THE STUDY ON THE FEASIBILITY OF USING IN THE FOOD CHAIN SOME BATCHES OF WHEAT AND CORN CONDITIONED AND KEPT AT CEREAL SILOS IN OLTENIA

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ABSTRACT

In Romania, cereals, and especially wheat and corn, are traditional crops with a high nutritional value of seeds, which are the primary source of food for humans and animals and because they enjoy, in most areas, the conditions. favorable soil and climate.

The use of seeds contaminated with fungi or microorganisms during vegetation, during harvesting, during the conservation period, every year brings considerable losses that affect both the yield and the quality of the harvested and stored production.

In the presented paper we tested some batches of wheat and corn in order to determinate the presence and levels of mycotoxins as: Aflatoxin B1(AFB) and total aflatoxin (AFT), Ochratoxin A (OTA), Fumonisin B1 (FUMO), T-2/HT-2, Zearalenon (ZEA), Deoxinivalenol (DON) and heavy metal residues (Pb and Cd).

INTRODUCTION

Cereals have always been and will be the most important group of plants for human existence and activity, due to the chemical composition of the grains. About 60% of protein, 15% of fat and 70% of carbohydrates, in general, 55 - 60% of calories consumed worldwide come from cereal grains. The basic production of these plants is also the main material for ensuring meat, milk, eggs and has a wide use in the food and other goods industry.

Cereal grains (wheat, corn, barley, oats, rye) provide an ideal nutritional environment for the multiplication of micromycetes and the development of mycotoxins. They can be contaminated with fungi and mycotoxins, both during the vegetation phase of the plants, and especially during the conservation period, when the conditions of harvesting and storage are favorable for germination of the resistance forms of the micromycetes and the multiplication of the mycelial apparatus (Mirela Paraschivu et all, 2014). One of the main causes that generates these shortcomings can be the poor knowledge of the problems related to the health of the seeds, as well as the neglect of the measures of prevention and control of the main phytopathogenic agency that affect the crops (Răducanu Florentina et all., 2008).

Since the discovery of these toxins, research has focused on ways of detecting and determining the degree of toxicity induced in humans and animals, establishing favorable conditions for the development of fungi that produce mycotoxins, establishing maximum permissible limits of mycotoxins in food and feed.

At European level it is estimated that the level of mycotoxin contamination is 20%, even in developed countries, but this percentage is certainly higher when practicing less competitive agriculture. In the developed countries of Europe, a

harvest loss of 5-10% due to the fungal attack is estimated.

Because they have toxic effects on humans and animals, the maximum levels at which mycotoxins can be admitted into food and feed have been established in many countries. As a result of the health risks mentioned above, contamination with mycotoxins may reduce the price of products or, in extreme cases, may lead to the withdrawal of food and feed lots from the market (Wu, 2006).

MATERIAL AND METHOD

Contamination of agricultural products by mycotoxins occurs when they meet the environmental conditions for their occurrence, as well as inadequate harvesting, storage and processing processes, when they are cumulated.

Based on the fact that cereals, especially wheat and corn, make up the basic diet in our country, *we set out to investigate the degree of contamination of these raw materials of vegetable origin and to determine the causes that generate the contamination, in order to avoid the effects. unwanted contamination of consumers' staple foods*.

The National Surveillance Program initiated by ANSVSA aims to collect by survey and free analysis of these basic raw materials, in order to detect the contaminated ones and to prevent the disease of the population.

In order to achieve our goals, we use some reference documents and laboratory devices, as follow:

- SR EN ISO / IEC 17025/2005 - General requirements for the competence of testing and calibration laboratories;
- Ridascreen Kit User Guide Total Aflatoxin, Aflatoxin B1, Zearalenone, Deoxynivalenol, Fumonisin, Toxin T2/HT2, Ochratoxin A - Quantitative determination of residues by immunoenzymatic analysis;
- Commission Regulation (EC) no.1881/2006 - establishing the levels of mycotoxins in food;
- ELISA immunoenzymatic affinity assay;
- Specific standards for each determinate mycotoxins;
- Atomic absorption spectrophotometer AANALYST 200;
- Perkin Elmer Analyst 100 atomic absorption spectrophotometer, with recording and measuring system.

