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Microbiological Analysis of Coolant Used in Machining

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ABSTRACT

The subject of microbiological tests of coolants presented in the work is the cutting fluid (emulsifying oil) of Fuchs ECOCOOL 68 CF3. The liquid was taken from the tanks of three different numerically controlled machines (Lasertec 65, MillTap 700, CTX beta 1250 TC), used with different intensity. The five most characteristic and popular media were used in the study: nutrient agar – plain, TSA medium (tryptone-soy-agar), medium with dichloran, rose bengal and chloramphenicol, Sabouraud agar with Chloramphenicol, Czapka agar. The incubation time was dependent on the type of microorganisms. After the specified time, the plates inoculated on the medium were subjected to visual observations and under the Leica DM 500 microscope. As a result of the observation, the following types of microorganisms (fungi and bacteria) were detected: *Aspergillus Niger Tiegh, Candida Albicans, Saphylococcus Aureus, Micrococcus Luteus* and *Escherchia Coli and Citrobacter Freundia*. The assessments were made in fixed periods. The results are presented as a function of the intensity of the use of cutting fluids. Microbiological analysis of machining fluids will allow for optimizing the time periods of using coolants and will also contribute to the protection of the operator's health and indirectly "extending the life cycle" of the technological machine.

Keywords: metalworking fluids, CNC machining, cooling and lubricating properties, microorganisms.

INTRODUCTION

Microorganisms and the substances they produce can pose a threat to humans in many working environments, including the machine and metallurgical industry [1]. In recent years, there has been an increase in interest in biological risk factors, which are influenced not only by physical but also chemical factors. Microorganisms which develop in oils and other liquids used in metalworking machines and devices pose the greatest threats to people employed in the machine and metallurgical industries. Microflora most often develops in cooling and lubricating liquids (water-oil emulsions).

Cooling and lubricating fluids, also called CCS machining fluids (and in English nomenclature MWFs – metalworking fluids), are used in the metal industry primarily for cooling and lubricating tools and workpieces during various machining processes [2, 3]. The most commonly used cooling and lubricating fluids are water-oil emulsions and oils made from concentrates. Oils have very good lubricating properties but not good cooling properties. In the case of cooling properties of cutting fluids, water performs this role very well, but it does not have the required lubricating properties and favors corrosion. The concentrate is the base of an oil-water emulsion, which is miscible with water in any ratio. The oil content in the concentrate is usually not less than 60%, and the remaining 40% are components such as emulsifiers and modifying additives (e.g. stabilizers, corrosion and oxidation inhibitors, anti-wear, anti-seize additives, biocides or biostats, fragrances, antifoams and other). A commonly used biostable ingredient are esters (borate esters) or other boric acid derivatives and other boron compounds. In recent years, esters and other carboxylic acid derivatives have been used as biostable components. Some coolant components are allergenic, toxic and even carcinogenic, which is important when aerosols are formed during machining.

Microbial growth in CCS is possible due to the presence of organic substances in their composition. In such an environment, various types of fungi and bacteria thrive. The number and type of microorganisms in CCS depend, among other things, on the type of technological process [4]. One of the first studies on CCS microbiological analysis found high concentrations of microorganisms, up to about 10^7 CFU/ml [5]. The dominant, unfavorable components found in coolants include: bacteria, filamentous fungi, mold fungi and yeast. Referring to the literature, Cyprowski et al. [7] cite a list of 105 species of bacteria and 26 species of fungi found so far in cutting fluids. Due to their pathogenic properties, 29 species of bacteria and 3 species of fungi from this number are included in the second and third risk groups according to the Regulation of the Minister of Health [8]. The most common are the following bacteria: *Pseudomonas, Flavobacterium, Achromobacter, Coliform bacilli, Escherichia coli, Desulfovibro, and fungi and yeasts: Fusarium, Penicillium, Aspergillus, Candid*a. Sometimes pathogenic organisms such as *Streptococci, Straphylococci, Salmonella, Dermatophytes* also develop under certain conditions. In recent years, aerobic bacteria found in CCS have been dominated by Gram-negative bacteria, which are able to use all organic components present in coolants [3,6]. Anaerobic bacteria appear when machinery is not used for prolonged periods. Fungi accompanied by high concentrations of mycotoxins are also a serious microbiological hazard found in CCS [4,6].

In addition to live microorganisms, bacterial endotoxins – *lipopolysaccharides (LPS)* – are also detected in the processing fluids. The concentrations of endotoxins determined in CCS correlate with the number of gram-negative bacteria found [6]. The growth of microorganisms in CCS causes deterioration of their physical and chemical properties (e.g. loss or reduction of viscosity, lowering of heat resistance, lowering of pH), which leads to acceleration of the corrosion process of machining machines, loss of tightness of their cooling and lubrication systems and accelerated destruction of machining tools.

