Newsletter

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> EDITORIAL

In the past I was very often confronted with the question "What is the best method of mycotoxin analysis in grain or feed?". Well, to answer this directly it can be said that the most frequently and widely used method is HPLC (high-performance liquid chromatography). This method, which is quite sensitive and has reasonably low levels of detection, has been developed for most of the major mycotoxins. Thus HPLC is a very good quantitative method. However, despite the continuous improvement of analytical techniques for the detection of mycotoxins in the past, it has to be mentioned that a reliable (i.e. an accurate and correct) result does not only depend on the selected method of analysis. In fact, nearly 90 % of the error associated with mycotoxin assays can be attributed to how the original sample was collected! "Take a sample and send it to the lab". This really sounds easy and indeed, it can be if we think for instance of tap water. But in the case of mycotoxins, taking a sample which will give a satisfactory analytical result at the end is for sure not easy. Since mycotoxins are not evenly distributed in grain or feed, correct sampling is actually a real challenge. If a commodity has become contaminated by moisture leaks, for example, mycotoxin-containing particles may be located in isolated pockets, so called "hot spots". If the sample is drawn from a single location, contaminated particles may be totally missed ("false negatives") or too many may be collected ("false positives"). This newsletter was written following good advice of the RomerTM Labs' Guide to Mycotoxins - Sampling and Sample Preparation for Mycotoxin Analysis, Vol. 2, 2000. Enjoy reading!

Dian Schatzmayr

Mycofix® Plus product line

As mycotoxins are invisible, odorless and tasteless, the only way to determine if grains or feeds contain these undesired compounds is to analyze for them. However, although excellent analytical methods are available, it is difficult to estimate accurately and precisely the mycotoxin concentration in a large bulk lot because of the large variability associated with the overall mycotoxin test procedure.

Sampling for mycotoxins – *do we care enough?*

Testing for mycotoxins is a complicated process that generally consists of three steps: (1) several small samples are taken at random from the lot and composed into one larger "lot sample", (2) the entire lot sample is ground to a fine particle size and a representative subsample, the "analytical sample", is removed for analysis, and (3) the mycotoxins are extracted from the analytical sample and finally quantified.

Analytical techniques for the detection of mycotoxins continued to improve in the past. However, even when using accepted test procedures there is variability associated with each of the above mentioned steps. Studies by several scientists have shown that sampling usually is the largest source of variation associated with the mycotoxin test procedure [1, 2, 4, 5, 6]. For example nearly 90% of the error associated with aflatoxin testing can be attributed to sampling.

As time and money are being spent for the analyses of mycotoxins, the extra time for proper sampling is crucial for replicable mycotoxin results.

Sampling must be monitored and proper techniques

implemented. Sampling procedures must be written, reviewed and followed by everyone involved.
The high sampling error when testing for mycotoxins is due to two main factors: low concentration of mycotoxins in a given commodity (the "ppb-problem") and the unequal distribution in the lot.

The ppb-problem

Despite extremely high levels of mycotoxins in some kernels, the overall concentration of mycotoxins in a lot of grain is usually very low. The unit of measurement is commonly "parts per billion" (ppb). To illustrate the meaning of these low levels, some examples are given in table 1. [3] Always remember: mycotoxins already affect human and animal health at these low concentrations!

Tab. 1: *What is 1 ppb?*

1 ppb is...

- **1 part in 1.000.000.000**
- **1 second in 32 years**
- **1 grain of sand in 22 kg**
- **1 corn plant in 40.000 acres of corn**
- **1 kernel of corn in 3.5 railcars**

Uneven distribution

Unlike proteins or moisture content in corn or wheat, mycotoxins do not occur in every kernel. In extreme cases mycotoxins may only be present in a few ears or heads in an entire field. This means that some kernels may contain high toxin levels while others contain no toxin at all.
This is due to the fact that fungi do not grow evenly throughout a field or a bin of grain. Thus, mycotoxins tend to be concentrated in certain spots, so called "hot spots" or "nuggets", whereas the remainder of the lot is free of toxins (Fig.1). However, the greater the extent of contamination, the more likely is a more even distribution. Conversely, when the overall concentration of a toxin in a lot of grain is low, uneven distribution is accentuated [3].

