Effect of Grain Storage Conditions on the Accumulation of Mycotoxins

T. Akhaladze, A. Chkuaseli, A. Chagelishvili, M. Khutsishvili-Maisuradze

***Abstract*— Creating an optimal environment during grain storage has only one goal - maintaining grain yield/quantity and quality.**

For full/effective grain storage, it is necessary to create environmental conditions that prevent the development of pests and microorganisms. The storage conditions are directly related to the issues of maintaining the quality of grain as raw material, because it is the bacterial and fungal organisms on the surface of the grain that begin to develop as soon as the conditions are favorable for it, on the basis of which the quality of the grain deteriorates, the storage capacity decreases and the yield/quantity of the grain decreases. The growth and development of fungal organisms leads to the accumulation of toxic compounds - toxins in the contents of the grain.

The article discusses the impact of different methods of grain storage on the level of highly dangerous agents mycotoxins and at the same time on the growth of colony-forming units of pathogenic microflora (mesophilic aerobic and facultative anaerobic bacteria).

The research was carried out in the grain storage and drying complex of LTD "Chirina", the largest poultry meat products producer in Transcaucasia.

***Index Terms*— Cereals, storage conditions, mycotoxins, pathogenic microflora.**

I. Introduction

Modern technologies and technical means of grain storage give us more and more opportunities to store grain without loss and with preservation of quality for a long time.

The optimal regime is achieved by maintaining specific indicators of humidity, temperature and oxygen supply. In order to store grain, to maintain its mass without loss and quality, it is necessary to adjust at least one of the above-mentioned parameters.

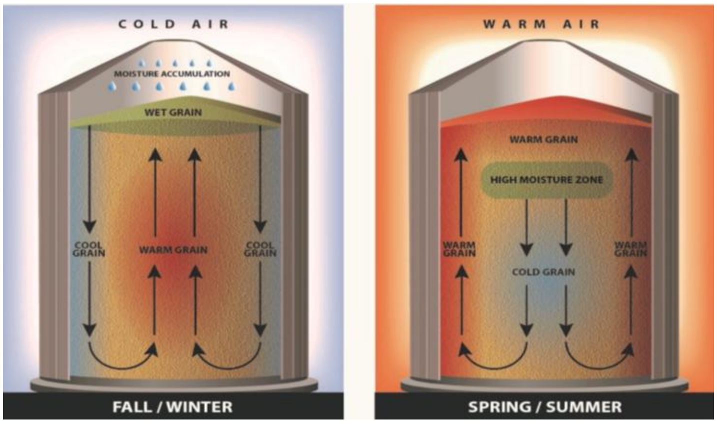
Postharvest losses of grain are 20 to 40%, and storage losses are 5%. The study showed that the world practice uses 4.931 million ha of agricultural land out of the total 14.894 million ha of land surface of the earth. In addition, agricultural production consumes 2.5 trillion m3 of water per year [1].

Traditional storage practices in developing countries do not guarantee grain protection, leading to 20-30% losses, especially post-harvest, due to the proliferation of pests and other pathogenic microorganisms. In addition to causing quantitative losses, grain-borne pests are also associated with aflatoxin contamination and poisoning. To avoid these problems, metal storage facilities called "silos" were created. It is a cylindrical structure building made of galvanized iron sheet and hermetically sealed. It ensures the storage of clothes without loss as much as possible and maintaining quality. Such types of grain storage facilities enable the storage of grain with minimum losses, which at the same time helps to strengthen food security. This is especially important in developing countries. [2]

Grain storage practices and technologies in developed countries are on the verge of transformation. It is driven by advances in information and computing technologies that offer unprecedented opportunities to farmers and grain storage technologists. [4]

A factor to consider when storing grain is seasonality, that is, the influence of winter and summer regimes on the storage of grain mass, especially in the case when the climate cannot be regulated. So, for example, at low ambient temperature, the grain placed in the central part of conveyor-type metal storages/silos keeps heat for a long time, while the layer of grain placed near the wall is cold.

