RESEARCH ON ACTIVE AND PASSIVE MONITORING AEROMICROFLORA IN THE MILK UNITS PROCESSING

Gheorghe PUCHIANU[1], Valentin NECULA[1], Dorin Valter ENACHE[1]

Abstract: *For monitoring aeromicroflora in the milk processing units we have proceeded to sampling procedures for monitoring using active and passive methods that have become an essential tool for environmental monitoring, both in food (workspaces, warehouses, etc.) and in testing laboratories.*

Microbiological examinations were performed: total number of aerobic bacteria detection (TNG) / m3 of air and number of yeasts and molds (Y+M) / m3 air.

We made a passive monitoring using sedimentation method by exposing Petri dishes with culture medium for collecting solid particles settle by gravity. To achieve active monitoring have taken defined amount of air passed through a sampling device directly on a solid culture medium.

After sampling proceeded to incubating the plates at different temperatures, depending on the target organism and then we determined the microbiological load of air through estimating the number of microorganisms expressed as cfu / m3 ,, using calculation formulas and Barzoi Omelianski

In the use of the two methods we concluded that sedimentation method allows an approximate quantitative determination, while the vacuum method allows for accurate quantitative determination

Keywords: microaeroflora, passive and active monitoring, Total Number of Germs - TNG, Yeasts and Mold (Y+M)

Introduction

Microbiological control of air workspaces addresses of processing units, storage, marketing, etc., of foodstuffs for human consumption and premises of testing laboratories.

There are two principal means of monitoring microaeroflorei: passive monitoring and active monitoring. Both are equally important, now becoming an essential tool for environmental monitoring in the food sector.

Depending on how sampling can use two methods - sedimentation and aspiration.

[1]Transilvania University of Brasov, *Mailing adress: Faculty of Food and Tourism,, Castle Street, nr. 148, cod 500123, Brasov, Phone/Fax: 0268472222, E-mail: gheorghe.puchianu@unitbv.ro

Sedimentation method is suitable to detect microbial particles which are deposited passively due to the force of gravity. For passive monitoring can use two methods: sedimentation Koch, standard Polish PN 89/Z-04008/08 and sedimentation method as "microbiological methods for laboratory examination of food products of animal origin". [1]

The disadvantages of sedimentation are: the method is non-dimensional (not provide information on the volume of air that collects particles) does not allow a quantitative determination of the exact applicability of the plates is limited, it may monitor only the particulate biologically viable settle out of the air and are deposited on a surface in a given exposure time, can not detect small particles suspended in the air, are influenced by interference and contamination of other sources, agar plates can deteriorate if exposure too long, the pads can become very busy in environments heavily contaminated and data interpretation becomes difficult. [2,6]

Advantages sedimentation are the plates are inexpensive and easy to use, requires no special equipment, use more qualitative analysis microaeroflorei, the data can detect trends and provide an early warning of problems. In an environment with low risk (ex. Food production facilities), the plates can provide a suitable means of microbiological monitoring of air quality.

The method aspiration can be achieved by: impact according to ISO 16000-18 - suitable for cases where expected only low concentrations of microbial, for example. clean rooms, food production facilities, etc; filtration according to ISO 16000-16 - suitable for highly contaminated environments, the technique can cause impaction plate over filling. The method allows the use of decimal dilutions to determine the real number of microorganisms.

It is an active way, by volume (the volume of air provides information from which the particles are collected), and so allow accurate quantitative determination, requiring the use of a device that absorbs a known volume of air.

There are two types of sampling devices commercially available: the slot and the screen. The slot device, air is drawn through a narrow gap, and the particles are deposited on a rotating agar plate. The sieve devices, air is drawn through a perforated plate (sieve) with holes with known diameter and the particles deposited on an agar plate fixed underneath. There is the possibility of grouping several sites with different sizes.