The mycotoxins are extracted from samples with 70% methanol aflaroxin b1 and total aflatoxin, fumonisin, zearalenone and T2 / HT2, ochratoxin A is extracted with 0.13 M sodium bicarbonate and deoxynivalenol with water. Stir well, centrifuge, filter and dilute specific to each kit, then pipette into the wells in the kit.

The cereal samples for the determination of heavy metals are burned in the calcination furnace at 450^{o}C until complete calcination, dissolved in nitric acid, filtered, diluted and read at the SAA with graphite furnace.

RESULTS AND DISCUSSIONS

The wheat and maize samples were analyzed at the Veterinary Sanitary and Food Safety Laboratory of Craiova, in 2017, by the ELISA immunoenzymatic method for mycotoxins and by the SAA for the determination for heavy metal residues, on a number of 13 silos in Dolj County (table 1).

Table 1

Investigations regarding the presence of mycotoxins in wheat and maize Batches at some silos in Dolj County

SILOS	BATCHES (tons)	MATRICE	INVESTIGATION
Segarcea 1	2,48	Wheat	AFB_1, AFT, DON, ZEA, OTA, T-2/HT-2
Segarcea 2	120	Maize	DON
Banu Mărăcine	14	Wheat	OTA
Băileşti 1	60	Wheat	AFB_1, AFT
			DON, ZEA, OTA, T-2/HT-2
Băileşti 2	50	Maize	AFB_1
	50	Maize	DON
	50	Maize	FUMO
Leu 1	50	Wheat	DON, OTA, ZEA, T-2/HT-2, AFB_1
	100	Maize	Pb, Cd
Leu 2	100	Maize	AFT, AFB_1
	100	Maize	DON
	100	Maize	FUMO
Moţăţei	800	Wheat	Pb, Cd
	800	Maize	AFB_1, AB_1, B_1, FUMO
Cârcea	800	Wheat	DON, ZEA, OTA, T-2/HT-2, AFB_1
	750	Maize	AFT, AFB_1
Pleniţa	66	Wheat	Pb, Cd
	120	Maize	DON, FUMO
	45	Maize	ZEA
Bechet 1	75	Wheat	AFB_1, AFT, DON, ZEA, OTA, T-2/HT-2, Pb, Cd
Bechet 2	100	Maize	AFB_1, AFT, DON, FUMO,
Portăreşti	80	Maize	DON

European Commission Recommendation 2006/583 of 17 August 2006 regarding the prevention and reduction of Fusarium toxins in cereals and cereal products, including maize and maize products, contains general principles for the prevention and reduction of *Fusarium toxin contamination*: zearalenone, fumonisins and trichothecene. Thus, the Commission Regulation (EC) No 1881/2006 of 19 December 2006 establish maximum levels for certain contaminants in foodstuffs establishes maximum levels for *Fusarium toxins* in certain foods.

Aflatoxins are the metabolic product of the molds *Aspergillus flavus* and *Aspergillus parasiticus.* The following aflatoxins are known: B_1, G_1, B_2, G_2, B_{2a}, G_{2a} and from aflatoxin metabolism results others, such P_1, M_2, Q_1 or aflatoxicol.

Related the AFB_1 – *figure 1* – we obtained for wheat batches values of the aflatoxine presence under the MRL admitted for wheat, of 2 ppb. The values range from 0.5 ppb at Segarcea 1 to 0.8 ppb recorded at Băilești batches of analyzed wheat.

For maize, in the two batches analyzed samples, the levels were under 30% of MRL of maize established in European Reg. 1881/2006 of 5 ppb.

