII – XII); see GAP section below
    b. MT aspects include:
        i. Establishment of risk mapping as a predictive tool, for both decision making for targeting interventions to different areas, and as an early warning tool for emerging aflatoxin hotspots despite best efforts
        ii. Establishment of mycotoxin testing and response laboratories in each province, forming a hub-and-spoke network as prioritized in the national stakeholder meeting
        iii. Enhancing GAP training and tools in the national system, beyond efforts already underway
    c. LT aspects include:
        i. Sustainable establishment of a food systems-level mycotoxin mitigation and monitoring system serves as a model for research for development approaches to further food safety threats
3. Good agricultural practices should be promoted (this should form the basis of the agriculture component of an aflatoxin/mycotoxin risk reduction program, spanning short-to-long terms as capacity and knowledge emerges from 1 and 2):

a. Production level (generally, practices that increase yield and reduce biotic and abiotic stress also reduce risk of mycotoxin contamination in the field):
    i. Reducing residues of past crops in the field, where mycotoxigenic fungi can reside and contaminate the next season's crop
    ii. Proper tilling practices, where appropriate
    iii. use of improved, adapted seed (appropriate variety, especially one that will be more drought tolerant if necessary for the agroecological area)
    iv. appropriate use of fertilizer
    v. proper weeding and pest management
    vi. avoiding drought stress by proper irrigation where possible
b. Peri-harvest
    i. Timely harvesting at physiological maturity
    ii. Avoid harvesting directly onto the soil (reservoir of mycotoxigenic fungi)
c. Post-harvest
    i. Avoid heaping for extended periods of time, since lack of airflow and moisture are conducive to fungal growth and mycotoxin accumulation
    ii. Sorting out and disposing of damaged and moldy cobs/chilies/grains, so they do not contaminate the rest of the harvest
    iii. Proper drying immediately after harvest (note that xx% of the households surveyed sun-dried maize on the ground, so there is essentially no even marginally improved drying in practice); this is a highly effective step in reducing aflatoxin accumulation, and was prioritized highly at the PHLIL Nepal Mycotoxin Stakeholder Workshop
        1. Low cost: dry on a clean tarp rather than on bare ground
        2. More advanced on-farm or coop level: dryers such as the BAU-STR dryer (for rice, maize wheat; donated by PHLIL Bangladesh and successfully piloted by HKI in Nepal) or the Horticulture Innovation Lab chimney dryer (chilies,…; piloted by HortLab in Nepal)
        3. Industrial scale: larger scale dryers are available for mills, warehouses, national stores,…
        4. Moisture measurement to ensure proper drying is key (options include: low-tech/lower accuracy DryCard; high accuracy, fast, multiple commodity calibrated PHLIL GrainMate moisture meter; lower cost/slower to equilibrate FPLIL hygrometer approach)

iv. Proper storage; this is a highly effective step in reducing aflatoxin accumulation, and was prioritized highly at the PHLIL Nepal Mycotoxin Stakeholder Workshop

    1. For grains, clean metal silos or better yet hermetic storage bags (eg, ZeroFly Hermetic, GrainPro, PICS) are highly effective at reducing aflatoxin accumulation in grains; need to be paired with proper drying

v. Mycotoxin surveillance:

    1. Risk mapping: the PHLIL/CSIRO risk mapping tool produced in this project could be refined to help inform

        a. where to deploy different interventions, as a decision-support tool, using historical climatic data

        b. where emerging aflatoxin hotspots are at harvest, to help target mitigation measures to those areas most likely to be affected due to in-season climatic conditions when crops were in the field

    2. A network of testing capacity:

        a. Hub reference laboratory (NAST PHLIL Mycotoxin Laboratory, established by this project)

        b. Satellite more basic laboratories, in each province

        c. Mobile testing capacity, linked to mitigation options with economic value to the grain/food/feed owner when it is found to be over the limit for aflatoxin (eg, gas-mediated decontamination and use as feed)

Note: all of these were discussed as options by stakeholders at the national workshop. See annex VIII for further detail.

Detailed Project Report

This section is organized to present a cohesive and building set of findings. Since many activities under the specific objectives cut across more than one, and are complementary, this section is not organized by Specific Objective.

II. MYCOTOXIN SURVEYS

This objective encompassed an ambitious scope, in terms of both commodities and geography, as well as with the end goal of producing information on mitigation options (overlaps with Specific Objective 4). The approach was designed to gather a broad set of information across the 20 contiguous-district ZOI, on aflatoxin contamination in a range of food and feed commodities, and associated agricultural practices and agricultural economic factors to inform suggested mitigation options and policies. As PHLIL outlined in the Scope of Work: *"PHLIL is seeking to design a tiered, integrated survey, allowing both: a robust assessment of mycotoxins in the specified commodities; as well as information gathered on postharvest practices and associated economic, gender and nutrition-related factors (on a scale commensurate with the already ambitious scope of the survey and available funding. This will provide a more conclusive snapshot of contamination within the seasons surveyed, and enable the team to draw more specific conclusions to inform short-, medium- and longer-terms measures to be taken in mycotoxin mitigation by the Mission, GON, researchers, development agents, and other actors."*

Previous studies

Researchers within the national system and internationally had established some understanding of mycotoxin contamination of food in Nepal, including aflatoxin. Overall, their scope was fairly limited to better understand contamination across the food system, as is typical of most mycotoxin surveys since costs and complexity associated with broader surveys can be prohibitively high. Nonetheless, these studies were an important part of the considerations for design of the PHLIL surveys, and included information on contamination in the Kathmandu Valley.

The United Nations Food and Agriculture Organization and United Nations Environmental Program's Regional Monitoring of Food Contaminants Project conducted the first survey of aflatoxin in Nepal. This study collected and analyzed samples from Nepal, as well as India, Pakistan and Sri Lanka, from 1980-87. Karki and Sinha (1989) at the Central Food Research Laboratory, Ministry of Agriculture, reported the results of aflatoxin contamination of maize, groundnut and poultry feed in Nepal, as part of this study. The report does not specify the methodology for sampling, processing or analysis. Overall, they found aflatoxin levels exceeding 30ppb in 6% (35/582) of the food commodities tested.

Koirala *et al.* (2005) reported on aflatoxin in samples from Eastern Nepal, collected from 1995-2003. They used thin layer chromatography (TLC) to measure aflatoxin, a method that provides less accurate values (arguably semi-quantitative) than other methods – for example Enzyme-Linked Immunosorbent Assay (ELISA), High Pressure Liquid Chromatography (HPLC), Immunocapture Fluorometry, and others. Nonetheless, TLC can provide invaluable information, especially when a food system is virtually uncharacterized, or when exact measurements are not needed (eg, screening a field trial or *Aspergillus* isolates for relative toxigenicity). Of the 832 samples, 32.8% were contaminated with aflatoxin, with 18% estimated to be above the then-maximum limit of 30ppb.

Peanuts (34%), peanut butter (42.5%), maize grit/flour (31.9%), and cornflakes (31.5%) had the highest levels of aflatoxin contamination. These results indicated that like in other countries, maize and peanuts/groundnuts are particularly susceptible to aflatoxin contamination in Nepal.

Gautam *et al.* (2008) found aflatoxin contamination in maize, groundnuts and chilies from the Kathmandu valley; 14 chili (54%), 18 maize (60%) and 24 groundnut (75%) samples were above the maximum permissible level in Nepal. The sample size and geographic focus, as well as single sampling time, were relatively modest, however together with additional studies outside Nepal, and the PHLIL team observations during scoping visits (particularly observations by Distinguished Professor John Leslie), it does suggest these three commodities should be included in the survey.

These disparate results across studies demonstrate the skewed and stochastic nature of aflatoxin contamination that calls for surveys of broader scope across the food system. Because of this highly skewed and seasonally variable nature of mycotoxin contamination, a risk mapping approach would transform our ability to assess and monitor aflatoxin risk across geographies and seasons, well beyond the capacity of and complementing physical surveys and monitoring. Based on this, PHLIL integrated this aspect as an addition to the initial draft of the SOW.

Collectively, these previous studies do help provide an important foundation for the design of the PHLIL survey, and their relatively modest scope and other sampling/analytical consideration further validates the need for a broader food system survey capturing multiple commodities over more than one season or agroecology/district. While we are not questioning any individual food/feed aflatoxin survey, when considering mycotoxin results from any set of studies there are some key methodological considerations: sampling error can account for up to 90% of the error in aflatoxin test results (Whitaker 2003); robust validation of laboratory Standard Operating Procedures is necessary and too frequently not conducted, leading to possible false negatives/positives or inaccurate measurements; and institution of a regular technician proficiency testing regimen, to detect any human or equipment/reagent-based error, is often not conducted. Establishment of a central hub/reference laboratory, accessible to researchers across the national system, would greatly enhance the capacity of the Nepali national system.

Human exposure to aflatoxin was the basis for the PHLIL project. The Feed the Future Nutrition Innovation Lab (NIL) reported that aflatoxin exposure was detected in 94% of pregnant women in Banke District, by detection of AFB1-lysine adducts in blood serum (Andrews-Trevion et al., 2019). With this finding, investigation of sources, drivers and intervention strategies in the food and feed system was prioritized as a next step.

While aflatoxin was the primary focus of the PHLIL Buy-In, and the survey scope was already ambitious with that focus, there is reason to suspect other mycotoxins in the food supply that may similarly impact nutrition and health. Desjardins *et al.* (2000) reported that several mycotoxigenic (mycotoxin-producing) *Fusarium* species are present in maize and wheat in Nepal. They used immunoassays and HPLC, reliable quantitative methods. Fumonisins were above the common maximum limit of 1000 ng/g in 22% of maize samples, as well as nivalenol (NIV) and deoxynivalenol (DON) in 16%. It is notable that only 74 maize samples were tested, a low number to do anything but establish whether a mycotoxin is present in a food or feed commodity, but certainly a basis for more extensive surveying. Mycotoxin levels in wheat were comparatively low. In terms of practices that might reduce mycotoxin levels, they also found that 12 Nepalese women using traditional sorting of discolored, moldy or broken grains of maize successfully reduced mycotoxin levels. Of course, it is of paramount importance in the food system to consider the ultimate end-use of the highly contaminated grains that were sorted out.

It is striking that Karki and Sinha (1989) noted that the "aflatoxin problem as revealed by this study is of no serious magnitude." The work from NIL, as well as the results of the PHLIL mycotoxin survey and other studies clearly indicate that this is not correct, and that detrimental levels of aflatoxin exist in the food system in Nepal, warranting strong action that the national system is now galvanized to take due to the leadership from the Government of Nepal as well as the USAID Mission and Feed the Future. Historical context is also important, and we must consider that statement in its time. Mycotoxin- and aflatoxin-related global awareness in the research and development communities, as well as the evidence base itself, has grown vastly since 1989. All key stakeholders now recognize aflatoxin as a key issue to address.

PHLIL survey design

For the PHLIL survey, the main objective was to cast as wide a net as possible to identify the sources and potential interventions for avenues of human exposure to aflatoxin consumption. The project team also sought to avoid expanding the scope so wide that any particular set of samples was so small that it would undermine the ability to draw statistical conclusions or conduct risk mapping.

Accordingly, key design considerations included:

- multiple times of year (multiple survey rounds), capturing food at different times after harvest to maximize the opportunity to capture potential contamination issues; also, NIL had observed seasonal fluctuation of AFB1-lysine adducts in maternal serum, further underpinning the need for more than one timepoint
- broad coverage of districts across agroecologies, across the Zone of Influence
- capturing the food system in all districts, at households and different types of markets (a survey covering households in all districts would be a tremendous undertaking, well beyond resources available; markets were used as a more easily accessible catchment of the local food system, ensuring sample collection in each of the 20 districts within the resources available)

- multiple food and feed commodities (the design employed a phase approach, adapting as more information was gathered from each round)
- a design in which the Nepal Academy of Science and Technology could play a leading role in sample processing, with University of Nebraska-Lincoln taking the lead on capacity building and final analysis of many samples (otherwise it would not be possible to establish the NAST lab, train the team and analyze all samples)

After extensive discussion and scenario-planning around these considerations, the broad ZOI surveying was comprised of three phases (1.1-1.3):

1.1) pilot snapshot market survey early in the project, to inform food and feed commodities to be included in the full-scale survey.

1.2) A subsequent first round of full-scale surveying markets (in municipalities and rural municipalities) in each of the 20 districts in the ZOI, and households in four "sentinel districts" across the hills and terai (representative of other districts, since a full household survey across the ZOI would be prohibitively expensive and involved to undertake in the scope of the Buy-In).

1.3) A second round of market and household surveying, similar to (2), but informed by the aflatoxin levels in food and feed commodities collected in (1).

1.4) In addition to these surveys, PHLIL collaborated with NIL to assess levels of aflatoxin in potentially contaminated food and feed commodities in a subset of AflaCohort II households. (see Banke/NIL below)

Banke District/Nutrition Innovation Lab study:
conducted in parallel (designed after ISPA pilot snapshot, and before full-scale ZOI survey and ranking of food/feed commodities for relative aflatoxin contamination risk)

Conducted two surveying rounds in Aflacohort II Banke households, in collaboration with NIL and with HKI. Given that a smaller subset of households had maize, and especially groundnut, in AflaCohort experience, the survey team first targeted households that previously had produced/stored groundnut in earlier Aflacohort survey rounds; this helped ensure as many potentially contaminated samples (especially groundnut) from households were collected as possible. After this purposive sampling, other households were randomly selected from the as-yet-not surveyed Aflacohort II households with infants who had not aged out.

Mycotoxin survey findings – ZOI-wide assessment:

1.1 Pilot snapshot market survey to inform food and feed to be included in full-scale survey:

The project team conducted a pilot snapshot multi-mycotoxin, multi-commodity survey round to inform food and feed commodities for inclusion in full-scale market and household surveys. Pilot snapshot samples were collected from markets in a subset of readily accessible markets in the Zone of Influence, to facilitate collection and analysis as early in the project as possible. Sample types were selected based on knowledge of the PHLIL technical team, including visual observations on site visits and commodities previously reported to potentially have mycotoxin issues; maize and groundnut were excluded since they are "usual suspects" that commonly have aflatoxin contamination issues globally. This initial pilot snapshot succeeded in identifying chilies and soybean cake/nuggets as additional commodities for inclusion (not "usual suspects").

Results from the ISPA project report (Antonio Logrieco, Ceronica Lattazio, Giancarlo Perrone):

The samples were screened for major mycotoxins, namely: deoxynivalenol, T2/HT2 toxins, zearalenone, aflatoxin B1, fumonisins, ochratoxin A, 3/15 acetyl deoxynivalenol, enniatins (A, A1, B, B1) and beauvericin. For these purposes a method based on solid phase extraction clean up followed by liquid chromatography – high resolution mass spectrometry has been used.

LC-HRMS method detection limits are:
DON: 100 μg/kg
3/15-AcDON: 10 μg/kg
HT2/T2: 10 μg/kg
ZEA: 10 μg/kg
AFB1: 1 μg/kg
OTA: 4 μg/kg
FB1/FB2: 10 μg/kg
ENNA, ENNA1, ENNB, ENNB1: 10 μg/kg
BEA: 10 μg/kg

The analysis of fungal contamination of the samples was done by diluting 10 gr of grounded samples in 90 mL of sterile distilled water, 100 μl aliquots of appropriate serial decimal dilutions were plated, in triplicate, on DRBC medium (Dichloran Rose-Bengal Chloramphenicol Agar - DRBC, Oxoid). DRBC plates were incubated at 25 °C for 3-5 days.

Results of chemical and mycological analysis are reported (additional tables in Annex III).

General comments:

- Aflatoxin B1

The incidence of positive samples per each analyzed commodity is reported below. Soybean cake and chilies (green and dry) showed the highest incidence of AFB1 contamination.

commodity	no. positives/total samples
Rice	0/27
Chili – green	9/25
Chili – dry	6/26
Cumin	1/26
Coriander	0/28
Cardamom – small	1/25
Cardamom – large	0/27
Soybean cake	16/27

Table 1: Aflatoxin positives by food type.

A good agreement between aflatoxin contamination and *A. Flavi* presence was observed, with a few exceptions. AFB1 contamination levels in positive samples ranged from 0.3 and 6.1 μg/kg (the EU maximum permitted level in spices is 5 μg/kg), however 3 highly contaminated samples were identified (NPJ 4-1, cumin, **99.2** μg/kg; BGL 3-1, chili dry, **25.8** μg/kg; KLG 6-2, cardamom small, **17.2** μg/kg).

- **Ochratoxin A**

Generally no OTA contamination was revealed, with the exception of 2/26 chili dry samples and 1/25 cardamom small sample.

- *Fusarium* toxins

No *Fusarium* toxins were detected in all analyzed commodities but Cardamom small. Interestingly 16/25 cardamom small samples were found to be contaminated by fumonisins. These findings will be further investigated.

Full scale ZOI survey, general design

Following this pilot snapshot, conducted a first round of full-scale surveying across the Zone of Influence, in: households in 4 sentinel districts, and markets in all 20 districts (municipalities and rural municipalities).

Activities included:

1. Semi-structured questionnaire
    a. Demographic information
    b. Characterization of pre- and post-harvest agricultural practices (household survey)

c. Characterization of storage, cleaning, sorting and storage practices (market survey)

2. Sample collection (using sampling SOPs to help ensure representative samples due to highly skewed nature of aflatoxin contamination across and within samples)

Sampling Strategy – selecting market stores and households

1. Store selection: (76+20) x (1 ~ 3) stores
   a. **All municipalities (76)** will be selected
   b. Randomly select **one rural municipality** from each district **(20 rural municipalities).**
   c. For each selected municipality and each selected rural municipality, find the center of the main market place.
   d. Spin a bottle and follow the bottle's direction (or road's direction that is closest to the bottle's direction).
   e. Visit the first store
      i. Municipality: Visit the second store. If all three commodities (maize, dry chili, and groundnuts) are sampled finish the survey with the second store. If not, visit the third store (the third store can be nearest push cart from the third store).
      ii. Rural municipality: Finish the survey if all three commodities (maize, dry chili, and groundnuts) are sampled. If not, visit the second store (the second store can be nearest push cart from the third store).

