Argentine: Mycotoxin in Grains of Stored Corn

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ABSTRACT

Argentina consolidated the role of the second world exporter of corn. At the same time, the world consumption projects in continuous expansion, this is due to the economic yield and demographic growth in the developing countries. The mycotoxins are compounds produced by the secondary metabolism of toxicogenic fungi. The contamination of the grains and by-products of corn with fungi is a problem that worries the exporters and importers in the whole world for its impact on the human and animal health. In Argentina, there are few studies that estimate the levels of contamination of corn grains with mycotoxins.
The aim of this work was to evaluate the results of mycotoxins in stored corn. A sampling based on "The good practices" recommended by ISO 950-1979 was carried out in the frame of project PNCER3353. The samples taken during 2005, 2006 and 2007 (n=102, n=86 and n=105, respectively) were gathered after having entered to the silo from Buenos Aires, La Pampa, Santa Fé, Córdoba and Entre Ríos. The Laboratory of Chemical Contaminants of the ITA - INTA determined the concentration of Fumonisin (FB_1, FB_2, FB_3), Aflatoxin (AB_1, AB_2, AG_1, AG_2), Deoxynivalenol (DON), Zearalenone (ZEN) and Ochratoxin A (OTA), which are some of the most powerful carcinogenic agents. The HPLC-MASS methodology was applied for the analysis and the results obtained are in agreement with the legislation in force.
The monitoring shows a significant decrease in the analyzed mycotoxin across the studied years. The monitoring of stored corn grains contributed with the identification of the risk of mycotoxin contamination.

1. INTRODUCTON

Argentina consolidated the role of the second world exporter of corn[1]. At the same time, the world consumption projects in continuous expansion, this is due to the economic yield and

[1] Informe Diario 16-01-2009 Mercado de Granos Dirección de Mercados Agroalimentarios. SAGPyA Secretaría de Agricultura, Ganadería, Pesca y Alimentos.

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demographic growth in the developing countries (Ricca, A.P. 2006). The mycotoxins are compounds produced by the secondary metabolism of toxicogenic fungi. The contamination of the grains and by-products of corn with fungi is a problem that worries the exporters and importers in the whole world for its impact on the human and animal health (53rd WHO Meeting)[2]. In Argentina, there are few studies that estimate the levels of contamination of corn grains with mycotoxins.

The unpredictable and heterogeneous nature of the mycotoxin production and contamination may not be possible due to the destruction of mycotoxins in all food systems. However, it is considered that the use of Good Agricultural Practices (GPAs) before harvest and Good Manufacturing Practices (GMP) after harvest would minimize mycotoxin contamination (Dohlman, 2004). HACCP- based hurdle systems, in which contamination is monitored and controlled throughout production and postproduction operation, may be effective. The development of suitable integrated mycotoxin management systems may be controlled at various points from the field to the consumer (Narayanasamy, 2002).

The aim of this work was to evaluate the results of mycotoxins in stored corn. A sampling based on "The good practices" recommended by ISO 950-1979 was performed in the frame of the PNCER3353 project.

The samples taken during of the years 2005, 2006 and 2007 (n=102, n=86 and n=105, respectively) were gathered on having entered the silo from Buenos Aires, La Pampa, Santa Fé, Córdoba and Entre Ríos.

2. MATERIALS AND METHODS

The monitoring was designed by SENASA and INTA technical personnel and with the operative support of CIARACEC. The samples were analyzed during 2005, 2006 and 2007 (n= 102, n=86 and n=105, respectively). The samples of different zones of Argentina were gathered on having entered the silo from Buenos Aires, La Pampa, Santa Fé, Córdoba, Entre Ríos, etc (Table 1). The Laboratory of Chemical Contaminants of the ITA - INTA determined concentration of FB_1, FB_2, FB_3, AB_1, AB_2, AG_1, AG_2, DON, ZEN and OTA, which are some of the most powerful carcinogenic agents. For the treatment of the samples immunoaffinity columns were used (r-Biopharm). The analyses were performed in an HPLC consisting of quaternary pump, column heater and autoinjector (Waters Alliance 2695), with Mass Detector Waters Micromass ZQ, with the parameters optimized for FB_1, FB_2, FB_3, (AB_1, AB_2, AG_1, AG_2)*, DON and ZEN. In case of OTA, the determinations were made with a Fluorescence Detector Waters 2475. The mobile phase conditions were adjusted for every micotoxin using a C18 column 3,5um 2,1x100mm (Xbridge) with pre-column of the same filling. The analysis were realized in agreement with the legislation in force. The concentrations for each mycotoxin were ranged to find homogeneity. Non parametric tests were used for statistical analysis. SPSS v12 software was used.

