World Academy of Science, Engineering and Technology
International Journal of Marine and Environmental Sciences
Vol:5, No:4, 2011

Microbiological Contamination of Outdoor Air in Marine Durres's Harbour, Albania

Laura Gjyli[1], Pirro Prifti[1], Lindita Mukli[2], Silvana Gjyli[3], Irida Ikonomi[1], Jerina Kolitari[4]

Abstract—Microbial air contamination of the outdoor air in Marine Durres's Harbour (Durres, Albania) was estimated by sedimentation technique in August-October 2008. The sampling areas were: Ferry Terminal (FT), Fishery Harbor (FH), East Zone (EZ), Fuel Quay (FQ) and Apollonian Beach (AB). The aim of this study was to measure the number of aerobic plate count (mesophilic aerobic bacteria) and fungi (yeasts and molds) in the outdoor air in these areas. The number of colonies that were formed determines the number of cells at the moment in the outdoor air; respectively the number of mesophilic aerobic bacteria and yeasts and molds. The measure of bacteria and fungi used is CFU (Colony Forming Units) per Petri dish. It is said that marine harbours are very polluted areas. The aim of study was the definition of mesophilic aerobic bacteria and yeasts and molds number, and the comparison of microorganisms number in air sampling areas.

Keywords—Air microbiology, colony forming units, Marine Durres's Harbour, mesophilic aerobic bacteria, outdoor air, yeasts and molds.

I. INTRODUCTION

MICRORGANISMS present in the air originate from soil, plants and water and atmospheric air is not a convenient environment for the their growth. Their concentration is high and they find proper conditions for growth, such as high humidity and suitable temperature. However, spore-forming bacteria and fungi are able to survive in bioaerosols and stay viable for a long time in the air. Many microorganisms present in the air, including viruses, bacteria, fungi, yeasts and protozoa, are associated with diseases occurring in humans, plants and animals [1]. It is generally known that microorganisms present in the air can affect human health, causing mainly respiratory and related diseases transmitted via respiratory route. Many species of bacteria as *Streptococcus pyogenes, Mycobacterium tuberculosis* or *Legionella pneumophila* or viruses may cause severe human infection diseases. However, particularly significant and simultaneously burdensome for humans are filamentous fungi – moulds occupying sometimes 70% of all microorganisms present in the air [2-4]. They can cause many health problems, including allergic and toxic reactions. Nowadays, allergic respiratory diseases have become common. The number of allergic respiratory diseases is increasing and therefore allergies are called " the epidemic of the 21st century" [3]. The basic reason for this dramatic rise of allergic diseases is urban air pollution and therefore it is observed first of all in the industrialized world [2, 3, 5, 6]. Many genera of fungi can cause allergic responses, but particularly important are some species of *Cladosporium, Alternaria, Penicillium,* and *Aspergillus*. *Cladosporium* and *Alternaria* are frequent and predominant genera present mainly in the outdoor air and found throughout the world, whereas *Penicillium* and *Aspergillus* species are generally isolated from indoor environments [7, 8]. The concentration of microorganisms in atmospheric air and their qualitative composition is very much dependent on different atmospheric factors [9-12]. Wind, rain, sunlight, ozone – all these natural forces have a dispersing and sometimes even bacteriocidal affect on microorganisms [10]. Also, chemical air pollution is associated with microbiological contamination [6, 11]. The diesel engines at harbours, which power ships, trucks, trains, and cargo-handling equipment, create vast amounts of air pollution affecting the health of workers and people living in nearby communities, as well as contributing significantly to regional air pollution [13, 14]. Nowadays people spend most of their lives (80-90%) indoors, so indoor air quality is important for human health and comfort. On the other side, in an ideal world, microorganism composition of indoor air would reflect qualitatively that of outdoor air with quantitative difference only. Therefore outdoor air is thought to be an important source of microorganisms indoors and monitoring of outdoor air may become a significant element necessary for the estimation of indoor air quality [12, 15, 16]. The aim of our work is to perform a observation of microbiological quality of outdoor air in the city of Durres, especially in Marine Durres Harbour and impact of this area in outdoor air of Durres. Durres's Marine Harbour is located in the southern part of Adriatic Sea, in south of Durres, north of Durres Bay. Durres bay is approximately 18 km long from north to south, with a coastline of 7 km in the east [17].