The situation is opposite in summer, the grain placed in the central part of the silo is cold, and the layer near the wall is hot. The temperature of the wheat grain close to the wall is always lower during the cold period of the year and vice versa. The temperature difference in the grain mass causes the air flow to move first towards the central part and then towards the ceiling. Condensation is formed as a result of humid warm air reaching the surface of the cold grain. This leads to an increase in moisture in the grain mass and focal spoilage. (Image #1)



Pic I. The effect of seasonal temperature on grain storage in a modern grain store

During the migration of moisture between the cold and hot layers and as a result of the condensation, a high humidity layer can be formed in the upper part of the storage during the

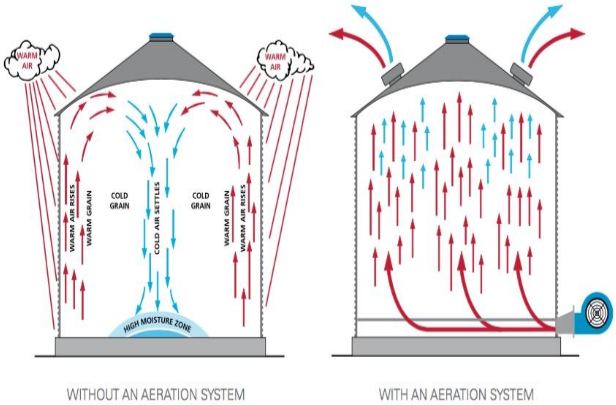
Tamar Akhaladze, Agrarian University of Georgia, Tbilisi,Georgia,
Amrosi Chkuaseli, Agrarian University of Georgia, Tbilisi,Georgia
Avtandil Chagelishvili, Agrarian University of Georgia
Maia Khutsishvili-Maisuradze, Agrarian University of Georgia

winter, and in the middle part during the summer.

During grain storage, the moisture content in different parts of the silage is not the same. Due to the change in ambient temperature, it is possible to create zones with high humidity.

One of the downsides to condensation is that there is no real way to get rid of it. High humidity significantly increases the risk of condensation. It is because of the formed condensate that sometimes it is not possible to store grain until the planned time. The way out of these circumstances, as a lifeline, is the presence of a proper aeration system.

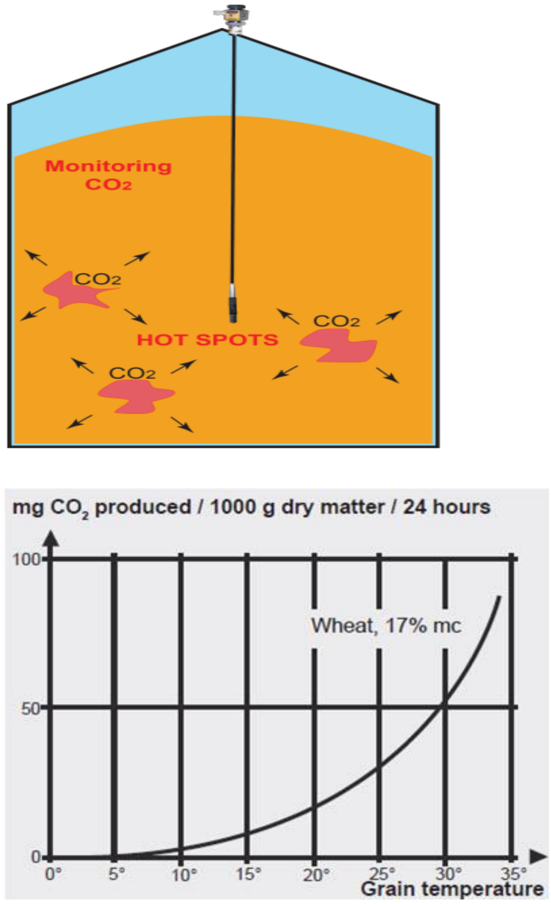
During seasonal temperature changes, it is important how much it is possible to store grain in a cool environment. Processing grain with ventilation (aeration) with a small flow of heat is one of the important measures. The main purpose of aeration is to maintain an even temperature at different points of the grain mass, and it also prevents moisture from migrating to the top (or bottom) of the storage silo based on natural convection. The possibility of ventilation helps to increase the duration of storage (Figure #2) [4] [5]



Pic II. Effect of ventilation mode on grain weight during long storage period

Very often, during grain storage, a so-called "hot spot" - a localized high temperature zone - is formed in the grain mass. Hot spots can occur anywhere in the reservoir (silo). Usually caused by an infestation that produces excessive amounts of gas in the form of carbon dioxide (CO2).

Grain is a fairly poor conductor of heat, making these hot spots difficult to detect. It is impossible to fix it from the outside. Therefore, thermal imaging is one of the methods used to detect hot spots. This non-contact method involves the installation of several sensors in the silo, which will detect high moderate readings reached up to a certain point (on the basis of which the service operator will take appropriate action). Modern sensors are inserted through the silo roof and its end must be embedded in the grain mass (pic. #3) [5] [6]



Pic III Emergence of hot spots in the granary under the influence of carbon dioxide

A grain, like a living organism, breathes and releases carbon dioxide. As the temperature rises, the respiration process is activated, both for the pathogenic aerobic microorganisms present in the grain and in the mass. Grain respiration is significantly higher than microbial respiration. This physiological process has long been used to measure the metabolic activity of a stored product.

