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«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

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URGENT ISSUES OF THE MYCOTOXIN PROBLEM IN KAZAKHSTAN

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Mycotoxins which are poisons produced by microscopic fungi form a vast group of substances which contaminate food products [1,2,3,4,5,6]. More than 200 mycotoxins have been isolated from food products and identified as belonging to differing classes of chemical compounds and exerting different effects on an organism [7,8,9,10]. A range of biological actions of mycotoxins have been described including general toxic effects, embryotoxic, teratogenic, mutagenic, oncogenic and allergising [8,10,11,12,13,14].

There is a host of data about the diseases of man and farm animals caused by mycotoxins and covered by a common term of mycotoxicoses. Mycotoxins, owing to their great spread, the hazard they present to human health and the considerable economic damage, which they cause are regarded now as a worldwide problem, the main aspects of which are studied in the framework of a number of international organisations (WHO, FAO, UNICEF) and many national health systems.

Aflatoxins which are very potent toxic and carcinogenic substances with a well expressed hepatotropic action hold a special place among mycotoxins [12, 14, 15]. According to published data, they are capable of inducing tumors in many species of animals which belong to a wide range of evolutionary stages [5,10]. Tumors of aflatoxic genesis have been registered in monkeys, too [15,16]. It is believed to be proved that primary
1457 cancer of the liver in humans in a number of regions of the word where the population consumes food contaminated with the mentioned agent is of aflatoxic origin [17].

Aflatoxins are capable of contaminating practically any food product of vegetative or animal origin [2,5,6,10,19], which is due to the wide range of the adaptive capabilities of their producers-Aspergillus flavus and A. parasiticus.

It follows from a study of published data that mycotoxins, primarily aflatoxins, present a danger for the interests of health and economy of the majority of countries. Their importance for our country, in general, and for Kazakhstan, in particular, still requires further study. Considering numerous information on a considerable incidence and degree of contamination of food products with aflatoxins and other toxic metabolites of fungi in North and South America Europe, Africa and Asia, including countries which border directly on the USSR and have similar climatic and geographic conditions, one can suggest that the relevant problem might be real for our country, too.

The final conclusion, however, about the extent of urgency of the problem for our country, about the ways and means of resolving it, can be only formulated on the basis of results of regular studies covering a variety of climatic and geographical regions. The necessity of this type of approach is indicated by the early research carried out by the laboratory of health-and-food mycology of the Kazakh branch of the Institute of Nutrition (the USSR Academy of Medical Sciences) which has made clear some of the aspects of the problem of mycotoxins in Kazakhstan [19,20,21,22].

The objective of the mentioned research programme was the elucidation of the extent of contamination of grain, its products and other food with microscopic fungi, aflatoxins and their producers in two large areas of Kazakhstan - North and

South-East, to be able to assess the potential health risk for the population and to elaborate recomendations for prevention.

The studied matter was samples taken from 14 districts of six regions of the mentioned areas of the Republic which differ substantially in their climatic and geographic conditions. Samples were taken at elevators, grain delivery centres, bakeries and from retail trade. A total of 1629 samples of products were examined, including 657 samples of wheat, 366 - flour, 301 - noodles, 97 - different groats, 27 - bread, 161 - rice, 20 - milk. Besides this, 120 samples of imported products were examined; primarily products for the confectionery industry - peanuts, cashew nuts, filberts, almonds, soya meal, coffee and cocoa beans. The ability to produce aflatoxins has been studied in 406 strains of different fungi which were isolated from local and imported products*).

Fungi were identified and registered in keeping with the conventional techniques of cultivation, specific identity was determined in pure cultures obtained by the method of dry isolation on elective media, using rules fixed for each species. Special identifiers were used in each case [23,24,25]. The degree of wheat grain infestation was determined by a technique of calculating the percentage of infected kernels, and that of flour and noodles - by inoculating nutritive media with serial dilutions of one gramm of the product with a subsequent count of the grown colonies [26]. The identification of aflatoxins in food products and in fungal cultures was done by thin layer chromatography with additional tests to confirm the presence
1457 of aflatoxins [27].

*) Participating in collecting and examining the samples were Fadeeva L.M. and Bukharbayeva A.S.

The analysis of the obtained results leads to the following conclusions. Practically all the examined products in the mentioned areas proved to be contaminated with microscopic fungi, representing many of the known families and genera of the relevant microworld. The variety of the micrflora is attested to by the fact of isolation of 99 species of fungi which belong to 36 genera.

The fungi which belong to the Aspergillus and Penicillium genera were particularly frequent in both areas. Their representatives, within the confines of the regions of the two areas, were found in 60-99 per cent of the examined samples of wheat grain (Table 1), in 37-100 per cent - of flour and noodles (Table 2). The species belonging to the Fusarium genus were noted in 4-70 per cent and 0-7 per cent of the samples, respectively. It should be stressed that the mentioned genera include a large number of species which owing to their toxicity are dangerous for man's health. The latter incorporated particularly often A. flavus, A. fumigatus, A. niger, A. nidulans, A. luchuensis, P. notatum, P. cyclopium, P. martensii, P. gibbosum, etc. The mentioned micromyces were found in all the examined food products.