During the operation of machine tools, the cutting fluid is released into the air in the form of oil mist, on the particles of which biological factors are transported – mainly bacteria and endotoxins. Concentrations of bioaerosols sometimes exceed the limit values given in the standard (Regulation), posing a threat especially to the proper functioning of the respiratory system of people exposed to their impact. The emitted oil mist is characterized by variable concentrations, which largely depend on the type of machine tool in operation. Referring to the literature, as shown by Wang et al., higher rotational speeds of machines favor an increase in oil mist concentrations [9, 11]. It should be mentioned at this point that in Poland the value of the highest permissible concentration (MAC) for oil mist is set at 5 mg/m^3 [10].

Counteracting the presence of excessive amounts of bacteria and fungi in cutting fluids is possible not only by specially developed fluids (e.g. ecological coolants), but also by technical solutions using ultraviolet light or ozone. Blaser's Blasocut coolants, which are produced in an SKF factory that produces 20,000 rolling bearings a day, are an example of such coolants that can be used in an environmentally effective way. The temperature that occurs directly in the cutting zone requires the use of cooling and lubricating liquids, i.e. machining fluids. The main purpose of using these types of fluids is to dissipate heat from the machining zone and thus limit overheating of the tool and the workpiece. The temperature in the contact zones of the tool and the workpiece reaching values up to 900°C, which promotes the release of volatile compounds affecting the health of the CNC (Computerized Numerical Control) machine operator. When machining hard-to-machine materials, e.g. heat-resistant nickel and cobalt alloys, the cutting temperature reaches 750–1020 °C [12]. Such a high temperature can lead to accelerated tool wear and have an adverse effect on the condition of the workpiece (due to surface oxidation processes or changes in the surface layer structure, such as grain growth or change in chemical composition). As previously indicated, cutting fluids are used primarily to reduce the temperature in the cutting zone, as well as to ensure adequate lubrication at the points of contact between the cutting tool tip and the workpiece and chip. The flow of cooling and lubricating liquid also facilitates the removal of swirls from the machining area and ensures protection of the surface of the processed material against corrosion. However, it should be emphasized that the use of cutting fluids has minimal impact on the health of the machine operator. The main goal of this paper is microbiological analysis of coolant used in machining – emulsion type Fuchs ECOCOOL 68 CF3. Quantitative and qualitative identification of fungi and bacteria in the analyzed fluids (used in CNC machine tools with different frequency) will allow for an optimal strategy for the replacement of metalworking fluids and reduce many risks resulting from the development of fungi and bacteria in them. This is extremely important not only from the point of view of protecting the health of the CNC machine operator, but also from the point of view of the effectiveness of cooling and lubrication and the quality of these processes during machining.

MATERIALS AND METHODS

The subject of microbiological tests of the coolant presented in the work is the cutting fluid (emulsifying oil) of Fuchs ECOCOOL 68 CF3, taken from the tanks of three different numerically controlled machines (Lasertec 65, MillTap 700, CTX beta 1250 TC), used with different intensity. The coolant adopted for the tests is water-soluble in the form of a biostable, semi-synthetic emulsion, used for universal machining of carbon and alloy steels, cast irons and aluminum alloys. Five most characteristic and popular media were used in the study: nutrient-plain agar, TSA medium (tryptone-soy-agar), dichloran, rose bengal and chloramphenicol medium, Sabouraud agar with chloramphenicol and Czapek agar. For microbiological tests of coolants, ready-made culture media in Petri dishes were used, which minimize the time needed to prepare the substrate for determinations and significantly facilitate work. The dishes were stored under refrigerated conditions in a Haier laboratory fridge. A TX4 Digital IR Vortex Mixer was used to homogenize the fluid with the physiological fluid. The inoculations were made using automatic pipettes, loops and spreaders in a laminar chamber with vertical air flow by Alpina, BIO 130 model. The plates with the inoculation were incubated in the UF260 incubator by Memmert. The incubation time depends on the type of microbes. After the specified time, the plates inoculated on the medium were subjected to visual observations and under the microscope Leica DM 500. As a result of the observation, the following types of microorganisms (fungi and bacteria) were detected: *Aspergillus Niger Tiegh, Candida Albicans, Saphylococcus Aureus, Micrococcus Luteus, Escherchia Coli and Citrobacter Freundii.* The evaluations were made in fixed periods. The results were presented as a function of the intensity of use of cutting fluids. The effect of the development of microorganisms in the cooling system of a numerically controlled CNC machine tool is the gradual decomposition of the coolant components. This is accompanied by the following symptoms: decrease in the pH of the coolant, increase in microdroplets of the suspended phase (oil, loss of emulsion stability and oil separation (emulsion delamination)), deterioration of the lubricating properties of the coolant, deterioration of the quality of machined surfaces, increase in the corrosion aggressiveness of the coolant as a result an increase in the concentration of acidic metabolic products of microorganisms, such as: *lactic acid, acetic acid, formic acid,* and the emission of an unpleasant odor by the coolant due to the emission of reduced, volatile sulfur and nitrogen compounds. This is particularly often observed after a period of production downtime (days off from work – Saturday, Sunday), deterioration of work hygiene conditions; and manifests itself in the form of allergies and infections among employees, clogging of filters in circulation systems as a result of the formation of yeast and fungus clusters. The above symptoms result in shortening the life of coolants, increasing operating costs, production downtime related to the replacement of the coolant. Microbiological analysis of machining fluids will allow for optimizing the time periods of using coolants, and will also contribute to the protection of the operator's health and indirectly "extending the life cycle" of the technological machine.