[1]Department of Medicine, University "Aleksander Moisiu", Durres, Lagjia nr. 1, Rruga e Currilave, Durres, Albania (phone: +355 52 39161; fax: +355 52 39163; e-mail: lauragjyli@yahoo.com, priftipirro@hotmail.com, irida.ikonomi@yahoo.com).
[2]Department of Mathematics, University "Aleksander Moisiu", Durres, Albania (e-mail: mukli_lindita@yahoo.com).
[3]Department of Chemistry, Tirana University, Tirana, Albania (e-mail: vanagjyli@yahoo.com)
[4]Institute of Fishery Research, Durres, Albania (e-mail: jerina_juka@yahoo.com)

II. MATERIALS AND METHODS

The number of airborne microorganisms was evaluated by culture settling plate technique, known as sedimentation technique. The following stations were evaluated: Ferry Terminal (FT), Fishery Harbor (FH), East Zone (EZ), Fuel quay (FQ) and a beach area as control, Apollonia Beach (AB). The numbers of aerobic plate count (mesophilic aerobic bacteria) and yeasts and molds were determined using, respectively, Plate Count Agar(PCA) and Chapeck [18, 19]. The intervals between sampling were two weeks during the period of August-October. Open Petri dishes containing 20 ml of culture media (PCA, Chapeck) were distributed at the proccesing areas and exposed for 15 minutes. Then the Petri dishes were closed and incubated at 37^{o}C. The reading of plates are made after 24 and 48 hours for mesophilic aerobic bacteria and after 5-7 days for yeasts and molds. The results were expressed as CFU per Petri dish. The culture medium used in this study are Plate Count Agar (PCA) and Chapeck. The culture mediums are prepared from dry mediums in vessels with a covering thread and are sterilized in 121^{o}C for 15 minutes [20, 21]. There are counted the colonies that are formed in culture medium. The number of colonies gives the number of microorganisms that have been fallen in Petri plate from air. If the number of colonies is till 200, the air is pure. When there are up 200, the air is considered impure [22, 23].

A. Statistical Data-processing

There are used these statistical indicators: average, variance, standard deviation and variation coefficient [24, 25].

B. The investigated area

Durres's Marine Harbour is very ancient and has a very important geographical position. It is not only the biggest harbour in Albania but also one of the most important harbours in the South Eastern Europe. It is situated 39 km away from the capital city, Tirana. It is located in the southern part of Mediterranean Sea. The entrance canal is 8.5 m deep. It has a length of 3.65 miles and a width of 60-195 meters. The general surface of the harbour is 1,467,000 m^2, where the water surface is approximately 674,000 m^2. There are 11 quays and the depth near the quays is 6.5-10.2 m. The length of the operation quays is 2.2 km [17].

III. Results and discussions

A. Aerobic Plate Count (Mesophilic Aerobic Bacteria) in all Air Sampling Stations along the Coastal Zone of Durres Area

This study is made in Durres's Harbour in these places:

Ferry Terminal, (FQ) Fishery Harbor, (FQ), East Zone, (EZ), Fuel Quay (FQ) and in Apollonia Beach (AB) in August-October 2008.

The number of Mesophilic Aerobic Bacteria (Fig. 1, Table 1) in air presents changes in months. The richest month with Mesophilic Aerobic Bacteria is August. This confirms that high temperatures in the Summer favors the growth of bacteria in the air. There are evidences in literature that the temperature affects the microbial number in the air [26]. Rely on each station where the samples are taken, August has the highest number of presence of Mesophilic Aerobic Bacteria in FQ with 170 CFU. Then are ranked FH, FT and AB. In the end is EZ with 93 CFU. But, in any case, the air is within standards to consider clean in all stations (the limit for clean air is 200 CFU per Petri plate) [20, 22, 23].

