Romanian Journal of Ecology & Environmental Chemistry, 5(1), 2023
https://doi.org/10.21698/rjeec.2023.105

Article

Microbiological status of anti-SARS CoV-2 protective face masks

ALINA ROXANA BANCIU, LUOANA FLORENTINA PASCU, DRAGOS MIHAI RADULESCU, CRISTINA IFTODE, ANCA HARABAGIU, ANA FULGHECI, DANIEL RUDARU, MIHAI NITA-LAZAR*
National Research and Development Institute for Industrial Ecology- ECOIND, Bucharest, Street Drumul Podu Dambovitei 57-73, 60652, Romania
**Corresponding author: mihai.nita@incdecoind.ro*

Received:	*Accepted:*	*Published:*
29.06.2023	*06.07.2023*	*10.07.2023*

Abstract
The SARS-CoV-2 pandemic situation put extreme pressure on the worldwide medical system from medical personnel to medical equipment. The protective face mask became the front line of anti-SARS-CoV-2 prevention methods, used by most of the world population, more than 7 billion people. The massive demand for face masks activated the world market, where countries from different continents increased their mask production and commercialization worldwide. Unfortunately, the focus on face mask production mostly relied on their ability to filter small-size molecules and less on their microbiological sterile status. Since bacterial structural communities could differ between various large population groups or continents, in this study, we analysed the microbial quality of face masks received from various countries/continents. There were analysed the microbiological load of four types of masks, three from China and one from Romania. The bacterial density from masks was analysed by membrane filtration and swabbing bacterial collection techniques. The results showed the Romanian mask had a smaller bacterial load than the Chinese masks. In addition, the bacterial identification showed a wide range of bacterial strains, quite different between face mask types.

Keywords: *SARS-CoV2, pandemic, protective face masks, microbiology*

INTRODUCTION

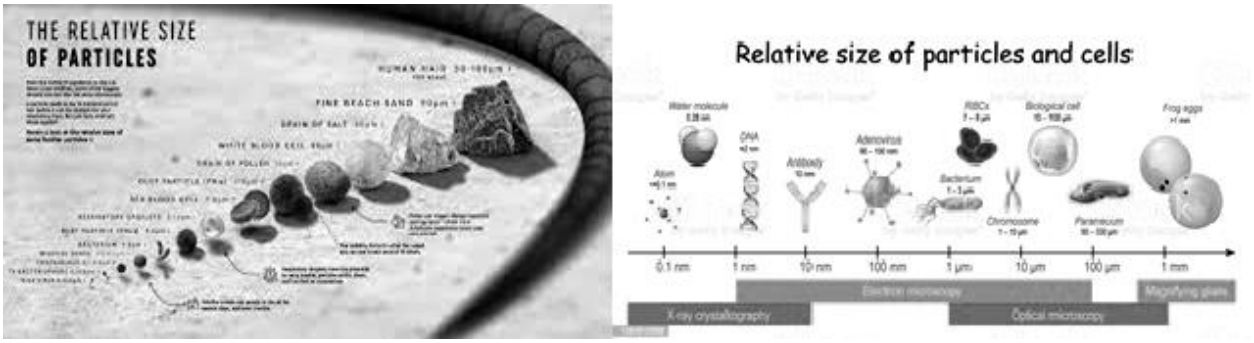
During the COVID-19 pandemic, the use of face masks become increasingly recommended and even mandatory in the community settings. They have become in the front line for protection against the spread of Sars-CoV-2 virus worldwide [1, 2]. A shortage in supply of masks was resulted in response to the greater demand of World Health Recommendations. For these reasons, uncertified and homemade masks were manufactured and sold in open markets as compliant devices at overestimated prices [3- 6].

There are different types of face masks offering different levels of protection against COVID-19 pandemic. The extent of COVID-19 pandemic level has led to a global shortage supply of these medical devices, including their raw materials. At the same time, a quest to improve the quality and performance of protective masks has been accelerated aiming to, starting from how to wear them from a public health perspective, technical marketing details and engineering advances regarding the disinfection and durability of these devices [2].