RESEARCH RESULTS

Culture media, i.e. clusters of nutrients in the right amount and quality needed for the cultivation of microorganisms, were incubated to check their quality after the appropriate incubation time. The presence of microorganisms was not demonstrated in the clean, freshly taken coolant, which allowed for conducting tests with their use. Table 1 presents the microbiological reagents used in the research.

The liquid sample taken from the barrel stored in the manner recommended by the manufacturer after inoculation on media and incubation did not show any microflora as shown in Figure 1.

The coolant that was pipetted from the tanks into the sterile containers (Fig. 2), signed in accordance with the date of collection and the name of the machine, differed significantly in appearance, despite being replaced after the same period of time. The reason for this could be inaccurate cleaning of the liquid tank along with the entire flow system and various interruptions in the operation of the machine. There was a significant difference with the CTX beta coolant compared to the others. In this case, stratification of the liquid and sediment accumulated at the bottom of the machine are visible (Fig. 2d). The appearance of the zero, pure coolant marked as Ch0 and the coolant working in each of the devices is shown in Figure 2.

The results of the number of microorganisms obtained for individual machines are presented in Table 2 and Figure 3.

Figure 3 shows graphically the number of microorganisms in the cutting fluid from the individual tested machines: LASERTEC 65, MILLTAP 700 and CTX beta 1250 TC. The greatest values and diversity of microflora are found in the case of CTX beta 1250 TC. The microorganisms present in each of the machines are: *Aspergillus Niger Tiegh* and *Candida Albicans*, these are fungi characterized by an unpleasant odor, which is typical of the microflora of coolants. These microorganisms can cause blockages in fluid flow systems and clog sprays, leading to unintentional downtime.

Figure 4 shows the media together with the grown microflora. The vast majority and variety of microflora is also present in the tank liquid of the CTX beta machine. In the case of the coolant from the CTX beta machine, a significant variety and amount of microorganisms are visible at the lowest concentration. Both fungi and pathogenic bacteria are present here (Fig. 4). A slight growth of the mycelium was observed after 8 days from inoculation and staying in the incubator (Fig. 5a). The grown mycelium only after 13 days from the inoculation reached considerable size, which is shown in Figure 5b.

This shows a slow growth of microorganisms in the first days of incubation in relation to their growth after 8 days and a significant – intensive growth after the next 5 days.

In some cases, no growth of any microflora was noticed on the media with low concentrations (Fig. 6). The reason for which could be too diluted coolant for a given medium and the addition of bactericides used for microbiological stabilization of machining coolants.

When inoculating the most concentrated dilutions, a large number of microbial colonies of one *Aspergilus Niger* species is noted, as shown in Figure 7.