3. RESULTS

The samples were analyzed during 2005, 2006 and 2007 (n= 102, n=86 and n=105, respectively). The samples size were considered proportional in the years evaluated ($p>0.05$) according to the *ji* square test.

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2 WHO (World Health Organization) (2000) Inocuidad de los alimentos. Resolución WHA53.15 World Health Organization. http://www.who.int/foodsafety/publications/biotech/WHA53.15_sp.pdf

* unshown data

The samples were classified per year, in agreement to the limit of quantification of the laboratory method (MQL) for FB_1, FB_2, FB_3, DON, ZEN and OTA, as not detected (ND), lower (≤ MQL) and higher (>MQL) than MQL (Table 2).
Then the samples ≥MQL were qualified and evaluated according to its concentration and year. Table 3 shows an overview on the concentration range of all mycotoxins used. According to Kruskal Wallis's test the ranges were significantly different ($p < 0.001$) and the year effect was not statistically significant.
The concentration of total fumonisins (TF) and total aflatoxins (TA) were classified in agreement to the EU limits (EUL) fixed by Regulations (CE) Nº 1881/2006 o 1126/2007 (Table 4).
Figures 1 and 2 show the results of the frequencies of mycotoxin concentrations during 2005, 2006 and 2007. Figure 1 shows, in 2005, 5 samples (TF) with ≥EUL were observed. In 2006, 3 samples with concentration ≥EUL were observed. In 2007, 36 and 69, ND samples and samples with concentration <EUL were observed, respectively. In addition, in 2005, 4 samples (TA) with ≥EUL were observed. In 2006, 5 samples with concentration ≥EUL were observed. In 2007, 99 and 6, ND samples and samples with concentration <EUL were observed, respectively.
In 2007, 76 samples (DON) with >MQL were observed (Figure 2), but only 5 samples showed a concentration ≥EUL.
In 2007, all the samples (OTA) were ND.
In 2005, 2006 and 2007 for ZEN, were observed 6, 3 and 35 samples with a concentration >MQL, respectively. In 2005 only 5 samples ≥EUL were found.
The number of samples observed with >MQL increase for FB_1 (24, 43 and 62, in 2005, 2006 and 2007, respectively). Figure 3 also shows for FB_2 16, 29 and 37 samples with >MQL concentration in 2005, 2006 and 2007, respectively. In 2005, 2006 and 2007, 6, 3 and 1 samples overcame 2000 µg/kg of FB_1. Nevertheless, the number of ND samples also increased in 2007 (35% and 42% for FB_1 and FB_2, respectively). On the other hand, in 2005 and 2007 all analyzed samples were ND (FB_3). In 2006, the 72% of the samples with >MQL concentrations of FB_3 were found.
Figures 4 to 6 and Table 5 show means and standard deviations of FB_1, FB_2, FB_3, DON, ZEN, TF and AB_1. In addition it is possible to observe a red dotted line that indicates the limits fixed by the EU.
The observed samples (Figure 7) that overcome the limits fixed by EU located in following groups:

2005:

- Group B: 5 samples with concentrations of top TF to 4000µg/kg (one of them was 15779µg/kg).
- Group F: 1 sample with concentrations of ZEN higher than 350µg/kg.

2006:

- Group D: 1 sample with concentration of TA higher than 10µg/kg and 2 samples with concentrations of AB_1 major to 5µg/kg.
- Group B: 1 sample with concentration of TF higher than 4000µg/kg
- Group A: 1 sample with concentration of TF higher than 4000µg/kg and 2 samples with concentrations of AB_1 major to 5µg/kg.
- Group J: 1 sample with concentrations of AB_1 major to 5µg/kg.