During the storage of grain, groats, flour noodles a decrease in the incidence of identification of the majority of fungi and a replacement of the "field" species by the "storage" ones have been observed. For instance, Monila sitophila and some xerophytic aspergilli multiply in food products during storage and suppress such fungi as Alternaria, Helminthosporium, Fusarium, etc.

It is known, however, that the frequency of identification of fungi is not the decisive indicator for the evaluation of the

Table 1. **Frequency of Micromyces Detection in Wheat Grain in the South-Eastern and Northern Areas of the Republic**

Area and region	Harvest season and year	Number of samples	Percentage of samples infected with fungi of genera											
			Mucor	Rhizopus	Penicillium	Aspergillus	Alternaria	Stemphylium	Helminthosporium	Fusarium	Trichoderma	Monilia	Trichothecium	Others
South-East														
Alma-Ata	1980, autumn	128	51	30	73	97	95	-	23	36	-	2	-	8
Taldy-Kurgan	1980, autumn	51	81	78	97	97	83	-	-	6	14	-	-	12
North														
Tselino-	1981, autumn	77	100	99	96	99	100	14	44	56	14	-	41	-
grad	1982, spring	39	72	43	87	77	100	-	27	15	3	92	-	8
Kokchetav	1981, autumn	82	94	71	95	95	100	-	8	17	-	13	17	4
	1982, spring	49	67	77	96	94	92	4	-	42	4	86	-	-
	1982, autumn	39	90	85	85	67	85	26	10	46	3	13	15	24
Kustanai	1981, autumn	102	78	72	86	8	98	8	1	6	9	4	-	7
	1982, spring	44	93	98	99	98	97	-	3	30	-	54	-	8
	1982, autumn	65	78	57	86	75	100	17	21	43	-	12	18	21
North-Kazakhstan	1981, autumn	53	98	49	94	64	100	4	19	4	-	-	30	-
	1982, spring	56	82	94	96	96	100	-	-	36	-	78	-	9
	1982, autumn	20	100	100	60	70	100	-	10	70	-	-	10	10

Table 2. Frequency of Micromyces Detection in Flour and Noodles in the South-Eastern and Northern Areas of the Republic

Area and region	Type of product	Number of samples	Mucor	Rhizopus	Penicillium	Aspergillus	Alternaria	Fusarium	Monilia	Trichochecium	Stachybotris	Others
South-East												
Alma-Ata	Flour	121	39	15	77	86	4	7	9	9	3	32
	Noodles	102	21	4	77	88	1	3	-	4	-	-
Taldy-Kurgan	Flour	45	37	20	89	81	18	5	-	7	-	24
	Noodles	40	43	39	83	87	14	2	3	5	-	28
North												
Tselinograd	Flour	36	44	50	72	89	-	5	19	-	-	5
	Noodles	22	50	73	50	37	-	-	4	-	-	19
Kokchetav	Flour	35	37	34	34	60	-	6	14	-	-	-
	Noodles	33	33	36	51	69	-	-	3	-	-	6
Kustanai	Flour	8	37	37	100	100	-	-	25	-	-	37
	Noodles	66	35	29	71	97	3	1	-	-	-	4
North-Kazakhstan	Flour	45	50	22	72	62	4	-	28	-	-	28
	Noodles	15	13	13	80	73	-	6	-	-	-	7

safety of food products. In real conditions, and specifically during monitoring, it may serve but as an approximate test in determining the health-mycological situation and in defining the priority trends in its elucidation. A more important criterion is the intensity or the extent of infestation of products with fungi, specifically with potential toxin producers. It follows from an analysis of our findings that the extent of wheat grain infestation with different fungi is not uniform, it varies greatly even in the confines of one and the same area (Table 3). Thus, the intensity of contamination of wheat of the 1981 harvest with aspergilli in the North-Kazakhstan region was 5.5 and in the Tselinograd region - 44.0 per cent. In the Alma-Ata and Taldy-Kurgan regions, the extent of infestation of the same product with aspergilli in 1980 was 25.7 and 32.8 per cent, respectively. The extent of contamination of wheat grain of the 1981 harvest with penicilli varied from 14.6 to 35.7 in the Northern area and from 10.6 to 38.1 in the South Eastern area. On the other hand, the infestation with the fusarii spores was low. An exeption was the wheat of the 1980 harvest in the Alma-Ata region where the relevant indicator was as high as 10.3 per cent. It follows from the Table that a more relevant dependence of the extent of infextation is one based on the time of storage rather than on the nature of the area. For instance, the concentration of spores of Alternaria and Helminthosporium species proved to be much higher in freshly harvested grain than in grain which had been in storage for a long time. More than that, fungus Trichothecium roseum
1457 was identified only in this grain. As for Aspergillus and Monilia species - the representatives of the so-called "storage" moulds, they were found in large quantities in spring samples.

