Protection of Public Health from Microbial and Chemical Hazards in Swimming Pool Environments

Yuli Ekowati

PROTECTION OF PUBLIC HEALTH FROM MICROBIAL AND CHEMICAL HAZARDS IN SWIMMING POOL ENVIRONMENTS

Yuli Ekowati

This research was conducted under the auspices of the SENSE Research School for Socio-Economic and Natural Sciences of the Environment

CRC Press/Balkema is an imprint of the Taylor & Francis Group, an informa business

Published by:
CRC Press/Balkema
Schipholweg 107C, 2316 XC, Leiden, the Netherlands
Pub.NL@taylorandfrancis.com
www.crcpress.com – www.taylorandfrancis.com
ISBN 978-0-367-25135-2
Cover image: The Arab Baths, Girona, Spain (photo by Yuli Ekowati)

Protection of public health from microbial and chemical hazards in swimming pool environments

Bescherming van de volksgezondheid tegen microbiologische en chemische gevaren in en om zwembaden

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties in het openbaar te verdedigen
op maandag, 11 maart 2019 des middags te 2.30 uur

door

Yuli Ekowati

geboren op 8 juli 1978
te Pati, Indonesië

Promotoren : Prof.dr. A.M. de Roda Husman
Prof.dr. M.D. Kennedy

Copromotoren : Dr. F.M. Schets
Dr. G. Ferrero

This thesis was accomplished with financial support from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No. 619116.

Table of contents

1

Introduction

Swimming is a popular recreational activity, also recommended for its health benefits. However, besides the health benefits derived from swimming and recreational water activities, the swimming pool environment exposes people to different health risks. These health risks may be associated with exposure to microbial and chemical contaminants. Swimming pool environments have to be maintained, controlled and monitored for the protection of public health.

Disinfection technologies

Typical water treatment processes in swimming pools consist of coagulation, filtration and disinfection (Barbot and Moulin, 2008; Glauner et al., 2005; WHO, 2006) (Figure 1). However, the treatment train may vary since some swimming pools do not apply coagulant and/or pH correction. The disinfectant applied can be a single disinfectant (e.g. chlorine or bromine) or a combination of disinfectants (e.g. ozone/chlorine, ultraviolet (UV)/chlorine). Considering the continuous microbial contamination introduced by bathers in swimming pools, water treatment processes should be able to provide a multi-barrier to prevent waterborne outbreaks. While disinfection is the most crucial step for inactivation of pathogens, each treatment step also contributes to the reduction of microorganisms in swimming pools. Consequently, inadequate pool operation, maintenance, and water treatment can result in waterborne outbreaks in swimming pools (Craun et al., 2005; Yoder et al., 2008).

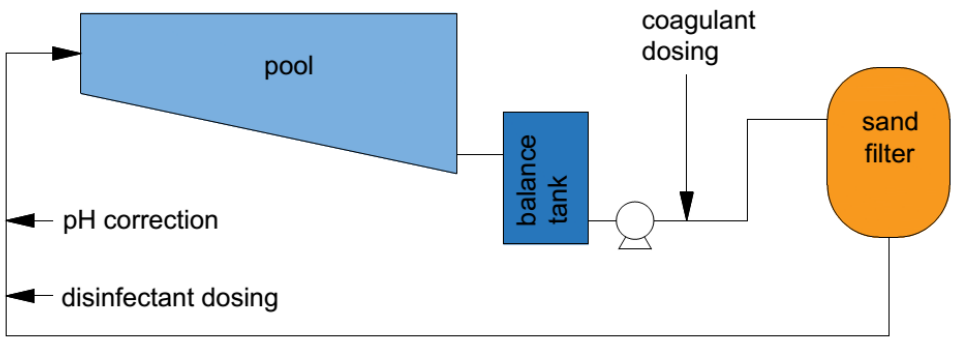

Figure 1. Typical water treatment processes in an exemplary swimming pool

Chlorine is the most widely applied disinfectant in swimming pools and similar environments. However, some pathogens have proven to be resistant to chlorine (e.g. *Cryptosporidium* oocysts and *Giardia* cysts) (Korich et al., 1990) and therefore can still pose a threat to the health of bathers, even when proper chlorine disinfection is in place. Additionally, chlorine based disinfectants and other chemical disinfectants, such as bromine based disinfectants and ozone, have been found responsible for disinfection byproducts (DBPs) formation, due to their reaction with organic and inorganic constituents in water. Numerous studies have discussed the potential negative health