For filtration method, filter membranes obtained after harvesting, are resuspended in 5 ml of saline with 0.01% polysorbate 80 (Tween), this being the initial suspension of the decimal dilutions are prepared. 0.1 ml of these dilutions is dispersed on the surface of the agar plates. [3,4,5]

Whatever the process of harvesting (by impact or by filtration), the number of germs is calculated by reporting the number of colonies developed on the culture medium surface to the volume of air. The calculation for the two methods they were carried out in accordance with ISO 16000-17. [7,8,9,10]

Material and method

The methodology of diagnosis was performed in accordance with EN ISO 4833-1 / 2009 - Horizontal method for the enumeration of microorganisms, Part 1, Colony-count technique at 30° C and SR ISO 21527-2: 2009 Horizontal method for the enumeration of yeasts and molds, part 2, colony-count technique in products with water activity less than or equal to 0.95.

Equipment used to make the diagnosis were: oven, incubator (25 ± 1° C) incubator (30 ± 1° C or 37 ± 1° C) water bath (45 ± 1° C), cabinet protection laminar flow, the colony counting equipment, pH - 0.1 meter accuracy pH unit at 25° C, using a microscope, gas burner, Petri dishes with a diameter of 90-100 mm, disposable unit for the sampling air samples;

For effective monitoring and passive we used Petri dishes standard containing culture media (PCA and DG18), which after opening we have exhibited in spaces controlled by 2 plates with the medium for total plate count by 2 plates with medium yeasts and molds for 10 minutes.

The plates were placed in workrooms at work surfaces in deposits - a plaque on pavement and a board height of 0.8 - 1.0 m, in order to collect sediment particles by their gravitational force. After 10 minutes the plates were covered with lids and were transported immediately to the laboratory.

After incubating the plates at different temperatures, depending on the target organism (NTG or DM), we determined the microbiological charge air by estimating the number of organisms expressed as CFU / m^3.

To perform active monitoring have taken a defined amount of air after passing through a sampling device, the particles in the air is collected directly on a solid culture medium using devices sieve, allowing air to be pulled through a plate perforated (sieve) with hole diameter known particles are deposited on an agar plate fixed underneath. After harvesting the plates were identified from the sampling site, packaged, sealed and shipped under refrigeration (2-4°C) to the laboratory, where have been incubated immediately upon arrival.

If yeasts and molds we proceeded to identify yeasts and molds. Their colonies or smear preparations we made and we examined under a microscope with 40 x objective

Results and discussions

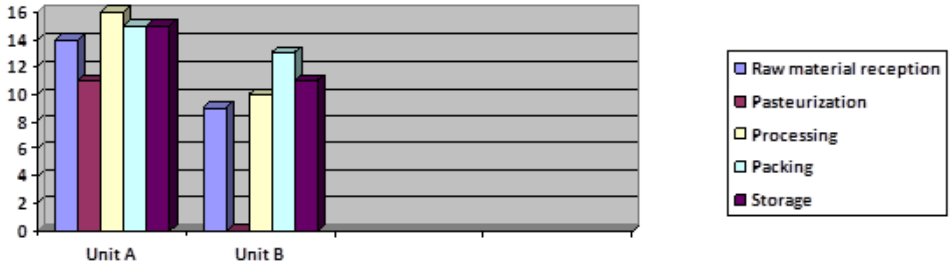
Samples were taken from different workspaces two milk processing units in the county of Brasov. The total number of samples collected was 183, of which 114 were obtained by using the sedimentation method, and 69 the suction method.

The 114 samples collected by sedimentation technique, were taken from the following workspaces: (Table. 1 and Chart no. 1)

Table. 1. The number of samples examined by sedimentation method

Nr. Crt.	Name of establishment	Total samples	Space name				
			Raw material reception	Pasteurization	Processing	Packing	Storage
1.	A	71	14	11	16	15	15
2.	B	43	9	-	10	13	11
	Total	114	23	11	26	28	26

Chart no. 1. The number of samples examined by sedimentation method



For taking air sampling we used standard Petri plates containing culture media - TNG PCA and DG18 to Y + M. The culture media were prepared as described and were then cast in Petri dishes having a diameter of 9 cm. Subsequently Petri dishes in number 4 (2 TNG and 2 to Y + M), were positioned in such spaces in table. 1 over an area of 1m2 on the floor and other surfaces located at different heights in relation to the (1 - 2m) for 15 minutes.

After this contact, the Petri dishes were covered and immediately transported to the laboratory where they were incubated at 37 ° C in the case of NTG and 25 ° C for the Y + M. The incubation period was 72 hours in TNG and 4-5 days for Y + M after incubation culture media were examined to identify potential colonies developed. (Fig. 1,2,3,4,5,6).