Medical/ surgical mask N95 respiratory mask

Figure. 1. Types of protective face masks [7]

A distinction must be made between surgical and respiratory protection masks (figure 1). Surgical masks cover the nose and mouth, but are not airtight. They are designed to provide protection especially against exhaled particles. Respiratory devices are airtight creating a face seal and they are classified according to filtration efficiency. In the Sars-CoV-2 pandemic context the most concerning particle has been the Sars-CoV-2 virus which most of the time is inhaled. The virus has a size range from 60 to 140 nm, which is smaller than other air contaminants such as bacteria, dust or pollen (Figure 2). Therefore, the masks selection was mainly taking into consideration pore size criteria of the protective mask and not so much the material/fabric composition [8].



a. **b.**

Figure 2. The relative size of particles [8, 9]

Therefore, masks and respirators made of materials with larger pore size, such as cotton or synthetic fabric were not be able to effectively filter these viruses or tiny virus-laden droplets as compared with the tight-filtering N95 respirators [2]. Respirator masks, in standard and surgical/medical varieties, filter particles with a diameter of 0.3 µm with an efficiency of 95%. They consist of a fan and are made of four layers of non-woven polypropylene for the inner/outer layers and fused polypropylene for the middle ones. However, respirators do not provide effective protection for particles smaller than 300 nm in diameter [10-12]. The infectious droplet and aerosol filtration ability of MAR can vary significantly based on many aspects, such as filter media materials, the number of filter media layers, fit, filtration methods, water resistance, and antimicrobial activity [13]. Before the pandemic, very little research had been done on improving different MAR. Generally, MAR can filter out droplets (>5–20 µm), but some pathogen-bearing aerosols (≤5 µm) can pass through the filter media [14, 15]. Generally, cloth masks have less filtration efficiency than nonwoven-based surgical MAR [16]. Unfortunately, there were in 2020-2021 some reports on side effects or secondary infections due to wearing protective masks, effects for which researchers have performed studies, without a precise cause being discovered yet. Very few studies were carried out on the supervision and control of quality biological conditions in the manufacture and sale.

In Romania, starting from 2020, there was an influx of imported masks manufactured in Asian countries, and the National Authority for Consumer Protection (ANPC) carried out checks to ensure the compliance of these products commercialized on the national market and issued alerts regarding the withdrawal from the sale of certain batches of masks based on quality conditions. Although the ANPC intensified the program for monitoring the quality of protective masks right from the beginning of the pandemic, the controls were carried out randomly, so that the consumer also received products that did not fully meet the quality conditions imposed by national and international standards or, even moreover, they can also cause the appearance of some pathologies secondary to their use.

Most of the checked parameters were related to the size filtration of the mask and very little (micro)biological parameters were analysed. The present study, focused on the (micro)biological analysis studies on masks from the Romanian national market, such as the (micro)biological potential contamination of unused protective masks.

EXPERIMENTAL PART

Samples

4 batches of face masks of different types were analysed: because China is the producer of half the world's face mask even before Covid-19, three of them were from China and one from Romania [17] (Table 1)

Table 1. Characteristics of masks batches microbiological tested.

Nr. crt.	Mask type/ Symbol study	Characteristics	Valability	Batch/ Certificate/ Proveniency
1.	MA –FFP2 mask Model TX-NO1	- FFP2 mask , BFE≥98%; - 20 buc/ package, individually wrapped; - Production standard EN 149:2001+A1:2009 FFP NR; - according to EN 14683:2019; - suitable for protection against harmful particles such as dust, droplets and other particles with a size of at least PM2.5 (air and bacteria); -made of melt-blown, non-woven fabric, having an electrostatic PP-filter.	12.2020-12.2023	Batch: TX202012 Certificate: 2163-PPE-952/01 Production: China, Zhejiang
2.	MB –FFP2 mask Model BT-005	- FFP2 mask; - 20 buc/ package, individually wrapped; -according to EN 149:2001+A1:2009 (EU); GB2626-2006 (China); - it is made of melt-blown, non-woven fabric.	11.2020-11.2022	Batch : 2020110901 Certificate: CE 2163 Production: China, Shandong
3.	MChi – Blue protective mask with three layers	- Blue protective mask with three layers; -50 buc/ package, packed in set with protective film; - according to GB/ T 32610-2016; - made of three non-woven layers with 90% filtration efficiency.	02.2020 08.2021	Batch: YL20200416 Certificate: - Production: China, Fujian
4.	MRO – Blue protective mask with three layers Model MII	- type II medical mask in 3 layers and 3 folds; -50 buc/ package, packed in set without protective film; -according to SR EN 14683+AC:2019 și Directiva 93/42/EEC; - made of 3 layers of PP.	04.2025	Batch: MII.0001 Certificate: CTC 43 Production: România, Galati