Microorganisms were observed under the Leica 500 optical microscope. Fig. 7a shows the mycelia of *Aspergillus Niger* after 8 days from inoculation, and in Fig. 7b, strongly developed colonies registered 13 days after inoculation. The observed mycelium of *Aspergillus Niger* is spherical and has a diameter of approximately 4 mm. It reproduces asexually as a result of the secretion of spores by the head. Noticeable in Fig. 8a characteristic hyphae allow the growth and spreading on the substrate. However, the mycelium of *Candida Albicans* (Fig. 8b) subjected to microscopic observation is characterized by a large number

Table 1. Microbiological reagents

Name	Application	Company
Nutritional Agar	Universal medium for culture, multiplication, determination of total number of microorganisms	BTL Catalog No.: PP 0049
TSA medium (Tryptone-Soy-Agar) (without KB)	Universal medium for culturing microorganisms	BTL Catalog No.: PP 0090
Dichloran Rose Bengal Chloramphenicol (DRBC) medium	Medium for determining the number of yeasts and molds	BTL Catalog No.: PP-0399
Sabouraud's Chloramphenicol Agar	Selective medium for cultivation, isolation and identification of fungi, including pathogenic fungi	BTL Catalog No.: PP-0135
Agar Czapka	Medium proposed by Czapek and Dox for the cultivation and identification of fungi	BTL Catalog No.: PP0003

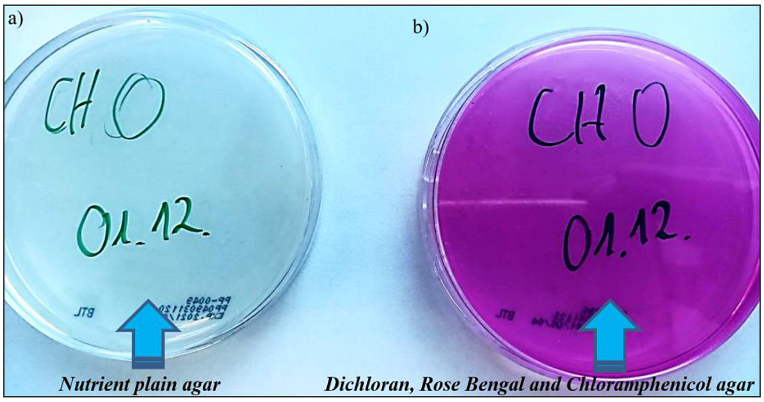

Fig. 1. Nutrient plain agar (a) and Dichloran, Rose Bengal and Chloramphenicol agar (b)

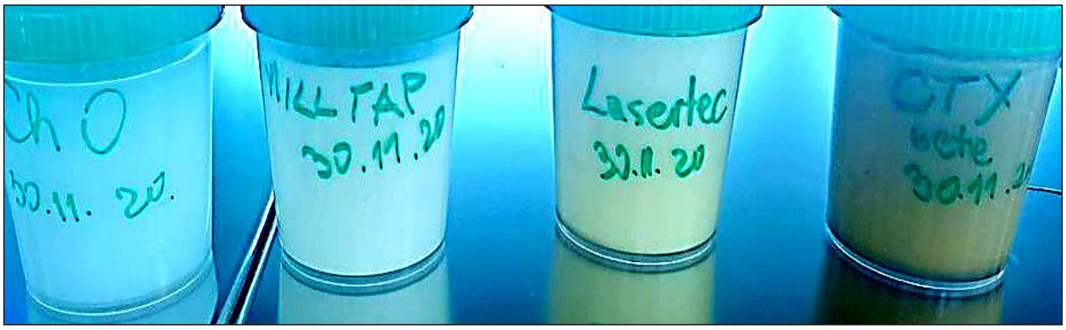

Fig. 2. Samples of pure coolant (a) not used in machining (Ch0) and used respectively from the tanks of the following machines: (b) four-axis milling machining center MILLTAP 700, (c) five-axis milling machine LASERTEC 65 SAUER, d) numerically controlled lathe CTX beta 1250 TC

of dark round heads with a diameter of about 0.1–0.3 mm, surrounded by numerous branched bright hyphae. Hyphae are very characteristic elements of the mycelium that make up the body of the fungus, allowing them to spread.

Mesophiles and thermophiles shown in Fig. 9–11 are characterized by the fact that they need high temperature for growth. The optimum temperature required for their growth and multiplication is between 30°C and 40°C. The shape of the colony is round, punctate with a smooth edge, about 0.5 to 1 mm in diameter.

Machining fluids are commonly used during the mechanical processing of metals. The presence of water and organic substances causes the development of microorganisms in the liquids – mainly bacterial ones. Among them are fungi and bacteria potentially dangerous to human health. During the operation of machine tools, the machining liquid is released in the form of oil mist, on the particles of which biological factors are transported.

Referring to the work [9] in which the author presents the size distribution of airborne particles in the form of fog and particles containing endotoxins in metalworking fluid environments, this is very dangerous for the machine tool operator and requires appropriate safeguards.