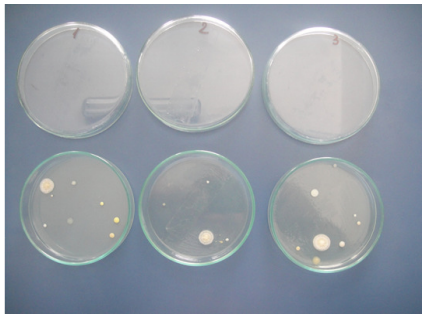


Fig. nr 1. Petri dishes with PCA medium – TNG (non-compliant samples)

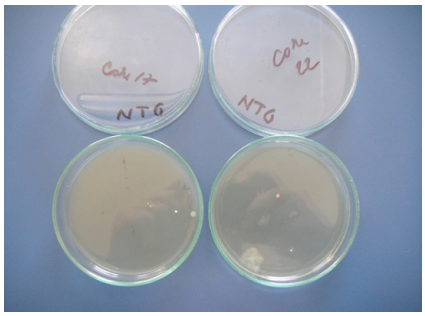


Fig. nr 2. Petri dishes with PCA medium - TNG (compliant samples)

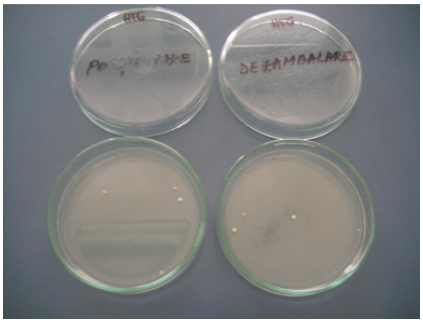


Fig. nr 3. Petri dishes with PCA medium - TNG n-compliant samples)

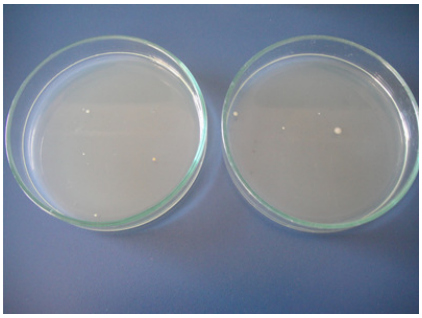


Fig. nr 4. Petri dishes with PCA medium - TNG (compliant samples)

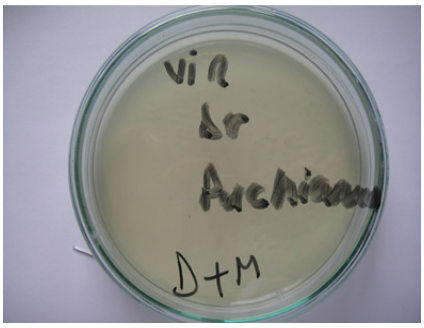


Fig. nr. 5. Petri dishes with DG 18 medium - Y + M (compliant sample)

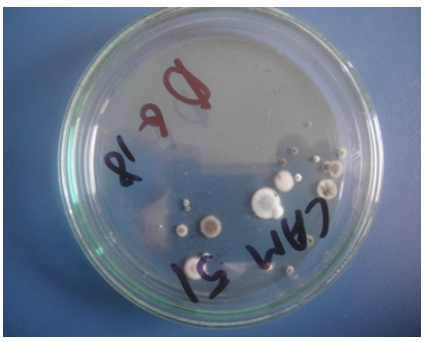


Fig. nr. 6. Petri dishes with DG 18 medium - Y + M (non-compliant sample)

Depending on the target organism (TNG or Y+M), we determined the microbiological load by estimating the number of microorganisms air expressed as CFU / m^3.