Microbiological analyses

Bacterial sample extraction using immersion method

Four mask samples from each batch (Table 1) were analysed in accordance with the EN 14683:2019 and AC: 2019 standard [18]. The mask samples consisted of a full mask as well as inner, middle and outer mask layers obtained under aseptic conditions (microbiological hood) from

commercialized packages. Each individual sample (full mask, inner, middle or outer layers) was immersed in a volume of 300 mL buffered peptone water – AP (Oxoid, UK) for 5 min, under 250 rpm agitation. A volume of 100ml from each immersed sample was used to quantify bacterial densities.

Bacterial sample extraction using swabbing method
Inner and outer layers of each mask were wiped with a swab moistened in sterile distilled water and Sodium Lauryl Sulphate Broth, SLSB, (Oxoid, USA) by applying firm pressure to the surface passing each swab 2-3 times through the same place of one mask, in different directions (the second pass perpendicular to the first and the third oblique over the second). Each collected swab was immersed in 100ml of SLSB suspension solution.

Bacterial quantification using colony counts (Colony Forming Units, CFUs)
A volume of 100 mL suspension from the immersion bacterial extraction method was filtered through a 0.45-µm pore diameter membrane, which was then placed in aerobic conditions, on a solid bacterial growth medium Tryptic Soy Agar (Oxoid, UK), Sabouraud Agar (Oxoid, UK) or Czapek-Dox Agar (Oxoid, UK). The samples were incubated 48 hours at 37℃ for Tryptic Soy *A*gar and Sabouraud Agar or 5 days at 25℃ for Czapek-Dox Agar. The colonies developed after the incubation time were counted and recorded as CFUs/mL.
Positive and negative controls with standardized reference strains, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus stearothermophillus* ATCC 7953, *Staphylococcus aureus* ATCC 6538, were analysed for all culture media used. Also, before being used, the culture media also went through the blank control test.
All tests to collect and analyse the total number of microorganisms that develop at 25℃-37℃ and the identification of bacterial species were performed in triplicate.

RESULTS AND DISCUSSION
Our work, performed during 2021 and 2022, was a case study on the potential presence of potentially pathogenic microorganisms in non-sterile protective masks purchased from various authorized suppliers. Although it is known that bacteria and fungi are widely present on the surface of the materials used in our daily activities, the study hypothesis was based on a sterile product condition that it shouldn't contain viable microorganisms [19, 20], and on a non-sterile medical device condition that shouldn't have any microorganisms that endanger the health of the user. The selection of the 4 mask batches (Table 1) was made by taking into account the batch purchase frequency and the reported mold smell issues and their packaging options.
The membrane filtration technique from the immersed full masks samples revealed a higher degree of contamination of the MB –FFP2 mask Model BT-005 (MB) samples compared to that of the other batches of tested masks (Figure 3). MRO – Blue protective mask with three layers Model MII (MRO) and MChi – Blue protective mask with three layers (Mchi) had a very low CFUs counts, up to 0.025 CFU/ml) compared to MA –FFP2 mask Model TX-NO1 (MA), 0.095 CFU/ml. The MB CFUs (0.165 CFUs/ml) was almost twice as MA (Figure 3).
The samples grown on Sabouraud Agar culture medium incubated at 20-25℃ for 7 days favoured the development of filamentous fungi. Thus, it was possible to correlate the strong mold smell of the specimens with the presence of fungal development on the seeded culture medium.
Also, the development and multiplication of fungi on the tested masks could be argued, both by the packaging conditions – in the case of the MRO lot packed only in the cardboard box without any other additional protection, and by the origin of the material and the manufacturing conditions – in the case of the individually packed masks.

