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# Microbial evaluation of sandboxes located in urban area

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ABSTRACT

This paper presents the results of a study on the degree of bacteriological pollution of sandboxes situated in fenced and unfenced housing estates located in an urban area in Olsztyn, Poland. Heterotrophic plate counts (HPC22, HPC37), *Enterobacteriaceae*, *Escherichia coli*, *Enterococcus* spp., *Staphylococcus* spp. and *Clostridium perfringens* determined by cultivation and fluorescence in situ hybridization (FISH) methods were used as indicators of the sanitary state. Their maximum number in the sand samples reached values of up to $5.4 \times 10^7$, $2.6 \times 10^6$, $3.3 \times 10^4$, $2.1 \times 10^3$, $1.8 \times 10^4$, $1.9 \times 10^1$ and $1.2 \times 10^4$ CFU/g, respectively. It was found that values of culture-independent method were two–four orders greater than those obtained by the cultivation method. Among identified *Enterobacteriaceae*, *Pantoea* spp. and *Enterobacter cloacae* were the most numerous, whereas *Escherichia* cells were detected only occasionally. Pathogenic bacteria of the genus *Salmonella* sp. were isolated from sandboxes also when *E. coli* were absent. Bacteria from *Staphylococcus* genus were isolated irrespective of the site and time of sampling. Additionally, the presence of molds and yeasts was studied. Maximum counts of these microorganisms amounted to $1.0 \times 10^5$ and to $3.5 \times 10^4$ CFU/g. *Aspergillus*, *Penicillium*, *Alternaria* and *Trichoderma* genera were most numerous among molds, whereas *Trichosporon* was detected most frequently among yeasts. Sandboxes in the fenced housing estate and those located in the area which is not close to trees were less polluted than the sand collected from sandboxes in the unfenced housing estate. Potentially pathogenic bacteria of the genus *Salmonella* spp. were identified in analyzed sandboxes, also when *Toxocara* and *E. coli* were absent. It seems that assessing the contamination of children's play areas basing only on fecal bacteria counts and by monitoring number of parasites' eggs may be insufficient to evaluate microbial pollution of sandboxes and may not fully reflect their safety for children.



## 1. Introduction

Natural environment, being biologically contaminated, may harbor various kinds of pathogenic microorganisms. These may pose a threat to humans if contact occurs. The risk is greater for young children as their immunological, neurological and digestive systems are immature (Nwachuku and Gerba, 2004). Furthermore, preschool children have not acquired proper rules of hygiene and tend to eat sand while playing in sandboxes (geophagia), which makes them prone to various infections (Overgaauw and van Knapen, 2013). Young children often spend time in sandboxes, which may have been polluted with domestic (dogs, cats) and wild animals' droppings (rodents, birds). This is also the reason why the incidence of most enteric infections is the greatest among children. Heaney et al. (2012) analyzed 144 wet sand samples and completed 4999 interviews among beachgoers. Authors observed a positive relationship ($p < 0.05$) between fecal microbial pollution of beach sand and incidence of enteric illnesses among people (almost 6% of interviewers) who dig or bury themselves in sand. They also found that gastrointestinal illness and diarrhea incidence were highest among children younger than 5 years (9.5% and 5.2%) and lowest among those aged 55 years and over (5.5% and 4.3%). Hernandez et al. (2014) in their studies of sand beaches, found that the average monthly enterococci loads from animals ranged from $8 \times 10^8$ to $7 \times 10^{11}$. Moreover, the presence of enterococci in the sand has also strongly and significantly correlated with the incidence of pathogens, including fecal coliforms ($r=0.83$; $p < 0.001$), yeasts ($r=0.76$; $p < 0.001$) and nematode larvae ($r=0.83$; $p < 0.001$) (Shah et al., 2011), what further indicates how potentially significant it is for the public health to measure fecal indicator bacteria levels in the sand. The main microorganisms responsible for human enteric infections are some viruses (enteric adenoviruses, astroviruses, human caliciviruses, rotaviruses) and several bacteria, such as *Campylobacter jejuni*, a variety of opportunistic pathogenic and pathogenic *Escherichia coli*, several *Shigella* species, various *Salmonella* and *Vibrio* strains.

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These bacteria are mainly found in the digestive tract of humans and animals. They could be transmitted from person to person through contaminated (by feces) water, food or soil. Enteric infections are usually caused by strains (mainly from nosocomial infections) which produce exo- or/and endo-toxins and which are responsible for almost 1.7–2.5 million deaths per year, mostly in young children and infants in developing countries (Girard et. al., 2006).

In Poland, as well as in many other countries, sand from sandboxes is analyzed to detect presence of eggs of pathogenic parasites (*Toxocara canis* and *Toxocara cati*), which are carried mainly by young dogs and cats (Blaszkowska et al. 2013; Deplazes et al. 2011). However, lack of parasite eggs in tested material does not exclude the possibility that it has been contaminated with feces and consequently contains pathogenic microbiota. Certain microorganisms which serve as sanitary state indicators are reliable bioindicators of the sanitary conditions and the degree of contamination of a given environment. Human and animal digestive tract bacteria e.g. *E. coli*, *Enterococcus* spp. and *Clostridium perfringens* are such microorganisms – their occurrence in an analyzed sample suggests that the material has been contaminated with feces and may contain other pathogenic forms (*Shigella, Salmonella, Klebsiella*) originating from carriers and ill human individuals.