For the calculation we used comparative three formulas: Polish standard PN 89 / Z-04008/08 Omelianski formula and method Barzoi as follows:

1. According to the Polish standard PN 89 / Z-04008/08:

CFU/m^3 aer = a · 10.000 / p · t · 0.2

where:
a = number of colonies in the Petri dish
p = area in cm^2 Petri plate (for a diameter of 9 cm, S = 3.14 x R 2 = 63.5 cm^2)
t = exposure time of the Petri plate

2 According to the formula Omelianski

……..

where:
a = number of colonies in the Petri dish
p = area in cm^2 Petri plate (for a diameter of 9 cm, S = 3.14 x R 2 = 63.5 cm^2

CFU/m^3 aer = N x 10.000 / S x K

where:
N = the number of colonies in the Petri dish
S = surface area in cm^2 Petri plate (for a diameter of 9 cm, S = 3.14 x R^2 = 63.5 cm^2)
K = coefficient exposure time (factor) (5 minutes = 1, 10 = 2 min, 15 min = 3)

3. According to the method Barzoi

CFU / m^3 air = n x 50

where:
n = the average number of colonies on the two Petri dishes
For this method uses two Petri dishes with a diameter of 10 cm, exposed for 10 minutes

Calcuation method	Formula
Polish standard PN 89/Z-04008/08	CFU/m^3 air = a · 10.000 / p · t · 0.2
Omelianski formula	CFU/m^3 air = N x 10.000 / S x K
Bărzoi method	CFU/m^3 air = n x 50

Taking in considering the number ufc developed on the surface of media culture plates used for sedimentation of micro-organisms and comparing them to the maximum permitted levels set in the procedure National Sanitary Veterinary Authority on sampling we considered inconsistent all samples were above 600 cfu / m3 in the case TNG and 300 cfu / m^3 for Y + M. For the calculation we used

standard formulas provided in Polish and Omelianski formula (Table no. 2 and chart no. 2 and 3)

Table. 2. Number of samples compliant and non-compliant

Name of establishment	Total samples				Space name													
					Raw material reception			Pasteurization			Processing			Packing			Storage		
		C	N		C	N		C	N		C	N		C	N		C	N	
			No	%	No	No	%	No	No	%	No	No	%	No	No	%	No	No	%
A	71	66	5	8	12	2	14	11	-	-	15	1	6	13	2	13	15	-	-
B	43	41	2	5	8	1	11	-	-	-	10	-	-	12	1	8	11	-	-
Total	114	106	7	6	20	3	13	11	-	-	25	1	4	25	3	11	26	-	-

C = compliant

N = non - compliant

Chart no. 2. Probe compliant and non-compliant - Unit A

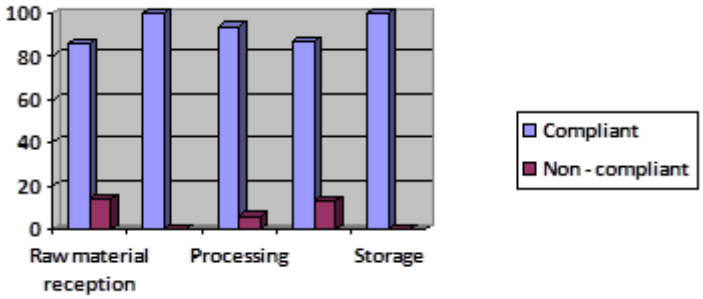
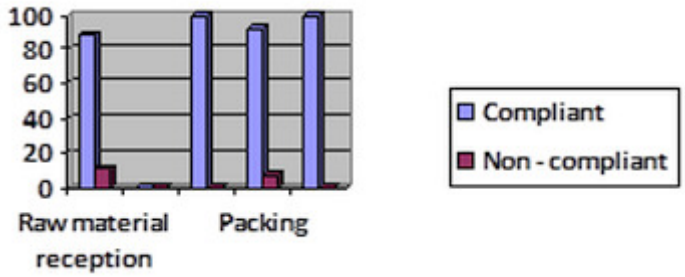


Chart no. 3. Probe compliant and non-compliant - Unit B



Regarding on the three formulas accepted and used it is noted that the results are identical for standard formula Polish and Omelianski and differ slightly from the results obtained after applying the formula Barzoi.

Thus, if two petri dishes and placed on the surface of the culture medium from each plate is developed two cfu, yielding an average of 2 colonies, the results achieved by the three formulas are as follows:

Polisch standard	Omelianski formula	Bărzoi method
2 x 10.000/63,5 x 15 x 0,2 = 105	2 x 10.000/63,5 x 3 = 105	2 x 50 = 100

Result that TNG are consistent when the culture medium on the surface of the 2 plates develops an average exceeding 11 cfu (12 cfu results being true).