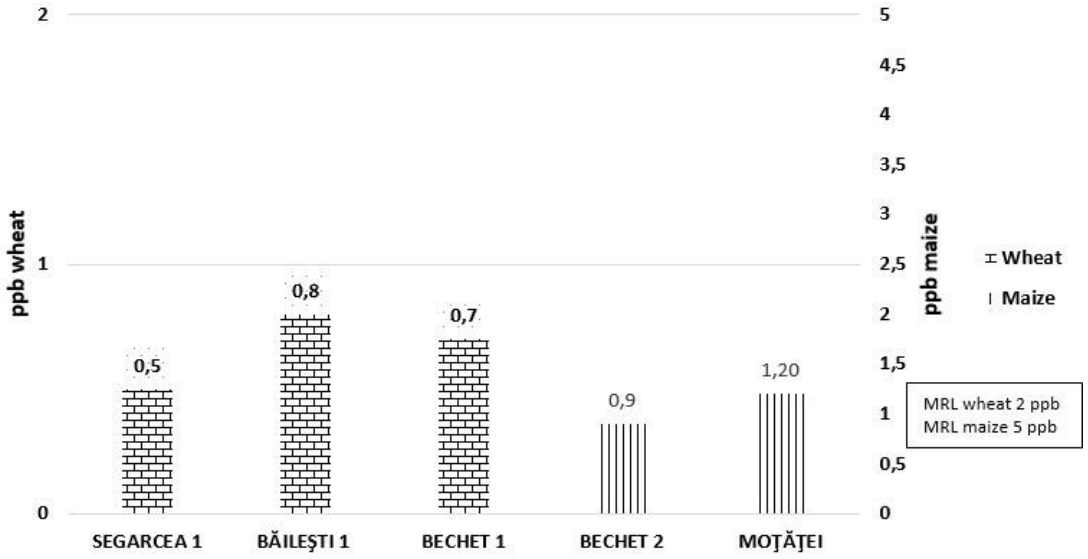

Figure 1 – The concentration (ppb or mg/kg) of AFB_1 on samples of wheat and maize determinate in 2017 on some silos in Dolj County

Analyzing the samples extracted from the batches of wheat and corn regarding the ***AFT content*** (figure 2) it was found that this mycotoxin was detected, but without exceeding the maximum allowed limit, with values between 0.2 ppb in the wheat batch in Bechet silo and 3.9 ppb in batch 2 of maize from the Leu silo. The MRL for this mycotoxine in the European Reg. 1881/2006 is 4 ppb for wheat and 10 ppb for maize.

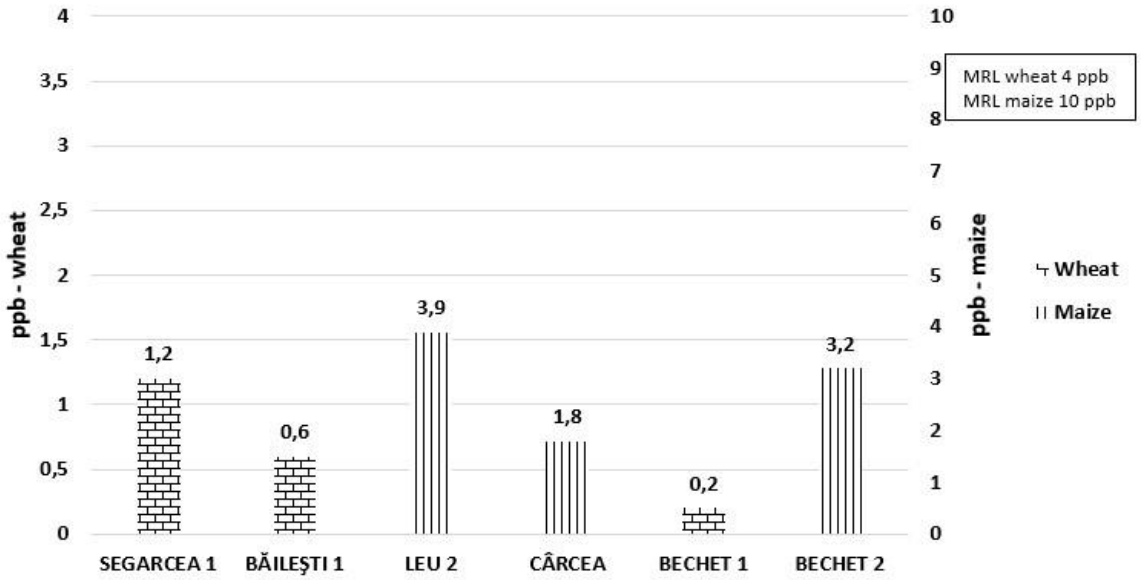

Figure 2 – The concentration (ppb) of AFT on samples of wheat and maize determinate in 2017 on some silos in Dolj County