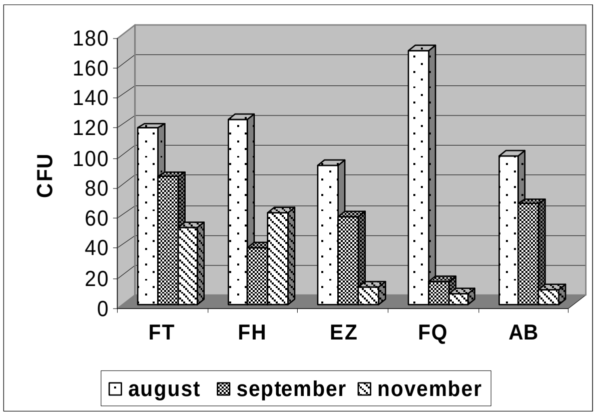

Fig.1. Mesophilic Aerobic Bacteria Number according to months in "FT, FH, EZ, FQ, AB".

In September it is noticed that has a downfall number of Mesophilic Aerobic Bacteria. This confirms that with coming of Autumn, the temperatures are not favorable for growth of bacteria in air. The richest area with bacteria is FT with 85 CFU, whereas the poor area is FQ with 15 CFU per Petri dish.

In October there is a drastic downfall of Mesophilic Aerobic Bacteria, this for the reason of decrease of temperatures. An exception is FH, where the number of bacteria is increased compared with September, that signifies that there are others factors, as garbage from fishery boats in this period, that brings an increase of bacteria in air in this station, where the number of Aerobic Plate Count is 61 CFU, and then are ranked FT, EZ, AB and in the end FQ with 7 CFU.

Table I Absolute Frequency (CFU) And Relative Frequency (%) Of Mesophilic Aerobic Bacteria Of "FT, FH, EZ, FQ, AB" According To Months

Station		FT		FH		EZ		FQ		AB	
Month	Sample	ab. freq.	rel. freq.	ab. freq.	rel. freq.	ab. freq.	rel. freq.	ab. freq.	rel. freq.	ab. freq.	rel. freq.
Aug.	air	118	46	123	55	93	57	170	89	99	56
Sept.	air	85	34	38	17	58	36	15	8	67	38
Oct.	air	51	20	61	27	12	7	7	4	10	6

Note: ab. freq= absolute frequency, rel. freq.= relative frequency

TABLE II AVERAGE, STANDARD DEVIATION AND VARIATION COEFFICIENT OF AEROBIC PLATE COUNT IN SAMPLING AREAS IN MONTHS AUGUST-OCTOBER

Place	Average (CFU / Petri plate)	Standard Deviation	Variation Coefficient
FT	90	44.16	0.49
FH	60	37.63	0.63
EZ	62	35.52	0.57
FQ	65	84.13	1.29
AB	68	45.07	0.66

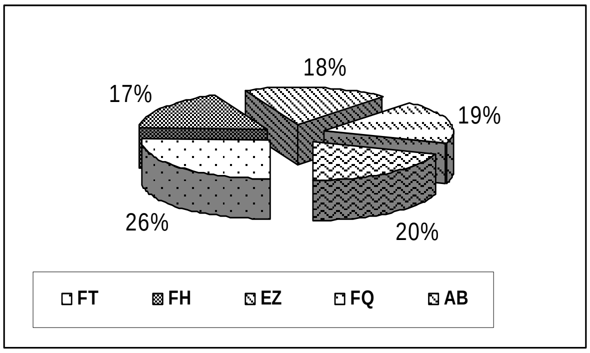

Fig. 2. The average of Aerobic Plate Count in "FT, FH, EZ, FQ, AB"