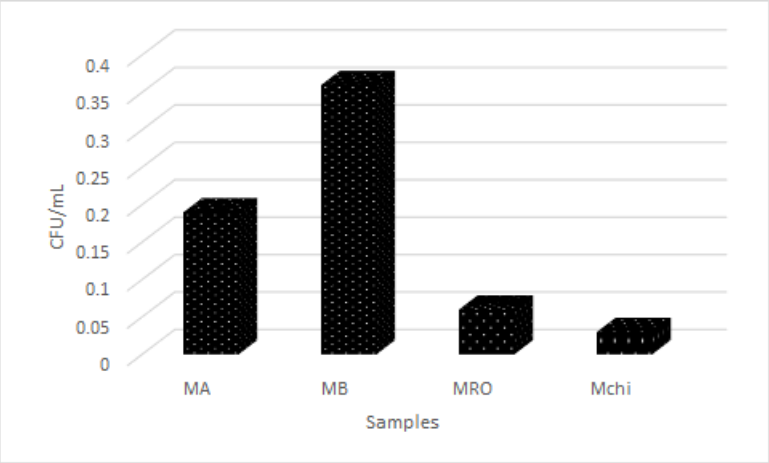
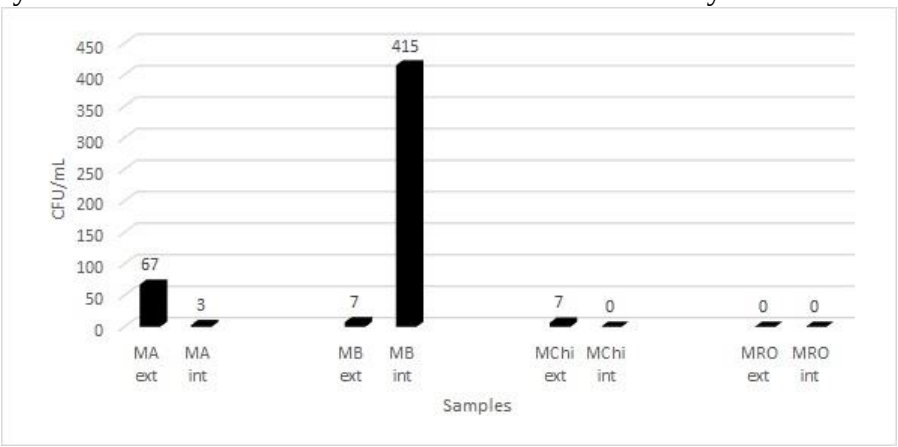
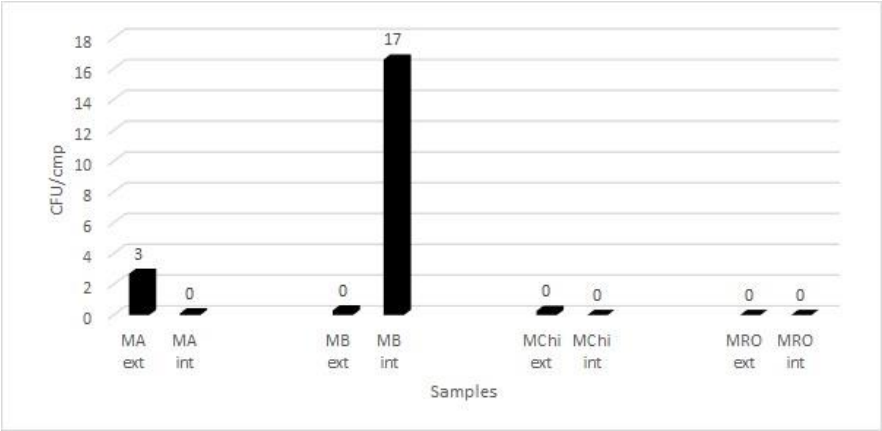


Figure 3. The density of microorganisms (CFU/mL) obtained on TSA (Tryptic Soy Agar) culture medium incubated at 30°C for 3 days after filtering 100 mL of bacterial suspension through a membrane with a pore diameter of 0.45 µm. MA, MA –FFP2 mask Model TX-NO1; MB, MB –FFP2 mask Model BT-005; MRO , MRO – Blue protective mask with three layers Model MII; MChi, MChi – Blue protective mask with three layers.