2. Store selection: 4 x 2 x 2 x20 = 320 households.
   a. Decide **4 districts**
   b. Randomly select **one municipality and one rural municipality** from each selected district.
   c. Randomly select **2 wards from each selected municipality** and from each selected rural municipalities.
   d. Divide each ward into **four quadrants.**
   e. Go to the center of each quadrant.
   f. Spin a bottle and follow the bottle's direction (or road's direction that is closest to the bottle's direction).
   g. Visit the first household.

h. Ask the first household to give **the list of 4 nearest neighboring households**. Survey **5 households** total in each quadrant – **20 households** total in each ward.

Executed ZOI-wide survey summary:

1. Market Survey
    - Two rounds: Round 1 (March - April 2018) and Round 2 (October - November 2019)
    - A total of 71 municipalities present in the all 20 districts in ZOI were surveyed. In every municipality, 2 stores were surveyed. One rural municipality was chosen randomly from each district. In every rural municipality, 1 - 2 stores were surveyed. We ended up with 270 stores in Round 1 and 249 in Round 2 (No overlapping stores across the rounds).
2. Household Survey
    - Two rounds: Round 1 (March - April 2018) and Round 2 (October - November 2019)
    - Four districts: Dang, Salyan, Kailali and Dadeldhura (Banke – assessed separately as a collaborative project with NIL, distinct research questions and design elements; not included in the agricultural economic analyses)
    - We randomly choose 20 households/ward, 2 wards/local unit, and 2 local units/district: 320 households are surveyed.
    - We track same households over the rounds (Attrition: 14 households).

Total analyzed samples:
Of the 3,215 samples collected in the full-scale surveys, a total of 3,197 were analyzed (in addition to the 211 in the pilot survey). The 0.56% (18) samples that were collected but non-analyzed were mainly due to exclusion of green chilli (so few were collected that it did not warrant the effort/expense to validate the analysis Standard Operating Procedure), and a few samples where weights were too small to analyze. Given that the Banke households were surveyed in collaboration with NIL, and needed to follow their timeline of engaging the cohort households, these were opportunistically sampled; the sampling ended up out of sync with the main survey, which on its own was a massive logistical undertaking and could not be shifted, so the two sample sets were analyzed and are reported separately. Given that aflatoxin can accumulate progressively the further from harvest it is stored, the best practice for conclusive results involves synchronized collection and comparison.

Round	Location	Commodity							Sub/totals
		Comp. food	Maize	Rice	Soy nugget	Chili	Ground nuts	Feed	
R1	Households		305			157	13	262	
R1	Market		147			225	163		
R1*	Banke (HH)	123	33	165		149	2		
R1 subtotals		123	485	165	-	531	178	262	1,744
R2	Households		298		20	175	21		
R2	Market		135		191	187	166		
R2*	Banke (HH)		68		27	139	26		
R2 subtotals		-	501	-	238	501	213	-	1,453
Overall	subtotals	123	986	165	238	1,032	391	262	3,197
									Overall total

Table 2: Number of samples analyzed by survey round, location and commodity. Notes: 1) green chilies were excluded since so few were collected and developing a Standard Operating Procedure would have been laborious, 2) the NAST laboratory received and processed (milled/subsampled) all samples, and all groundnut samples were analyzed for aflatoxin levels at NAST, 3) the Banke HH samples were collected in collaboration with NIL, out of sync with the two main survey rounds and therefore considered separately (*). Complementary (Comp.) food.

ZOI-wide survey – mycotoxin results:

1.2 full scale ZOI survey, round 1

Based on previous reports (see Previous Studies, above), stakeholder consultations and the pilot snapshot survey results, the first round of market and household surveying across the ZOI was conducted from March-April, 2018.

Banke district households are from the collaboration with NIL and are presented within here, alongside the ZOI-wide household and market results for comparison (ie, how indicative may exposure levels in mothers and infants – and others – be across the ZOI in comparison with Banke, based on levels of contamination in food and feed elsewhere).

The following food and feed commodities were collected:

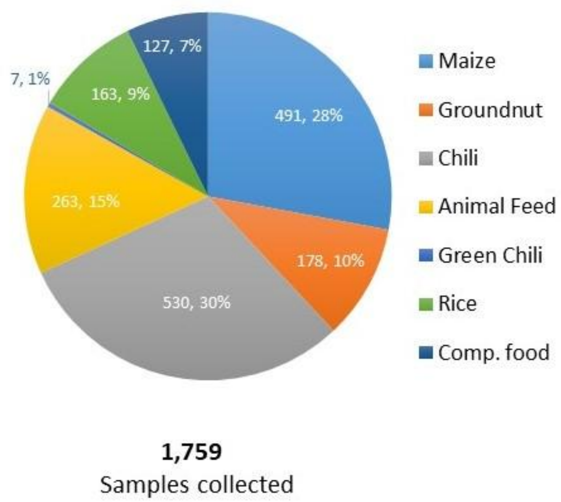


Figure 1: Round 1 full-scale ZOI survey samples collected.

Notes:

- Maize and groundnut were included since they are known for being commonly susceptible to aflatoxin contamination
- chili (dry) and green chili were included based on pilot snapshot survey, and other reports; due to the small number of green chili samples and the need to develop/validate an extraction and analysis SOP for each separate matrix (sample type), these were not analyzed
- animal feed was included per the SOW and reports that feed and consequent animal-source foods can be contaminated with aflatoxin
- rice was included at the direction of the Mission, to provide the Government of Nepal with specific evidence that, similar to reports from elsewhere, rice is not necessarily a high-risk food for aflatoxin contamination
- complementary food was included given that it is a potential source of exposure for infants, and the association of aflatoxin contamination with stunting children's development.

Full scale ZOI survey, round 2

Based on the results from round 1 of the full survey:

1) rice and complementary food were removed, given that there were low/undetectable levels of aflatoxin in the vast majority of samples; these were deemed as a low risk source of aflatoxin exposure.

2) Maize, groundnut and chili were retained as samples to be collected, given that they exhibited high levels of aflatoxin prevalence and concentrations from households and markets.

3) Soy nuggets were added as a sample to be collected, given that removal of rice and complementary food allowed for more commodities to be analyzed within the time and resources available to the project; soy nuggets were the next most important commodity (a food, complementary food and feed component) to be assessed after the round 1 commodities.

Note: The total number of samples collected relied on availability of that commodity at the households and market vendors visited, as well as the commodities sampled in round 1 vs. round 2; as a result of these design differentials and reliance on availability of a given commodity at a survey site, the number of samples across the two rounds differed.

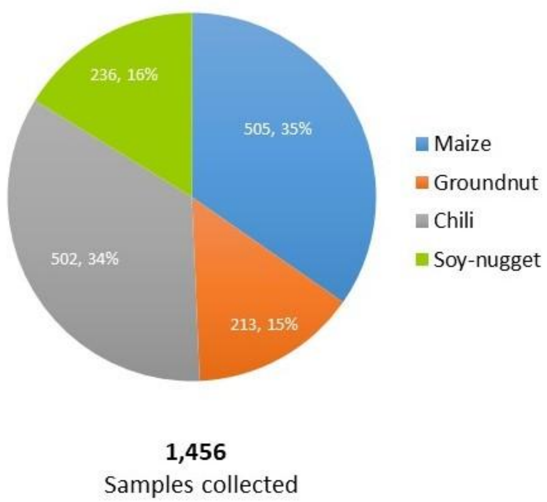
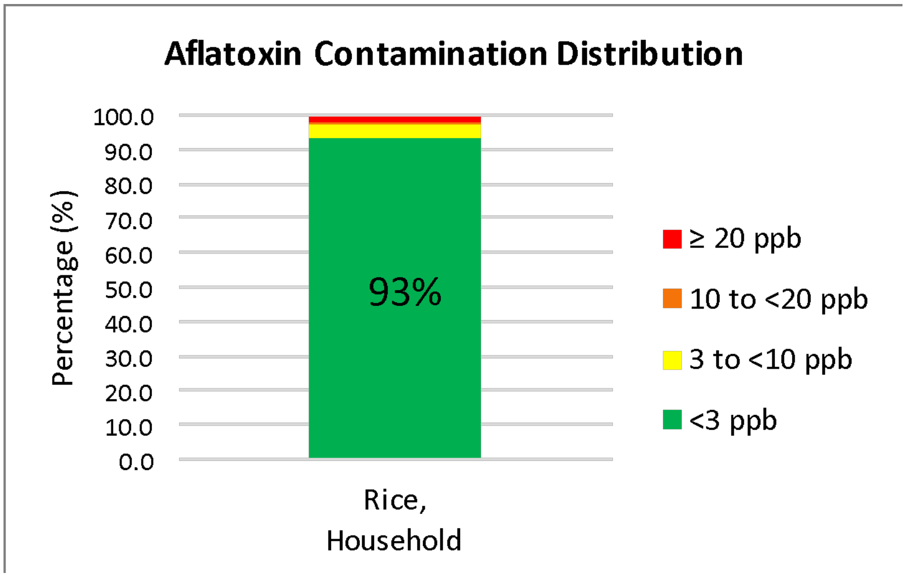


Figure 2: Round 2 full-scale ZOI survey samples collected.

Dr. Andreia Bianchini (University of Nebraska-Lincoln) led the technical area of mycotoxin diagnostics. This included intensive training of the Nepali team in her lab, validation of laboratory Standard Operating Procedure protocols, oversight of sample analysis at UNL and virtually/site visits at NAST and synthesis of overall results.

<u>Aflatoxin levels from ZOI-wide survey (households and markets), rounds 1 and 2:</u>

Figure 3: Aflatoxin contamination of rice

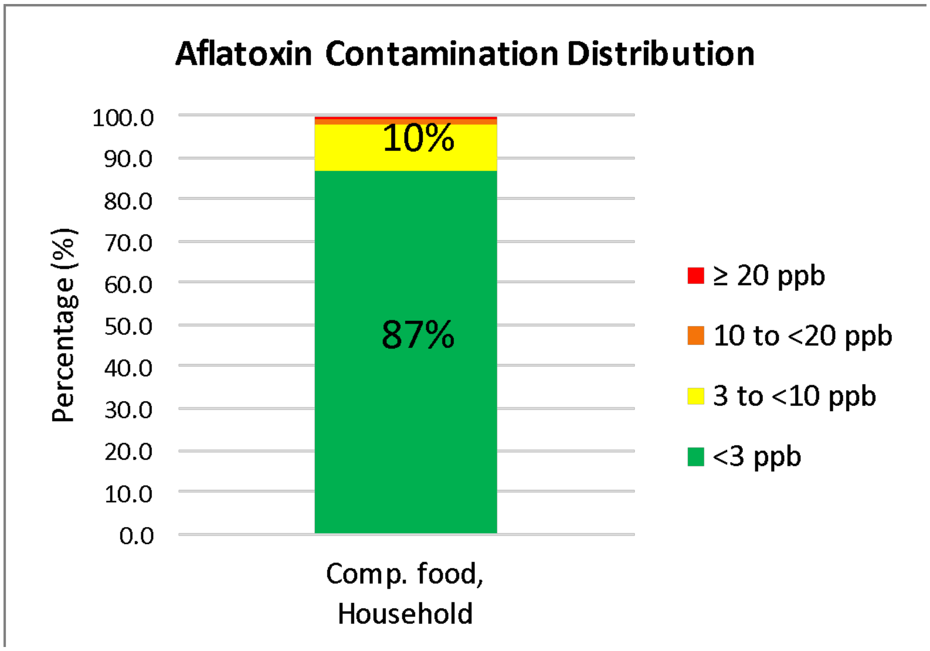


		Household
Aflatoxin (ppb)	Min*	3.5
	Max	25.0
	Mode	0.0
	Median	0.0
	n	165

* above LOD (3 ppb)

20ppb regulatory limit for food (DFTQC)

Figure 4: aflatoxin contamination of complementary food

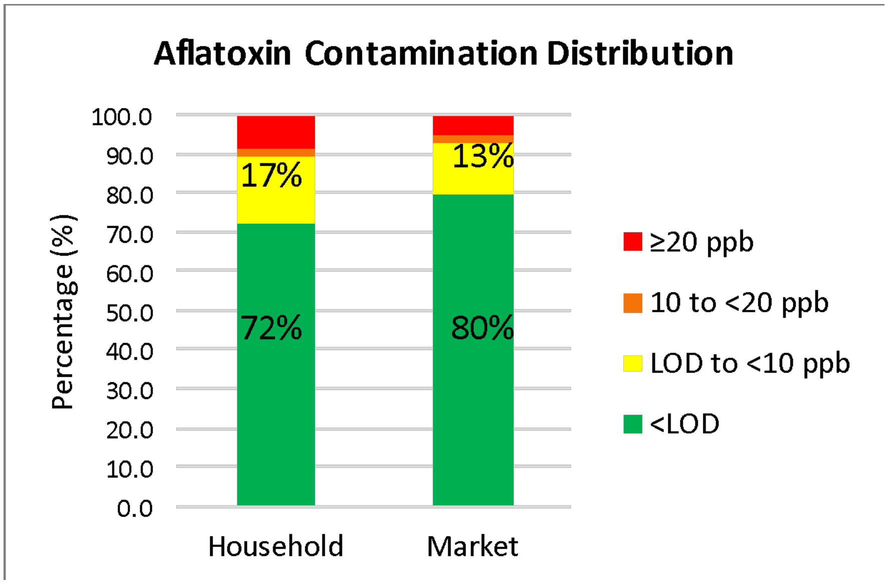


		Household
Aflatoxin (ppb)	Min*	3.3
	Max	39.0
	Mode	0.0
	Median	0.0
	n	123

* above LOD (3 ppb)

20ppb regulatory limit for food (DFTQC)

Figure 5: aflatoxin contamination of Soy nuggets

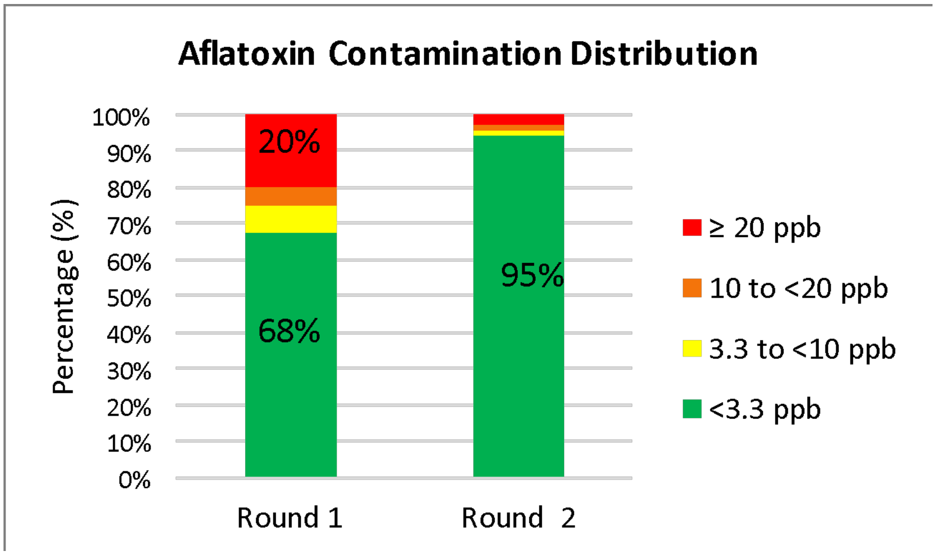


		Household	Market
Aflatoxin (ppb)	Min*	0.8	0.7
	Max	42.1	44.4
	Median	0.0	0.0
n		47	191

* above LOD (AFB1: 0.22 ppb, AFB2: 0.06 ppb)

20ppb regulatory limit for food (DFTQC)

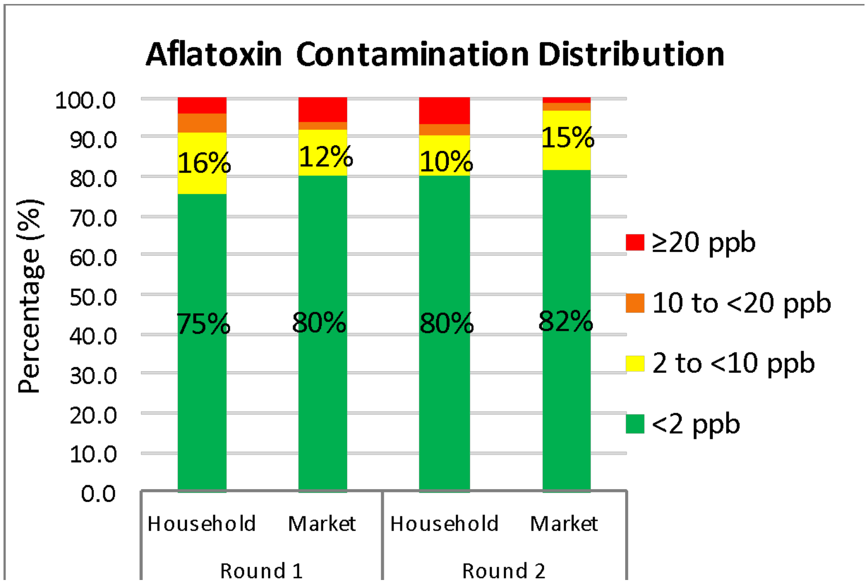
Figure 6: aflatoxin contamination of groundnut



	Total number of samples	% of Samples ≤ LOD[1]	% of Samples > LOD and < Regulatory Limit[2]	% of Samples > Regulatory Limit	Aflatoxin (ppb) range (Samples > Regulatory Limit)	Aflatoxin (ppb) mean (Samples > Regulatory Limit)
Groundnut	391	59	10	27	21-5400	440

20ppb regulatory limit for food (DFTQC)