The respiration rate can vary depending on the variety, age and quality of the grain. The loss of dry matter is obtained as a result of the use of carbohydrates during respiration. Consequently, respiration data can also be used as a measure of dry matter loss.

The rate of loss of dry matter can become an indicator of grain suitability. It is considered that with a loss of 1% during 12 months, the grain is suitable for animal feed. [6] [7] [8]

Due to the fact that there are many opinions regarding the determination of the need for aeration cooling and aeration drying, it is necessary to compare how these two processes differ from each other. A relatively low air velocity is required to cool the grain. About 2-4 liters of air per second/ton. Whereas grain drying requires much higher air flow rates, more than 15 liters of air per second/ton.

When using cooling aeration, the grain can be cooled even by 10º degrees Celsius, which significantly reduces the risk of fungi and mycotoxins.

When storing grain mass, moisture is of crucial importance, because excess moisture is a favorable condition for the activation of all vital processes, including pathogenic microflora. [9]

Reproduction of stored grain fungal organisms, accumulation of toxins, and grain spoilage are possible when the water activity (moisture) in the grain mass exceeds a critical threshold, which in turn promotes the growth of mycotoxin-producing fungi. (Some mushroom species are known to produce harmful mycotoxins). The most harmful toxigenic species include xerophilic species (genera Aspergillus and Penicillium, Fyzarium)). Because mycotoxin contamination of grain is a worldwide problem, ongoing monitoring of mycotoxin content and product detoxification is a constant concern for public health and the grain food industry. This is why tolerable levels of mycotoxins are strictly regulated worldwide.

Based on the above, the integrated management of fungal organisms in stored grain is based on four pillars:

1. Prevention of the development of fungal organisms - by keeping the grain moisture below the critical limit of fungal growth;
2. Accurate monitoring of grain and temperature changes during the storage period, which is related to the monitoring of early indicators of respiratory activity of storage fungi;
3. Cleaning of grain mass from mechanical impurities;
4. It is possible to use biocompetitive strains of fungi or bacteria to prevent the development of mycotoxigenic fungi in grain bulk - although this is a topic for future research.

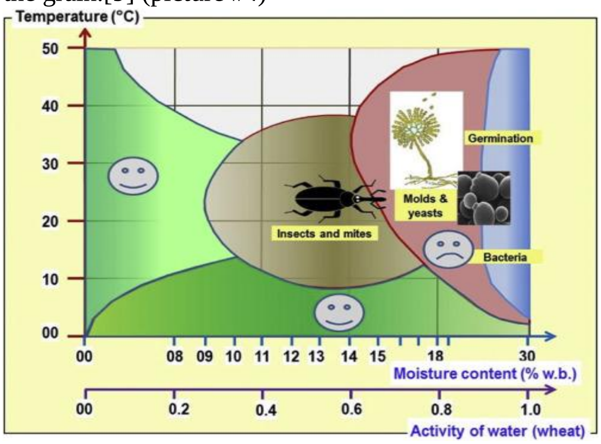
Pests, fungi, bacteria are one of the biggest problems in grain storage.

The first measure to be taken is prevention. Which means maintaining a healthy surface of the grain and the absence of fungal diseases.

In most cases, the grain mass is more or less damaged by fungal diseases. At this time, it is very important to know the characteristics of the development of fungal diseases and grain pests.

From 0 to 50 ^{0}C the grain mass is ideally stored when the humidity is 9%.

From 9% humidity to 20^{0}C pests begin to develop well. For the development of fungal organisms, a moisture content higher than 15% and a temperature of 20 to 50 ^{0}C are needed in the grain.[9] (picture #4)



Pic IV. Humidity and temperature range of distribution of pests and pathogenic microorganisms.

https://www.sciencedirect.com/science/article/abs/pii/S0022474X16300911 (Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins – An update - Journal of Stored Products ResearchVolume 71, March 2017, Pages 22-40)

When the desired moisture level is violated, one of the main measures is to dry the grain mass to the standard moisture content.

When drying the grain, it is necessary to take into account the fact that all metabolic reactions in the grain are related to its moisture content. Through micro and macrocapillaries in the grain, moisture circulates in the form of a liquid to the surface of the grain and vice versa.