Applying the method using sterile swabs, the samples tested for bacterial load showed results only for the samples suspended in Broth Sodium Lauryl Sulphate (Figure 4) since sterile distilled water has no capacity to extract bacterial cells fixed in the mask material by cell adhesion.



a)



b)

Figure 4. The density of microorganisms obtained on Czapek-Dox Agar incubated at 25°C for 48 hours after seeding 1 mL of microbial suspension obtained by sampling with a sterile swab from the protective mask and homogenized in Sodium Lauryl Sulfate Broth: a) Density of microorganisms measured in CFU/mL; b) Density of microorganisms measured in CFU/cmp.

From the analysis of the quantitative results, it can be observed significant degree of microbial load in the volume of 100 mL taken in the analysis of the MB mask on the inner layer (415 UFC/mL – Figure 4a) compared to the values obtained for the other tested batches. At the same time, the microbial density recorded in relation to the surface of the protective material (Figure 4b) indicates a higher contamination potential for the MAext and MBint samples, with an obviously high value in the case of MBint.
Also, after seeding and incubating the BLS suspension on solid media with a higher specificity than those used in the membrane filtration method, microbial growth was observed only on the Czapek-Dox Agar medium (Figure 5). This result indicates the specificity of the microorganisms present, which is why isolation of the microbial strains was carried out in order to identify them.

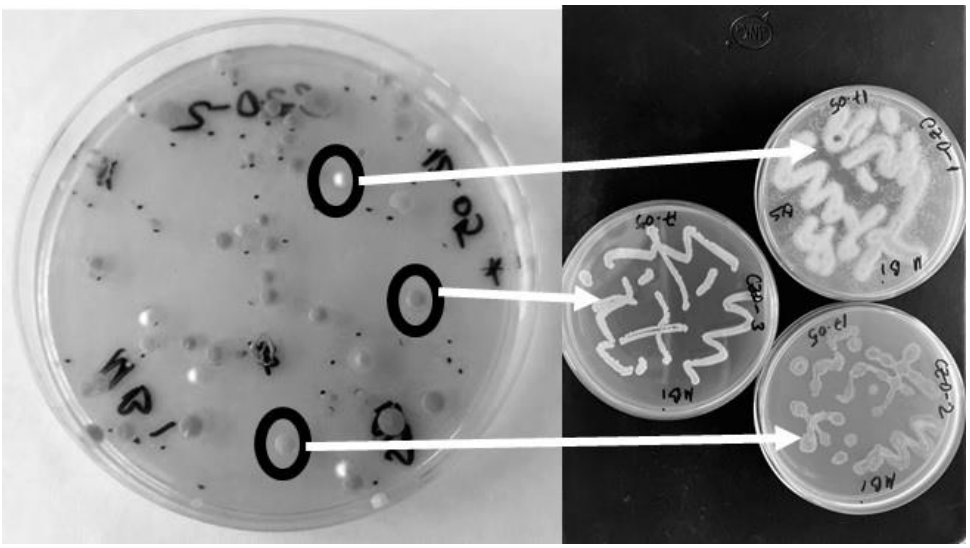


Figure 5. Microorganisms growth on Czapek-Dox Agar medium after 5 days of incubation at 25°C of sanitation samples collected from the external and internal layers of face masks before use.

While no microorganisms were identified on the MRO mask, the presence of filamentous fungi was detected on the MChi mask that can cause respiratory ailments with constant and prolonged wearing of these devices.
The immersion technique favoured the identification of more microorganisms than the method based on sterile swab sampling, this fact indicating the ability of the immersion medium to combat the cell adhesion force to the mask material.
The bacterial density (Figure 6) determined using swab method on Czapek-Dox Agar indicated the higher degree of contamination of the MB samples.