Figure 7: aflatoxin contamination of chilies

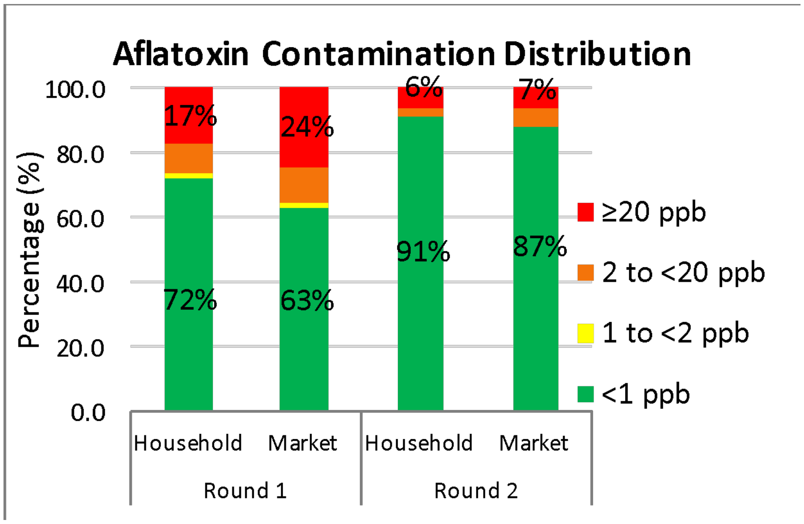


		Round 1		Round 2	
		Household	Market	Household	Market
Aflatoxin (ppb)	Min*	2.0	2.3	2.0	2.1
	Max	80.0	100.0	130.0	45.0
	Median	0.0	0.0	0.0	0.0
n		277	214	307	196

* above LOD (2 ppb)

20ppb regulatory limit for food (DFTQC)

Figure 8: aflatoxin contamination of maize

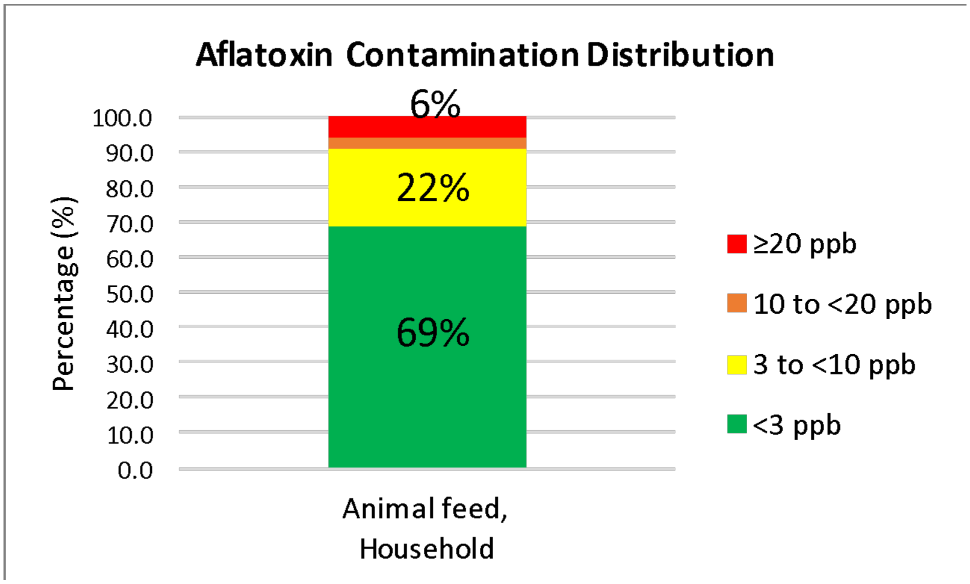


		Round 1		Round 2	
		Household	Market	Household	Market
Aflatoxin (ppb)	Min*	1.3	3.4	1.2	1.7
	Max	2250	1050	7248	1850
	Median	0.0	0.0	0	0
N		338	147	364	135

* above LOD (1 ppb)

20ppb regulatory limit for food, 50ppb for feed (DFTQC)

Figure 9: aflatoxin contamination of animal Feed



		Household
Aflatoxin (ppb)	Min*	3.2
	Max	650.0
	Mode	0.0
	Median	0.0
	n	262

* above LOD (3 ppb)

50ppb regulatory limit for feed (DFTQC)

Conclusion: ZOI-wide aflatoxin survey food category aflatoxin risk levels

Based on ZOI-wide survey results, ranked aflatoxin risk level in tested food and feeds based on results as follows:

a) High aflatoxin risk (up to thousands of ppb aflatoxin contamination – up to more than 100x the maximum limit): maize and groundnut

b) medium aflatoxin risk (up to several times the maximum limit for aflatoxin contamination): soy nuggets (an infant complementary food and feed component) and chilies

c) low aflatoxin risk (samples below the maximum limit for aflatoxin with very few exceptions, which were not far above the maximum limit): rice and wheat-based complementary infant food.

d) Feed is also at risk for aflatoxin contamination

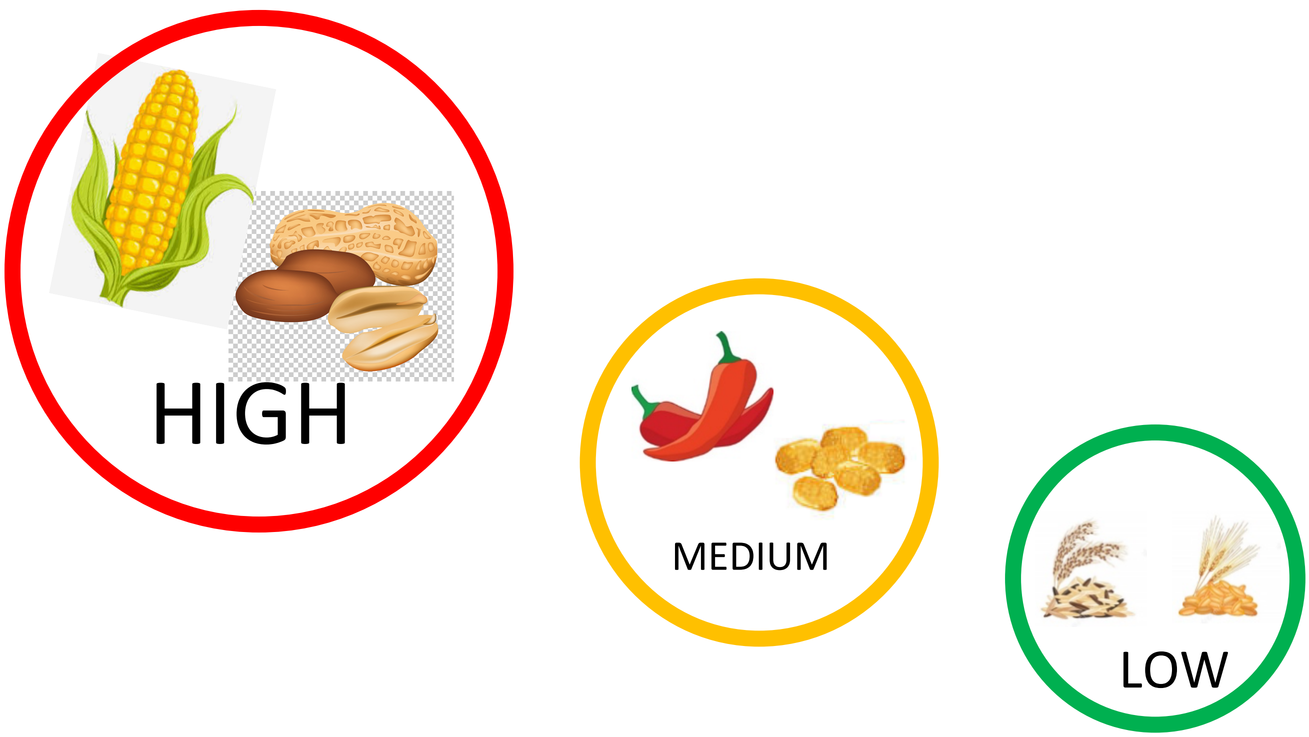


Figure 10: relative risk levels of aflatoxin contamination, based on the PHLIL survey.

ZOI-wide mycotoxin survey:
Trends, drivers of aflatoxin risk and other insights

Statistical analysis of survey answers and aflatoxin levels in collected samples revealed the following, including associations that can underpin aflatoxin mitigation plans.

Market Survey

Statistical analysis (annex VII) prioritized maize and chilies, since these had larger sample numbers and higher occurrence and levels of aflatoxin contamination.

Descriptive information – general:

1. 519 market stores were surveyed, across the 20 districts in the ZOI.
2. In 2018, 27.4% of interviewees were female; in 2019, 30.5% of interviewees were female.
3. Education level and age group were also captured.
4. In terms of storage conditions of the collected samples, for those that reported one or the other, 29.9% were storing the food in "dark with poor ventilation/moist" conditions, versus 70.1% storing food in "enough light with ventilation/dry" conditions.
    a. Note: Similar to what PHLIL has seen in Ghana and elsewhere, perceived "adequate" storage conditions and "dry" grains or other food/feed commodities can actually still frequently fail to reach the standards for safe storage.

Descriptive information – maize:

1. 282 maize samples were collected from markets (2-17 samples per district).
2. 97% of maize collected from stores was kept in plastic/jute bags. (others included hanging on poles, in bins, and open room/floor)
    a. This type of storage has issues with potentially promoting accumulation of inadequately dried maize grains.
    b. Jute bags can promote carry-over of toxigenic *Aspergillus* fungi from one use to the next, wherein a "clean/safe" source of maize can become contaminated with aflatoxin (Mars Global Food Safety Center study, Cui et al., 2018).
3. The amount of time the maize had been stored, as reported by the interviewee, was:
    a. 2017: 56.7% 1 month, 21.3% 2 months, 8.7% 3 months, 9.3% 4-6 months, 2.7% 7-9 months, and 2% 10-12 months.

b. 2018: 50.7% 1 month, 34.6% 2 months, 7.4% 3 months, 7.4% 4-5 months.

4. Reported variety of maize was also captured.

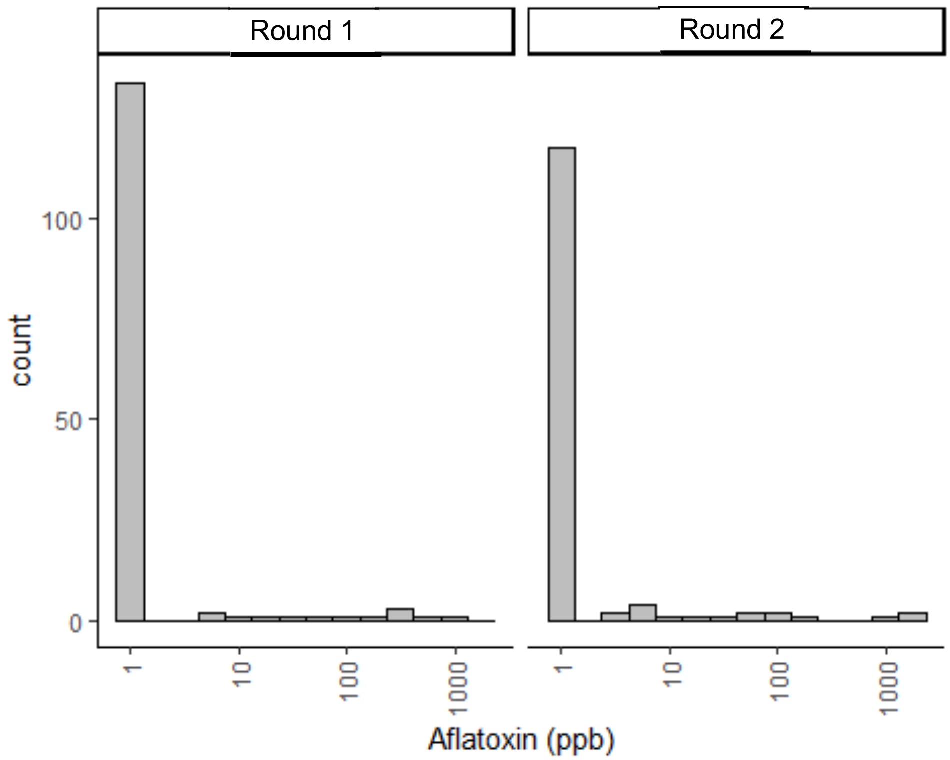


Figure 11: *aflatoxin contamination of maize, distribution of sample numbers by contamination level.*

Aflatoxin occurrence associations – maize (given that so many samples were below the limit of detection for diagnostics, statistics were conducted on the occurrence of aflatoxin: on presence or absence of detectable aflatoxin in the collected maize):

1. As altitude of the market increased, fewer samples had detectable aflatoxin ($p = 0.003$).
2. Poor storage conditions were associated with higher occurrence of aflatoxin ($p = 0.018$).
3. Aflatoxin contamination was an issue even with only 1 month of storage.
    a. Under poor storage conditions, aflatoxin can accumulate within a matter of just a few days.
4. No evidence for difference in aflatoxin occurrence among age groups.

Descriptive information – chilies: 409 chili samples were collected from markets (4-24 samples per district).

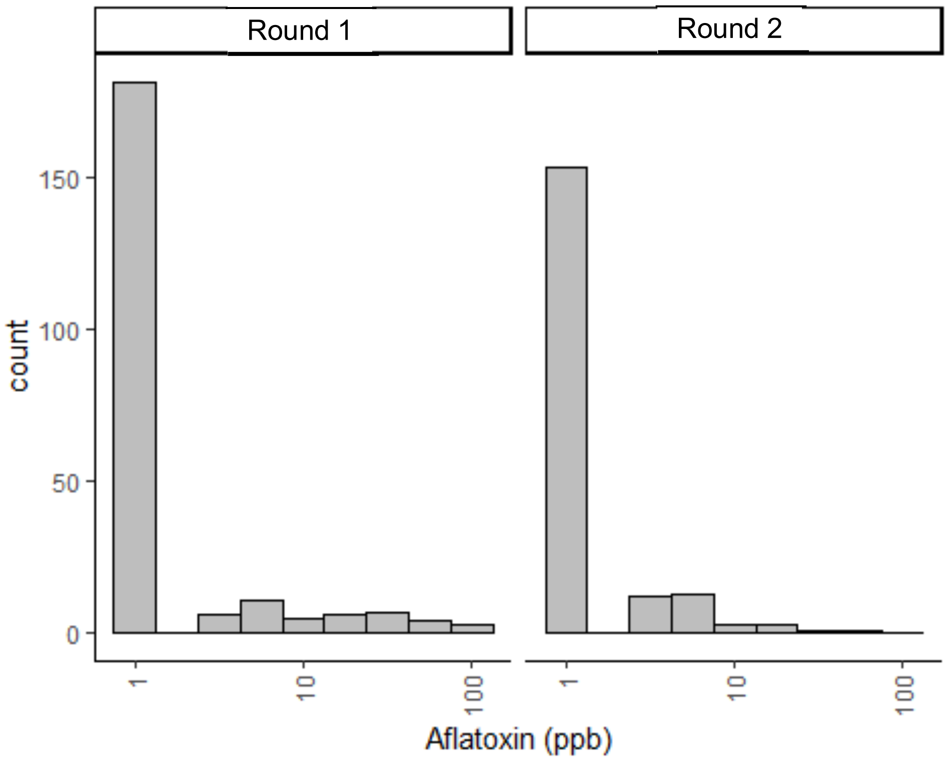


Figure 12: *Occurrence of aflatoxin detected in chili samples from markets for round 1 and round 2.*

Aflatoxin occurrence associations – chilies (as for maize, presence or absence of detectable aflatoxin in the collected chilies was used for statistical analysis):

1. Significantly higher prevalence of aflatoxin contamination in chilies from lower elevation markets (negative association between elevation and aflatoxin contamination prevalence; $p < 0.001$).
2. Significantly higher prevalence of aflatoxin contamination of chilies in stores with good conditions versus poor conditions ($p < 0.001$).
    a. This suggests that drying and storage of chilies along the value chain is inadequate, even under "good" conditions in the store. Poor drying and storage anywhere along the value chain can result in aflatoxin contamination, and the ISPA snapshot market survey analysis revealed that even fresh green chilies had aflatoxin contamination.
3. No evidence for differences of aflatoxin prevalence in chilies among age groups.

<u>Household Survey</u> (PHLIL survey sentinel districts – Dadeldhura, Dang, Kailali and Salyan; Banke reported elsewhere; complete information available in annex VI)

Statistical analysis (annex VI) prioritized maize, chilies and groundnuts, since these had larger sample numbers and higher occurrence and levels of aflatoxin contamination.

Descriptive information – general:

1. 629 households were surveyed, in Dadeldhura, Dang, Kailali and Salyan districts. 519 market stores were surveyed, across the 20 districts in the ZOI.
2. In 2017, 59.9% of interviewees were female; in 2018, 67.5% of interviewees were female.
3. Education level and age group were also captured.
4. When asked whether they clean their storage, 95.4% responded that they did.
    a. How well they clean the storage, and whether the storage technology itself is adequate is another issue. An extension program with proper training and technologies can address this.

Descriptive information – maize:

1. When asked whether they harvested maize early, late, or at the right time: 94.3% right time, 4.4% early, and 1.4% late.
    a. Farmers' impressions about what constitutes the "right time" can differ from the actual Good Agricultural Practice timing for harvest.
2. 99.8% of surveyed farmers used sunlight to dry (one farmer reported "other").
    a. This is a clear indication that improved drying technologies are not in use by these Nepali farming communities.
3. The location for drying maize was: 57.8% uncovered ground, 28.4% tarp, 12.7% roof, and 1.1% other.
    a. It is troubling and readily addressable that over half of the households are not even using a tarp to protect the drying maize from soil contamination (*Aspergillus* and other food safety hazards reside in the soil, so the farmers are inoculating their own harvest with mycotoxigenic fungi before storage). Using a tarp represents a basic, inexpensive measure to reduce risk of aflatoxin contamination.
4. Households that sorted maize to remove perceivably discolored, moldy, damaged or otherwise perceived poor quality grains or cobs: 82.7% sorted, 17.3% did not sort.

   a. While many households may have reported sorting out "bad" maize, the end use for that sorted and potentially highly contaminated grain is important. Often, it may be used as feed, or potentially eaten first or by specific members of the family.

5. In terms of how households stored maize: 58.2% hanging, 31.5% sacks, 5.7% open room, 2.0% metal bins, 0.7% clay bin, 0.2% bamboo mat, and 1.8% other.
   a. Similar to drying technologies, improved storage technologies that prevent post-harvest losses including aflatoxin contamination are essentially not present in these farming communities.
6. Reported duration of storage: 5.3% <1 month, 1-3 months 36.7%, 3-6 months 29.8%, 6-12 months 20.2%, do not know 8.1%.
   a. Improper drying and storage practices, coupled with long term storage, are ideal conditions to increase risk of aflatoxin contamination, potentially to very high levels.

<u>**Maize aflatoxin findings and associations:**</u>

Generally, aflatoxin was found to be an issue in maize across current practices and storage durations.