Table 3. Degree of Micromyces Infestation of Wheat Grain in the South-Eastern Northern and Areas of Kazakhstan

Area and region	Year and season	Number of samples	Average (min. and max.) indices of infestation with fungi (per cent)											
			Mucor	Rhizopus	Penicillium	Aspergillius	Alternaria	Stemphylium	Helminthosporium	Cladosporium	Fusarium	Trichoderma	Monilia	Trichothecium
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
South-East														
Alma-Ata	1980	128	14.5 (3–70)	23.6 (5–62)	10.6 (2–27)	25.7 (5–80)	64.3 (10–100)	-	1.5 (0–7)	-	10.3 (2–18)	-	2.4 (0–6)	-
Taldy-Kurgan	1980	51	46.3 (10–90)	38.8 (3–70)	38.1 (5–68)	32.8 (2–90)	11.7 (0–32)	-	-	-	0.2 (0–3)	-	-	-
North														
Tselinograd	1981, autumn	77	49.0 (13–97)	52.0 (6–96)	35.0 (3–98)	44.0 (2–98)	57.0 (8–98)	2.0 (0–8)	-	0.2 (0–10)	5.7 (0–55)	3.0 (0–12)	-	3.6 (0–23)
	1982, spring	39	25.6 (2–80)	5.1 (5–54)	10.5 (6–32)	48.0 (6–84)	27.3 (6–78)	0.5 (0–2)	0.05 (0–2)	3.7 (0–20)	0.6 (0–9)	0.05 (1–2)	28.9 (0–50)	-
	1981, autumn	82	23.7 (3–100)	16.0 (8–72)	35.7 (6–92)	29.4 (6–400)	25.7 (2–100)	-	0.6 (0–20)	1.4 (0–40)	1.1 (0–2)	1.5 (0–10)	1.6 (0–22)	1.5 (0.20)
Kokchetav	1982, spring	49	44.7 (10–60)	24.4 (9–60)	22.9 (2–80)	39.1 (8–80)	14.6 (3.56)	0.3 (0–6)	-	0.6 (0–6)	1.7 (0–6)	0.2 (0–6)	36.5 (3–100)	-
	1982, autumn	39	49.7 (2–100)	33.8 (5–100)	22.5 (6–100)	15.1 (5–80)	56.8 (9–100)	1.5 (2–12)	0.6 (3–9)	0.9 (2–9)	1.9 (2–9)	0.6 (0–20)	1.4 (3.15)	5.0 (12.6)

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Table 3 (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Kus-tanai	1981, autumn	102	76.6 (5–100)	53.4 (10–90)	17.9 (2–72)	12.1 (5–50)	49.5 (30–96)	-	0.02 (0–3)	2.2 (0–8)	0.3 (0–6)	1.0 (0–38)	1.2 (0–40)	-
	1982, spring	44	24.8 (6–70)	35.3 (3–100)	11.3 (5–60)	17.0 (10–60)	35.7 (10–100)	-	0.2 (0–6)	0.4 (0–8)	1.1 (0–8)	-	30.3 (4–100)	-
	1982, autumn	65	28.4 (4–100)	20.7 (2–72)	9.8 (2–90)	13.6 (5–78)	63.6 (6–100)	0.9 (0–12)	1.4 (0–18)	3.0 (0–27)	3.9 (0–21)	-	-	3.0 (0–48)
North-Kazakh-stan	1981, autumn	53	52.0 (6–90)	6.7 (5.34)	14.6 (6–42)	5.5 (2–24)	82.0 (69–97)	0.3 (0–8)	0.4 (0–3)	-	0.3 (0–2)	-	-	3.2 (0–23)
	1982, spring	56	14.9 (10–36)	23.0 (9–50)	17.2 (2–54)	16.0 (2–32)	42.0 (14–92)	-	-	0.01 (0–3)	1.6 (0–10)	-	16.0 (0–80)	-
	1982, autumn	20	30.5 (10–68)	57.2 (30–100)	5.4 (0–18)	6.8 (0–12)	65.6 (33–82)	0.2 (0–2)	0.3 (0–10)	-	3.6 (0–12)	-	-	1.61 (0–10)

At the same time in both areas, the concentration of spores of penicillia in wheat grain varied in a broad range both in autumn and spring which, most likely, was dependent on the conditions of storage.

The extent of contamination of grain with mucor fungi (Mucor, Rhizopus), as it follows from the same Table, was comparatively high regardless of the time of sampling. The samples taken from the Alma-Ata (1980) and Tselinograd (1981) regions are an exception.