Table 2. The results of the amount of microorganisms in the coolant for each machine

<table>
<tr><td rowspan="2">LASERTEC 65</td><td colspan="2">Aspergillus Niger Tiegh</td><td colspan="2">Candida Albicans</td><td colspan="2">Micrococcus Luteus</td></tr>
<tr><td colspan="2">$3.8 \cdot 10^4$</td><td colspan="2">$4.8 \cdot 10^3$</td><td colspan="2">$1.4 \cdot 10^4$</td></tr>
<tr><td rowspan="2">MILLTAP 700</td><td colspan="2">Aspergillus Niger Tiegh</td><td colspan="2">Candida Albicans</td><td colspan="2">Saphylococcus Aureus</td></tr>
<tr><td colspan="2">$2.6 \cdot 10^4$</td><td colspan="2">$4.5 \cdot 10^3$</td><td colspan="2">$2 \cdot 10^2$</td></tr>
<tr><td rowspan="2">CTX beta 1250 TC</td><td>Aspergillus Niger Tiegh</td><td>Candida Albicans</td><td>Saphylococcus Aureus</td><td>Micrococcus Luteus</td><td>Escherchia Coli</td><td>Citrobacter Freundii</td></tr>
<tr><td>$4.2 \cdot 10^4$</td><td>$4.9 \cdot 10^4$</td><td>$2.1 \cdot 10^2$</td><td>$1.9 \cdot 10^4$</td><td>$1.6 \cdot 10^2$</td><td>$3.9 \cdot 10^4$</td></tr>
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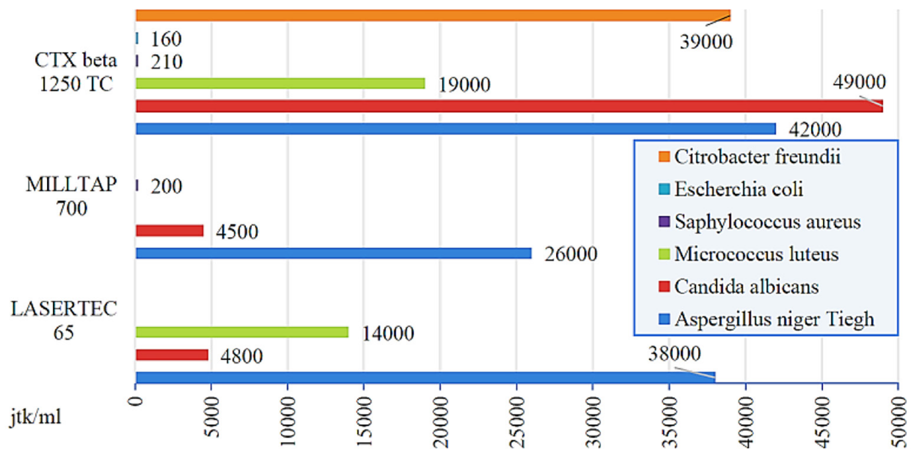

Fig. 3. The number of microorganisms in the cutting fluid from individual machines

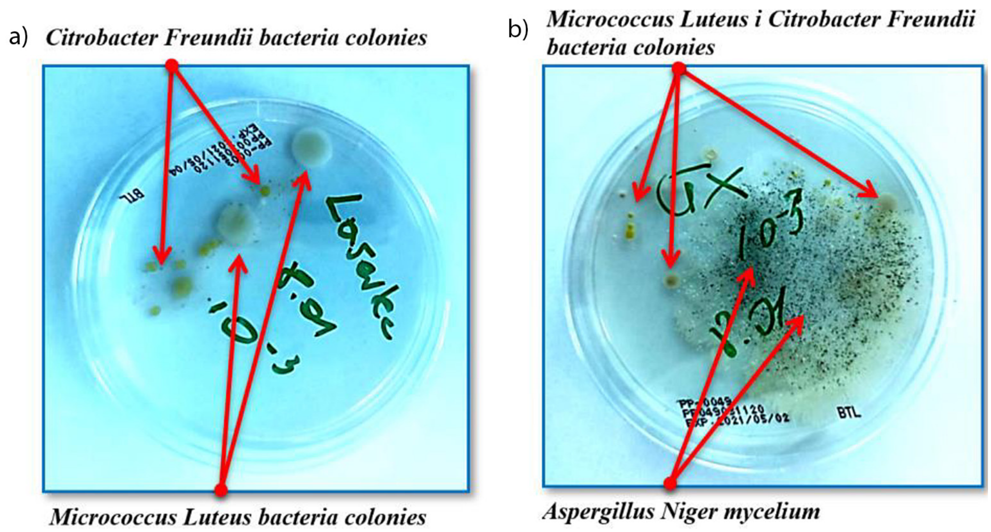

Fig. 4. Colonies of aerobic bacteria on Agar czapka obtained from a 10–3 dilution, for a cooling lubricant used on: (a) five-axis milling machine LASERTEC 65 SAUER, (b) numerically controlled lathe CTX beta 1250 TC