1. Statistical analysis revealed no evidence that there were differences in aflatoxin occurrence for maize, groundnut or chili among different storage time ranges (annex VI). The highest concentration of aflatoxin in maize (7,248ppb) was found in maize that had been stored 1-3 months rather than up to 12 months like others. Likewise, the highest amount of aflatoxin in chilies was from chilies stored less than one month. This reflects poor pre- and post-harvest Good Agricultural Practices, and the absence of proper drying and storage technologies in these farming communities. It also reflects the skewed distribution of aflatoxin contamination – one of the things that makes it challenging to characterize in agricultural systems.
2. For maize storage, sacks and hanging were the most common methods. No evidence for differences in aflatoxin occurrence between those methods was found. (Neither is a particularly good post-harvest practice, for various losses including from aflatoxin contamination.)

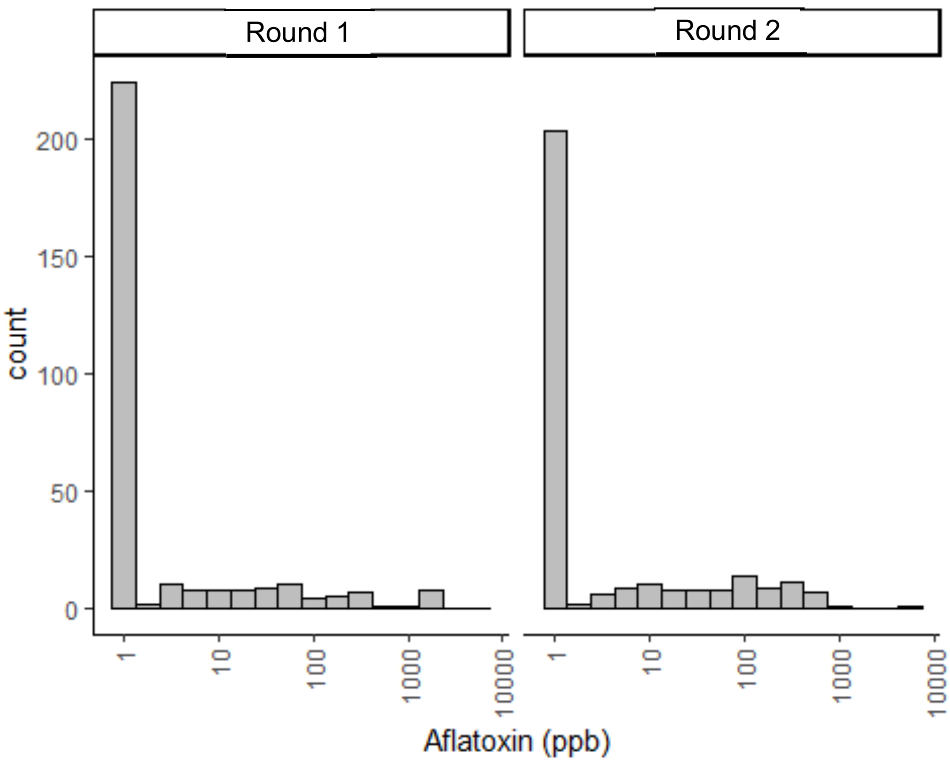


Figure 13: *Distribution of aflatoxin in maize samples from households in both seasons.*

Descriptive information – groundnuts:

1. When asked whether they harvested groundnuts early, late, or at the right time: 76% right time, 16% early.
2. 100% of surveyed farmers used sunlight to dry groundnuts.
3. 100% of surveyed farmers used sunlight to dry chilies.
4. The location for drying groundnuts was: 81.8% tarp, 15.2% uncovered ground, 3% roof.
5. Households that sorted groundnuts to remove perceivably undesirable or "bad" groundnuts: 59.2% sorted, 40.8% did not sort.
   a. Sorting groundnuts has been documented to be effective at reducing aflatoxin contamination of the "good" portion. However, during site visits, household members recounted that they sort out discolored or moldy groundnuts in order to roast and eat them. This is actually an effective and dangerous way that these household members are concentrating their intake of aflatoxin from an enriched source, all at once.
6. In terms of how households stored groundnuts: 87.5% sacks, 4.2% clay bin, 4.2% hanging, 4.2% open room.
7. Reported duration of storage: <1 month 42.9%, 1-3 months 4.8%, 3-6 months 14.3%, 6-12 months 38.1%.

    a. Long storage periods in which aflatoxin contamination can accumulate, especially with improper drying and storage.

Descriptive information – chilies:

1. When asked whether they harvested chilies at the early, late, or at the right time: 84.9% right time, 3.4% late and 12.2% early.
2. The location for drying chilies was: 62.9% tarp, 28.5% roof, 7.5% uncovered ground, 1.1% other.
3. Households that sorted chilies to remove discolored, moldy or otherwise perceived poor quality grains: 61.2% sorted, 38.4% did not sort.
    a. During site visits, household members recounted that they sort out discolored or moldy chilies in order to grind and use them in cooking first. This is actually an effective and dangerous way that these household members are concentrating their intake of aflatoxin from an enriched source, all at once.
4. In terms of how households stored chilies: 79.0% sacks, 2.6% hanging, 1.6% bamboo mat, 1.6% clay bin, 1.6% metal bin, 1.1% open room, and 12.6% other.
5. Reported storage duration: <1 month 8.0%, 1-3 months 17.1%, 3-6 months 29.6%, 6-12 months 10.2%, do not know 35.2%.
    a. Long storage periods in which aflatoxin contamination can accumulate, especially with improper drying and storage.

Mycology and risk mapping: Characterizing the fungus and towards anticipating hotspots of aflatoxin contamination

Contamination of a given food or feed depends on colonization with mycotoxin (including aflatoxin)-producing fungus, followed by production of mycotoxins (aflatoxin) themselves. For any plant-microbe interaction, the three parts of the disease triangle affect the dynamics and ultimate outcome of disease: host (how susceptible is the host to the fungus, or is it resistant), pathogen (is a pathogen population present that can colonize and cause disease (and/or produce mycotoxin) on a susceptible host, and environmental conditions (including temperature/degree days, rainfall and relative humidity). In order to come at the host-fungal interactions that result in food and feed becoming contaminated with mycotoxins (aflatoxin) from several angles within this, **a four-tiered approach was taken** to more broadly assess mycotoxigenic fungal species present, conduct risk modeling of conducive climatic conditions for aflatoxin production, and follow up with more extensive characterization of identified fungal species from the initial snapshot. Together with the actual survey results, this complementary information further supports our understanding of which crops/commodities are at risk of contamination by mycotoxins (especially aflatoxin) in which areas.

Specific Objective 2/3 achievements:

I. ISPA Snapshot market survey mycology

Assessed a range of mycotoxigenic fungal species in the wider range of food and feed commodities analyzed by ISPA in the initial market snapshot.

This included:

a. mycotoxigenic fungi in *Aspergillus flavus*, *Aspergillus nigri*, other *Aspergillus*, *Alternaria*, *Penicilium*, and *Fusarium*
b. *in* rice, green and dry chili, cumin, coriander, small and large cardamom and soybean cake.

Results:

Mycological analysis

In the snapshot survey (collected from markets), data confirm the higher and wider incidence of fungal contamination in chili samples respect to the other spices. *Aspergillus* Sect. *Flavi* and Sect. *Nigri* are the predominant potential toxigenic fungi occurred; but *Alternaria, Penicillium* and *Fusarium* strains were also found in chili. Most of the commodities sampled had flavi, nigri, alternaria and penicillium contamination in at least one of the samples; they were generally but not universally present. Exceptions were no *Alternaria* on rice, soybean nuggets, cumin or large or small cardamom.

II. UNL and KSU mycology

Aspergillus and *Fusarium* are well-documented and rank as the most problematic in contaminating various food and feed commodities, and produce some of the most prevalent and harmful mycotoxins. Further, recent evidence has suggested that Fumonisin (a Fusarium toxin) may play a role in stunting children's development. Based on this, a set of animal feed and household flour (weaning food) samples were assessed for presence of Fusarium and Aspergillus. *Aspergillus* species isolation and characterization in terms of species present and aflatoxin production ability by cultural methods at UNL; and *Fusarium* species were isolated and characterized by cultural and molecular methods at KSU.

Fusarium analyses from feed and flour (KSU):

Samples of animal feed (267) and household flour used to make weaning food (140) were tested for the presence of *Fusarium* species. Material was placed on a peptone-PCNB medium selective for *Fusarium* and incubated at 25°C for 7-10 days. Twelve colonies were recovered from the household flour and 444 colonies from the animal feed. Colonies were purified through subculturing a single spore, preferably a microconidium, and then transferred to carnation leaf agar for morphological identification and to Czapek's liquid medium for growing material from which DNA could be extracted. DNA was extracted by using a CTAB method and portions of the translocation elongation factor 1-α (TEF) gene amplified by using PCR with standard primers. The TEF sequences were checked against NCBI database to identify species. Morphological examinations were made of all strains, and DNA sequences tested for all of the strains from household flour, but only about a quarter (102) of the animal feed strains.

In the household flour, there were four strains each of *F. proliferatum*, *F. verticillioides* and *F. incarnatum*. In general the contamination in the household flour was very low, with < 10% of the samples having any *Fusarium* species present at all. Both *F. proliferatum* and *F. verticillioides* are common pathogens of maize and likely were in samples in which the household flour included maize flour. Strains of these species are known for their ability to synthesize fumonisins and a few other toxins, including moniliformin and beauvericin. If the fungi detected are indicative of potential toxin problems, then no more than 5% of the household flour samples are likely to be contaminated with fumonisins.

In the animal feed samples the most common species were *F. proliferatum* (41%), *F. verticillioides* (15%), *F. fujikuroi* (13%) and *F. equiseti* (11%). Other *Fusarium* species represented in the animal feed by one to a few colonies included: *F. chlamydosporum*, *F. circinatum*, *F. flocciferum*, *F. incarnatum*, *F. mangiferae*, *F. nygamai*, *F. oxysporum*, *F. pseudocircinatum*, *F. reticulatum*, and *F. solani*. Amongst the feed samples, the common fungi probably are consistent with material included in the feed – maize for *F. proliferatum* and *F. verticillioides*, rice for *F. fujikuroi*, and as a secondary invader of other lesions for *F. equiseti*. Over half of the samples are at risk for fumonisin contamination based just on the presence of *F. proliferatum* and *F. verticillioides*. Some species recovered are reported to produce trichothecenes, but the number of strains here is small and they probably are not a major problem if the frequency of the species recovered here can be used as a guide.

Aspergillus analyses from feed and flour (UNL):

Aspergillus flavus or *parasiticus* (AFP) and *A. niger* isolates were recovered from the feed, maize, soy nugget, and chili samples using AFP agar. Of these, preliminary toxigenicity data was collected from a subset of fifty AFP isolates, out of which 34% (17/50) came from samples which tested positive for total aflatoxins and 66% (33/50) from samples negative for aflatoxins. Once isolated and purified, AFP isolates were grown on Yeast Extract Sucrose broth at 25°C for 18-24 h at 80 rpm, followed by an addition of chloroform and a 2 h agitation (180 rpm). Each extract was filtered, and the organic phase was filtered and evaporated to dryness. Samples were reconstituted with fresh chloroform and place onto a pre-made Think Layer Chromatography (TLC) silica plate with later addition of trifluoroacetic acid, allowing 5 min to react. Each TLC plate was ran using Acetone:Chloroform 85:15 as developing reagent, and read under UV light. Out of the 50 AFP isolates tested for toxin production, 72% (36/50) of them were positive (blue/green fluorescence) for aflatoxin production.

Conclusion:

UNL mycology results indicate that a subpopulation of atoxigenic *Aspergillus* should be present in Nepal (as suspected) which could potentially be exploited for biocontrol. Atoxigenic members of Aspergillus populations should be present essentially anywhere Aspergillus is found, given natural mutation and the fact that aflatoxin production is not required for growth in various habitats (eg, soil, maize kernels,…).

III. Metabolomic deep profiling of maize and soil from Nepali farms

A metagenomics assessment was designed and co-funding from KSU and the Mars Global Food Safety Center (and technical collaboration) was secured. A MSc student is currently using this to broadly assess mycotoxigenic fungal populations in maize and soil samples from fields in Nepal, to complement the mycology and risk mapping work.

Paired soil and maize samples were collected from twelve maize farmers in Nepal, from two different agroecological zones. The maize had been grown in the field in which a pooled sample of soil was collected – a natural reservoir for the fungi that ultimately contaminate the maize. A partnership and co-funding from the Mars Global Food Safety Center was initiated, which was introduced by PHLIL AOR Ahmed Kablan. Next generation sequencing of the ITS marker gene from fungi in the soil and maize populations are being conducted to assess the taxonomic diversity present. This is being combined with analysis of >400 being analyzed in the maize samples at BOKU, Austria; this second analysis will give a broad sense of which mycotoxigenic fungi are present, together with the genetic analysis. Analysis to date has found aflatoxin in 3/12, and ***fumonisin in 9/12 maize samples, adding to the body of evidence that fumonisin is a mycotoxin that should be studies and addressed further in Nepal.*** Given that co-funding and a graduate student were required for this fourth approach, analysis is still ongoing. Additional results are being written up in the MSc thesis.

IV. Risk mapping: building an updated aflatoxin risk model

While aflatoxin is commonly recognized as a post-harvest issue, fungal infection and aflatoxin contamination of grains can also occurs before harvest; pre-harvest fungal infection is a key stage of infection by *A. flavus*, preceding and contributing to the post-harvest phase of fungal infection/growth and aflatoxin contamination. Statistical models of aflatoxin risk can be used to predict the likelihood of aflatoxin given certain weather conditions during grain fill and altitude, should weather medium range forecast data be available. These forecasts could be useful at the provincial and national level. Higher spatial resolution requires accurate weather information as similar spatial granularity.

Risk mapping (the readout of these statistical models) was conducted to assess where within the ZOI climatic conditions are conducive to aflatoxin contamination of maize at harvest. The risk algorithms were used to further predict aflatoxin risk levels in maize during the two seasons when the PHLIL household survey was conducted. The occurrence of aflatoxin in grain samples can be related to drivers of aflatoxin production, drivers associated with plant stress during grain fill such as degree days and the number of dry periods as well as altitude.

In this study dry maize grain samples were collected from small scale farmer households in the Dadeldhura, Dang, Kailali and Salyan after the September harvests of 2017 and 2018. The collection of maize samples took place several months after 2017 harvest (march 2018) but only a few weeks after the 2018 harvest (October 2018). The weather data collected was daily summary information for precipitation, mean and maximum temperature and relative humidity from weather stations located in and around the ZOI districts. The maize samples tested for aflatoxin were grown during the 2017 and 2018 growing seasons, planted sometime during the months of March, April or May. Weather information was extracted from the stations for the 120 days leading up to harvest, nominally, the 30^{th} September for each year. This aligns approximately from silking to maturity. The degree-days for each weather site was calculated as well as the number of dry periods during grain fill. 85 weather stations had 120 days of rainfall data in 2017.

The distribution of aflatoxin level is often seen to be right skewed but a large number of non-detectable or zero observations. For the co-kriging modelling we used the gstat package (Gräler, Pebesma, and Heuvelink 2016) of the R statistical environment (R Core Team 2019).

The statistical distribution of aflatoxin (Figure 14) is extremely right skewed. Seventy one percent of samples were aflatoxin free with a small number of high values and 88% of all samples having less than 10 ppb and the maximum being higher than 6000 ppb. Statistical inference based on linear regression models rely on the distribution of samples being reasonably symmetric and with 79% of all values less than 1 ppb, the log transformation was not sufficient to normalize the distribution of the highly skewed response. Reducing the aflatoxin measurement to a binary response (presence/absence or occurrence) and fitting a logistic regression model is an alternative that avoids some of the distributional properties needed to fit linear regression models.

The model was built using the following from the 2017 maize growing season: number of dry periods during grain fill, degree days during grain fill and elevation. Note that in the risk map, areas outside of actual maize production (eg, high elevations) will be filtered out in the final risk maps for circulation.

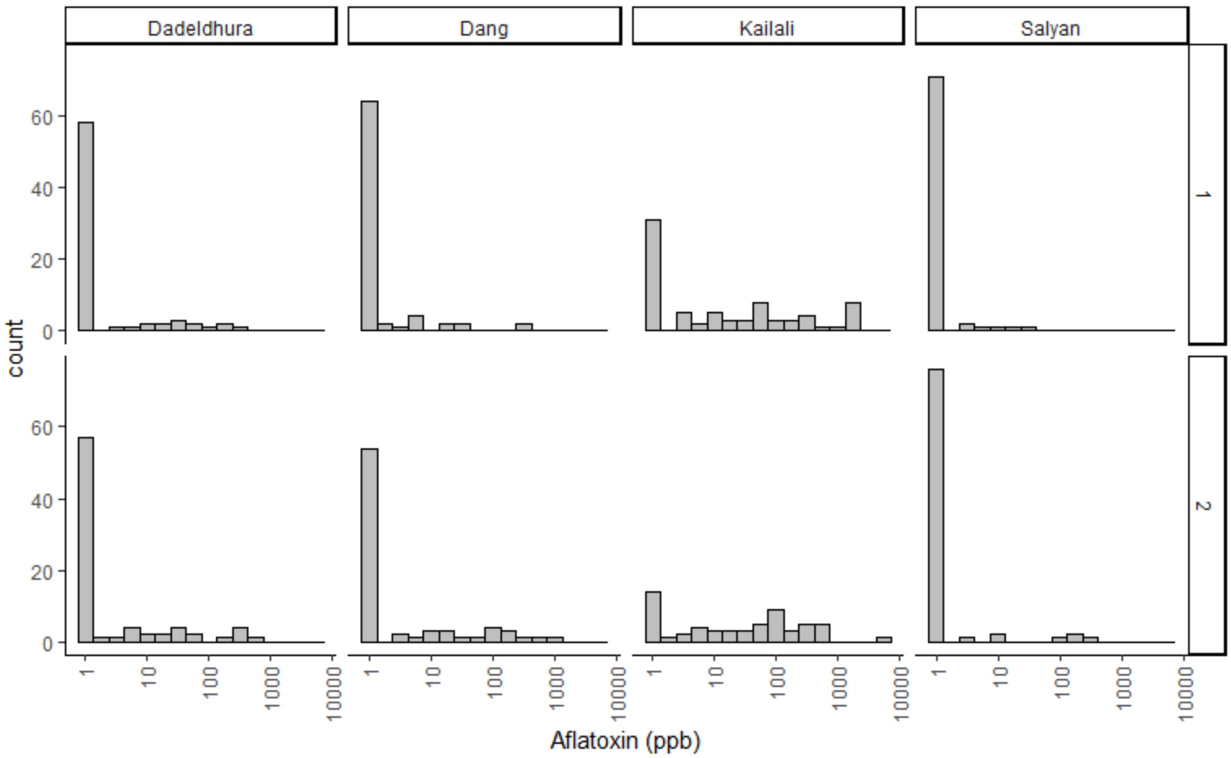


Figure 14: Histogram of aflatoxin for each district and season. Horizontal axis is a base 10 scale, adding one to enable transformation of values <LOD (limit of detection). 1 and 2 on the right correspond to the first (March-April 2018) and second (October-November 2018) rounds of the survey.