The comparatively high level of contamination of grain with representatives of the Aspergillus and Penicillium genera should be emphasised. These two genera, as noted earlier, incorporate many species which are producers of different mycotoxins. This renders more importance to information about considerable contamination of wheat with species of the Fusarium genus, in the Alma-Ata region.

The information in Table 4 sums up the results of studying the intensity of infestation of flour and noodles with micromyces. This indicator varies, subject to the type of product and place of sampling. Thus, the extent of infestation is higher in the Taldy-Kurgan region where the average number of spores in one gram of flour was 6456, and in one gram of noodles - 5897. The infestation of some of the samples was as high as 53566 spores in one gram. The extent of infestation of flour and noodles in the Kustanai, Kokchetav and North-Kazakhstan regions was somewhat lower.

The most important factor of the possible influence of mycotoxins of vegetative origin on man's organism is bread.

We have undertaken a programme of research into the nature of mycoflora and the extent of infestation in the process of bread baking (flour, dough, baked bread) and storage (Table 5). It has been found that flour samples regularly contained spores of mould fungi. The number of spores in one gram of first grade flour was 1859 on the average, second grade flour - 2054. The infestation of wheat-whole flour is much higher (3407).

The spores of Alternaria, Mucor, Rhizopus and Penicillium fungi made up the bulk of all spores in all samples of flour of different grades. The fungi of the Aspergillus genus were found in all flour samples. They were represented more frequently by the A. fumigatus and A. flavus species and rarely - by A. niger and others.

Table 4. The Extent of Infestation of Flour and Noodles with Microscopic Fungi in the South-Eastern and Northern Areas of Kazakhstan

Area and region	Type of product	Year and season of sampling	Number of samples	Number of spores in 1 g of product		
				average	minim.	maxim.
South-East						
Alma-Ata	Flour	1980	121	2629	183	9750
	Noodles	1980	102	2100	40	10000
Taldy-Kurgan	Flour	1980	45	6456	500	46000
	Noodles	1980	40	5897	830	54566
North						
Tselinograd	Flour	autumn	11	3905	1500	5000
		spring	12	2030	120	3395
	Noodles	autumn	12	1660	1100	2866
		spring	22	1529	0	3925
Kokchetav	Flour	spring	35	3176	86	7800
	Noodles	autumn	7	3413	2266	4330
			26	1683	150	5100
Kustanai	Flour	autumn	12	5112	2550	8150
		spring	25	2300	220	4500
	Noodles	autumn	37	4344	1650	9400
		spring	29	1135	880	3225
North-Kazakhstan	Flour	autumn	21	2964	1000	8700
		spring	26	3398	65	9000
	Noodles	autum	14	2907	463	5850
		spring	15	1443	180	3100

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Table 5. Indices of Contamination with Spores During Bread Baking

Grade of product	FLOUR			DOUGH			BREAD		
	number of samples	number of samples with fungi	average number of fungi spores per 1 g	number of samples	number of samples with fungi	average number of fungi spores per 1 g	number of samples	number of samples	average number of fungi spores per 1 g
Ist grade	9	9	1859	8	3	1976	9	9	1718
IInd grade	9	9	2054	8	8	1864	9	9	1556
Wheat-whole	9	9	3407	8	8	1677	9	9	2148

Interesting results were obtained in the study of dough directly before baking. Only in three out of eight dough samples made of first grade flour did we find fungi spores. As for the other samples, there was an abundant growth of yeasts which were used for dough leavening. The average number of spores in one gram of dough made of second grade flour was 1854, made of wheat-whole flour - 1577. It should be emphasised that alongside a considerable growth of fungi in the Petry dishes, the growth of yeasts was much less intense in the samples of dough made of the second grade flour and the wheat-whole flour. The species of fungi found in dough samples were the same as in the starting product.

The average content of fungi in one gram of bread made of first and second grade flour immediately after baking differed but slightly and was 1718 and 1556, respectivaly. However, the infestation of bread baked from wheat-whole flour proved to be much greater (2148).

As a result of evaluation of the extent of infestation of bread with fungi spores immediately after baking, a conclusion can be made that there is no clear dependence on the product grade. After the passage of one day there was a clear drop in the number of spores in the wheat-whole bread and in the bread made of second grade flour (the average indicators were 534 and 899, respectively). Less prominent shifts were found in the bread made of first grade wheat flour. It may be suggested that this decrease is associated with the inhibiting influence of spore aerobes.

The studies bread was infested with fungi belonging to the following genera: Aspergillus, Penicillium, Mucor, Rhizopus and Monilia. The prevailing fungi were those of the Aspergillus genus and specifically - the A. fumigatus and A. flavus species. The mentioned species were found most frequently in the bread made of first grade wheat flour. The generic and specific composition of fungi in different grades of bread shows that it originates from the microflora of initial products. It should be noted that the species of the Aspergillus genus which were identified both in the initial products and in bread, according to published information, are potentially toxigenous, and some of them are aflatoxin producers.