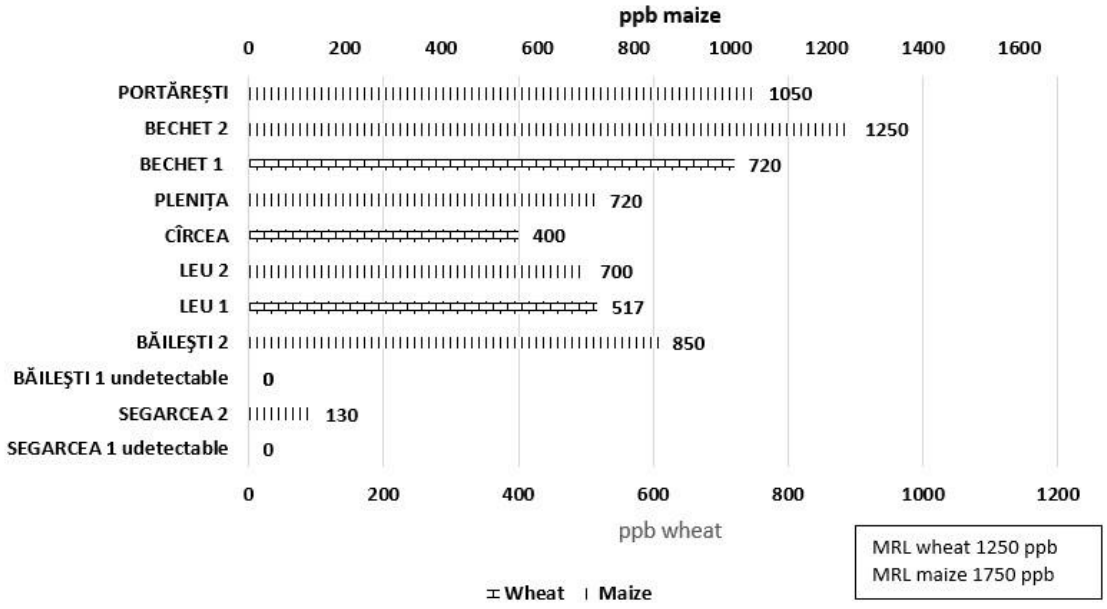

Figure 3 – The concentration (ppb) of DON on samples of wheat and maize determinate in 2017 on some silos in Dolj County

DON (Deoxynivalenol) – is a mycotoxin produced mainly by *Fusarium graminearum* and which is very present in our area, especially on durum and common wheat. DON could be also present in triticale, maize and rye crops.

In the 10 batches belonging to the silos shown in figure 3, samples were found in which mycotoxin *Deoxynivalenol (DON)* were identified, but these had values that were below the maximum allowed limit (MRL) for these raw materials, with variations included. between 400 and 720 ppb for wheat and 720 and 1250 ppb for maize. We note that in batch Băileşti 1there were no residues of this mycotoxin.