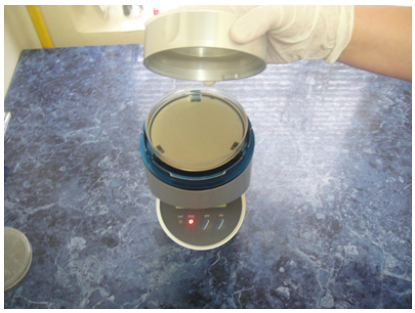
If Y + M, if we refer to the maximum possible limit of 300 cfu / m^3, the results are in line when the surface of the culture medium of the 2 plates develops an average of more than 5 cfu (from 6 cfu results being true) .

Using aspiration method it was collected 70 samples from the same number space of the processing units A and B of milk from which samples were taken and the sedimentation method, as follows: (Table no. 3)

Table 3. The number of samples by aspiration method

Nr. Crt.	Name of establishment	Total samples	Space name				
			Raw material reception	Pasteurization	Processing	Packing	Storage
1.	A	35	8	5	7	7	8
2.	B	35	9	3	6	10	7
	Total	70	17	8	13	17	15

To perform active monitoring we have taken a defined amount of air that is 100 liters (0.1 m^3) using a device that allows the collection of airborne particles directly on a culture medium - DG 18 using mesh devices that allow air to is drawn through a perforated plate (sieve) with holes with known diameter and the particles deposited on an agar plate fixed underneath. (Fig. no. 7 and 8)



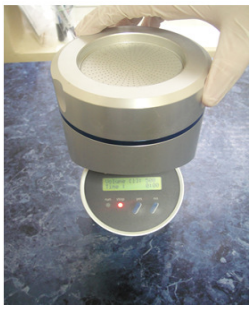


Fig. no. 7 and 8. Air sample equipment

After harvesting, the plates were identified, packed, sealed and transported to the laboratory where they were incubated immediately after arrival at 37 ° C in the case of TNG and 25 ° C for the Y + M. The incubation period was 72 hours in TNG and 4-5 days for D + M after incubation culture media were examined to identify potential colonies developed. (Fig. No. 9, 10, 11 and12).

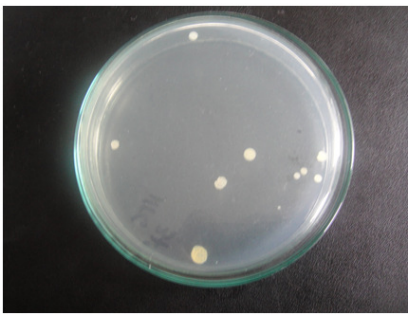


Fig. no.9. Petri dishes with PCA medium (compliant sample)

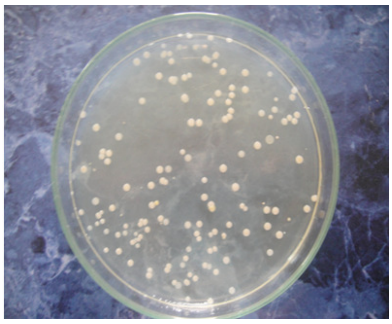


Fig. no.10. Petri dishes with PCA medium (non-compliant sample)

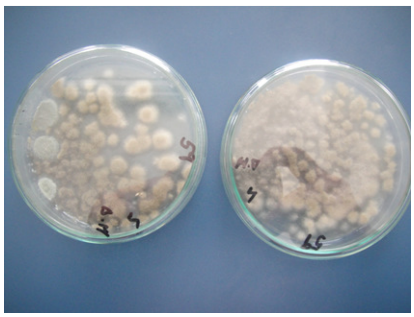


Fig. no.11. Petri dishes with DG 18 medium (non - compliant sample)

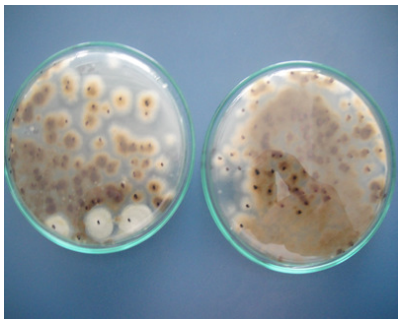


Fig. no.12. Petri dishes with DG 18 (non-compliant sample)

.