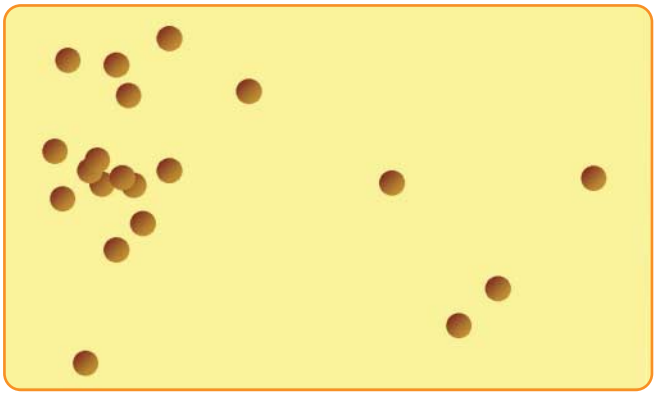

Fig. 1: *Uneven distribution. Brown circles indicate "hot spots".*

Be aware of "false negatives" and "false positives"

Correct analysis means determining the average contamination of the whole lot. If the proper sampling procedures are not followed it is likely that the analytical results will either under-estimate the true mycotoxin concentration (i.e. if only the non- or less-contaminated areas are sampled) or over-estimate it (i.e. if the samples are taken from hot spots).

False negative results are very common in mycotoxin testing, largely due to improper sampling and sampling preparation. When too few incremental samples are taken or the total lot sample is too small, it is much more common to "miss" one of the contaminated kernels than to "hit" it. This type of result is also common when the entire sample probed is divided or split prior to grinding. The number of false negatives can range from 5%, which is normal, to about 90%!
On the other hand, false positives reflect a higher than representative answer. This type of result is not as common as a false negative one.
However, both false negative and false positive results are detrimental as they can cause substantial financial losses (table 2). [3]

Tab. 2: *Consequences of false negatives and false positives.*

False negatives...

- **Expenses for mycotoxin testing are wasted.**
- **Additional transportation costs are incurred when the grain processor subsequently finds mycotoxin contamination, rejects the load and returns it to the seller.**
- **The seller loses credibility and confidence.**
- **Costly lawsuits may follow if a commodity is processed and detrimental health effects result from the consumption of the food or feed.**

False positives...

- **Good grain is sold at lower prices.**
- **Blending or treating good grain incurs unnecessary costs.**
- **Inaccurate results reflect poorly on the overall testing program and deters potential grain sellers to offer grain for purchase.**

Careful sampling is crucial for correct analytical results

Sampling is defined as the process of removing an appropriate quantity for testing from a larger bulk, in such a way that the proportion and distribution of the factors being tested are the same in both the whole (lot) and the part removed (sample). The importance of proper sampling becomes clear when we realize for example that most railcars contain around 55 to 80 t and trucks approximately 20 t of corn and we ultimately

analyze only 50 g of ground sample that must represent the entire lot.
To ensure that the test sample is representative, proper sampling techniques must be used. A "boot" sample from the exposed layer of grain in a hopper car or truck, or a "bucket" sample as truck or railcar is unloaded is NOT representative of the lot as a whole and therefore should never be used. Also, people collecting grain samples can influence how well the sample represents the lot of grain by sampling only a portion of the grain steam. For this reason, scoop sampling and hand-grab sampling is not allowed for official inspections.
The distribution of constituents, such as broken kernels or foreign material, is generally not uniform throughout the load. As grain is loaded into a container (truck, wagon, railcar or storage) constituents of the grain segregate depending on size, density and shape.
During loading fine particles tend to concentrate in the area near the center and larger-sized materials migrate to the outside of the storage container. When unloading, a reverse segregation occurs. This explains why the number of incremental samples and the proper sampling pattern is crucial to ensure that the sample is truly representative of the lot.
The importance of an adequate sample size for the accuracy of the analytical result is shown in the following study (table 3). The samples were taken from a truck containing corn contaminated with 20 ppb aflatoxin. By taking a sample that is too small, the toxins are either missed completely or found at much lower levels than truly present. [3]

Tab. 3: *Variability of test results in relation to the sample size (study by Romer® Labs)*