2007:

- Group A: 2 samples with concentrations of DON higher than 1750µg/kg.
- Group B: 2 samples with concentrations of DON higher than 1750µg/kg.
- Group K: 1 samples with top concentrations of DON higher than 1750µg/kg

4. CONCLUSION

In 2007 it was possible to observe a decrease of the cases that exceed the regulations of the EU, except for DON.
It is necessary to increase the control and the investigation in order to know the appropriate crop management to obtain healthy food for human consumption and preservation of the environment.

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5. FIGURES

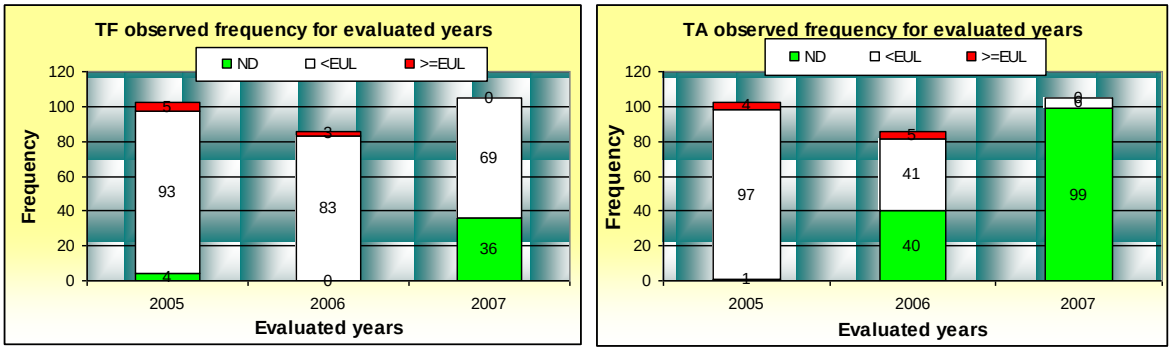

Figure 1. Classification for TF and TA concentrations during 2005, 2006 and 2007. ND: Non detected, lower and higher concentrations than European Union Limits (EUL) (< EUL and ≥EUL, respectively)