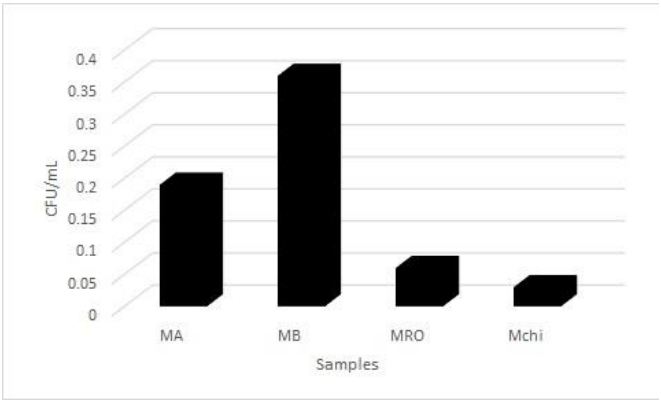


Figure 6. The density of microorganisms obtained on Czapek-Dox Agar incubated at 25°C for 48 hours after seeding 1 mL of microbial suspension obtained by the sterility control method by immersion.

By supplementing the control tests with the analysis of the medial filter layer of the masks, it emerged that microorganisms can transit the layers externally or internally and reach the medial layer that they colonize. In the case of Micrococcus luteus, contamination of all three layers of the MB mask tested can be observed. Also, the immersion test method highlighted, on the MChi mask, in addition to the presence of fungi detected on the outer layer.

CONCLUSIONS

The results of the membrane filtration method prove the specificity of its application in the evaluations and monitoring of the asepsis conditions of sterile masks. Considering the pore diameter of the filter membrane of 0.45µm, it has the specificity of retaining microorganisms according to the size, and the culture media plays the role of nutrient support for the biological particles retained by the membrane. Thus, the use of general growth media, without enzyme supplements dedicated to certain groups or microorganisms, supports the narrow range of bacteria identified, but provides important information regarding the degree of microbial contamination of protective masks.

The results obtained following the application of the immersion method showed an even greater specificity compared to the method using the swab, since the use of the entire material from which the mask is made eliminates the risk of omitting important portions of the tested specimen. At the same time, the separate analysis of each component layer indicates the selectivity of the method, because in most cases, microorganisms differ from one protective layer to another.

Following the application of the two methods for evaluating the degree of microbial contamination of protective masks for medical use, it resulted that the MB batch has the highest microbial load, with a diversity of bacterial strains.

In addition, the presence of filamentous fungi on all types of mask tested proves the source of the reported musty smell and indicates that the way of individual packaging in plastic material or protecting the entire set in plastic material does not create any difference compared to the batch packed only in a cardboard box. Inhalation of the spores by users may cause mainly widespread respiratory conditions after prolonged exposure to these agents.

The presence of microorganisms in the batches of tested masks highlights the improper conditions of the steps that non-sterile protective products go through from the manufacturer to the user. While there are standardized techniques for testing mask materials to determine filtration efficiency for non-sterile masks, no method is regulated or standardized for assessing contamination potential.

The results of this study highlight the existing problem, both at the national and international level, regarding the risks to which people are exposed through the widespread use of protective masks, without prior documentation. Although they were recommended to reduce the risk of infection with Sars-Cov-2, the masks from the analyzed batches show susceptibility to endangering the health of the population through infection with bacteria and fungi. This situation should raise an alarm signal regarding the materials from which the masks are made, their manufacturing and storage conditions, emphasizing microbiological control before use.

REFERENCES

[1] DELANGHE, L., CAUWENBERGHS, E., SPACOVA, I., DE BOECK, I., VAN BOECK, W., PEPERMAN, K., CLAES, I., VANDENHEUVEL, D., VERHOEVEN, V., LEBEER, S., Front. Med., **8**, 2021, https://doi.org/10.3389/fmed.2021.732047.

[2] NAUREEN, Z., CAPODICASA, N., PAOLACCI, S., ANPILOGOV, K., DAUTAJ, A., DHULI, K., CAMILLERI, G., CONNELLY, S.T., GASPARETTO, A., BERTELLI, M., Eur. Rev. Med. Pharmacol. Sci., **25** (1 Suppl), 2021, p. 74.

[3] RUBIO-ROMERO, J.C., PARDO-FERREIRA, M.C., TORRECILLA-GARCIA, J.A., CALERO-CASTRO, S., Saf. Sci., **129,** 2020, https://doi.org/10.1016/j.ssci.2020.104830.