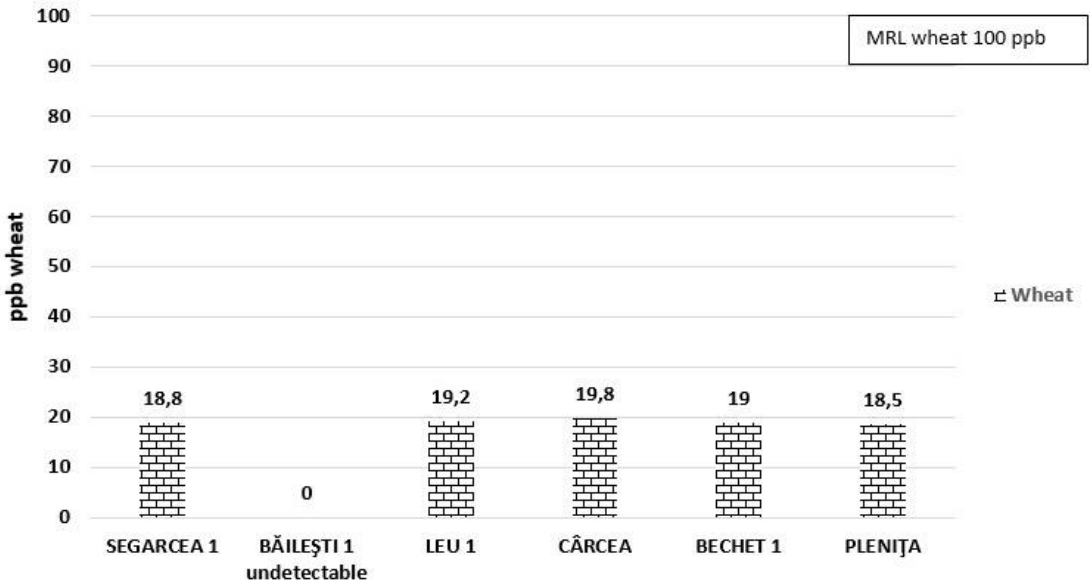

Figure 4 – The concentration (ppb) of ZEA on samples of wheat determinate in 2017 on some silos in Dolj County

In the wheat samples of batches of the 6 silos presented in figure 4, we found mycotoxin **Zearalenone (ZEA)**, without exceeding the maximum allowed limit, maximum being of 100 ppb, with values between 18.8 ppb at Segarcea 1 batch to 19.8 ppb recorded at Cârcea Silo.

The sample from Băileşti silo 1 did not show residues of this mycotoxin, the mycotoxin being undetectable.

Ochratoxin A (OTA) – is a toxin produced by several fungi, such as *Aspergillus* and *Penicillium* and which, incidentally, is found in nature in different varieties and samples of cereals, coffee beans, beans and dried fruits all over the world.

In the wheat batches of the 6 silos shown in figure 5 the samples analyzed for *Ochratoxin (OTA)* had values between 1.4 ppb at Segarcea 1 silo and 2.9 ppb at Leu 1 silo.

It is also important to highlight the fact that in the sample 1 from the Băileşti silo this mycotoxin was not detected.

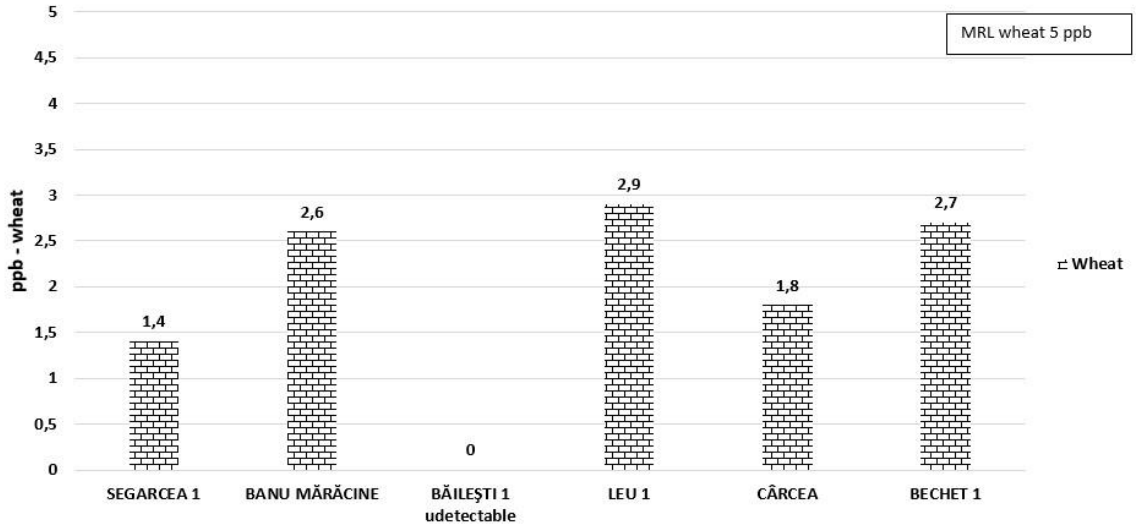

Figure 5 – The concentration (ppb) of OTA on samples of wheat determinate in 2017 on some silos in Dolj County

Estimates of cereal consumption indicate that the presence of T-2 and HT-2 toxins may be a public health problem.