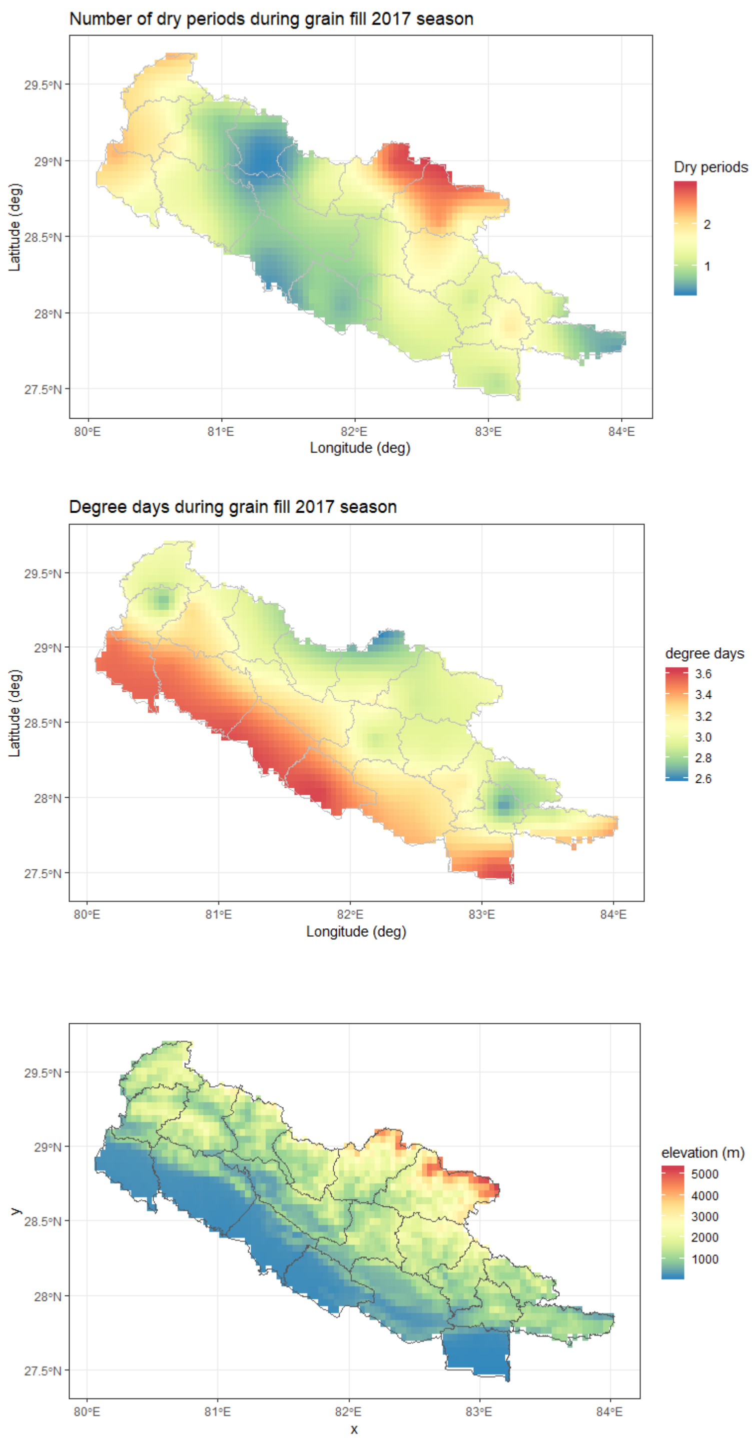


Figure 15: *Input components of the aflatoxin risk mapping, including dry periods, degree days and elevation.*

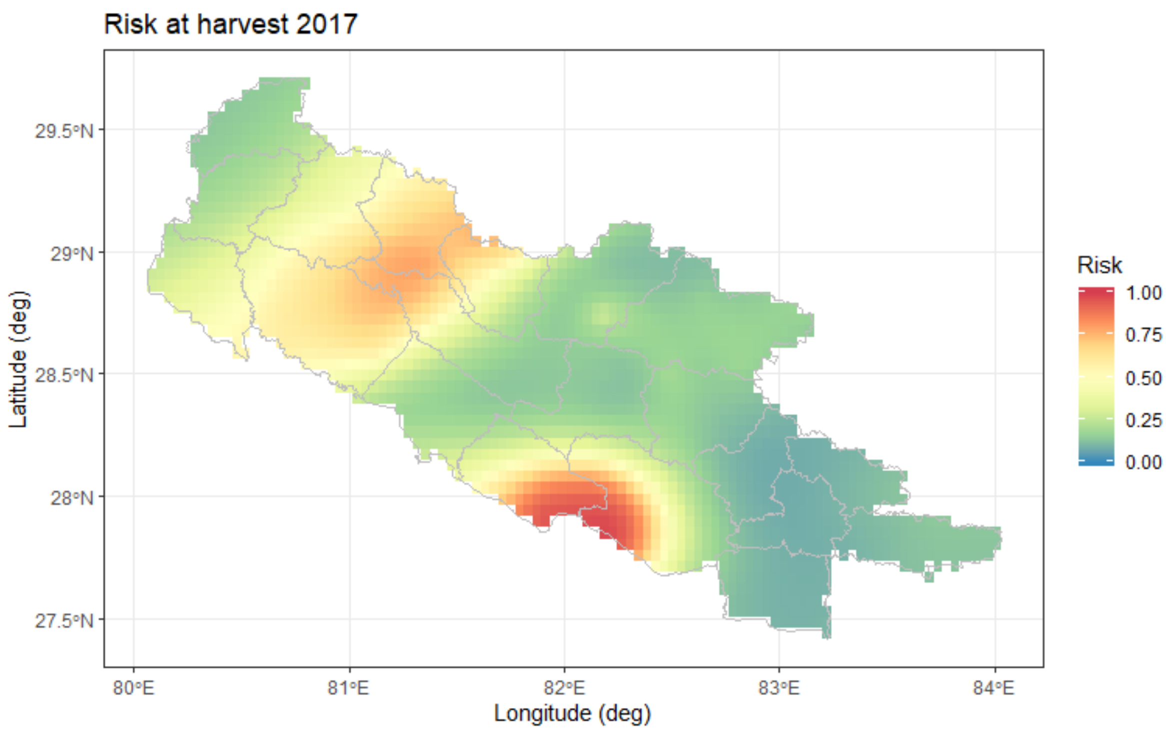


Figure 16: *Risk mapping output – predicted risk at harvest 2017.*

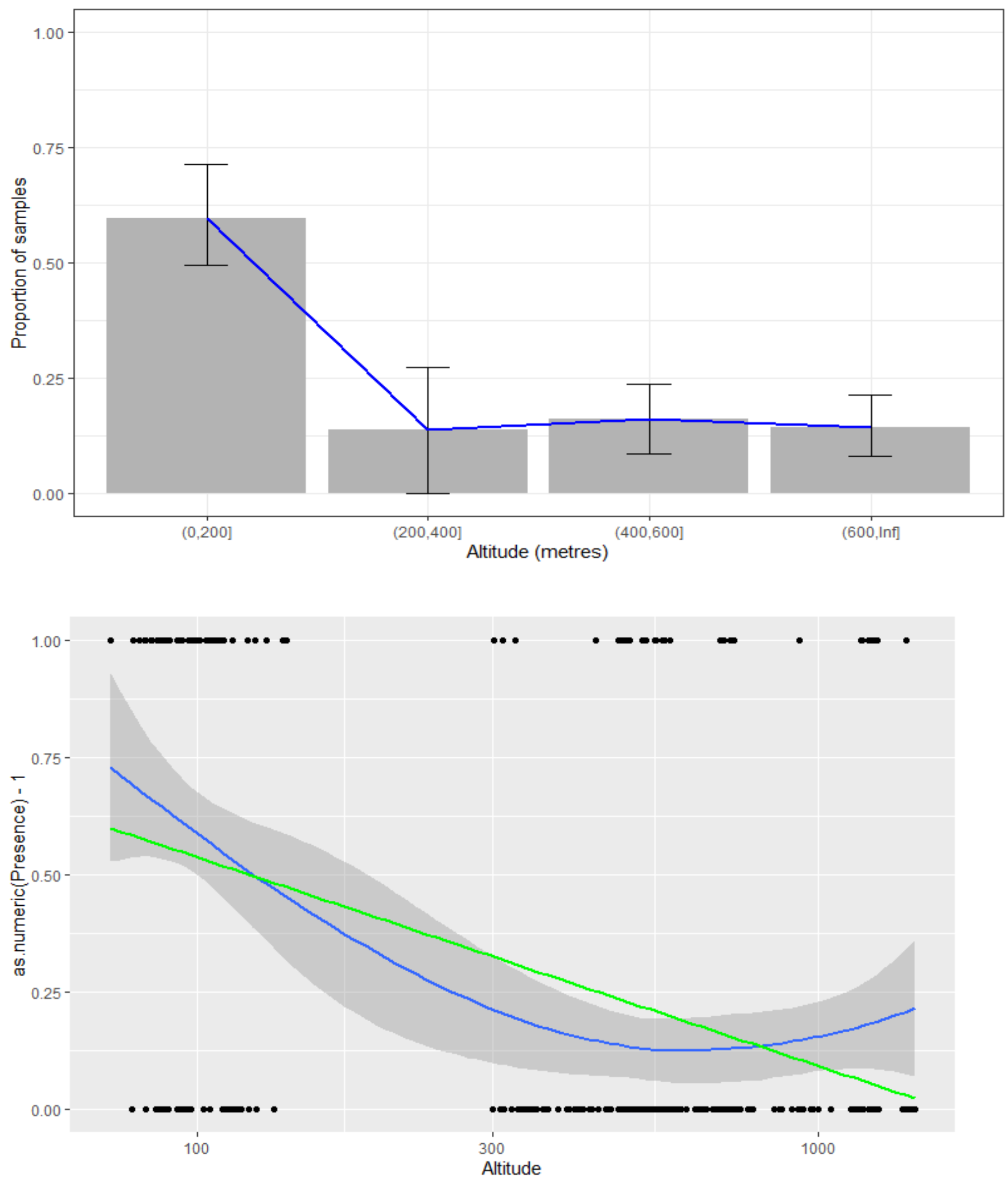


Figure 17: *aflatoxin positive samples by altitude.*

Towards a dynamic forecasting model, future work:

We modelled the occurrence of aflatoxin in maize using a Bayesian logistic regression model for the two seasons 2017 and 2018. The regression estimates that relate aflatoxin occurrence, Z, and the relationships with the drivers, denoted here as θ, can be estimated in a dynamic way, and updated each season.

$$P(\theta|Z_{2017}, Z_{2018}) \propto P(Z_{2018}|\theta)P(Z_{2017}|\theta) \; P(Z_{2018}|\theta)P(\theta|Z_{2017})P(\theta)$$

Initial results show that this modelling framework improves the precision of forecast risk, $P(\hat{Z}|\hat{\theta})$, as additional seasonal weather information is introduced.

The equation can be extended to include subsequent years as data is collected.

Risk modelling to inform mitigation and response to aflatoxin contamination in Nepal:

Risk mapping was highly regarded and ranked at the August National Stakeholder Workshop. Furthermore, Dr. Darnell presented his work to scientists from NARC, the Ministry of Agriculture, Nepali universities, and NAST at NDRI on December 11. There was substantial enthusiasm to engage further with Dr. Darnell on these efforts, as a key activity of the national partners, to move this work forward as a key tool to mitigate and monitor aflatoxin contamination in Nepal. We feel that the models here can be improved if a monitoring program is put into place and further effort to redefine the models based on expert opinion.

PHLIL Agricultural Economics Study Component:
Economic Factors Affecting Post-Harvest Practices and Food Safety in Nepal

Dr. Jisang Yu, Assistant Professor of Ag Econ at KSU led design and analysis of agricultural economic queries, to complement the broader project findings in informing recommended interventions. Understanding economic drivers behind post-harvest and management practices for better quality and improved food safety is crucial. Any interventions without considering economic incentives or constraints would lead to unintended consequences. We focus on maize since it has the largest coverage (although we collected information on other commodities).

Background

The key research questions of the agricultural economics component are:

1. Are there price premiums for high-quality maize?
2. Do government regulations (e.g. food safety inspection) associated with the quality of the product?
3. Are there differences in quality across different socio-economic groups?
4. What is the role of food safety perception in post-harvest practices?

While we do not have conclusive answers to the questions above due to the limited resources and the nature of the project, we have found interesting associations that show the necessity of future researches and policy designs and also can help implementing them.

Two surveys are implemented throughout the study period: market and household surveys, across the ZOI.

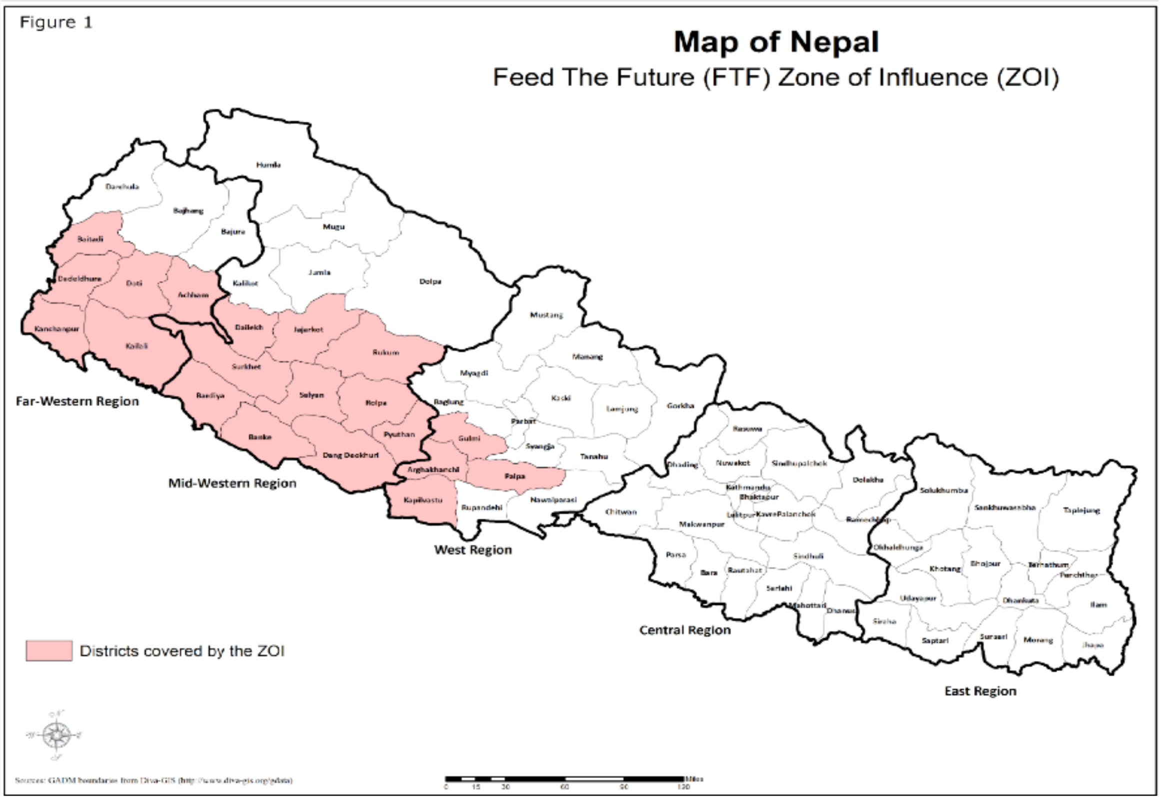


Figure 18: Map of the FTF Zone of Influence (source: USAID)

<u>Market Survey Findings</u>

Key findings:

- There exist price premiums for better quality maize (key factor to help address low quality/safety with adoption/scaling of interventions).
- Food safety inspector visits are correlated with higher quality (key factor to help inform regulatory enforcement strategy).

Both of these key findings are encouraging as footholds to help deploy and enforce interventions related to higher quality/more safe maize in Nepal.

Seasonality

Table 3 shows the summary statistics for the key variables. The average prices are 89.30NRP/kg and 97.78NRP/kg (we observe an increase in the maize price between the two period), the average degrees of damage, which are in self-stated 5-point scale measure (5 being most damaged) are 1.65 and 1.76, and the numbers of inspector visits during the last 12 months are 1.64 and 1.76, in rounds 1 and 2, respectively.

Table 3 Means and standard deviations of the selected variables (standard deviations are in the parentheses)

Variables	Round 1	Round 2
Maize price (NRP/kg)	89.30	97.78
	(51.79)	(44.49)
Degree of damage	1.65	1.76
	(0.75)	(0.82)
Number of inspector visits	1.64	1.76
	(1.96)	(2.21)
N	151	136

Given we observe differences across the two rounds in the means of the key variables, one of the questions assessed was whether there were differences in price and quality across the two times of year (seasonality) captured in the two rounds of surveying.

For price, we statistically reject the null of the equal distributions of the prices between the two rounds (p-value = 0.026; Kolmogorov-Smirnov test for distribution equality at 5% significance). Although we find the statistical difference between the two distributions, the mean difference is not statistically significant. When we limit our sample to the domestically produced maize, the difference is statistically significant. For the quality variable (i.e. the self-stated degree of damage in maize), the differences between the two rounds are not statistically significant. Overall, we observe the seasonality of the maize price and relatively constant quality throughout the sample period.

Price premium

Using our data, we estimate the association between price and quality to investigate whether there exist price premiums for the higher quality maize. Figure 19 shows the district-level average prices and quality and the negative correlation between the two.