Therefore, the active surface of the grain, where the exchange of moisture with the environment takes place, is almost a thousand times greater than the geometric surface of the grain. The moisture present in the grain is found in different forms and connections. This connection can be conventionally divided into three groups: chemical, physico-chemical and mechanical. In the process of drying, the moisture that is in mechanical connection with it is removed from the grain, often this applies to physical-chemical connections as well. Even after drying, there is always moisture in the grain, the amount of which depends on the ratio of dry matter to water mass. Grain moisture is expressed as a percentage of moisture relative to dry matter.

The process of moisture transfer between the grain and the drying agent is carried out through the surface of the grain, so the condition of the surface of the grain is of great importance. Which is more than 1 kg. The surface of the grain mass, the more efficient the drying process is, therefore, the finer the grain, the more intensively it dries.

The influence of the drying agent temperature on the organic substances in the grain (proteins, fats, carbohydrates, enzymes and vitamins) is very important. Grain with 14% moisture can withstand heating only up to 60-65 degrees. Due to the influence of higher humidity or high temperature, the sowing value of the seeds decreases, starch and proteins are destructured, the structure of the gluten structure changes, the number of fatty acids increases, the activity of enzymes is inhibited, and the commercial value of the grain decreases and deteriorates.

In addition to biochemical changes, structural and mechanical changes are taking place: the skin of the grain breaks and the grain is deformed. This breaks the integrity and structure. The content of the grain becomes easily accessible to pathogenic microorganisms.

II. THE PURPOSE AND OBJECTIVES OF THE RESEARCH

The aim of the research was to study the concentration of mycotoxins in grain samples taken from five grain-producing regions of Georgia (Kakheti, Kartli, Samegrelo, Imereti, Guria).

a) to observe the stored grain, whether the content of mycotoxins changed, taking into account the storage conditions, and to express all this in a numerical indicator.

b) at the same time, to study the dynamics of bacterial background changes in grain mass depending on grain moisture and duration of storage.

Scheme of the test:

Option 1 - The grain was stored in the form of embankment for 6 months, with an acceptable concentration of mycotoxins in it

Option 2 - The grain was stored in polyethylene film under vacuum

Option 3 - The grain was stored in modern granaries of Matal with ventilation mode

Option 4 - The grain was stored in a metal granary without ventilation.

Initial state of grain: normative (Mycotoxin Regulations For Feed in The EU EC No. 32/2002 Directive of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed)

III. RESEARCH MATERIAL AND METHODS

The research material was grain (corn, wheat), sampling was carried out taking into account the methodology, in accordance with the regulation. ("Approving the technical regulation on sampling and analysis methods for the determination of mycotoxins" Resolution of the Government of Georgia No. 497 of November 7, 2016, effective date 01/01/2018)

Samples for different mycotoxins were taken separately from each batch to be evaluated. Aokin spectrometer was used to determine the amount of mycotocins, Perten DA 7200 tool was used to determine the chemical composition of combined food. Spectral method was used for research.

Mesophilic aerobic and facultative anaerobic microorganisms were determined using the microbial cultivation method. The analysis will be conducted in the accredited laboratory of "Chirina" LLC.

Accumulation of mycotoxins in grain mass depending on storage conditions

Storage conditions	**DON (Microgram-ppb/kilogram)**				**ZON (Microgram ppb/kilogram)**				**AFLA B1 (Microgram ppb/kilogram)**				**T2 HT2 (Microgram ppb/kilogram)**			
Mycotoxin	Corn		Wheat		Corn		Wheat		Corn		Wheat		Corn		Wheat	
	№1	№2	№1	№2	№1	№2	№1	№2	№1	№2	№1	№2	№1	№2	№1	№2
Option 1 - the grain was stored in the form of embankment for 6 months	1200	860	1110	970	320	420	230	130	5	3.2	2.1	0.9	120	180	115	390
Option 2 - the grain was stored in polyethylene film under vacuum	400	620	2300	320	200	80	90	430	1.6	0.1	0.9	0.5	290	150	210	200
Option 3 - grain was stored in modern Matal granaries with ventilation mode	120	70	650	890	180	300	300	310	1,9	0.7	3.2	3.5	310	200	390	180
Vatiant 4 - grain was stored in a metal granary without ventilation.	120	80	2000	4200	210	380	180	200	0.6	1.7	5.1	2.2	90	140	500	270

IV. RESEARCH RESULTS IN PRODUCTION CONDITIONS

1. As can be seen from the table, the trend of increase of mycotoxins was observed in option 1, where the grain was stored in the form of embankment. Accordingly, the growth of fungal organisms in the bulk of stored grain is more pronounced, which is due to the activation of fungal organisms caused by the interaction of the large surface of the grain mass with air.