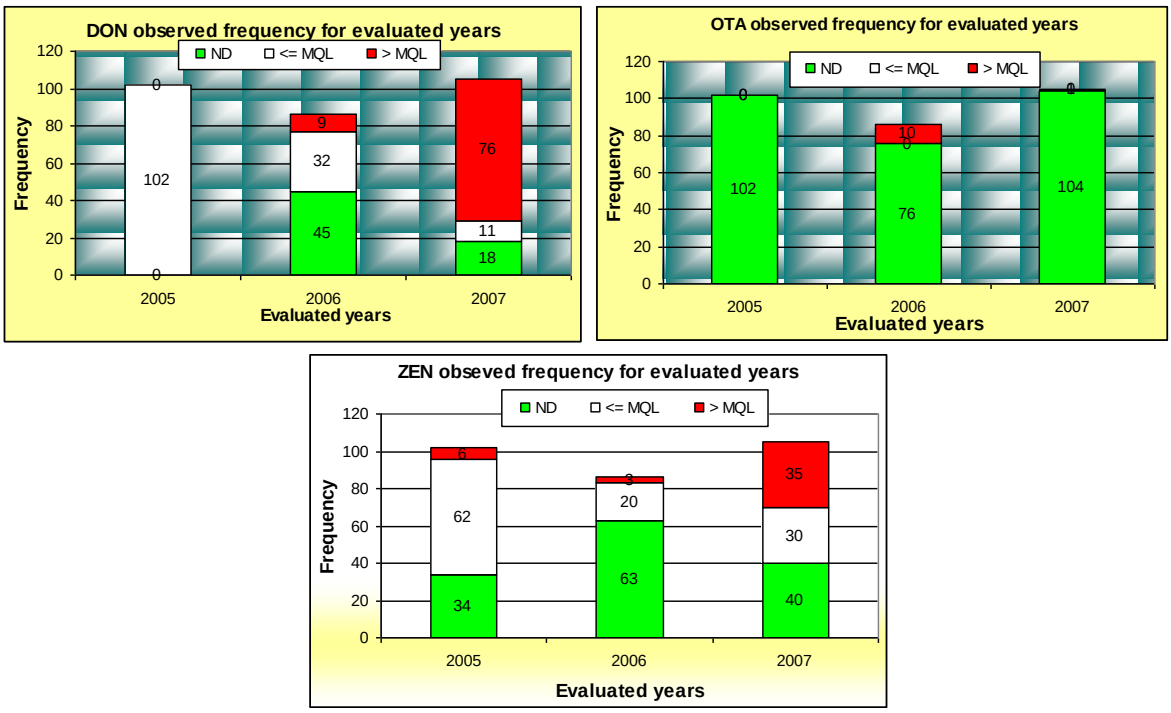

Figure 2. Classification of total samples analyzed of DON, ZEN and OTA during 2005, 2006 and 2007. ND: Non detected, lower and higher concentrations than MQL (≤ MQL and >MQL, respectively)

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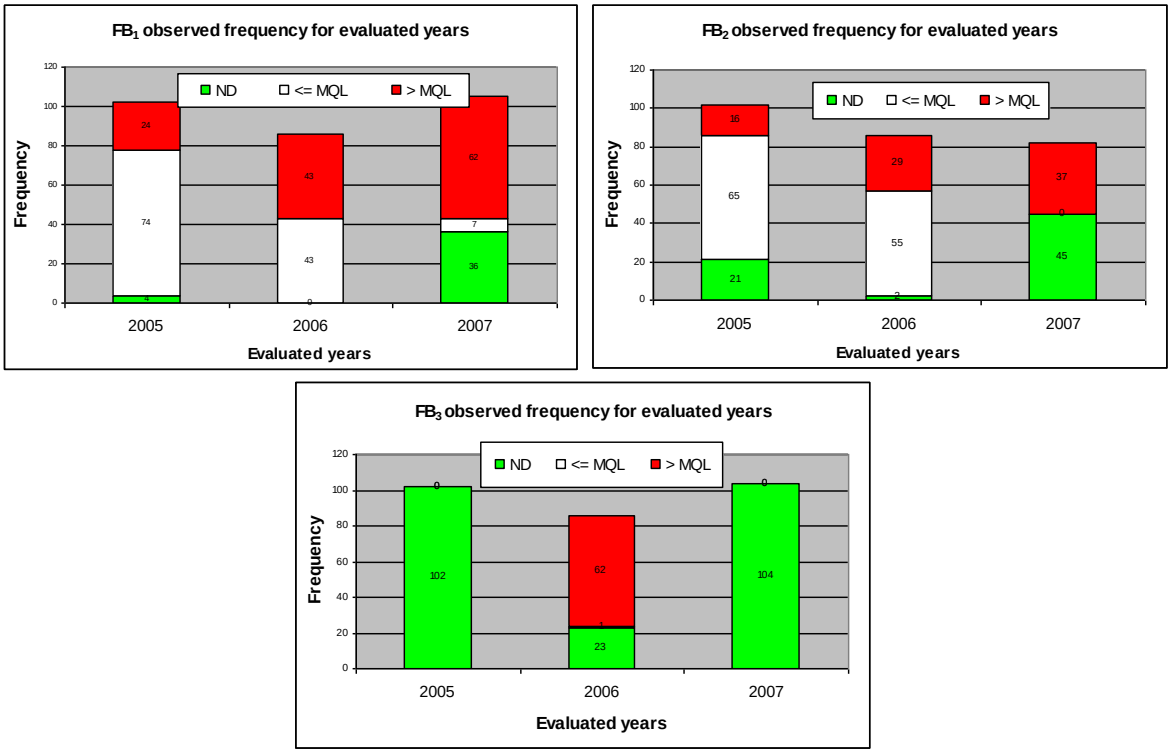

Figure 3. Classification of total samples analyzed of FB_1, FB_2 and FB_3 during 2005, 2006 and 2007. ND: Non detected, lower and higher concentrations than MQL (≤ MQL and >MQL, respectively)