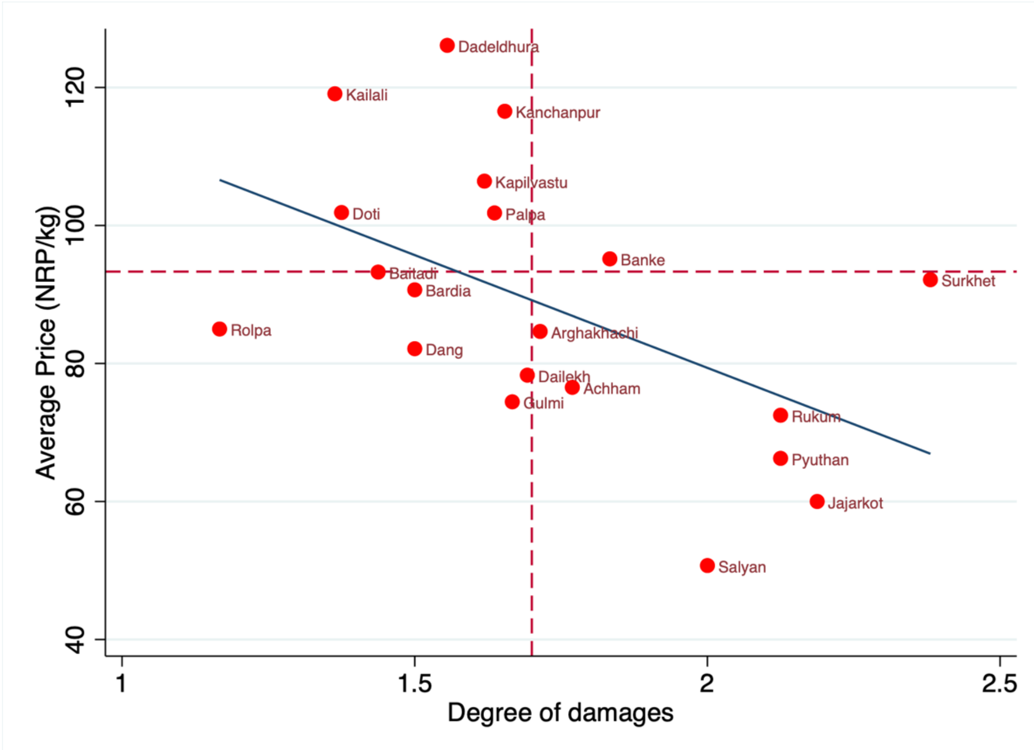


Figure 19 The relationship between price and quality

In order to further interrogate the difference in premium for perceived quality, we use regression analyses. Table 4 reports the estimated results and shows the positive and significant statistical relationship between the price and the quality variables. The results are robust with respect to the inclusion of additional control variables.

Table 4 The relationship between price and quality

Variables	(1)	(2)
Good quality	21.62***	12.20**
	(6.09)	(5.60)
Controls	No	Yes
Adj-R2	0.14	0.45
N	287	287

(Note: The dependent variable is NPR/kg and the variable "Good Quality" indicates whether the self-stated degree of damage is in the lowest category or not. Standard errors are in the parentheses and clustered at the district level)

Additional findings from the regression (2) are:

1. Indian maize is more expensive compared to domestic (estimated coefficient of 16.79 with p-value=0.07).
2. Maize sourced from wholesaler/traders is more expensive compared to own-produced maize (59.91 and 55.38 with p-value<0.01).
3. Storage duration is positively correlated with price: weak evidence on seasonal arbitrage? (3.11 with p-value=0.043)
4. The number of products stocked is negatively correlated with price: economies of scale? (-1.13 with p-value=0.013).
5. Store management practices do not seem to affect prices other than through quality.

The role of food safety inspection

The other key question we are interested in is on the role of food safety inspection. Figure 20 shows the negative relationship between the self-stated degree of damages and the number of food safety inspections during the last 12 months.

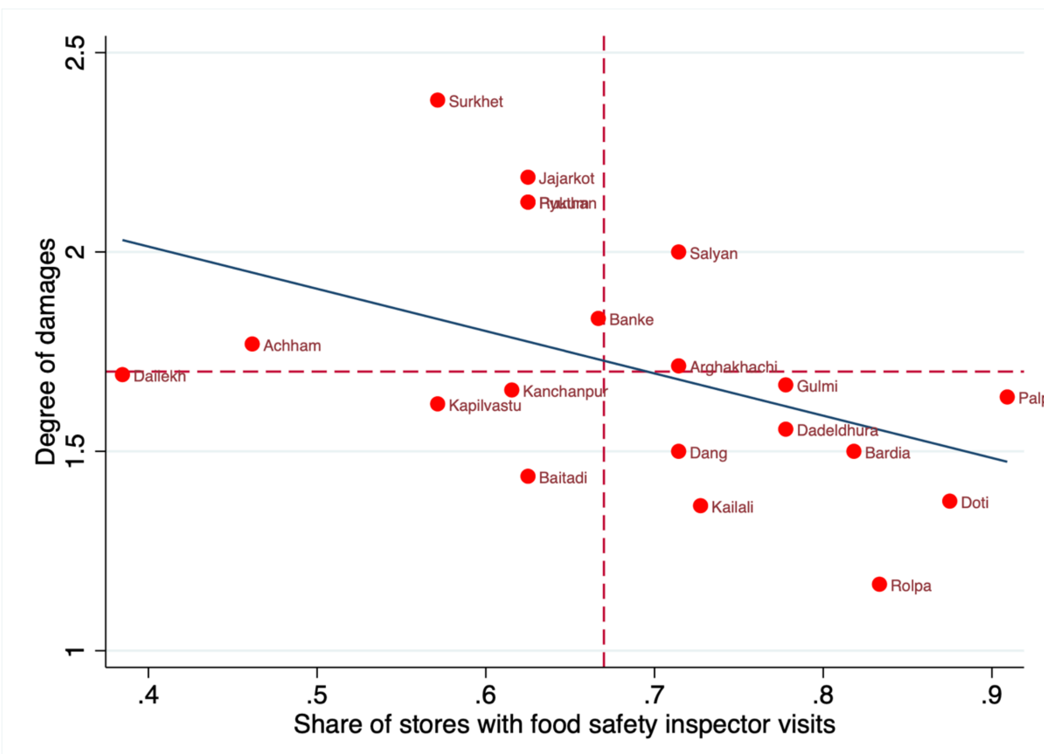


Figure 20 The relationship between the self-stated degree of damages and the number of food safety inspector visits

We also conducted regression analyses to investigate the relationship between the self-stated degree of damages and the number of food safety inspections during the last 12 months. In general, we find the positive relationship. However, we need to be cautious with the results given that there could be endogeneity issue of the number of inspector visits (e.g. reverse causality).

<u>Household Survey Findings</u>

Key findings:

- There exists little difference in observable quality across socio-economic groups.
- The frequency of checking the stored maize and cleaning storage are correlated with lower degrees of damage.
- Perceived food safety awareness is associated with longer storage duration but not to better practices.

Table 5 displays the summary statistics of the key variables from the household survey. The degree of damage in their maize is a self-stated 5-point scale measure (5 being most damaged), the duration of drying is in days, and the variables, sorting maize, cleaning storage, and perceived food safety awareness are indicator variables.

Table 5 Means and standard deviations of the selected variables (standard deviations are in the parentheses).

Variables	Round 1	Round 2
Degree of damage	2.54	1.87
	(1.05)	(1.02)
Duration of drying	6	4.28
	(5.95)	(3.95)
Sorting maize	0.91	0.80
	(0.28)	(0.40)
Cleaning storage	0.92	0.99
	(0.27)	(0.063)
Perceived awareness	0.35	0.67
	(0.48)	(0.47)
N	256	256

For the variables listed in the summary statistics above, they were significantly different between the two rounds of household surveying (two-sample t-test, at p-value <0.01). Further investigation is needed to understand the possible seasonality or the possible effect of the first round of survey on the variables.

Maize quality by region, and by socio-economic group

Figures 21 – 23 show that there is little difference in the self-stated degree of damage across regions and socio-economic groups. While it is important to acknowledge that the measure of the quality is imperfect and prone to have some measurement error, the information from the figures indicate that there is no particular group that consumes systematically low-quality food.

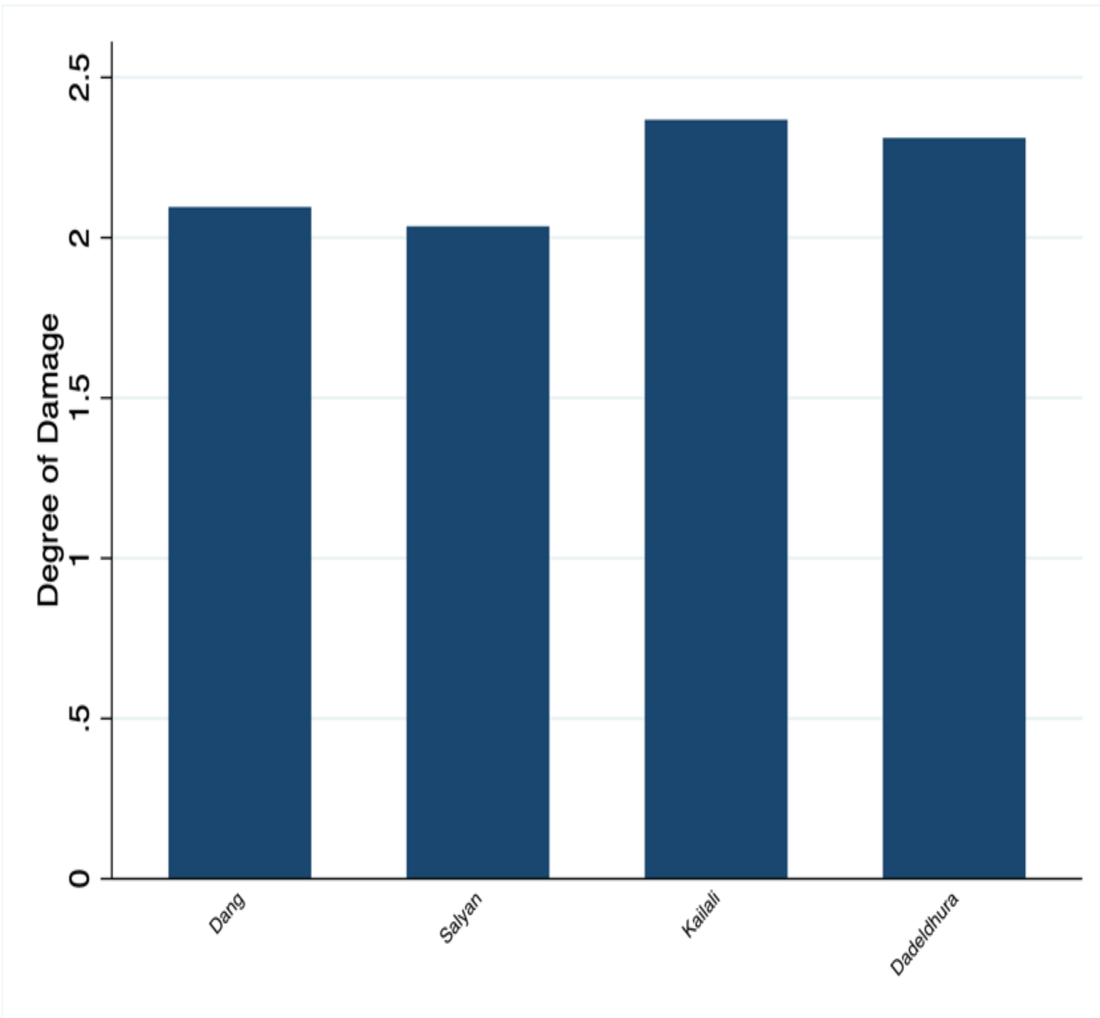


Figure 21 Quality by district

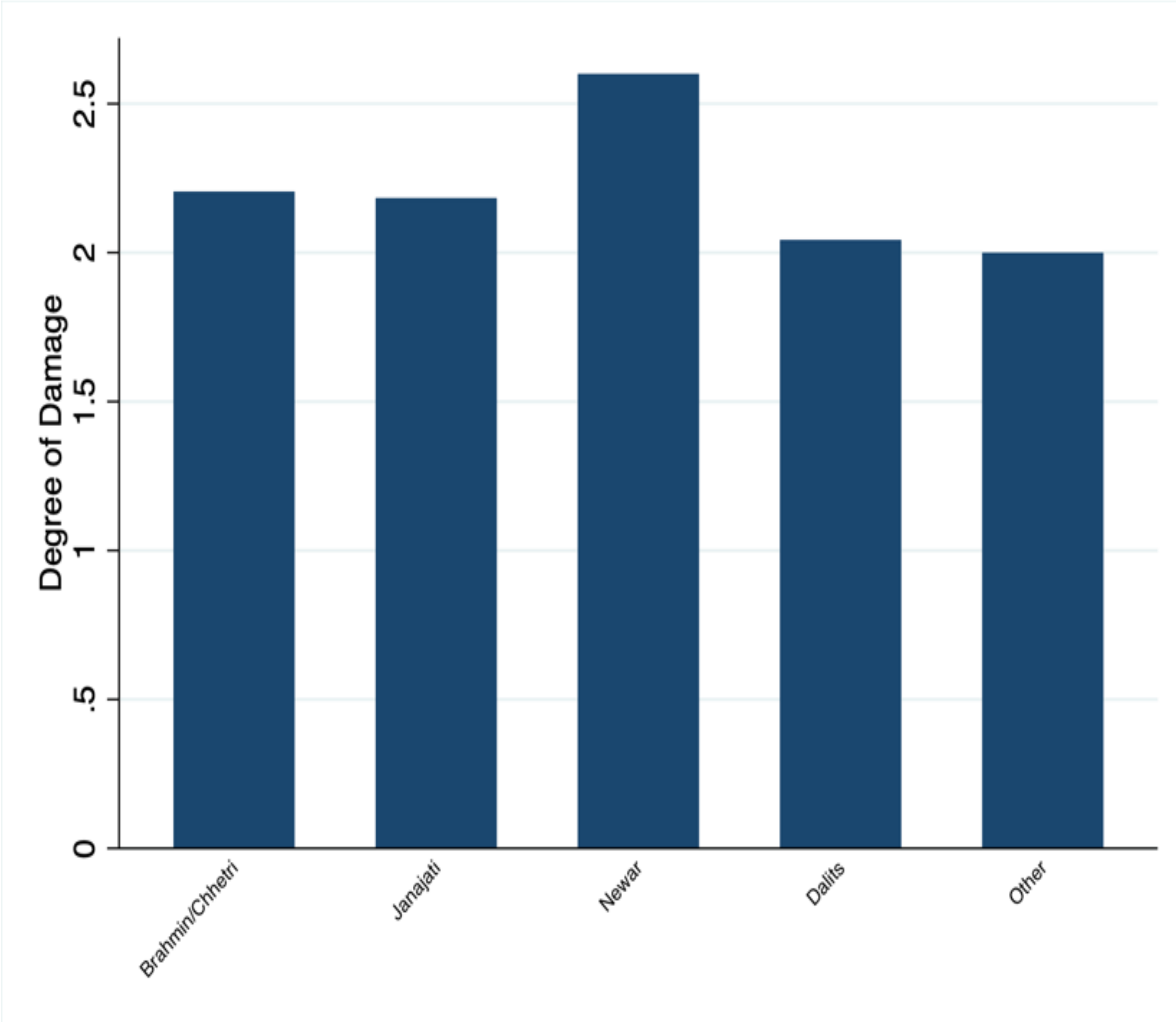


Figure 22 Quality by ethnic group

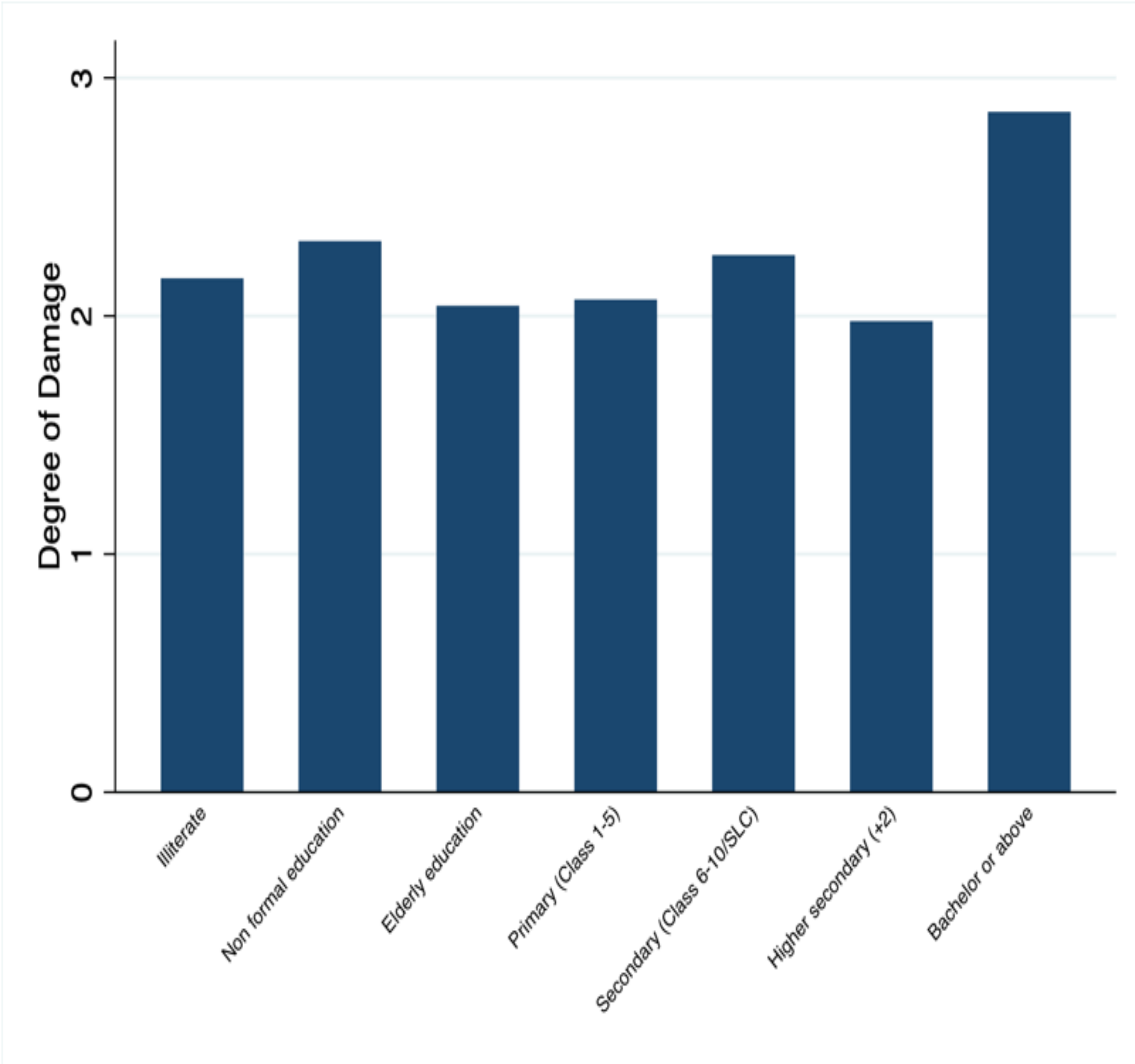


Figure 23 Quality by education level

Practices do matter

In order to further interrogate the factors/practices which may influence observable maize grain quality in households, we again conduct a regression analysis. We use Panel Fixed Effects model to control for unobserved heterogeneity across farms. For farm i, in district j, in round t,

$$Degree\ of\ damage_{ijt} = \alpha_{ij} + BZ_{ijt} + \varepsilon_{ijt}$$

where α_{ij} is household fixed effects, and Z_{ijt} is the vector of practice variables.