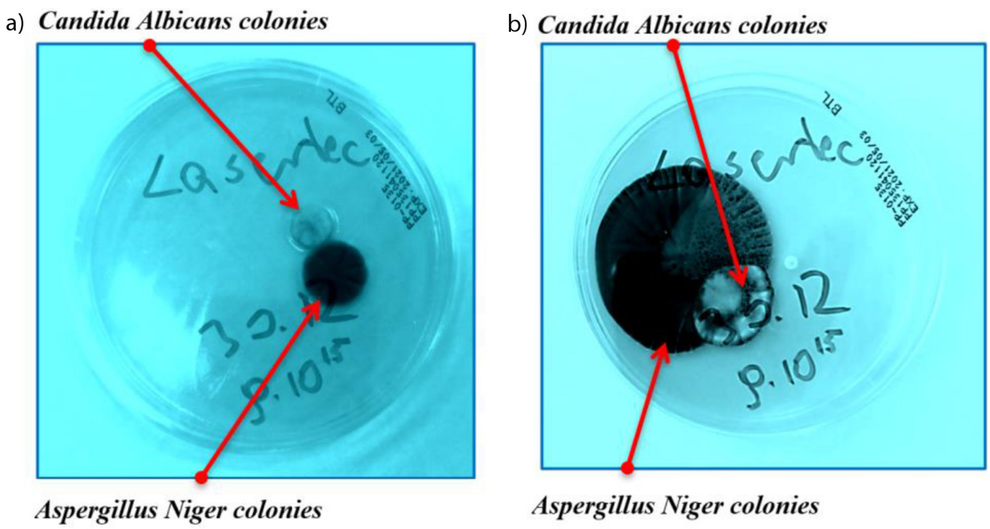

Fig. 5. A spilled colony of *Aspergillus Niger and Candida Albicans* on Sabouraud's Agar medium with chloramphenicol of the cooling lubricant used in the LASERTEC 65 SAUER five-axis milling machine: (a) 8 days after inoculation obtained from a control sample, (b) after 13 days after inoculation, obtained from a control sample, (c) 13 days after inoculation

CONCLUSIONS

Despite the actions taken by both the manufacturers of cutting fluids and users from the machine industry, the ability of microorganisms to grow and multiply in the specific environment of cutting fluids is still a very serious problem. Significant concentrations of bacteria or endotoxins suspended in the oil mist allow this work environment to be included in the group contaminated with organic dust, for which in the hygienic assessment one can use the classification of permissible concentrations of microorganisms in the air.

The observations show that the intensity of microbial growth depends on the intensity of the machine's operation, taking into account the coolant change after the same time. The greatest gains and diversity are seen in the device that has had the longest downtime, the CTX beta 1250 lathe. Already at the time of taking samples from the tanks of the machines, the color of the liquid and the resulting sediment from CTX beta 1250 differed from the others. The lack of circulation of the coolant in the system as a result of machine downtime creates favorable conditions for the development of microorganisms. Fluid in continuous operation flowing through the filters can be used for a longer period of time. The growth of microorganisms, especially fungi, has an adverse effect on the machine's operation. Microorganisms settling in the channels create a blockage in the inflow of the appropriate amount of liquid. This can result in lower heat dissipation, which destabilizes the entire cutting process. Machines which run more often can have their coolant changed much later than machines with lots of downtime. More frequent replacement generates high costs for liquid disposal. Despite the fact that some of the obtained microorganisms occur naturally in the human body or the environment, they become dangerous for people with reduced immunity. One of the most dangerous is the antibiotic-resistant *Staphylococcus Aureus* bacterium.

Fig. 6. No visible microorganisms on the Czapka Agar medium obtained from a 10–3 dilution of the coolant from the Milltap 700 machine

The use of fluid is an important element in the machining process. However, it should be remembered that working in such an environment for a long time carries a risk of complications,