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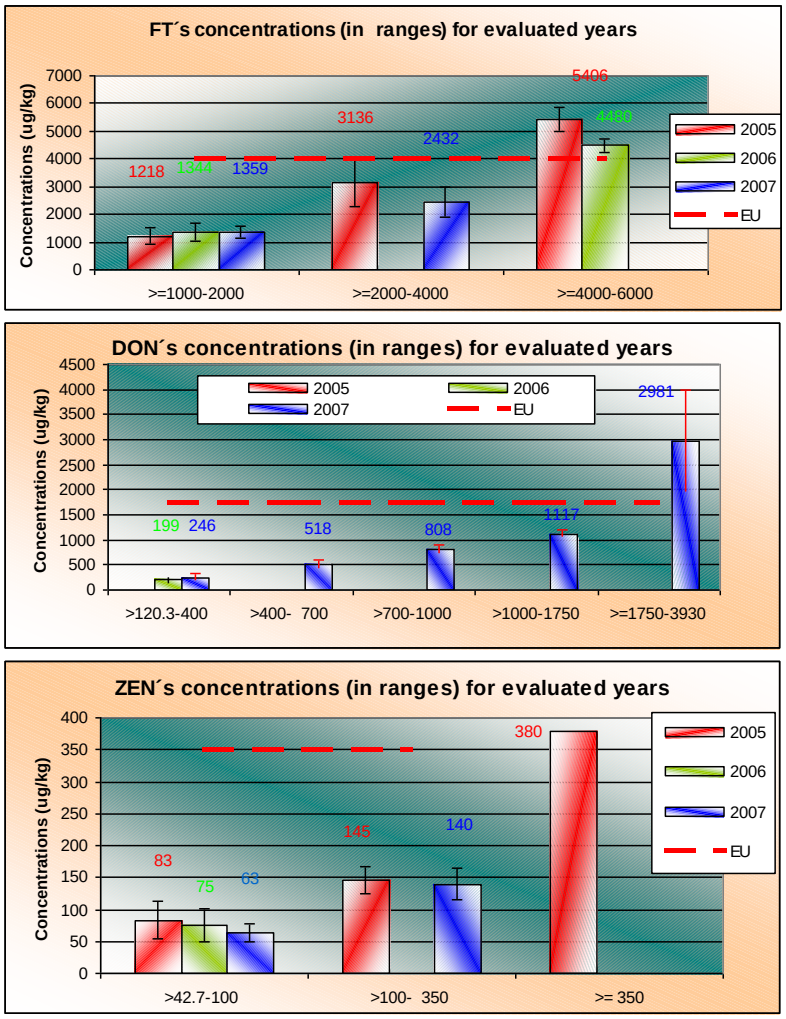

Figure 4. Means and standard deviations of concentrations TF, DON and ZEN with a red dotted line that indicates the limits fixed by the EU.

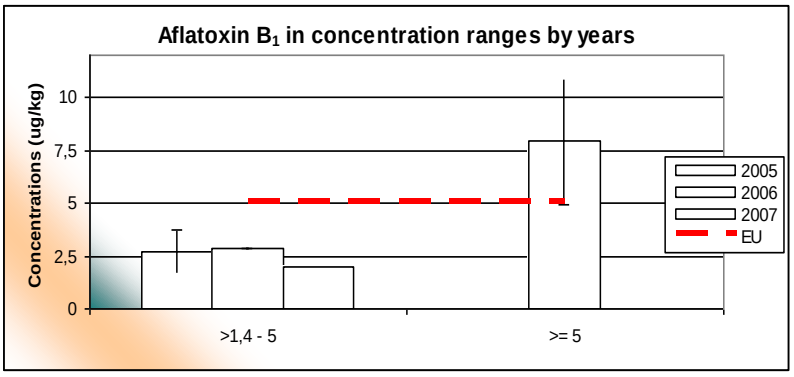

Figure 5. Means and standard deviations of concentrations AB_1 with a red dotted line that indicates the limits fixed by the EU.