The number of bacteria was calculated by reporting the number of colonies developed on the culture medium surface to the volume of air. The calculation for the two methods set out in ISO 16000-17. "The air inside. Part 17: Detection and enumeration of molds - based method cultivation. "Pct. 7.6.2 - impact method

- for each sample is analyzed in parallel two different volumes of air (ex. 1 m^3 and 2 m^3), thus requiring four Petri dishes to sample.
- concentration, expressed as CFU / m^3 air, is calculated by formula:

CI = nufc / VI

where:

nufc = total number of colonies on Petri dishes

VI = total sample volume in cubic meters.

Example:

On the DG18 medium, a sample is analyzed for two different air volume of 100 liters and 200 liters. The counts were obtained the following results: Sample volume of 100 liters (0.1 m^3) - shoulder cfu was developed colonies on the plate 21 1:19 colonies in the plate 2 and the sample volume of 200 liters (0.2 m^3) - the number of cfu was 34 în plate no. 3 and 39 in plate no. 4.

$$\mathbf{C_I = n_{ufc} / V_I = 40/0,2 = 200 = 2,0 \times 10^2}$$

$n_{ufc} = 21+19+34+39 = 113$

$V_I = 0,1+0,1+0,2+0,2 = 0,6$

$C_I = n_{ufc} / V_I = 113/0,6 = 188 = 1,9 \times 10^2$

Taking in consideration the number ufc developed on the surface of media culture plates used for active monitoring and comparing them to the maximum permitted levels set in the procedure of National Sanitary Veterinary and Food Safety Authority on sampling we considered inconsistent all samples were above 600 cfu / m^3 in the case NTG and 300 cfu / m^3 for Y + M. (Table. 4)

Table 2. Number of samples compliant and non-compliant

Name of establishment	Total samples				Space name													
					Raw material reception			Pasteurization			Processing			Packing			Storage		
		C	N		C	N		C	N		C	N		C	N		C	N	
			No	%	No	No	%	No	No	%	No	No	%	No	No	%	No	No	%
A	71	66	5	8	12	2	14	11	-	-	15	1	6	13	2	13	15	-	-
B	43	41	2	5	8	1	11	-	-	-	10	-	-	12	1	8	11	-	-
Total	114	106	7	6	20	3	13	11	-	-	25	1	4	25	3	11	26	-	-

Chart no. 4. compliant and non-compliant samples - Unit A

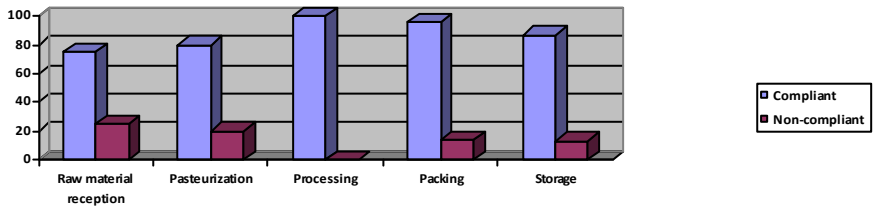
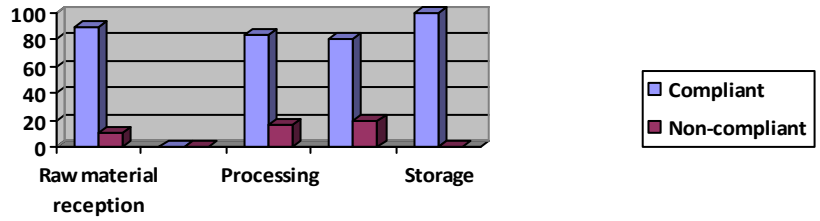


Chart no. 4. compliant and non-compliant samples - Unit B



Comparing the number of cfu that develops on the surface of media on air samples by sedimentation method and the air sample taken through active monitoring that when TNG value 12 cfu / m^3 corresponds to a total of 360 cfu / m^3 which develops on the surface of the culture medium of the four petri dishes and in the case of D + M, a value of 6 cfu / m^3 corresponds to an average of 180 cfu / m^3 which develops on the surface of the culture medium of 2 Petri dishes. Values greater than these, representing noncompliance, while the volume of the sample taken from monitoring active is 100 liters and 200 liters (0.1 m^3 and 0.1 m^3)

For this reason, we believe that if the formula for calculating sedimentation, should be amended, taking as a basis for calculating the maximum permissible correspondence value cfu of passive monitoring to active monitoring of the maximum permissible value.