Many authors (Bonilla et al., 2007; Edge and Hill 2007; Haack et al., 2003; Oshiro and Fujioka, 1995; Wright et al., 2009) reported that the contamination of recreational places with fecal indicator was primarily caused by animals (dogs, cats, birds) feces. It has been found that one gram of animals feces contains from a few hundred to several millions of intestinal bacteria such as enterococci or *E. coli* (Table 1). According to Alderisio and DeLuca (1999), even old and dried-up excrements of birds can contain from $8.2 \times 10^2$ up to $3.0 \times 10^5$ viable coliform bacteria per gram. Murphy et al. (2005) reported that the duck droppings are a rich source of bacteria (numbered at least $1 \times 10^6$ CFU/g) which are potentially pathogenic to humans. Whereas according Wright et al. (2009), dog's feces contain a higher number of enterococci than the birds feces (Table 1), and their droppings can be a major cause of pollution of recreational places where the animals stay.

Currently, there is a tendency to extend environment screening tests by adding assays of other microorganisms than just typical sanitary state indicators which originate from excrements and secretions of living organisms. As far as such microorganisms are concerned, *Staphylococcus* genus (e.g. pathogenic *S. aureus* including methicillin-resistant forms) and some species of yeast fungi (e.g. potential human pathogens *Candida tropicalis*, *C. albicans*, *C. parapsilosis*), which can be particularly hazardous to human health, draw much attention (Shah et al., 2011). The survival rate of those microorganisms in an extracorporeal environment depends on a number of physicochemical factors (temperature, pH, moisture, insolation, availability of nutrients) (Erickson et al., 2014; Garcia et al. 2010; Zhu et al., 2011). As a general rule, survival of microorganisms is greater in moist environments than dry environments (Lang et al. 2007), whereas increasing soil temperatures generally decreases pathogen survival (Garcia et al. 2010; Ongeng et al. 2011). Burkhardt et al. (2000) observed a pattern of reverse correlation between high temperature and the rate of *E. coli* death. Furthermore, they discovered directly proportional correlation between intensity of sunlight and inactivation of these bacteria. Some authors (Zhu et al., 2011), also confirmed that the rate at which allochtonous bacteria (important for the sanitary condition of environment) were inactivated was influenced by temperature and solar inactivation. This effect occurs both in soil and in water in which such bacteria typically die out sooner at higher temperatures (20, 25 or 37 °C) but survive for longer periods of time at 4 °C. Long-term survival of potentially pathogenic fecal bacteria in the environment at low temperatures poses the greater risk of infection (Baumgardner, 2012). Additionally, soil type and nutrients content are also important and they have the greatest impact on the survival of bacteria in soils. Paluszak and Ligocka (2003) suggested that *E. coli*, *Salmonella* and *E. faecalis* survive in alluvial soil an average of 30 weeks. According to experimental field studies, persistence of *Salmonella* spp., *E. coli* O157:H7 in soils rich with nutrients ranges from about 5 to 38 weeks (Erickson, et al., 2014; Franz, et al., 2011) and serves as justification for the latter conservative interval guidelines.

The ability of microorganisms to cause an illness is related to their infectious dose, virulence and resistance of an organism exposed to infection. Nonetheless, any contact with pathogenic microorganisms is a potential threat to human health. Symptoms of infection caused by dangerous microorganisms (*E. coli*, *E. faecalis*, *Salmonella*, *K. pneumoniae*) may be mild in character, limited to small gastrointestinal illness. However, they can also be very severe, such as inflammation and damage of various internal organs (inflammation of the urinary, respiratory and digestive tract, inflammation of bones, bone marrow, joints or meningitis, etc.). In extreme cases such infections can be fatal especially in infected children and infants (Mahon et al., 2010). Another source of serious risk is attributed to mycotoxins (which display carcinogenic, neurotoxic, teratogenic and mutagenic activities) produced by molds of the genera *Aspergillus*, *Penicillium*, *Fusarium* or *Stachybotrys* which have the greatest potential to evoke allergic reactions (Dynowska et al., 2006; Vujanovic et al., 2001). Their number depends typically on the humidity and temperature of the habitat as well as presence or absence of plants (trees, shrubs) (Gotkowska-Płachta et al. 2013).

The aim of this study was to analyze the qualitative and quantitative composition of microorganisms (bacteria and fungi) present in the children's sandboxes, located in urban area, depending on their use and management of adjacent land. The culture-dependent and culture-independent (fluorescence in situ hybridization – FISH) methods of quantification and identification

**Table 1**
Number (colony forming units – CFU per 1 g feces) *Enterococcus faecalis* and *Escherichia coli* from different animal feces.

| Type of animal | *Escherichia coli* | *Enterococcus faecalis* | References |
|---|---|---|---|
| Dogs | $2.3 \times 10^7$ | $9.8 \times 10^8$ | Oshiro and Fujioka (1995) |
| Small dogs (weight < 9 kg) | | $1.1 \times 10^5 - 1.0 \times 10^7$ | Wright et al. (2009) |
| Large dogs (weight > 9 kg) | | $5.7 \times 10^4 - 2.8 \times 10^8$ | Wright et al. (2009) |
| Cats | $7.9 \times 10^7$ | $2.7 \times 10^7$ | Oshiro and Fujioka (1995) |
| Birds (Ibis, Gull, Pigeon, Coot, Duck, Heron, Pelican) | | $8.0 \times 10^3 - 9.7 \times 10^5$ | Wright et al. (2009) |
| Duck | $3.3 \times 10^7$ | | Oshiro and Fujioka (1995) |
| Pigeon | $1.7 \times 10^8$ | $4 \times 10^5$ | Oshiro and Fujioka (1995) Alderisio and DeLuca (1999) |
| Gulls | $1.0 \times 10^5 - 1.9 \times 10^9$ | $2.0 \times 10^4 - 1.3 \times 10^8$ | Haack et al. (2003) |