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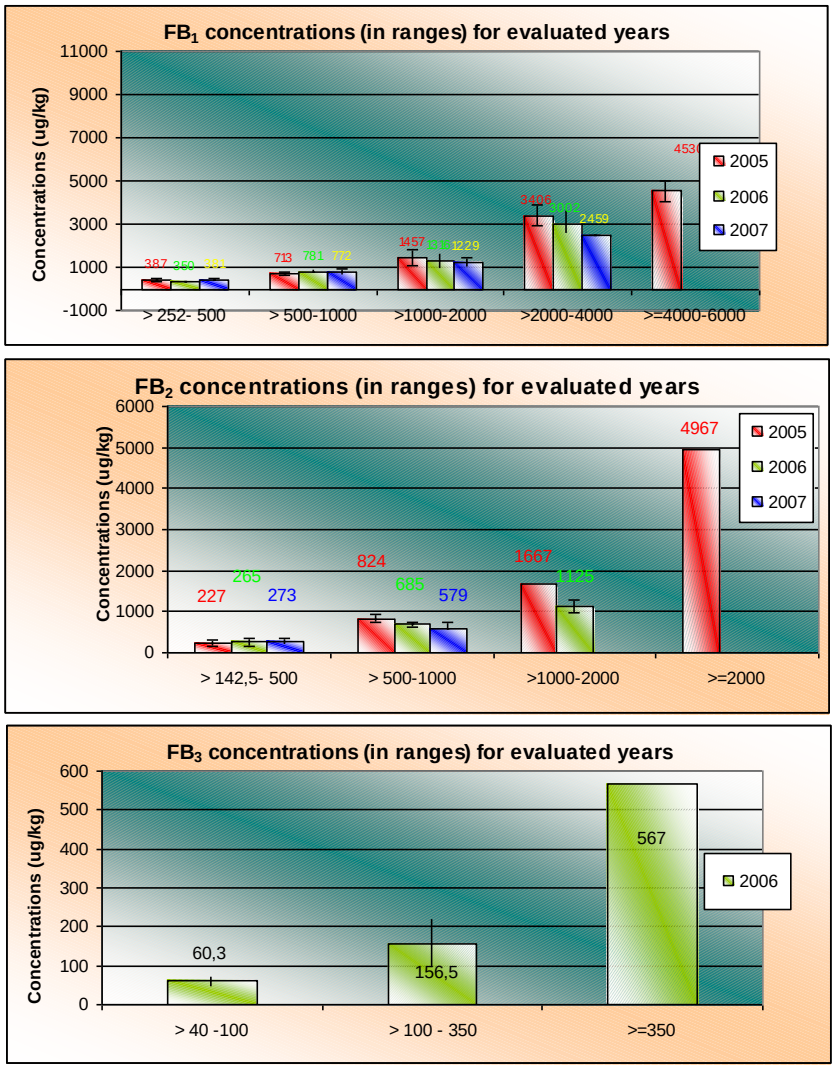
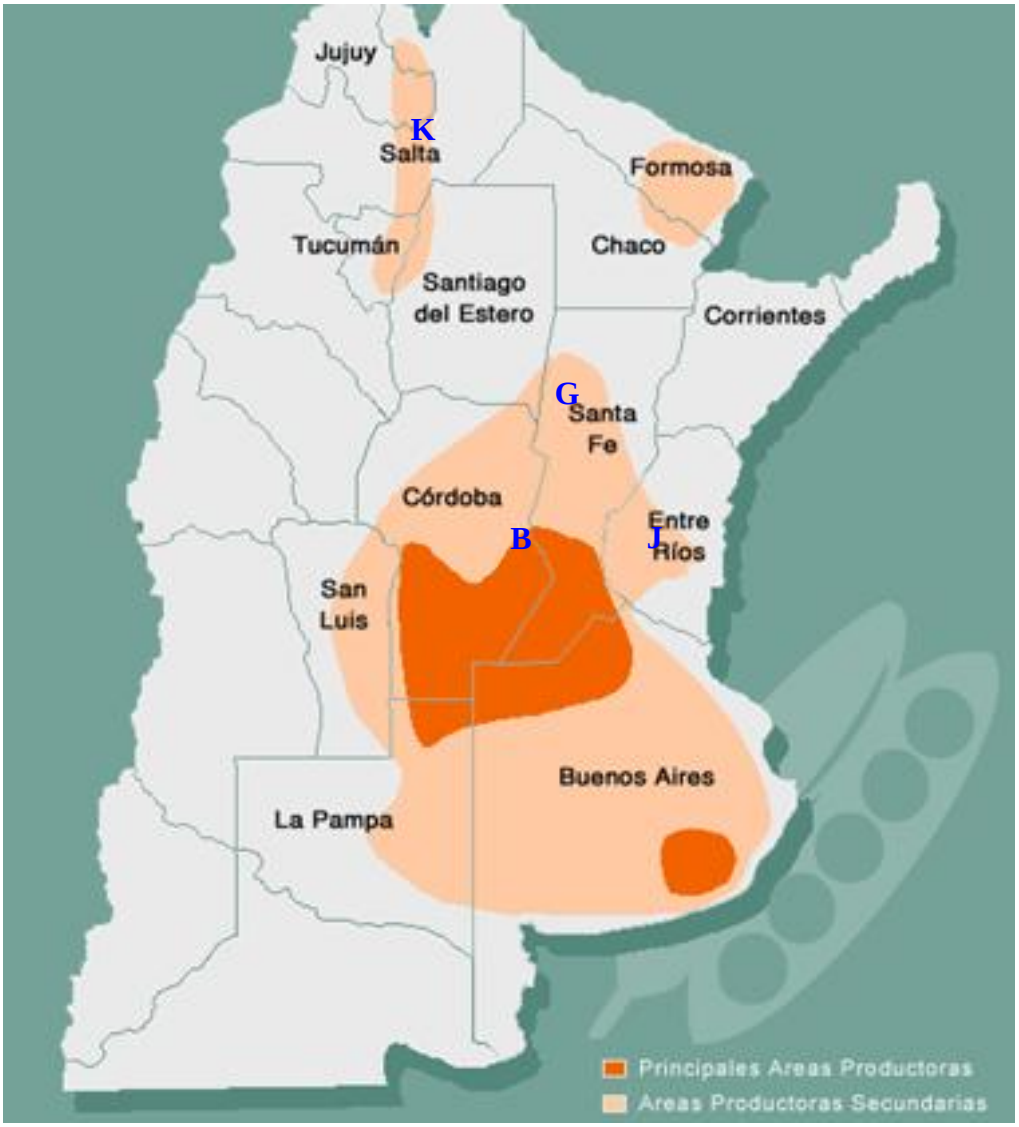