The study of some imported products used, in the main, in the confectionery industry, has shown that all the studied samples were infected with mould fungi. The icidence of infestation with aspergilli was 100 per cent. The spores of penicillia have been found not in all products - soya meal, almonds and filberts were found to . free of them. The presence of Mucor
1457 was noted in all samples with the exception of soya meal and cocoa. The Rhizopus spores were identified only in samples of cashew, those of Monilia - in cocoa.

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It follows from Table 6 that the overall number of spores per gram of product varied from 10 to 6500 in cocoa, from 130 to 20,000 in cashew, from 167 to 2200 in peanuts from 363 to 6803 in filberts, from 859 to 3405 in almonds, from 1152 to 4306 in coffee and from 2499 to 14065 in soya meal. The indicators of average infestation with fungi were the highest in peanuts from Turkey (5800), peanuts from Sudan (4390) and cashew from India (3247).

The largest share in the total of spores belongs to aspergilli. The quantitative representation of spores of mucor, penicilli and, specifically of other fungi species, was much lower. In the studied samples, the Aspergillus fungi included the following species: A. flavus, A. fumigatus, A. niger, etc. A. flavus prevailed among them.

Table 7 presents the results of research into aflatoxin-producing properties of fungi isolated from samples of food products in the mentioned areas of Kazakhstan. It is seen that in the Northern area 43 (51.2 per cent) out of 84 strains of A. flavus were producents of aflatoxin B_I. Ten of them were concurrently producing aflatoxin G_I, three - B_2 and one - B_2 and G_I. Aflatoxin synthesizing strains have been isolated from wheat grain, wheat flour and noodles. At the same time, the studied strains of Penicillium did not have this property. Some of them, however, produced a substance which by the nature of its luminescence in ultraviolet light, the Rf value, the reaction with trifluoroacetic acid and iodine was similar to aflatoxin B_I, but differed from it by a range of other physical and chemical properties.

It also follows from the same Table that aflatoxin producers in the South-Eastern area were found but among the A. flavus

Table 6. Infestation of Imported Products with Spores of Microscopic Fungi

Product and exporting country	Number of samples	Number of spores in 1 g of product			The average number of spores of fungi					
		average	min.	max.	Rhizopus	Mucor	Penicilliun	Aspergillus	Monilia	Others
Peanuts (USA)	17	2365	167	11200	-	579	63	1723	-	-
Peanuts (Sudan)	14	4390	270	22000	-	801	59	3279	-	251
Peanuts (Turkey)	3	5800	190	18000	-	2020	660	3120	-	-
Cashew (India)	25	3247	130	20600	246	700	300	2001	-	-
Filberts (Turkey)	18	2068	363	6803	-	1003	-	865	-	200
Almonds (Iran)	4	2334	1000	3405	-	1317	-	1017	-	-
Almonds (USA)	5	1203	859	1599	-	412	39	742	-	-
Cocoa beans (Brazil)	23	2828	100	6500	-	-	300	1920	608	-
Coffee beans (Brazil)	6	2443	1152	4306	-	1260	209	374	-	595
Soybean meal (USA)	5	7894	2499	14065	-	-	-	6809	-	1085

Table 7. Aflatoxin-producing Ability of Fungi Strains Isolated from Food Products in Norther and South-Eastern Kazakhstan

Areas and species of fungi	Number of strains		Products from which producers of fungi have been isolated					
	Total	Aflatoxin B_I producers	Wheat	Flour	Noodles	Groats	Rice	Others
North								
A. flavus	84	43	34	1	8	-	-	-
Penicillium sp.	53	6^+	6^+	-	-	-	-	-
South-East								
A. flavus	131	40	2	3	11	12	6	6
A. niger	7	1^+	-	-	-	-	-	-
A. nidulans	7	3^+	-	-	3	-	-	-
Penicillium sp.	53	16^+	7^+	-	3^+	4^+	1^+	1^+

strains. Out of the 131 studied strains, 40 (30.5 per cent) proved to be active there. Eight strains synthesized also alkatoxin G_I. Aflatoxin-producing strains were at the same time isolated from all the studied food products. As for the other species of the Aspergillus and Penicillium genera, aflatoxin producents were not found in the South-Eastern area just as it was in the Northern area. However, strains which synthesize a mentioned earlier substance similar to aflatoxin were also isolated there.

The results of studying the aflatoxin-producing ability of fungi isolated from imported production are illustrated in Table 8.