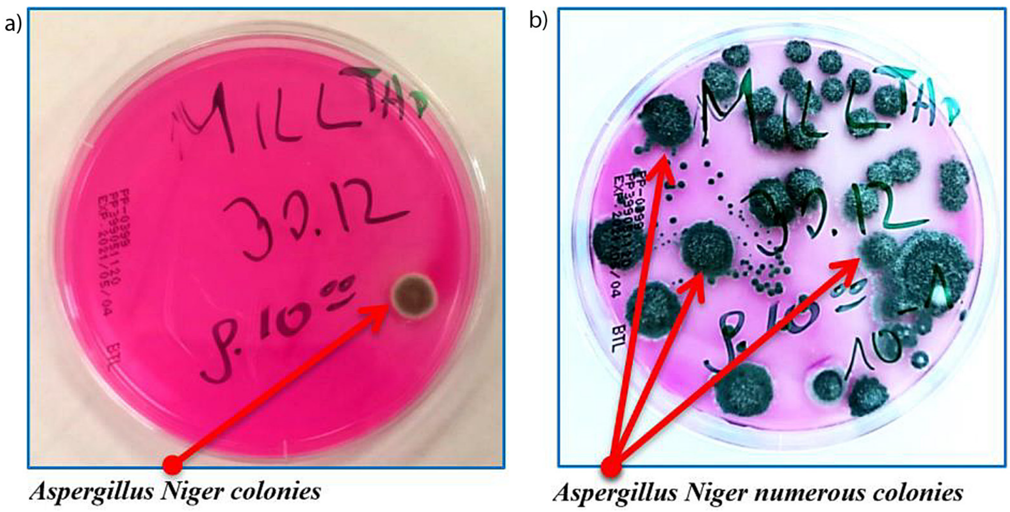

Fig. 7. Dichloran, Rose Bengal, and Chloramphenicol Agar Medium: (a) with a visible Aspergillus Niger colony (8 days after inoculation), (b) with numerous colonies of Aspergillus Niger (after 13 days from inoculation) for a dilution of 10–1

infections and even death. Microflora, despite the appearance in the coolant, is typical of the microorganisms that occur in it. When biocides are used in cooling lubricants, there is no large variety of microorganisms, which confirms the positive effect of these agents. The effect of the development of microorganisms in the cooling system of a CNC machine tool is the gradual decomposition

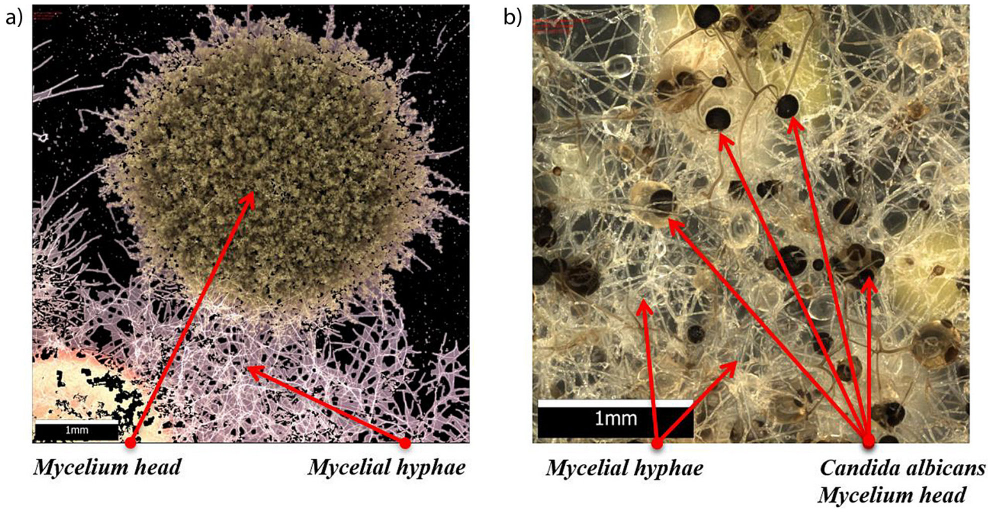

Fig. 8. Mycelia; (a) *Aspergillus Niger* on Rose Bengal medium, (b) *Candida Albicans* on TSA medium