Figure 6. Means and standard deviations of FBs concentrations.



Year	Detected zone groups										
	A	B	C	D	E	F	G	H	I	J	K
2005		TF (5)				ZEN(1)					
2006	TF(1) and AB_1(2)	TF(1)		TA(1) and AB_1 (2)					TF(1)	AB_1(1)	
2007	DON(2)	DON (2)									DON (1)

Year	Total zone groups										
	A	B	C	D	E	F	G	H	I	J	K
2005	43	12	10	10		10	6	11			
2006	36	26		13	3				4	4	
2007	26	26	1	18	8					6	20

Figure 7. Zone groups

D

F

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6. TABLES

Table 1. Different port zones evaluated in years 2005, 2006 and 2007.

Group	Port zones evaluated
A) Buenos Aires	9 de julio, America, Arrecifes, Ascensión, Balcarce, Bolivar, Bordenave, Bragado, Carlos Casares Colon, Coronel Dorrego, Coronel Granada, Coronel Suarez, Chacabuco Chivilcoy, Darregueira, Elevador America, General Arenales, General Pico, General Villegas, Guamini, Junin, Lincoln, Loberia, Los Toldos, Mar del Plata, Necochea, Pehuajo, Pergamino, Rojas, Salazar, Salliquelo, San Pedro, Tandil, Trenque Lauquen, Tres Arroyos and Tres Lomas.
B) Córdoba	Alejandro Roca, Arias, Arroyito, Bell Ville, Corral de Bustos, Dean Funes, General Cabrera, Juarez Celman, La Carlota, La Puerta, Manfredi, Marcos Juarez, Oliva, Oncativo, Rio Cuarto, Rio Segundo, Rio Tercero, San Francisco, Ucacha, Union and Villa María.
C) South of Córdoba and North of La Pampa	H. Lagos, Laboulaye, Mataldi and Rancul.
D) Santa Fé	Cañada de Gomez, Casilda, Estación Murphy, Firmat, General Villegas, Las Rosas, Roldan, Rufino, San Jeronimo, San Jorge, San Jose Esquina, Venado Tuerto and Villa Cañas
E) Argentine Northwest (NW)- Argentine Northeast (NE)	Rosario de la Frontera, Chaco, Salta, Santiago del Estero and Tucumán.
F) Santa Fé and Buenos Aires	Christophersen, General Arenales, Maria Teresa, San Gregorio and Teodelina.
G) Santa Fé and Santiago del Estero	Banderas and Ceres.
H) South of Santa Fé and adjacent zone	Cañada Seca, La Cesira, María Teresa, Rufino and Vivero.
I) Campo	La Cesira, NW de Buenos Aires and SW de Santa Fe, Rufino y Vagones Rufino
J) Entre Ríos	Crespo, Diamante, Gualeguaychu, Nogoya, Paraná and Victoria.
K) Another zone	Not registrer

Table 2. Method quantification limits (μg/kg).