Table 8. Aflatoxin-Producing Ability of Fungi Strains Isolated from Imported Products

Species	Number of strains		Products from which fungi were isolated; country				
	total	aflatox. B_1 producer	filberts (Turkey)	peanuts (Turkey)	cashew (India)	cocoa (Brazil)	soya meal (USA)
A. flavus	43	12	2	2	1	2	5
A. niger	5	-	-	-	-	-	-
A. nidulans	2	-	-	-	-	-	-
A. fumigatus	1	-	-	-	-	-	-
Penicillium sp.	20	6+	-	-	2+	2+	2+

+ producers of a substance which is similar to aflatoxin B_I

It follows from this information that the studied strains included species belonging to the Aspergillus and Penicillium genera. Aflatoxins were found in 12 (out of 43) strains of

A. flavus. They include two strains which produced both aflatoxin B_I and G_I. Producents were isolated from peanuts, cashew, cocoa, soya meal, filberts.

Table nine illustrates the results of a study of food contamination with aflatoxin B_I. It is seen that aflatoxin B_I has been detected in samples taken in the Northern and South-Eastern areas of the Republic. The distinctions are only in the incidence and extent of contamination. On the whole these indices are considerably higher in the South-Eastern area. Out of the 597 food samples studied there, 103 or 17.2 per cent proved to be contaminated, whereas in the Northern area aflatoxin B_I was found but in 23 (3.3 per cent) samples out of 850. The incidence of aflatoxin contamination among the studied samples of wheat grain, flour, noodles and groats in the South-Eastern area varied from 14.3 to 22.1 per cent, in the North-- from 2.3. to 3.8 per cent. It should be noted, however, that in both areas the aflatoxin content in the majority of contaminated samples was within the maximum allowable level - 5 μg/kg which has been fixed in our country. Another fact is also noteworthy, namely that many of the samples in both areas were contaminated with much greater intensity - in the range of 5.1-20 μg/kg and even - 20.1-40.0 μg/kg and more (in the South-East). The information of the Table shows also that aflatoxin contaminated rather frequently rice, sporadically coffee beverage and dry fruit studied in the South-Eastern area of Kazakhstan. There were samples of rice and dry fruit in which the contamination level was higher that the allowable standard.

Twelve samples of wheat grain in the Northern area, which were taken at the time of harvesting were found to contain not only aflatoxin B_I but aflatoxin G_I, also, and in two samples - G_2. There was no aflatoxin G_I or G_2 in the samples of the spring season.

Table 9. Aflatoxin B_1 Incidence and Extent of Contamination of Foodstuffs in the Northern and South-Eastern Areas of Kazakhstan

Area and foodstuff	Number of samples		Number of samples with toxin concentration (µg/kg)				
	total	containing aflatoxin, abs. %	up to 5.0	5.1-10.0	10.1-20.0	20.1-40.0	> 40.0
North							
Wheat	478	17 (3.5)	12	3	2	-	-
Flour	173	4 (2.3)	2	1	1	-	-
Noodles	159	6 (3.8)	3	2	1	-	-
Groats	40	1 (2.5)	1	-	-	-	-
T o t a l	850	28 (3.3)	18	-	4	-	-
South-East							
Wheat	106	17 (16)	14	-	3	-	-
Flour	112	16 (14.3)	14	-	1	-	-
Noodles	175	31 (17.7)	22	5	1	1	2
Groats	77	17 (22.1)	14	2	-	1	-
Coffee beverage	107	18 (16.8)	15	1	1	-	-
Dry fruit	8	2 (25.0)	2	-	-	-	-
T o t a l	12	2 (16.6)	-	-	2	-	-
	597	103 (17.2)	81	8	8	2	4

Table 10. Indices of Milk Infestation with Aflatoxins

Sample No.	Content of aflatoxin B_I µg/l	Content of aflatoxin M_I µg/l
1	-	-
2	-	-
3	-	-
4	0.1	-
5	0.2	-
6	-	-
7	0.1	-
8	0.4	-
9	0.2	-
10	0.2	-
11	-	-
12	0.2	-
13	-	-
14	-	-
15	0.2	0.4
16	-	-
17	0.5	-
18	-	-
19	-	-
20	0.5	0.35

The found extent of aflatoxin contamination was due to the violation of hatvesting practices, processing, transporting and storage of food products; all this has predetermined the development and spread of A. flavus fungus and of its toxigenic properties.

It is noteworthy that aflatoxin B_I contamination of food products in the South-Eastern area was much higher than in the North despite a comparatively lower percentage of aflatoxin-producing strains within the A. flavus species. We can suggest that the climatic and other geographical conditions as well as the conditions of storage in this area favour more the implementation of the aflatoxin-synthesizing function of this fungus. No final conclusions should be made, however, because the study of the products in the Northern area was held in the two dry (favourable) harvesting seasons, which could have affected the metabolic possibilities of the fungus. Further dynamic observations in the mentioned areas will enable a more substantiated comparison.