The richest station with Mesophilic Aerobic Bacteria (Fig. 2, Table 2) results FT with 26% or with 90 CFU. The presence of ferries in this station brings contamination of the air. In the second place is AB with 20% or 68 CFU that confirms the air in beach area is clean. Then come FQ, EZ, whereas the poorest station is FH with 18%. By all means rely on averages these stations have air within standards [22, 23], that signify air currents from sea clean air, despite the harbour activities [13, 14] and anthropogenic sources in these stations. After source of microorganisms is the human population, as commensal inhabitants of the skin, mucous membranes, and other body sites in humans and animals [4, 27]. The most variable station is FQ (Fig. 3, Table. 2), whereas the least variable station is FT. This confirms that in FQ, the contamination of air in this station may be connected with presence of oil-tanker crafts. FT is a station that has the least variability in number of bacteria, maybe the continuous activity of ferries, that brings a number of bacteria that does not change more considerably during the period of study comparing with other stations. In the AB the variation of growth of bacteria is higher than the other stations in Harbour, expect FQ. Perhaps the growth of bacteria is affected, not only by temperature, but also by other factors as difference of human population along these areas [24, 25].

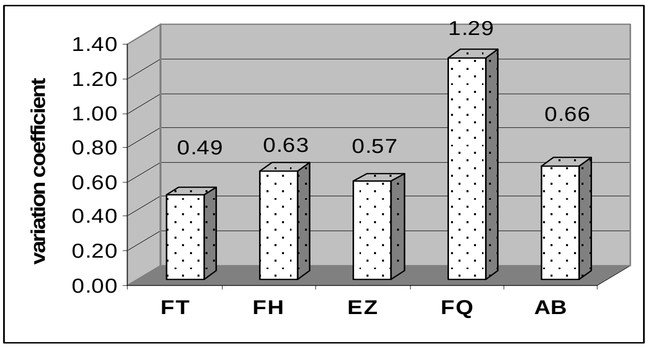

Fig. 3. Variation Coefficient of Total Number of Mesophilic Aerobic Bacteria in "FT, FH, EZ, FQ, AB"

B. Fungi (Yeasts and Molds) in all Air Sampling Stations along the Coastal Zone of Durres Area

The number of Yeasts and Molds per Petri dish (Fig. 4, Table 3) is different during the months. August is the richest month with fungi in outdoor air. There are evidences in literature that the temperature affects the microbial number in the air [26]. The richest station with fungi in August is EZ with 70 CFU, whereas the least station is AB with 17 CFU. Perhaps in EZ there is a big source of Yeasts and Molds as a result of the activity of the processing scrap and coal in this station. There is clearly evident that in the air it is formed a smoke-cloud. Then are ranked FH, FQ, FT and in the end AB.

In September there is a downfall of fungi in all stations, except the FQ where the number of fungi (41 CFU), not only is grown compared with August (21 CFU), but presents and the biggest number in all stations. Perhaps the high activity of oil crafts in this period, brings the biggest growth of Yeasts and Molds. The poorest station with Yeasts and Molds in this month is FH (7CFU).

In October it is noticed a drastic downfall of Yeasts and Molds, where the richest station is EZ with 15 CFU, whereas the least station is FQ and AB with 1 CFU.

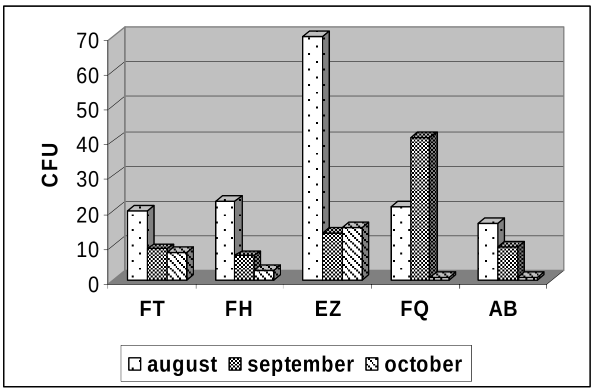

Fig. 4. Yeasts and Molds Number according to months in "FT, FH, EZ, FQ, AB".

Since the limit of 200 CFU is not passed, we can say that all the stations have pure air, in respect of Yeasts and Molds.