Sample size [kg]	Approx. number of kernels	Range[1] of analytical results [ppb]
4.5	30 000	11.6 – 28.4
2.2	15 000	8.1 – 31.9
1.1	7 500	3.2 – 38.8
0.4	3 000	0 – 46.9

[1] at the 95% confidence range

For a sample to be considered representative, it must be:

- **obtained with appropriate equipment, such as a probe for stationary grain and a diverter-type mechanical sampler or pelican sampler for moving grain.**
- **obtained using a sampling pattern and procedures designed to collect samples from all areas of the lot (see figure 2).**
- **of appropriate size, which depends on the lot size and the commodity. E.g. a 2.5 to 5 kg sample of corn and a 1.5 to 2.5 kg sample of wheat or barley should be taken from a truck or railcar of grain.**
- **adequate identified and labeled on the bag.**
- **handled in such a way as to maintain representativeness. This means that the samples should be stored in a cool and dry place and submitted in double or triple lined paper bags or breathable cloth bags. Never ship samples in plastic bags as these may promote mold growth if the sample moisture level exceeds 14%.**

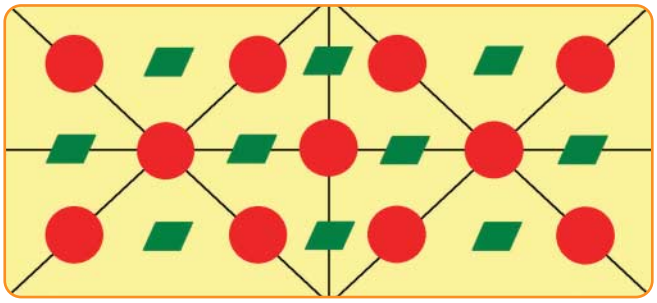

Fig. 2: *Sampling pattern. Red circles indicate spots that have to be sampled, green marks indicate optional sampling spots in case of very large lots. Incremental samples should be ground and accurately mixed. A subsample of about 2 kg should then be sent to the lab for analysis.*

Dictionary:

- **lot sample:** several small samples (= incremental samples) taken at random from the lot
- **analytical sample:** sample for analysis taken from the ground lot sample
- **random sampling:** every individual item in the lot should have an equal chance of being chosen
- **replicability:** the same result should be reached again and again when a specific lot is repeatedly analyzed

Sampling of mixed feed

When mixed feeds are sampled for mycotoxin analysis, two situations are possible:

1. **Mycotoxins were present in one or more of the feed ingredients when the feed was mixed:** The mycotoxins are more evenly distributed in the feed than they were in the contaminated ingredient, because the ingredient has been coarsely ground and mixed into the feed. A 1kg-sample of feed is sufficient to provide a representative sample.

2. **Mycotoxins were produced in the feed after it was mixed due to poor storage conditions (14% moisture or more):** The mycotoxins are usually less evenly distributed. The feed will first become moldy in the moist areas of the storage bin and the mold will slowly migrate to less moist areas as it grows. A good way to sample feed in this case is to take at least a 1-kg sample from the moist areas of the bin (usually the outer edges and corners) and a 1-kg sample from the center.

However, to be absolutely sure, you should always assume an uneven distribution!

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Doctoral thesis: Investigations of a microbial-based feed additive for the detoxification of trichothecenes with regard to viability, activity and safety aspects (Institute for Agrobiotechnology, Tulln)

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Poultry Focus Asia 2004
Bangkok, Thailand, February 23 - 25, 2004

Pig Focus Asia 2004
Bangkok, Thailand, February 23 - 25, 2004

26. Mycotoxin Workshop
Munich, Germany, May 17 - 19, 2004

XI International IUPAC Symposium on Mycotoxins and Phycotoxins
Maryland, USA, May 17 – 21, 2004

VII International Conference on Mycotoxins and Pathogenic Moulds
Bydgoszcz, Poland, June 28-30, 2004

18th Intern. Pig Veterinary Society Congr.
Hamburg, Germany, June 28 - July 1, 2004

Fifth International Conference on Bioaerosols, Fungi, Bacteria, Mycotoxins and Human Health
Saratoga Springs, NY, USA,
September 10 - 12, 2004

> Literature

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> Impressum

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