The study of mycotoxin contamination of imported products has brought to light the following (Table 11). Aflatoxin B_I has been found in three (our of 34) samples of peanuts. As a matter of fact it was found only in samples of lots of peanuts imported from Turkey. At the same time the samples from a lot of similar product imported from the USA and Sudan were not contaminated with it. Besides this, aflatoxin was found in 16 our of 25 samples of cashew. It was also isolated in one (out of six) sample of Brazilian coffee and in two samples (out of 23) of cocoa. Soya meal (USA), almonds (USA, Iran) and filberts (Turkey) did not contain any aflatoxin. As it follows from the Table, the samples of peanuts (Turkey) and cashew (India) contained a higher concentration of aflatoxine, at a level of 20-30 μg/kg and higher. Aflatoxin G_I was not found in any of the sampled products.

It follows that imported products are rather often contaminated with aflatoxin and the strains of A. flavus which produce it.

Table 11. Indices of Contamination of Imported Products with Aflatoxin B_I

Product and country	Number of samples		Toxin concentration, μg/kg		
	total	with aflatoxin	up to 5.0	5.1-20.0	20.1-30-0 and more
Peanuts (USA)	17	-	-	-	-
Peanuts (Sudan)	14	-	-	-	-
Peanuts (Turkey)	3	3	-	-	3
Cashew (India)	25	16	3	11	2
Soya meal (USA)	5	-	-	-	-
Coffee (Brazil)	6	1	-	1	-
Cocoa (Brazil)	23	2	2	-	-
Almonds (Iran)	4	-	-	-	-
Almonds (USA)	5	-	-	-	-
Filberts (Turkey)	18	-	-	-	-
T o t a l	120	22	5	12	5

It is noteworthy that the presence of aflatoxin B_I in food products is not always accompanied with the presence in them, at the time of examination, of aflatoxin-producing strains of A. flavus and vice versa (Table 12). There are instances when both aflatoxin producing and not producing strains are isolated from one and the same samples. This, most

Table 12. Identification of Aflatoxin-Producing Strains of A. Flavus in Local and Imported Foodstuffs Subject to their Contamination with Aflatoxin B_I

Product and exporting country	Total No. of samples	Samples with aflatoxin B_I	Samples containing strains		Samples free from aflatoxin B_I	Samples containing strains	
			producing aflatoxin B_I	not producing aflatoxin B_I		producing aflatoxin B_I	not producing aflatoxin B_I
Wheat	6	2	1	1	4	-	4
Groats	16	1	1	-	15	5	10
Flour	16	-	-	-	16	-	16
Noodles	26	7	2	5	19	6	16
Rice	23	2	1	1	21	5	25
Peanuts (Turkey)	3	3	-	3	-	-	-
Peanuts (Sudan)	5	-	-	-	5	1	4
Cashew (India)	7	3	1	3	4	-	4
Soya beans meal (USA)	3	-	-	-	3	5	2
Coffee beans (Brazil)	3	1	-	1	2	1	1
Cocoa beans (Brazil)	4	1	1	-	3	-	3
Filberts (Turkey)	6	-	-	-	6	1	6
Almonds crushed (US)	3	-	-	-	3	-	3
T o t a l	121	20	7	14	101	24	94

likely, is due to the specifics of the biocenosis which develops in the food substrate under concrete conditions of storage. This seems to be the explanation for the considerable variation of the frequency of identification of this charactaristic among the fungi strains isolated from different types of products or from samples of one and the same product but taken in different geographical zones. Therefore, the presence or absence of the signs of aflatoxin-producing property in A. flavus strains at the time of examination cannot be regarded as a reliable indication of the presence or absence of aflatoxin contamination of the food products from which they were isolated. However, any characteristic of the strain with respect to the mentioned indicator does not preclude the potential danger of aflatoxin contamination and this should be taken into consideration in the practice of the health and hygienic supervision over food products. Therefore, the supervision of the observance of obligatory conditions of the storage of products which are intensely contaminated with A. flavus should be made more stringent and the decision about permitting their use for food should be taken only after an analysis of their aflatoxin contamination.

Thus, a comparatively frequent contamination of food in the Northern or the South-Eastern areas of the Republic with A. flavus, many strains of which are aflatoxinogenic, lends itself to a conclusion about the presence of a regular potential of aflatoxin contamination in these areas. At the same time the identified aflatoxin contamination of food products at concentrations higher than the fixed MAC is an indication of a real danger, in both areas, of aflatoxicoses. This may be agravated by contaminated imported products.

In connection with all this it become necessary to further modernise the surveillance system in respect of the contamination of food products with aflatoxins and the prevention of this contamination.

The Kazakh branch of the Institute of Nutrition of the USSR Academy of Medical Sciences undertook a number of steps to arrange the health-mycological service in the Kazakh Republic. The facilities of the Laboratory for health-and-food mycology have been used for several years to hold annual seminars for bacteriologists and chemists from regional and town Sanitation and epidemiological stations of the Republic. The seminars are devoted to the fundamentals of health-and-food mycology, the methods of detection of toxigenic fungi and mycotoxins in foodstuffs. The Branch has the authority of a research-consultative and methodical centre in the Republic surveilling the contamination of food products with toxigenic fungi, mycotoxins and preventing mycotoxicoses.