The richest station with Yeasts and Molds (Fig.5, Table 4) results EZ with 38% (38 CFU). Maybe the presence of smoke-cloud in this station that brings the growth of Yeasts and Molds and the presence of spores of fungi. In the second place is FQ with 26% (27 CFU). Then come FT, FH whereas the poorest station is AB with 10% (11 CFU). This confirms that air in AB is pure from spores of fungi. Rely on averages, these stations have air within standards [22, 23], that signify air currents from sea clean air, despite the harbours activity and human population in these stations.

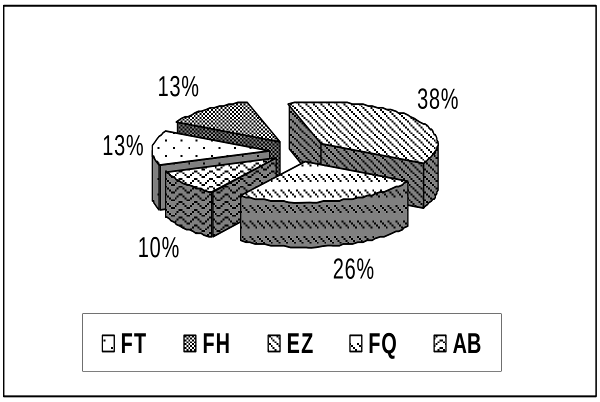

Fig. 5. The average of Yeasts and Molds in "FT, FH, EZ, FQ, AB"

TABLE III ABSOLUTE FREQUENCY (CFU) AND RELATIVE FREQUENCY (%) OF YEASTS AND MOLDS OF "FT, FH, EZ, FQ, AB" ACCORDING TO MONTHS

Station		FT		FH		EZ		FQ		AB	
Month	Sample	ab. freq.	rel. freq.	ab. freq.	rel. freq.	ab. freq.	rel. freq.	ab. freq.	rel. freq.	ab. freq.	rel. freq.
Aug.	air	20	54	23	69	70	71	21	33	17	61
Sept.	air	9	24	7	21	14	14	41	65	10	36
Oct.	air	8	22	3	9	15	15	1	2	1	4

Note: ab. freq= absolute frequency, rel. freq.= relative frequency

TABLE IV AVERAGE, STANDARD DEVIATION AND VARIATION COEFFICIENT OF YEASTS AND MOLDS IN SAMPLING AREAS IN MONTHS AUGUST-OCTOBER

Place	Average (CFU / Petri plate)	Standard Deviation	Variation Coefficient
FT	14	6.21	0.46
FH	13	14.32	1.09
EZ	38	50.36	1.33
FQ	27	33.17	1.24
AB	11	6.45	0.61

The most variable station is EZ (Graf. 6, Table. 4), whereas the least variable station is FT. This confirms that in EZ, the contamination of air in this station may be connected with presence of the activity of the crafts that process scrap and coal. FT is a station that has the least variability in number of Yeasts and Molds. In the AB the variation of growth of fungi is lower than the stations in Harbour, expect FT. Maybe that the growth of fungi is affected, not only by temperature and moisture, but and by other factors as result of activities in the Harbour and anthropogenic sources [22-25].

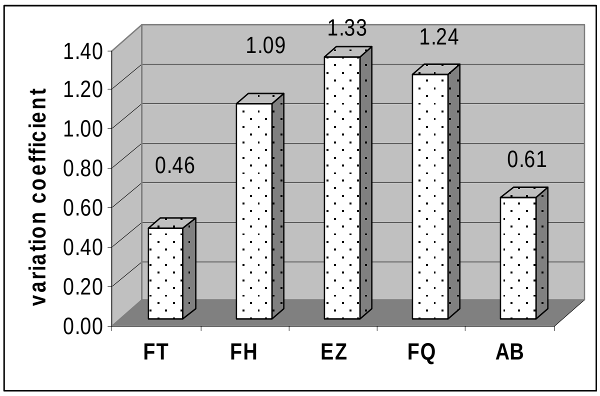

Fig. 6. Variation Coefficient of Total Number of Yeasts and Molds in "FT, FH, EZ, FQ, AB"

C. Correlation between the Growth of Yeast and Molds and Mesophilic Aerobic Bacteria

The correlation coefficient is $\rho=0.68$ (Fig.7). This confirms that between variable Y_1 "Yeast and Molds" and Y_2 "Mesophilic Aerobic Bacteria" there is a correlation over medium positive. The growth of Yeast and Molds is commensurate with growth of Mesophilic Aerobic Bacteria and conversely. The numbers of two groups of microorganisms interchange positively during the period of study. The concentration of both groups of microorganisms in atmospheric air is very much dependent on different atmospheric factors, and anthropogenic factors. [10, 11, 24, 25].