Therefore, it is necessary and a priority to develop a precise and safe method of analysis, to collect more data on the incidence of occurrence and to carry out further investigations/research on the factors that determine the presence of T-2 and HT2 toxins in cereals and cereal processed products.

In **mycotoxins (T2/HT2)** from 5 silos shown in figure 6, samples were found without exceeding the maximum allowed limit, these values ranging from 10 ppb the wheat batch of Cârcea silo and 47 ppb at Bechet 1 batches.

The analyzes performed on the samples extracted from batch 1 from the Băileşti silo did not reveal residues from these toxins.

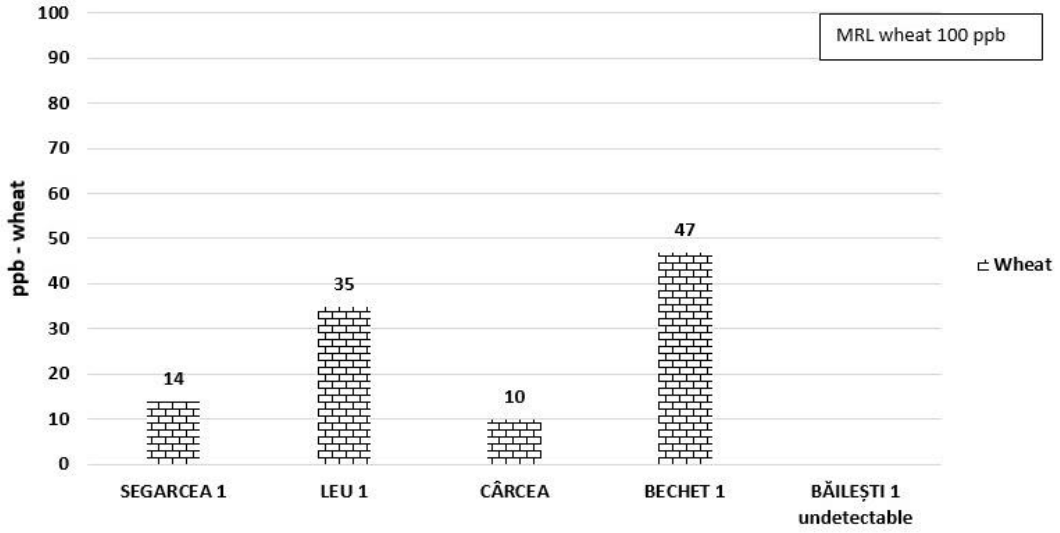

Figure 6 – The concentration (ppb) of T2, HT2 on samples of wheat determinate in 2017 on some silos in Dolj County

The fumonisins are a group of mycotoxins found in corn contaminated with *Fusarium fungus*. They are chains of about 20 carbons with acidic ester, acetylamino and sometimes other substituents. They inhibit ceramide synthetase conversion of sphingolipids to ceramides.

In figure 7 we describe the situation regarding the analysis of the presence in samples of **Fumonisin (FUMO)** in maize batches.

It is observed that, in the maize batches of the 5 analyzed locations, residues of this toxin have been identified without exceeding the maximum allowed limit (MRL), with values between 98.54 ppb in the Bechet silo and 515 ppb in the Moţăţăi and Plenița silos.