2. The lowest increase in mycotoxins was observed in option 3, where the grain was stored in a metal granary with the possibility of using an active mode of ventilation.

Based on the research results, we can make the following conclusions:

- The growth of mycotoxin-producing species is closely dependent on grain moisture content. The higher the humidity above +14^{0}C, the higher the overall bacterial contamination on the grain surface. Growth of aerobic bacteria and fungal organisms (Figure #5)

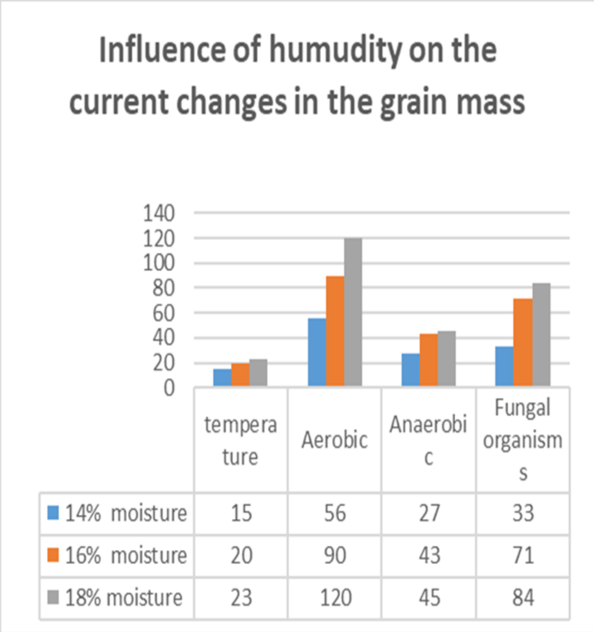


Fig. 4 Influence of humidity on changes in grain mass

1. The grain must be in the phase of physiological maturity. Harvesting of grain should be done in a tight time frame and taking into account the weather conditions to avoid excess moisture in the grain. (The ideal moisture content for harvesting is below 20%)
2. Recommended storage temperature is 15-20ºC temperature range.
3. It is recommended to keep the grain mass moisture at 12.5%. The upper limit should not exceed 14%. The increase of humidity above the mentioned index leads to problems during the storage period, the result of which is grain mouldy, heating red-hot and an increase in the concentration of mycotoxins.
4. Prevention of mycotoxigenic fungal spoilage of stored grain is possible with a preventive strategy that includes integrated approaches combining different methods, materials and practices

REFERENCES

[1] Olorunfemi B.; Kayode E., Post-Harvest Loss and Grain Storage Technology-Turkish Journal of Agriculture - Food Science and Technology , 9(1), 2021, Pages 75-83 https://www.researchgate.net/publication/348680799_Post-Harvest_Loss_and_Grain_Storage_Technology-_A_Review

[2] Tefera T.; Kanampiu F.; Hellin J.; and others -The metal silo: An effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing – Journal Crop Protection, Volume 30, Issue 3, 2011, Pages 240-245 https://www.sciencedirect.com/science/article/abs/pii/S0261219410003376

[3] Millls J.T. „Spoilage and heating of stored agricultural products" Research Station, Winnipeg, Man.Research Branch Agriculture and Agri-Food Canada. 1989 https://grainscanada.gc.ca/en/grain-quality/manage/guides/pdf/shsap-depae-eng.pdf

[4] Graham T. „Storage of Cereal Grains and Their Products" , Chapter 4 Alternative and emerging storage practices and technologies, Publisher: Elsevier 2022

[5] Kurt A. Rosentrater „Storage of Cereal Grains and Their Products", Chapter 3 Overview of storage systems for cereal grains and grain products, Publisher: Elsevier 2022

[6] Ramachandran R. P. Integrated approach on stored grain quality management with CO2 monitoring, Journal of Stored Products Research, Canada, Publisher:Elsevier 2022

[7] http://ecoursesonline.iasri.res.in/mod/page/view.php?id=904

[8] https://grainst.com.au/2021/02/26/why-is-temperature-so-important/

[9] https://www.sciencedirect.com/science/article/abs/pii/S0022474X16300911 (Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins, Journal of Stored Products ResearchVolume 71, March 2017, Pages 22-40)