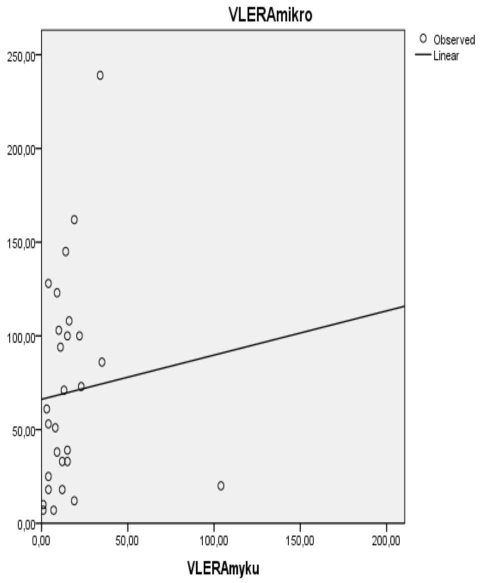

Fig. 7. Correlation between the Growth of Yeast and Molds and Mesophilic Aerobic Bacteria

IV. Conclusions

Experiments in airborne microorganisms were carried out at five varying types of areas by sedimentation technique in August-October 2008.

The number of Mesophilic Aerobic Bacteria in outdoor air is bigger then the number of Yeasts and Molds.

The richest area with Mesophilic Aerobic Bacteria is Ferry Terminal with 90 CFU, whereas the richest area with Yeasts and Molds is East Zone with 38 CFU.

The richest month with microorganisms comes in August, which signifies that high temperature favours growth of microorganisms. The growth of Yeast and Molds is commensurate with growth of Mesophilic Aerobic Bacteria and conversely.

The concentration of both groups of microorganisms in atmospheric air is very much dependent on different atmospheric factors and anthropogenic factors.

However all the areas taken in this study, including also the Beach Area as comparision with other areas, are within standards, which confirms that air in all sampling stations is cleaned from air currents, that brings Adriatic Sea.

Acknowledgment

This research was supported by Deparmement of Biology, Faculty of Natural Sciences, Tirana University. The authors gratefully acknowlegde Prof. Dr. Margarita Hysko, Prof. of Microbiology, Department of Biology, Faculty of Natural Sciences, Tirana University, for scientific contribution.

References

[1] Dowd S.C., Maier R. M. Aeromicrobiology. In: Environmental Microbiology, R. M Maier., I. L Pepper., C.P Gerba. (ed.), Academic Press, San Diego 1999

[2] Gutarowska B., Jakubowska A. The estimation of moulds air pollution in university settings. In: Problems of indoor air quality in Poland'2001, 103-112, ed. T. Jędrzejewska-Ścibak, J. Sowa, Publishing House of Warsaw University of Technology. Warsaw 2001, (In Polish);

[3] D'Amato G., Liccardi G., Russo M., D'Amato M. On the interrelationship between outdoor air pollution and respiratory allergy. Aerobiologia 16, 1, 2000

[4] Madigan, M.T., Martinko J. M., Parker, J., Brock, Biology of Microorganism, Eighth Edition, U.S.A., 1996, ISBN 0-13-520875-0, http:// prenhall.com; pp.911-12,930-31, 946, 982-84, 786-96

[5] Kurup V. P., Shen H. D., Vijah H. Immunobiology of Fungal Allergens. Int. Arch. Allergy Immunol. 129, 181, 2002

[6] Wong G. W. K., Lai C.K.W. Outdoor air pollution and asthma. Curr. Opin. Pulm. Med. 10(1), 62, 2004.