We find that a) the frequency of checking the stored maize (-0.0036 with p-value=0.097) and b) cleaning storage (-0.87 with p-value=0.01) are correlated with lower degrees of observable damage.

The practices do matter in a way that the self-stated quality measure does respond to better practices.

Perceptions of food safety risk, post-harvest practices, and intertemporal staple crop allocation

Finally, we are interested in how perceived awareness of food safety risk affects post-harvest practices and storage decision. This is from a working paper by Ralph Armah, Jisang Yu, and Ben Schwab.

We use panel data estimation methods such as linear probability model with fixed effects and Tobit model with correlated random effects. Our first estimation equation is $Storelen_i = \alpha_0 + \alpha_1$-$AWARE_i + X'\delta + \varepsilon_1$ where $Storelen_i$ is length of maize storage by respondent i, and $AWARE_i$ indicates self-stated food safety awareness of respondent i. The vector X is a vector of control variables (i.e. respondent characteristics--sex, age, education, and occupation) and household characteristics (e.g. average quantity of maize consumed by household, household food expenditure, household food insecurity, and whether household sold any maize). We also estimate the equation $PHpract_i = \theta_0 + \theta_1 AWARE_i + X'\gamma + \nu_i$ where $PHpract_i$ indicates post-harvest practice such as drying, sorting, and clean store.

Our preliminary finding is that farm households who perceive themselves to have better awareness of food safety risks tend to store produced maize longer than the other households. However, there are no statistical differences in post-harvest practices (except for the variable whether they hang maize or not) between the households with higher perceived-awareness and the others. Figures 24 – 25 show the means of storage length and various practice variables by awareness.

These highlight the importance of strengthening the research-extension link and providing agricultural extension officers and farmers information better information on post-harvest management. Perceived awareness without proper information and education does not empower farmers to reduce food safety risks, including mycotoxins, and may lead to unintended consequences.

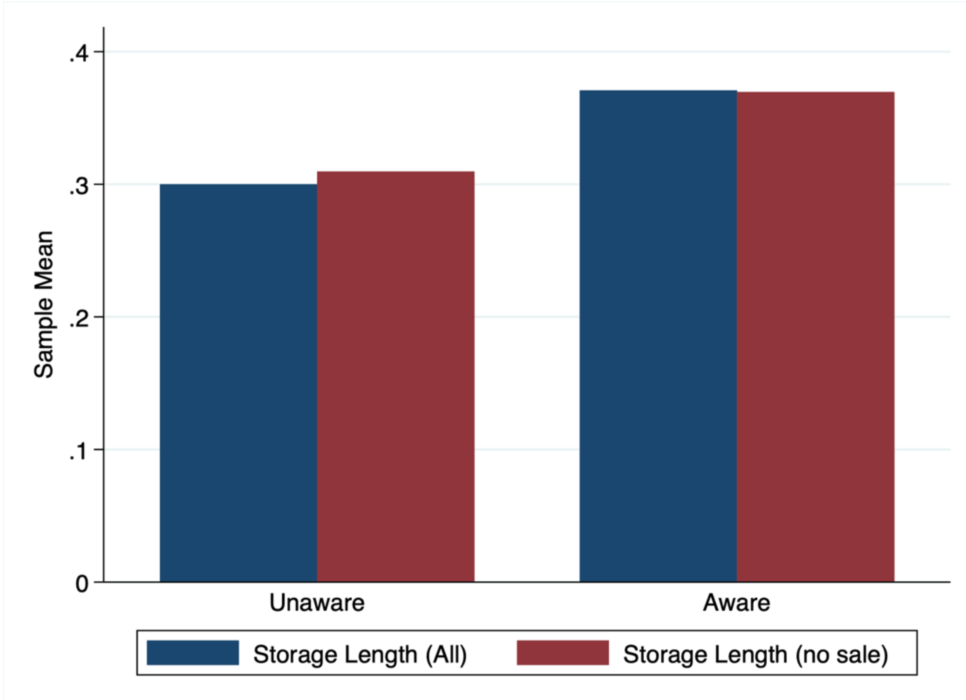


Figure 24 Perceived awareness and storage length

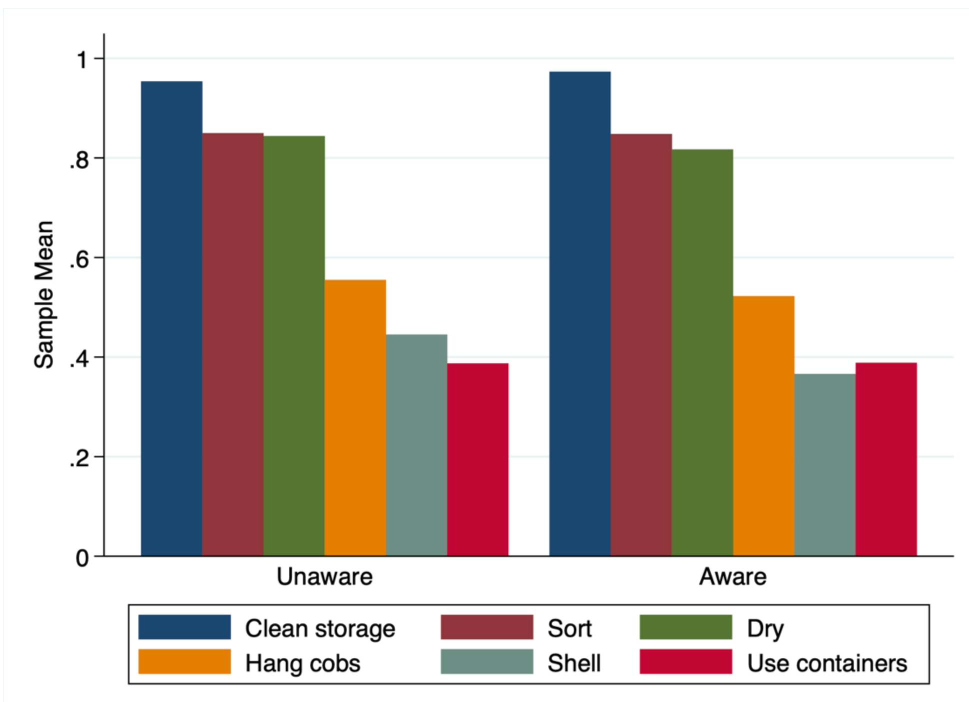


Figure 25 Perceived awareness and various storage practices

Concluding remarks – agricultural economics

From Market Survey, we find:

- There exist price premiums for better quality maize.
- Food safety inspector visits are correlated with higher quality.

From Household Survey, we find:

- There exists little difference in observable quality across socio-economic groups but better practices are correlated with lower degrees of damage.
- Perceived food safety awareness is associated with longer storage durations but not to better practices.

Market structure and institution matter. Any interventions, including trade policies, need to consider potential unintended consequences. To successfully encourage adopting technology/better practices, we need to understand what are the binding constraints (e.g., institutional, financial, informational constraints).

Additional research questions, which would build on these findings and towards a national mitigation strategy, include:

1. Estimating maize supply and price premiums: Do quality/safety-related price premiums improve post-harvest management practices or push low-quality maize to low-income households?
2. Exploring the roles of government and relevant institutions in promoting awareness and good practices, monitoring quality/safety, and enforcing regulations: What are the roles for Federal and local governments? How is the monitoring/inspection affecting the food market in rural Nepal, and how can it be improved?
3. Exploring the consequences of trade practices: How do changes in regulatory environment in potential trading partners affect the Nepalese food system?
4. Identifying constraints of technology adoption and developing effective designs for technology adoption: What are the constraints of adopting better post-harvest practices and the effective policy designs for mitigating such constraints?
5. Evaluating the impacts of better post-harvest practices: How does improved post-harvest practice affect economic and health outcomes of smallholders (e.g. income, nutrition, etc.)?

Banke household study/NIL collaboration: food and feed contamination and practices vs. exposure and stunting

A key objective of the Nepal project was a collaboration with the Nutrition Innovation Lab to explore associations between aflatoxin contamination of food, and aflatoxin and stunting levels in AflaCohort infants. Given the complexity of this collaboration, both in terms of the technical objective and in terms of a mixture of independent and collaborative objectives for each IL, NIL and PHLIL worked to develop a Note of Intent (similar to an MOU, however between the ILs rather than between the universities). Data sharing and other aspects of the collaboration have followed the NOI.

After extensive discussion, and a site visit to Tufts University by Dr. Harvey, the design of the food sampling from a subset of AflaCohort households (180 across two rounds of collection) was set. The survey tool was completed, approval gained from the Nepal Health Research Council, and activities undertaken with great help from common project partner Helen Keller International.

Power calculation:

In order to resolve the number of households to survey, Dr. Darnell (CSIRO) conducted a power calculation, which was shared with NIL.

The power of detecting a 0.2 correlation between blood levels and food contamination at a significance of 0.05 and power of 0.8 requires 194 samples. Based on our previous surveys elsewhere, this is our best guess given that there is not further information on contamination levels in Nepal. A correlation of 0.2 is quite low, so with a higher correlation, we are more likely to detect the correlation. The 0.8 value is a fairly standard power estimation, and 0.05 is a standard significance level used.

Statistical Test	Sample Size	Power	Correlation	Significance Level
Correlation	28.870	0.800	0.5	0.05
Correlation	46.570	0.800	0.4	0.05
Correlation	84.750	0.800	0.3	0.05
Correlation	193.780	0.800	0.2	0.05

Survey:

PHLIL worked with HKI on the sampling procedures for the food in the AflaCohort households. The plan was to collect food samples, then shortly thereafter NIL collect their samples/data from infants. This way, the aflatoxin levels in the food in those households and the serum aflatoxin levels would be related.

Timeline of collections:

Round 1:

June-July 2018: Round 1 Food samples were collected from Banke households June/July, but not biased towards groundnut households (hence only 2 groundnut samples collected that round).

June-August 2018: Biological samples (serum) was collected from households within a few weeks (18-22 months).

Round 2:

Nov-Dec 2018: food samples were collected from Banke households, with a bias towards getting the groundnut households based on the first round results.

NIL has a 24-26 mo infant timepoint after this, however they did not measure serum aflatoxin values.

Results:

Statistical analysis of aflatoxin levels in the food collected and serum aflatoxin levels and stunting have not revealed a significant association.

This study was designed before the relative risk ranking of Nepali foods was completed by PHLIL, so we now have more information (retrospectively) than when this was designed and undertaken. We now have a range of insights from this.

From the broader PHLIL survey, and groundnuts and maize were found to have high risk of aflatoxin contamination. At the December 2019 stakeholder workshop, NIL presented that consumption of groundnut based on AflaCohort recall of diet had the highest association with serum aflatoxin. This was followed by maize consumption, which had less significant association. Therefore, groundnut and maize were key likely contributors to aflatoxin exposure.

In the first round of the survey (for which serum aflatoxin levels were measured shortly after food collection), only two groundnut samples were collected, from 173 households. In the second round, surveying focused first on households that had previously had groundnuts in past AflaCohort work, and 24 groundnut samples were collected. Of the 26 total groundnut samples, 10 had detectable levels of aflatoxin. For maize, only about a quarter of the households had maize stored that could be collected.

Challenges/assumptions:

1. Even if more households had groundnut and maize, there would still have been assumptions about whether they were homogenously provided to the infants or if there was sorting beforehand (and which fraction the infants received), and any snacks such as groundnuts consumed immediately from the market would still be absent for sampling. By design, sampling, subsampling and lab analysis is meant to give as representative an aflatoxin value for a stored portion of grain/food as possible; the fact that PHLIL observed such a high percentage of households sorting maize, groundnut and chili before consumption biases this significantly.

2. Ultimately, in the first round of surveying when the food and serum/stunting measurements could be compared, too few households had groundnut and maize samples (especially groundnut). Were serum aflatoxin data available for the second round of food sampling, it would have increased the chances of detecting an association between collected food and aflatoxin exposure/stunting.

3. A further confounding factor to this is the fact that we have found aflatoxin in chilies, soy nuggets and other parts of the Nepali diet. Dietary diversity in Nepal further complicates capturing where the aflatoxin is coming in for exposure (compared to other countries where there is a primary staple, such as in Eastern Kenya).

Unfortunately, this all converged such that statistical analysis was unable to show an association between aflatoxin levels in food and serum aflatoxin levels and stunting.

Conclusions:

An association between aflatoxin in infants' food and blood serum aflatoxin levels and/or stunting was not established. However, the wealth of information gathered from the broader PHLIL study, as well as from NIL's extensive work, nonetheless charts a path forward to mitigating aflatoxin contamination and the negative health effects in the Nepali population. We do know from the broader work: risk levels for aflatoxin contamination in Nepal for various foods and feeds; geographic areas and climatic features that increase risk of aflatoxin contamination of maize (risk mapping and broader survey); current practices along the value chain, from farm to market, including those that are associated with higher aflatoxin prevalence in multiple food commodities and/or are known to increase risk of aflatoxin contamination; and stakeholder-informed and –formulated short, medium and long-term strategies to address aflatoxin contamination in food and feed in Nepal.

This is the first time an agriculture-health alliance has been forged to try to undertake the complex challenge of associating diet and aflatoxin exposure/stunting. The broader PHLIL Nepal project was ambitious and proceeding with all parts moving in parallel; this did not provide an opportunity for the work on establishing which foods are higher in aflatoxin contamination risk and other insights learned through this project to fully inform the Banke study design. With the lessons in hand, this positions the collaborative team to undertake a fully informed second attempt should the opportunity present itself in the future.

III. Drying and storage interventions:

See also Annexes X-XII, and further information in PHLIL Annual Reports and website (www.ksu.edu/phl)

For grains (eg, rice and maize), PHLIL has developed a forced-air furnace dryer (now with compressed natural gas) in Bangladesh. At the November 2018 NAST Mycotoxin Stakeholder Workshop and official lab launch, the PHLIL BAU team brought two BAU-STR dryers, gave a technical lecture at the workshop, gave technical training and handed over dryers to NARC and HKI. HKI has since piloted the dyers with potential end users. As a result, two parties are ready to take up the STR dryer through cooperatives with added investment in Kailali district, Province 7. One in Chure Rural Municipality, Sahajpur with training support from the Rural Village Water Management Program funded by the Finnish Government; the other is in Godavari Municipality which is developing a concept for the same. We will be providing the technical expertise for them through Nepal Agriculture Research Council in the near future, at a convenient time for all parties.

For horticultural crops including chilies, the UC Davis Horticulture Innovation Lab has developed a chimney dryer. HKI received seed funding from HortLab to validate the chimney dryer for chili drying.

In terms of storage, some hermetic technologies are available in Nepal. They have been extensively validated under a range of conditions, in many countries. Members of the private sector who sell these were present at the August stakeholder workshop, and a range of options were presented to and discussed by the stakeholders.

Discussions with the Ministry of Agriculture Postharvest Directorate in the first half of the project included discussions of drying and storage interventions. Their efforts included these already, so additional options were discussed.

The survey was designed to provide insight into which agricultural practices and other biophysical factors may be associated with increased risk of aflatoxin contamination. There is a wide body of research literature on this, however observing this directly in Nepal can be compelling confirmation for policymakers and other stakeholders, and help inform the selection of mitigation packages to be piloted and scaled to address the challenge of aflatoxin contamination in the food and feed supply.

For more extensive information and stakeholder prioritization, see Annex VIII for the national mycotoxin stakeholder workshop outputs.

IV. CAPACITY BUILDING

Specific Objective 5:
To build the capacity of, but not limited to, NAST, Nepal Development Research Institute, Tribhuvan University, the Agriculture and Forestry University, DFTQC, and the relevant research division in NARC. Primary partners will have intensive capacity building, given that they will execute the processing and laboratory analysis of survey samples; and others will receive more targeted information and training.