[4] AYDIN, O., EMON, B., CHENG, S., HONG, L., CHAMORRO, L.P., SAIF, M.T.A., Extreme Mech. Lett., **40**, 2020, https://doi.org/10.1016/j.eml.2020.100924.

[5] OECD, https://www.oecd.org/coronavirus/policy-responses/the-face-mask-global-value-chain-in-the-COVID-19-outbreak-evidence-and-policy-lessons-a4df866d/ [20.09.2022].

[6] MWEMA, F.M., NYIKA, J.M., Sci. Afr., **10,** 2020, https://doi.org/10.1016/j.sciaf.2020.e00563.
[7] Center of disease control, www.cdc.gov/niosh/npptl/pdfs/UnderstandDifferenceInfographic-508.pdf [20.09.2022].
[8] Visual capitalist, www.visualcapitalist.com/visualizing-relative-size-of-particles/, https://www.istockphoto.com/ro/vector/compararea-dimensiunii-relative-a-particulelor-%C8%99i-celulelor-la-scar%C4%83-biologic%C4%83-gm1284810583-381841905 [20.09.2022].
[9] iStock by Getty Images, Stock illustrations with Comparing the relative size of particles and cells at biological scale - Download image now - iStock (istockphoto.com) [20.09.2022].
[10] FARZANEH, S., SHIRINBAYAN, M., Polymers, **14**, no. 2, 2022, https://doi.org/10.3390/polym14020291
[11] HTWE, Y.Z.N., MAMAT, H., OSMAN, B., MAHMUD, H., Arab. J. Sci. Eng., **48**, 2023, p. 8349, https://doi.org/10.1007/s13369-022-06801-w.
[12] BHATTACHARJEE, S., BAHL, P., CHUGHTAI, A.A., MACINTYRE, C.R., BMJ Open Respir. Res., **7**, no.1, 2020, https://doi.org/10.1136/bmjresp-2020-000698.
[13] BATT, T., HERWIG, G., ANNAHEIM, S., CLEMENT, P., FURRER, L., HIRSCH, C., VARANGES, V., CAGLAR, B., MICHAUD, V., WANG, J., RICHNER, G, WICK, P., ROSSI, R., Chimia, no. **76**, 2022, p. 249.
[14] BALAZY, A., TOIVOLA, M., REPONEN, T., PODGORSKI, A., ZIMMER, A., GRINSHPUN, S. A., Ann. Occup. Hyg., **50**, 2006, p. 259.
[15] FENNELLY, K. P., Lancet Respir. Med., **8**, no. 9, 2020, p. 914, https://doi.org/10.1016/S2213-2600(20)30323-4.
[16] ZHANG, Z., JI, D., HE, HAIJUN, RAMAKRISHNA, S., Mater. Sci. Eng. R. Rep., **143**, 2021, https://doi.org/10.1016/j.mser.2020.100594.
[17] PULLANGOTT, G., KANNAN, U., GAYATHRI, S., KIRAN, D.V., MALIYEKKAL, S.M., RSC Adv., **11**, 2021, p. 6544, https://doi.org/10.1039/D0RA10009A.
[18] EN 149:2001+A1:2009 – respiratory protective devices – Filtering half masks to protect against particles – Requirements, testing, marking.
[19] ISO 1737-1:2018, Sterilization of health products – Microbiological methods, Part 1: Determination of a population of microorganisms on products.
[20] PARK, A.M., KHADKA, S., SATO, F., OMURA, S., FUJITA, M., HASHIWAKI, K., TSUNODA, I., Sci. Rep., **12**, 2022, https://doi.org/10.1038/s41598-022-15409-x.

Citation: Banciu, A.R., Pascu, L.F., Radulescu, M.D., Iftode, C., Harabagiu, A., Fulgheci, A., Rudaru, D., Nita-Lazar, M., Diatoms. Microbiological status of anti-SARS CoV-2 protective face masks, *Rom. J. Ecol. Environ. Chem*., **2023**, 5, no.1, pp. 42-49.

© 2023 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.Org/licenses/by/4.0/).