Mycotoxins	MQL (μg/kg)	Mycotoxins	MQL (μg/kg)
AB_1	1.8	FB_3	40.0
AB_2	0.6	ZEN	42.7
FB_1	252	DON	120.3
FB_2	142.2	OTA	1.8

Table 3. Concentration range (μg/kg)

Mycotoxins	Concentration ranges				
TF	>5-1000	>1000-2000	>2000-4000	>4000-6000	>6000
FB_1	>252-500	>500-1000	>1000-2000	>2000-4000	>4000-6000
FB_2	>142.5-500	>500-1000	>1000-2000	>2000	-
FB_3	>40-100	>100-350	>350	-	-
TA	>4-8	>13	-	-	-
ZEN	>42.7-100	>100-200	-	-	-
DON	>120.3-400	>400-700	>700-1000	>1000-1750	>1750
OTA	>1.8-3.0	-	-	-	-

Table 4. Maximum content (μg/kg) fixed by Regulation (CE) Nº1126/2007*and Regulation (CE) Nº1881/2006** of the EU.

Mycotoxins	**Maximum content fixed by EU** (μg/kg)
TA	10**
AB_1	5**
DON	1750*
ZEN	350*
OTA	5**
TF	4000*

Table 5. Means and standard deviation of mycotoxin concentrations range (μg/kg).

TF	>=5-1000		>1000-2000	F	>2000-4000	f	>=4000-6000		>6000	f
2005	181.3±215.4	88	1217.7±281.6	3	3135.5±869	2	5406±435	4	15779	1
2006	355.8±220.6	66	1349.6±290.4	13	2624.3±334.1	4	4480.0±226.6	3		
2007	546.7±207.6	35	1359.3±210.8	31	2432.3±525.9	3				

1 valor 15779 año 2005

TA	>=4 - 8	f	>13	f
2005	5.9±0.25	5		
2006	6.6±0.55	4	13.2	1
2007				

Table 5. (continued)

AB_1	>1.4-5	f	>5	f
2005	2.67±1.03	6		
2006	2.9±0	1	7.94±2.98	5
2007	2±0	1		

FB_1	>252-500	f	>500-1000	f	>1000-2000	f	>=2000-4000	f	>4000	f
2005	386.7±67.2	10	712.0±67.3	6	1457±356.4	2	3406±475.2	2	4529.7±488.0	3
2006	350.4±67.1	20	780.8±135.2	13	1316±336.4	7	3002±638.9	3		
2007	380.9±74.6	18	771.6±149.7	20	1229±205.3	23	2459±0	1		

1 valor 10812 año 2005

FB_2	>142.5-500	f	>500-1000	f	>1000-2000	f	>=2000	f
2005	226.8±81.4	9	823.8±99.2	5	1667±0	1	4967	1
2006	264.6±105.4	18	684.8±62.2	6	1125.4±162	5		
2007	273.0±75.4	36	579±0.0	1				

FB_3	> =40 -100	f	> 100 - 350	f	>350	f
2005						
2006	60.3±15.6	43	156.5±64	18	567±0	1
2007						

DON	>120.3-400	f	>400-700	f	>700-1000	f	>1000-1750	f	>=1750-3930	f
2005										
2006	199.±73.2	9								
2007	246±74.6	50	518.1±85.8	12	807.6±91.7	5	1117.2±68.3	4	2981±1020	5

ZEN	>42.7 -100	f	> 100 - 350	f	>=350	f
2005	83.3±28.9	3	145.5±21.2	2	380.0±0	1
2006	75.4±26.2	3				
2007	63.0±14.9	24	139.9±24.3	11		

7. REFERENCES

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