Core human and institutional capacity building of "primary partners" (NAST laboratory team; comprised of members from NAST, NDRI, Tribhuvan University):

Overall outline of human and institutional capacity development, centered around the NAST mycotoxin laboratory and research team (see also details in Annexes I and II):

1) Scoping visit and laboratory/capacity assessment at various institutions, by KSU, USAID (JH, JL, Ahmed Kablan - in coordination with Mission, facilitated by NDRI)
2) Scoping visit to field sites in Banke/Nepalgunj, by KSU, NIL, USAID (JH, JL, Ahmed Kablan, Shibani Ghosh, Johanna Andrews Trevino and Ashish Pokharel - in coordination with the Mission)
3) Partnership with Mars Global Food Safety Center, and adaptation of Mars factory x previous PHLIL mycotoxin analysis laboratories for NAST design; JH visits to Mars Pet Food factory in Matoon IL, Mars Global Food Safety Center in China; and Mars Global Food Safety Center lead food safety scientists to NAST, the PHLIL National Stakeholder Workshop, and the Nutrition Symposium in Nepal.
4) Training of NAST mycotoxin analysis team
    a) Surveying and sampling
        i) HKI and NDRI trained, and in turn led enumerator training for PHLIL survey
    b) Laboratory sample handling, processing and mycotoxin analysis
        i) At UNL: mycotoxin analysis
            (1) Five core NAST lab team researchers (see additional detail below)
            (2) Six month placement – Ram Kumar Shrestha(Tribhuvan University)
        ii) At Mars India: groundnut and maize sample processing and testing
        iii) In situ in the NAST lab
            (1) Weekly virtual lab meetings with US team
            (2) Site visits by US team to NAST lab
            (3) Nepal technical team carried out weekly technical meeting from beginning to end of the project and the team leader updated weekly progress in written form to Dr. Jagger, Director and his team members.
            (4) A project management committee (PMC) was formed by VC of NAST to monitor and facilitate the project works to be executed at NAST. The PMC meeting sat at

certain intervals, got update of the overall progress and cooperated to the team wherever and whenever needed for easy running of the lab works.

Additional capacity building in complementary areas and for other institutions (add institutions in attendance):

a. Tribhuvan University, Institute of Agriculture and Animal Science (IAAS) has included lectures and lab classes in its B.Sc.Ag. curriculum in the course of Plant Pathology, as well as in the syllabus of the third semester MSc degree in the Faculty of Education. ~~(Gopal sent the topics in previous emails).~~
b. Paper writing workshop x 2 (John Leslie)
c. Grant proposal workshop (JL)
d. Mycotoxin parallel workshop session: introduction to mycotoxins and mitigation options (PHLIL and NIL 2018 Nutrition Symposium)
e. Risk communication workshop (Jeffrey Morris)
f. Risk mapping lecture/discussion (Ross Darnell)
g. Technical training – theoretical lectures (Jagger Harvey, Bob Baker, Andreia Bianchini, JL, Jisang Yu, RD, and KC Gopal)
h. Technical training – laboratory ELISA training at NAST (NAST/NDRI/TU team)
i. STR dryer handover and technical training (PHLIL Bangladesh/Bangladesh Agricultural University team)
j. Technical training of Ministry of Agriculture in surveying techniques by HKI (planned following ongoing Post-Harvest Directorate leadership changes)
k. Immersive stakeholder workshop: technical presentations and focus group discussions over 4 days
l. Translation of Scientific Animations Without Borders aflatoxin and mitigation measures video into Nepali, presentation at national stakeholder workshop
m. MSc student research: Immaculate Wanjuki (ongoing, supported by KSU/Mars funds from here)
n. PHLIL Nepal team member (NDRI/TU/NAST) attendance at PHLIL 2017 and 2018 Annual Meetings: exposure to research and innovations under development across the active PHLIL six-country portfolio, presentation and involvement in discussions/networking.
o. Gopal also talked to Mr. Ram Kumar Shrestha, an Asst. Prof. of IAAS, Lamjung Campus, about possibility of mycotoxin lab establishment in Lamjung Campus. He talked to Campus Chief. Mr Shrestha then after told me that they have lab room where they can set mycotoxin lab if external support (cash/noncash) provided. The Campus Chief has also written a letter supporting this.

<u>Summary of human and institutional capacity building, centered around the NAST mycotoxin laboratory and research team:</u>

Establishing a highly trained core mycotoxin research team for Nepal:
Five technical research personnel from three Nepalese institutions were trained at UNL on mycotoxin analyses by Andreia Bianchini and her staff from 13-22 November 2017. These individuals were from NAST: Jaishree Sijapati and Rosa Ranjit; from the Institute of Agriculture and Animal Science (IAAS), TU: Gopal Bahadur K.C. and Ram Kumar Shrestha; and from Department of Agriculture, MoALD: Prakash Ghimire. Prior to training at UNL, none of the trainees had more than basic knowledge of mycotoxins and no experience in the analysis of mycotoxins. The personalized training covered theoretical and practical aspects of mycotoxins, their hazards to humans and domesticated animals, the identification of major mycotoxin-producing fungi and the crops and crop products with which they commonly are associated, mitigation strategies and detection techniques for aflatoxins and fumonisins in corn flour, raisins, chilies, and other foodstuffs by ELISA, fluorometer, HPLC and PCR. Techniques for culture, isolation and identification of mycotoxin-producing fungi associated with various foods also were taught. The NAST scientists (Jaishree Sijapati and Rosa Ranjit) Rabindra Dhakal from NDRI also received training from 3-7 June 2018 at a commercial Mars, Inc. testing laboratory in Hyderabad, India, on processing peanuts and maize for aflatoxin analyses in peanuts and maize in ELISA micro-wells. Ram Kumar Shrestha was seconded to the UNL mycotoxin lab from July 2018 to February 2019 to analyze samples collected during the large survey for aflatoxin. All of the trained staff now have in-depth knowledge of mycotoxin hazards and mitigation strategies. They also are proficient in the analytical skills required for aflatoxin and fumonisin analyses by ELISA and fluorometer. These staff can run an independent lab, if others are established outside NAST, and have already begun training others to conduct credible analyses and manage independent labs as well.

NAST mycotoxin laboratory establishment:

The mycotoxin lab established by the PHLIL project resulted from an investment of more than 10 million Nepalese rupees at NAST, Khumaltar, Lalitpur, and included a technical partnership with the Mars Global Food Safety Center (Beijing, China) as well. The NAST lab can efficiently analyze aflatoxins in peanuts and maize. Jaya Kumar Gurung and his team at NDRI led planning and oversight of the lab's timely establishment and initiated project operations, after which lab management was transferred completely to NAST staff. Great cooperation also was provided by the VC of NAST, Dr. Sunil Babu Shrestha, secretary Dr. Mahesh Kumar Adhikari, faculty and section heads and staffs of faculty of science, members of project management committee and heads and staffs of account and administration sections at NAST. Without their unwavering support for the lab establishment and the completion of the project, the results of the project would not have been as successful as they were.

The new lab at NAST can analyze aflatoxins in peanuts and maize and fumonisins in maize. The lab includes the following research apparatuses: Grain Drier, hand held moisture Meters, Computer hardware and software to generated bar-coded labels used for tracking and identifying samples, kitchen grinders food processors and blenders, laboratory scale Romer mill, humidity-temperature meters, chemical fume hood, coffee pulper (for peanut) 1 pc, -20°C freezers, refrigerator, Romer ELISA analysis equipment, fluorome shelling ter, digital balances (1 mg to 2 kg range), reverse osmosis water purifier, orbital shaker, air conditioners, chemical safety cabinets, Dell computers and printers, micropipettes (20 µl, 200 µl and 1000 µl), multi-channel pipettes, pH meters, timers, vacuum cleaners, vortexer, lab trolleys and glassware. The NAST lab will serve as a central lab for the country with plans being formulated for potential satellite labs being established at other locations throughout the country, as was identified as a high priority at the August 2019 national mycotoxin stakeholder workshop.

Teamwork uniting around the NAST mycotoxin research platform:

Given the ambitious scope of the Buy-In, and the strong desire of all team members to maximize the information produced to address aflatoxin contamination in the Nepali food system, it took the highest level of teamwork to succeed in this project. The PHLIL Nepal team was in the lead on delivering a very ambitious goal, analyzing an unprecedented number of diverse samples in a laboratory that was being established within the project itself. The UNL laboratory also had a tremendous task, in leading capacity building, training the Nepali team, and in receiving and analyzing thousands of different samples.

Throughout the busiest year when the NAST team were processing and analyzing the very large and diverse set of samples from the surveys, the PHLIL Nepal and U.S. team members held weekly laboratory meetings early in the morning/late in the evening. The Nepal team met the day before and presented a technical report to the US team each week, which formed the basis of discussion. These meetings underpinned the strong success and delivery of the Nepal team. Since the lab had to handle such a wide range of commodities, and it was not clear until one set of results was generated what the adapted plan would be, the team pulled together to confront some major operational challenges, especially considering the tight timeline. The US team members also made frequent trips to Nepal to help in lab assessment and planning, setup, troubleshooting and more. One of the most significant accomplishments of the project was how dedicated the team members were to delivering on this daunting task, and how they all pulled together to succeed despite the myriad challenges that invariably accompany setting up a lab, training a team, conducting a survey, analyzing samples and synthesizing results.

V. Stakeholder engagement

Formal major meetings, stakeholder consultations and workshops (all in coordination with the Mission):

1. NAST mycotoxin stakeholder workshop Nov 30, 2018
2. NAST official lab opening Nov 30, 2019
3. Core high-level government stakeholder pre-sensitization and consultation, before national mycotoxin workshop (called in)
4. National mycotoxin stakeholder workshop (Aug 2019)
5. National mycotoxin stakeholder meeting, participated in NIL-organized meeting (Dec 2019)
6. In addition to many iterative meetings with core government stakeholders, and other key scaling partners

VI. Key Policy Recommendations

Policy suggestions (NDRI/NAST/Stakeholder Workshop) for increasing the future provision of safe food to the Nepalese people (see also annex I):

1. Mycotoxin issues should be incorporated in Nepal government policy, so that they are recognized as an important issue by the people and are included in the mainstream of regular programming.
2. A mycotoxin testing lab should be established in each province. These labs would be managed by local provincial governments, can focus on toxins of local importance, and collectively provide first line coverage for the national domestic food supply. Mycotoxin tests should be conducted regularly on major products and suspected commodities regularly.
3. Education on food safety issues posed by mycotoxins should be incorporated into secondary school and university curricula.
4. Agricultural technicians, farmers, consumers and agricultural traders should be trained to provide awareness about mycotoxin hazards and mitigation.

   The government should provide a subsidy for recommended/improved drying and storage structures to enable adoption by farmers and/or farmers' cooperatives.
5. Importers and exporters should be made aware of existing mycotoxin regulations and asked to help enforce them to insure the safety of food being imported into Nepal.
6. A key recommendation from the stakeholder workshop was the call for formation of a national mycotoxin steering committee (potentially an adaptation of the Partnership for Aflatoxin Control in Africa).

Recommended priority areas for agricultural interventions, from research into use, with designations as short term (ST), medium term (MT), long term LT (see also national mycotoxin stakeholder workshop outputs, Annex VIII):

1. A comprehensive and targeted awareness creation, extension and training program should be established, following a stakeholder/government/USAID Mission-informed risk communication strategy.
    a. ST aspects include:
        i. experts in the national system already being subject-matter experts, empowered with knowledge generated by this and other projects
        ii. communications/extension materials already available, including the Nepali language SAWBO aflatoxin video, which was very positively received at the national stakeholder workshop
        iii. formation of a national mycotoxin steering committee, or adoption of mycotoxin as a key issue to be addressed by an existing committee

b. MT aspects include:

    i. national mycotoxin steering committee to synthesize, articulate and seek funding for a national mycotoxin reduction effort

    ii. Continued research to further understand interventions to reduce mycotoxin contamination and exposure at critical points of the food system

    iii. Deploy postharvest mitigation packages along the value chain and across the food system to reduce aflatoxin/mycotoxin contamination, as validated by further ST/MT research, integrated into nutrition and health intervention programs

    iv. Expand risk mapping work to be more predictive of hotspot emergence, and as a decision-making support tool to help stakeholders and the national steering committee identify the best intervention strategies for different target beneficiaries/food system actors

    v. Establishment of satellite mycotoxin laboratories in each of the provinces, with affiliated rapid response teams to measure and mitigate mycotoxin contamination at farms and markets, as issues and hotspots emerge.

c. LT aspects include:

    i. Deployment of a model agriculture-nutrition program into the Nepali national system, reducing the risk of mycotoxin exposure and responding to emerging outbreaks

2. Research and capacity should be further extended to further characterize multiple mycotoxin threats in the food system, and extend monitoring and interventions for aflatoxin and other priority mycotoxins across the food system

    a. ST Aspects include:

        i. continued research to expand our understanding of multiple mycotoxins in various foods and feeds across the food system

        ii. pilot interventions into use (as the PHLIL program does successfully in its core countries, which could provide a set interventions already validated for use in other Feed the Future countries, providing high-likelihood quick wins)

        iii. piloting the BAU-STR dryer into scaling for rice and maize in Nepal (note that the PHLIL program already used non-Nepal funds to bring the BAU-STR dryer from PHLIL Bangladesh for successful piloting)

        iv. Already identified good agricultural practices (pre- and post-harvest) promoted; the PHLIL survey revealed that almost no improved best post-harvest practices are being practiced, and many have already been validated based on broader research studies (see report and annexes I, VIII – XII); see GAP section below

    b. MT aspects include:

        i. Establishment of risk mapping as a predictive tool, for both decision making for targeting interventions to different areas, and as an early warning tool for emerging aflatoxin hotspots despite best efforts

    ii. Establishment of mycotoxin testing and response laboratories in each province, forming a hub-and-spoke network as prioritized in the national stakeholder meeting
    iii. Enhancing GAP training and tools in the national system, beyond efforts already underway
  c. LT aspects include:
    i. Sustainable establishment of a food systems-level mycotoxin mitigation and monitoring system serves as a model for research for development approaches to further food safety threats
3. Good agricultural practices should be promoted (this should form the basis of the agriculture component of an aflatoxin/mycotoxin risk reduction program, spanning short-to-long terms as capacity and knowledge emerges from 1 and 2):
  a. Production level (generally, practices that increase yield and reduce biotic and abiotic stress also reduce risk of mycotoxin contamination in the field):
    i. Reducing residues of past crops in the field, where mycotoxigenic fungi can reside and contaminate the next season's crop
    ii. Proper tilling practices, where appropriate
    iii. use of improved, adapted seed (appropriate variety, especially one that will be more drought tolerant if necessary for the agroecological area)
    iv. appropriate use of fertilizer
    v. proper weeding and pest management
    vi. avoiding drought stress by proper irrigation where possible
  b. Peri-harvest
    i. Timely harvesting at physiological maturity
    ii. Avoid harvesting directly onto the soil (reservoir of mycotoxigenic fungi)
  c. Post-harvest
    i. Avoid heaping for extended periods of time, since lack of airflow and moisture are conducive to fungal growth and mycotoxin accumulation
    ii. Sorting out and disposing of damaged and moldy cobs/chilies/grains, so they do not contaminate the rest of the harvest
    iii. Proper drying immediately after harvest (note that xx% of the households surveyed sun-dried maize on the ground, so there is essentially no even marginally improved drying in practice); this is a highly effective step in reducing aflatoxin accumulation, and was prioritized highly at the PHLIL Nepal Mycotoxin Stakeholder Workshop
      1. Low cost: dry on a clean tarp rather than on bare ground
      2. More advanced on-farm or coop level: dryers such as the BAU-STR dryer (for rice, maize wheat; donated by PHLIL Bangladesh and

successfully piloted by HKI in Nepal) or the Horticulture Innovation Lab chimney dryer (chilies,…; piloted by HortLab in Nepal)

3. Industrial scale: larger scale dryers are available for mills, warehouses, national stores,…
4. Moisture measurement to ensure proper drying is key (options include: low-tech/lower accuracy DryCard; high accuracy, fast, multiple commodity calibrated PHLIL GrainMate moisture meter; lower cost/slower to equilibrate FPLIL hygrometer approach)

iv. Proper storage; this is a highly effective step in reducing aflatoxin accumulation, and was prioritized highly at the PHLIL Nepal Mycotoxin Stakeholder Workshop

1. For grains, clean metal silos or better yet hermetic storage bags (eg, ZeroFly Hermetic, GrainPro, PICS) are highly effective at reducing aflatoxin accumulation in grains; need to be paired with proper drying

v. Mycotoxin surveillance:

1. Risk mapping: the PHLIL/CSIRO risk mapping tool produced in this project could be refined to help inform
    a. where to deploy different interventions, as a decision-support tool, using historical climatic data
    b. where emerging aflatoxin hotspots are at harvest, to help target mitigation measures to those areas most likely to be affected due to in-season climatic conditions when crops were in the field
2. A network of testing capacity:
    a. Hub reference laboratory (NAST PHLIL Mycotoxin Laboratory, established by this project)
    b. Satellite more basic laboratories, in each province
    c. Mobile testing capacity, linked to mitigation options with economic value to the grain/food/feed owner when it is found to be over the limit for aflatoxin (eg, gas-mediated decontamination and use as feed)

Note: all of these were discussed as options by stakeholders at the national workshop. See annex VIII for further detail.

VII. Additional achievements – towards sustainability

National research system commitments as a result of and beyond this Buy-In:

1. NAST has committed core institutional funds, on an annual basis moving forward, to continue running the mycotoxin laboratory. The first activity this is supporting is an aflatoxin survey of foods in the Kathmandu Valley.

2. According to Dr. Regmi, the province (province 6) on which he serves as a vice chair on the planning commission is preparing as below to initiate combat with mycotoxins:
    a. The province government has put mycotoxin issue in their annual plan, and wants to establish a lab in the state.
    b. The province government can provide some budget for the mycotoxin lab establishment and execution.
    c. Enough space including one room can be provided for the lab establishment.
    d. As detection of mycotoxin and associated fungi are identified from this project in that region, intervention for mycotoxin mitigation should be prioritized in the zone.
    e. As there is no skilled research technicians trained to work with mycotoxin issues at present, human capacity has to be developed.

VIII. National Mycotoxin Stakeholder Workshop: summary and output tables

"Building a better response" stakeholder workshop

On August 18-21, 2019, PHLIL gathered with a multi-sectoral, multidisciplinary group of stakeholders and researchers in Nepal to discuss the challenge of mycotoxins in the food and feed supply. Representatives from the Government of Nepal, universities, NGOs and other stakeholders, as well as USAID and international partners learned about and developed action plans to address this issue. This collaborative approach will pave the way for future collective action to protect the health of the people of Nepal.

The workshop was divided into five major working sessions, with each one comprised of topical introductory technical presentations, followed by 5-8 member focus groups discussing and ranking answers to a set of questions. This successfully raised awareness, forged buy-in, and produced a stakeholder-generated, rich set of qualitative and quantitative recommendations to address mycotoxins in Nepal in the short-, medium- and long-term.

The Outputs for each nominal group question, and the tables with rankings, are included as attached pdf files.

See Annex VIII for detailed information about the workshop, including the overall objectives and structure, program, attendees and nominal group discussion outputs.

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