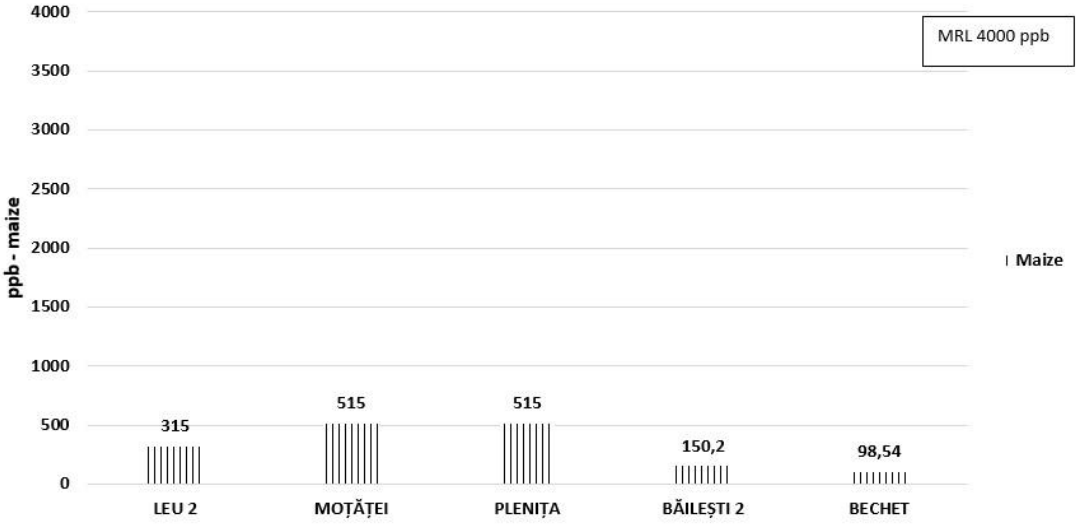

Figure 7 – The concentration (ppb) of FUMO on samples of maize determinate in 2017 on some silos in Dolj County

Heavy metals can be found as microelements in grains and in their by-products as a result of air pollution (Pb) and soil pollution (Cd). **Lead (Pb), cadmium (Cd)** and mercury (Hg) are not essential for sustaining life (animals and plants), so *they are considered as toxic metal contaminants.*

Pb and Cd are highly toxic and they were naturally "bioaccumulate" in the food and feed chain.

In the 4 analyzed silos in figure 8, samples with **Lead residues (Pb)** were identified which are below the maximum permissible limit, between 0.0017 mg/kg in the wheat batch in the Bechet 1 silo and 0.0080 mg/kg in the corn batch from Leu silo 1.

Samples where the **cadmium residue (Cd)** was analyzed were found in the 4 silos in figure 9 and had values between 0.002 mg/kg in the maize lot in the Leu 1 and Bechet 1 silos and 0.010 mg/kg in the lot of wheat from the Moțățăi silo, with lower values of these related the MRL established by the European Reg. 1881/2006 of 0.050 mg/kg.