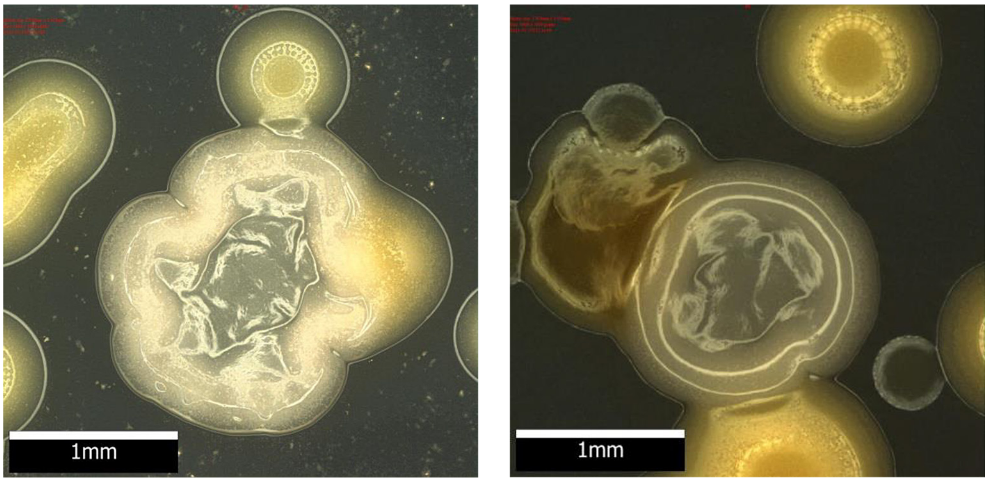

Fig. 9. Colonies of bacteria and fungi on an agar medium

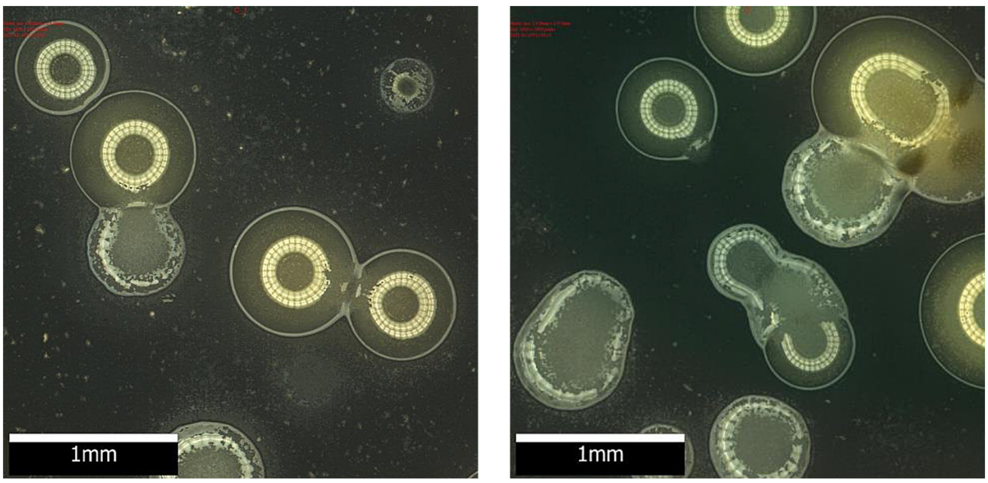

Fig. 10. Thermophilic and mesophilic bacterial colonies on agar medium

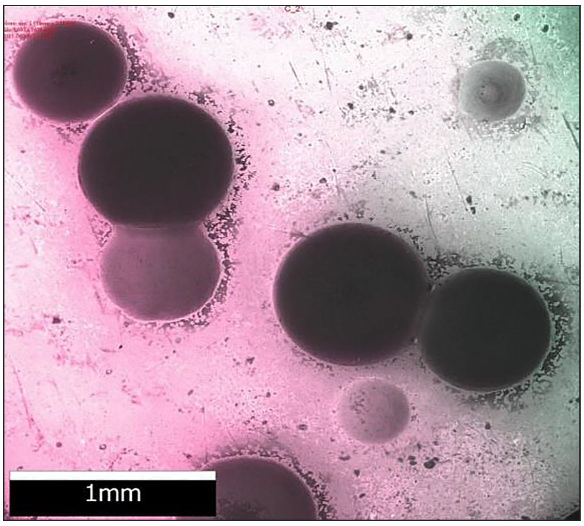

Fig. 11. *Aspergillus Niger* mycelial colonies

of coolant components. This is accompanied by the following symptoms: change – decrease in the pH of the coolant, increase in microdroplets of the suspended phase (oil, loss of emulsion stability and oil separation (emulsion delamination)), deterioration of the lubricating properties of the coolant, deterioration of the quality of machined surfaces, increase in the corrosive aggressiveness of the coolant as a result of increasing concentration acid products of the metabolism of microorganisms, such as: lactic acid, acetic acid, formic acid, and the release of an unpleasant odor by the coolant due to the release of reduced, volatile sulfur and nitrogen compounds. This is particularly often noticeable after a period of production downtime (days off from work – Saturday, Sunday), deterioration of work hygiene conditions; formation of allergies and infections among employees, clogging of filters in circulation systems by clusters of yeasts and fungi. The above symptoms result in shortening the life of coolants, increasing operating costs, production downtime related to coolant replacement. To sum up, the right quality of the liquid, both for the correct course of the process and for the health of employees, is crucial and very important.

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