In case of TNG:

$$\mathbf{CFU/m^3\ aer = 12 \times 50 = 600}$$

$$\mathbf{C_I = n_{ufc} / V_I = 360/0,6 = 600}$$

In case of Y+M

$$\mathbf{CFU/m^3\ aer = 6 \times 50 = 600}$$

$$\mathbf{C_I = n_{ufc} / V_I = 180/0,6 = 300}$$

Thus we intend to apply the following formula, which allows for the passive monitoring results more similar to active monitoring. Practical we apply Barzoi method a correction factor, 1.67, so if TNG as such and Y + M, which is the result of dividing the maximum allowable maximum number of cfu may develop on the surface of the four culture media that was They've taken defined amount of air, above which the sample is classed as non-compliant.

UFC/m^3 aer = n x Vmp/12 x Vma/360

where:

n = the average colonies developed on the culture medium inside surface of the second

Petri plates (cfu)

Vms = maximum allowable passive monitoring (cfu)

Vms = maximum allowable amount of active monitoring (cfu)

Example:

On the environment have been developed ATP 6 and 8 cfu, so an average of 7 cfu. What is the microbiological load of 1m^3 air.

1. Method Bărzoi

UFC/m^3 aer = 7 x 50 = 350

2. The calculation method proposed by us

CFU/m^3 aer = 7 x 600/12 x 600/360 = 7 x 50 x 1,67=584,5

It follows that the following formula proposed by us, a result inconsistent is when the surface of media number ufc is> 7 in the case of TNG and> 3 when Y + M, unlike the Barzoi method which is bringing non-compliant results when ufc number is> 12 in the case of TNG and> 6 if Y + M

Conclusions

(1) Monitoring passive identified a number of 6% non-compliant samples, while active monitoring 13% of the samples, while the air samples were collected from the same units and workspaces;

(2) Both methods detected microbiological air cargo highest in the same premises and reception areas namely raw material, 13% non-compliant samples for passive monitoring and 18% of the samples for active monitoring. And the other areas there is a correlation approximate, always with the predominance of non-compliant samples for air sampling defined in quantitative terms;

(3) The results obtained from the calculation formula proposed by us allows the number of non-compliant samples to be higher by about 50% in both quasi parameters (NTG and D + M) and thus closer to the number of samples non-compliant results from monitoring active. So after applying the calculation formulas known non-compliant samples ratio is 13: 6 know what the application zrma proposed new formula for calculating the ratio is 13: 12.

References

[1] Barzoi D., S. Apostu, 2002. Microbiology of food. Publisher Risoprint, Cluj Napoca.
[2]. Simona 2007. Ivana Veterinary Medical Microbiology, Vol I. Publisher Medical Sciences.
[3] Puchianu G. 2012. Microbiological and food safety criteria. Publisher Transilvania University of Brasov.
[4] Ştirbu C., D. Turcu, 1999. Getting bacteriology. Publisher Brumaire Timisoara.
[5] ANSVSA Order no. 35/2016. Detailed implementation rules Programme of the surveillance, prevention, control and eradication of animal diseases, those transmitted from animals to humans, animal protection and environmental protection, identification and registration of cattle, pigs, sheep and goats for 2012
[6] Piha, Rodica Dumitrache, 2015. Microbiological control of indoor air. Food microbiology training, Deva 30.09-2.10.2015
[7] ISO 16000-17. "The air inside. Part 17: Detection and enumeration of molds - based method cultivation. "Pct. 7.6.2 - impact method
[8] Procedure ANSVSA. Sampling food to microbiological testing.
[9] SR EN ISO 4833-1 / 2009. Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. Part 1. colony count technique at 30 ° C, pour plate technique;
[10] SR ISO 21527-2: 2009. Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of yeasts and molds. Part 2: Colony count technique in products with water activity less than or equal to 0.95