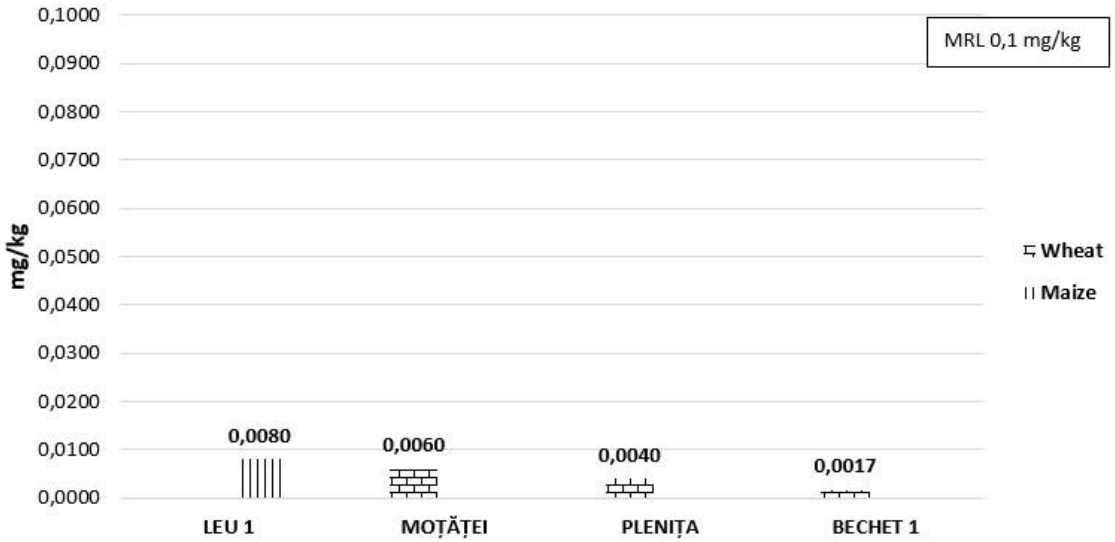

Figure 8 – The concentration (mg/kg) of Pb on samples of wheat and maize determinate in 2017 on some silos in Dolj County

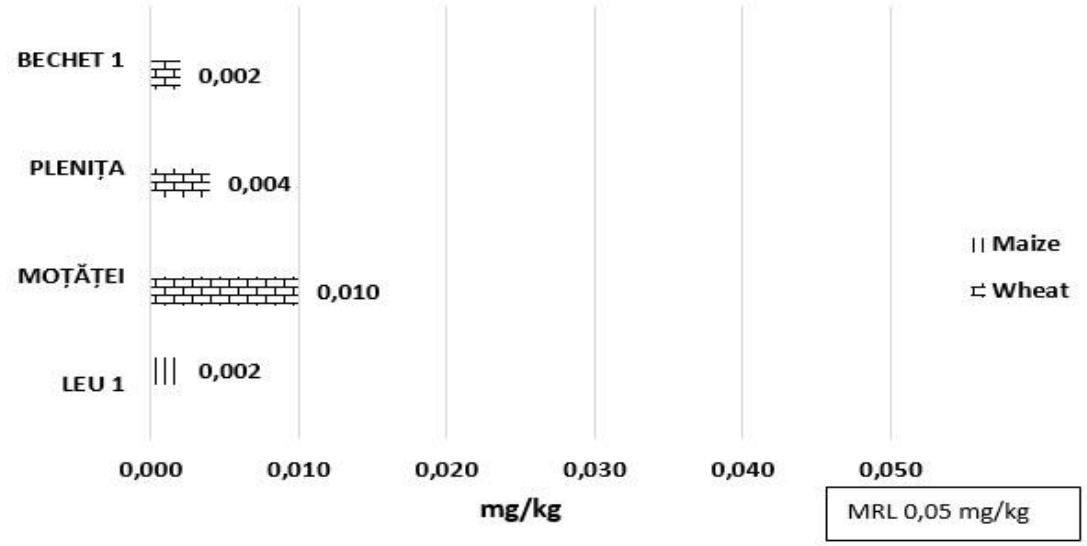

Figure 9 – The concentration (mg/kg) of Cd on samples of wheat and maize determinate in 2017 on some silos in Dolj County

COCLUSIONS

Now days, the phenomenon of extreme diversification of the categories of foodstuffs which, increasingly complex and colorful, are attracting more and more consumers, is manifesting on the domestic and international market.

In recent years there has also been an awareness of a significant number of consumers regarding the issue of food safety, as they are becoming more and more interested, not only in the quality, but also in the provenance of the agri-food products consumed (Mirela Paraschivu et all., 2015). In these conditions, it is necessary to eliminate any deficiency or weak point in the circuit through the agri-food products, from the farm gate, to the consumer's table.

The results show that in the analyzed samples, although almost all the mycotoxins targeted in the study were identified, they had low values, below the quality standards for use in the food manufacturing process.

Another very important aspect is ***the cumulative effect of these mycotoxins***, which can greatly influence the health of the final consumer. We pointed out that, although there were no exceedances of the maximum thresholds in the legislation, ***worrying is the situation of the presence in the same analyzed batch of 2-5 mycotoxins, which shows that measures are needed to prevent the presence and spread of these toxins in the producer chain - depositary processor***.

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*** *Fumonisins MeSH Descriptor Data 2020* - U.S. Department of Health & Human Services;

*** *Commission Regulation (EC) no.1881/2006*;

*** *SR EN ISO / IEC 17025/2005* - General requirements for the competence of testing and calibration laboratories;
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