[7] Corden J.M., Stepalska D., Stach A,. Milington W. M., Jackson F.A., Myszkowska D., Jozefiak M., . Sensational variation in *Alternaria* spores concentration in three European cities, Derby, UK, Cracow and Poznan, Pl (1995-2002). TESA Third European Symposium on Aerobiology, Proceedings 19, 2003

[8] Akerman M., Valentine-Maher S., Rao M., Taningco G., Khan P., Tuysugoglu G., Joks R., Allergen Sensitivity and Asthma Severity at an Inner City Asthma Center. J. Asthma 40(1), 55, 2003

[9] Mitakakis T., O'Meara T.J., Tovey E.R. The effect of sunlight on allergen release from spores of the fungus *Alternaria*. Grana 42, 43, 2003

[10] Mitakakis T., Clift A., McGee P.A. The effect of local cropping activities and weather on the airborne concentration of allergenic *Alternaria* spores in rural Australia. Grana 40, 230, 2001

[11] Lin W. H., Li C.S. Association of Fungal Aerosols, Air Pollutants, and Meteorological Factors. Aerosol Science and Technology 32, 359, 2000

[12] Flannigan B. Microbial Aerosols in Buildings: Origins, Health Implications and Controls. Proceedings of the III International Scientific Conference: Microbial Biodegradation and Biodeterioration of Technical Materials, 11-27, Łódź, Poland, 2001

[13] California Air Resources Board. Diesel Risk Reduction Plan, Oct 2000

[14] Pandya, R.J., Solomon, G.M., Kinner, A., Balmes, J.R.: "Diesel exhaust and asthma: Hypotheses and molecular mechanisms of action." *Environ Health Perspect* 110 (Suppl 1): 103-112, 2002

[15] Karwowska E. Microbiological Air Contamination in Some Educational Settings. Polish J. Environ. Studies 12(2), 181, 2003

[16] Rojas T.I, Martinez E., Gomez Y., Alvarado Y. Airborne spores of *Aspergillus* species in cultural institutions at Havana University. Grana 41, 190, 2002.

[17] www.apdurres.com.al Autoriteti Portual

[18] Sveum, W. H.; Moberg, L.J.; Rude, R.; Frank, J. F. Microbiological monitoring of the food processing environment. In: Vanderzant, C.; Splittstoeser , D.F. (eds). Compendium of Methods for the Microbiological Examination of Foods. 3rd APHA, Chapter: 3, 1992, p. 51-75

[19] USP 23-NF 18. The United States Pharmacopeial Convention, Inc. Microbiological evaluation of clean rooms and other controlled environments. *Pharmacopeial Forum* 1997; **23**: 5269–5295

[20] Hysko M., Manual of Microbiology, SHBLU Publishing Company, Tirana 2007, ISBN 99927-996-1-7; pp. 93-106; 240-241 (In Albanian)

[21] Whyte W. Sterility assurance and models for assessing bacterial contamination. *J Parenter Sc Technol* 1995; **40**: 188–197

[22] European Good Manufacturing Practices (EU GMP). *Guide to Manufacture of Sterile Medicinal Products*, 1997.

[23] Ren, T., J.,; Frank, F., J., Measurement of airborne contamination in two commercial ice cream plants, J. Food Protect., 55; 43-47; 1992b.
[24] Koni M.; Biostatistic, SHBLU Publishing Company, Tirana 2005; ISBN: 99927-845-8-X, pp.36-50,155-200
[25] Strum, Robert D., and Donald E. Kirk, First Principles of Discrete Systems and Digital Signal Processing, reading, Mass,: Addison-wesley Publishing Company,1988
[26] Heldman, D.R. Factors influencing air-borne contamination of foods: A. Review, J. Food Sci., 39: 962-969, 1974
[27] Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). *Color atlas and textbook of diagnostic microbiology* (1395 pp., 5th edn.) Philadelphia, New York: Lippincott