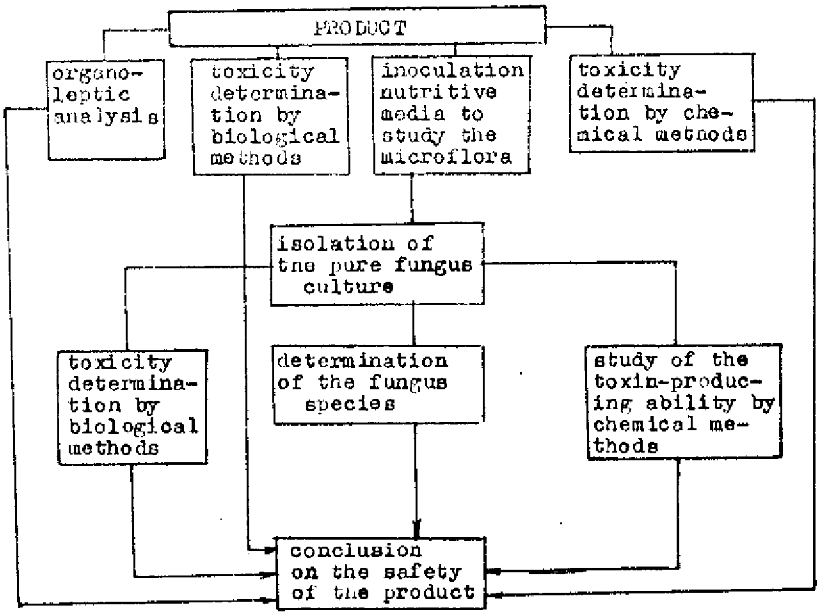
The techniques of mycological, toxicobiological and chemical analysis are applied to gain a complete health and mycological evaluation of food products. The latter is attainable if a definite pattern of a health and mycological analysis of products is applied: this pattern is shown in the appended chart (see next page).

Special conditions are necessary to carry out these analyses and they can be better provided in a specially equipped laboratory.

The following principles should be abided by in evaluating product quality.

Food products with organoleptic properties altered under the influence of fungi should be viewed as substandard and their use for food-prohibited.

CHART OF THE HEALTH-MYCOLOGICAL ANALYSIS OF
FOOD PRODUCTS



Food products which, by the results of the biotest are found to be toxic are prohibited for use.

If trials on animals show that the products are not toxic but a chemical test reveals the presence of mycotoxins in volumes over and above the MAC, such products are likewise banned for food use.

Whenever the results of a biotest show that a given product is not toxic but contains toxic fungi, the final conclusion depends on the results of the chemical analysis; if the product has no micotoxins in it or contains them in allowable amounts, it can be used as food. At the same time, the surveillance over the observance of storage rules has to be made more stringent and the time of storage - shortened.

The following conclusions can be drawn on the basis of the presented data:

1. Microscopic fungi are widespread in food products in the Northern and South-Eastern areas of Kazakhstan and are characterised by a considerable variety, numbering 99 species which represent 36 genera.

2. In both areas the prevailing fungi genera, in foodstuffs are Aspergillus and Penicillum, which are represented by many potentially toxigenic species: A. flavus, A. fumigatus, A. niger, A. nidulans, P. notatum, P. cyclopium. P. martensii, P. citrinum, P. viridicatum, etc.

3. 51.2 per cent of A. flavus strains isolated from food products in the Northern area are producents of aflatoxin B_I, the percentage in the South-Eastern area is 30.5. These figures predetermines the potential danger of aflatoxicoses in Kazakhstan.

4. 3.3 per cent of the examined food samples in the Northern area were found to be contaminated with aflatoxin B_I; the percentage in the South-Eastern area was 17.2. The contamination level in a considerable percentage of samples was found to be above the MAC which indicates the danger of aflatoxicoses.

5. Imported products which are primarily used in the confectionery industry are practically always contaminated with microscopic fungi, frequently with A. flavus, in many instances with aflatoxins in excess of the maximum allowable concentration.

6. It is necessary to modernise the system of health and mycological surveillance in the framework of the sanitation and epidemiological service of the country to prevent dangerous levels of contamination of food products with toxigenic fungi, aflatoxins, other mycotoxins and for preventing mycotoxicoses.

The following questions are recommended for discussion:

1. The problem of mycotoxicoses, its importance for different areas of the world.

2. Classification of toxigenic fungi and mycotoxins.

3. The spread of toxigenic fungi and mycotoxins in food products in different areas of the world.

4. Classification of the diseases of man caused by mycotoxins.

5. The system of health and mycological surveillance of foodstuffs.

6. Urgent tasks of the present day health and food mycology.

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