consequences due to long-term exposure to DBPs in drinking water and/or in swimming pools (Florentin et al., 2011; Nieuwenhuijsen et al., 2009; Nieuwenhuijsen et al., 2000; Villanueva et al., 2015; Zwiener et al., 2007). A possible solution for chlorine resistant pathogens and DBP formation is to apply alternative disinfection technologies which are more effective for microbial inactivation and at the same time contribute to very limited DBP formation.

Chlorination

Chlorine is widely used as a disinfectant in many water treatment applications (e.g. drinking water, wastewater) due to its efficacy against a wide range of pathogens and because of its availability and cost. Another advantage of using chlorine as disinfectant is that chlorine provides a residual disinfection effect for protection against microbial recontamination. In swimming pools, chlorine based disinfectants, e.g. chlorine gas, sodium and calcium hypochlorite are commonly applied disinfectants (Carter and Joll, 2017).

The addition of chlorine based disinfectants to water results in the formation of hypochlorous acid (HOCl). Depending on the pH of the water, HOCl can dissociate into hypochlorite ions (OCl^-). HOCl and OCl^- both have a disinfecting ability, however, HOCl is more effective than OCl^-. While both species are equally present at 25 °C and pH 7.54 (Morris, 1966), HOCl dominates at pH 5.5–7.5, and thus the disinfection is more effective in the lower pH range.

The level of adequate free chlorine in swimming pools based on WHO guidelines for safe recreational water should be 1 mg/L throughout the entire swimming pool and should not exceed 3 mg/L. In case combinations of chlorine with UV or ozone are applied, 0.5 mg/L or less free chlorine provides adequate disinfection (WHO, 2006). Different standards for free chlorine levels are applied in swimming pools in different countries, also depending on the operational conditions and the maintenance of the pools.

Ozone

Ozone is a very powerful chemical disinfectant which is able to oxidize organic and inorganic compounds, as well as killing microorganisms. The high oxidizing power is due to the weak bonds of the third oxygen atom causing instability of the ozone molecule. Ozone decomposes rapidly and thus does not provide a residual disinfection effect and has to be generated on-demand at the site of application. Ozone is a stronger oxidant than chlorine, with a higher redox potential (WHO, 2000).

In aqueous solution, ozone reacts with organic and inorganic compounds by means of two different mechanisms: direct oxidation by ozone molecules and oxidation by hydroxyl radicals during ozone decomposition (Glaze et al., 1987; Gottschalk et al., 2010;

Hoigné and Bader, 1976). Direct oxidation with an aqueous ozone is slower compared to oxidation by hydroxyl radicals, but the concentration of aqueous ozone is high, whereas the oxidation by hydroxyl radicals is fast but the concentration of hydroxyl radicals is small (U.S. EPA, 1999). Direct oxidation by aqueous ozone is dominant under acidic conditions while hydroxyl radicals are dominant under favourable conditions, such as at high pH, under exposure to UV or with the addition of hydrogen peroxide (Hoigné and Bader, 1976).

Ozone is often used in (waste) water treatment to remove organic matter and other pollutants from the source water (Chin and Bérubé, 2005). However, ozone breaks down natural organic matter creating smaller and biodegradable organic molecules which are easier for microorganisms to utilize as nutrients (Camel and Bermond, 1998; Miettinen et al., 1998; van der Kooij et al., 1989). Particularly in drinking water treatment, this might lead to bacterial re-growth in the distribution system. In swimming pool applications, ozone has been utilised to treat the pool water in addition to chlorine or bromine-based disinfectants. The recommended maximum limit of ozone in the air in swimming pools where ozone is used is 0.12 mg/m^3 (WHO, 2006).

UV irradiation

UV light is a non-visible light with a shorter wavelength than visible light (100–400 nm). The UV light spectrum is classified into 4 groups: UVA (315–400 nm), UVB (280–315 nm), UVC (200–280 nm) and vacuum UV (100–200 nm). UVC, with wavelength in the range between 245 and 285 nm, is considered as the optimal UV light for inactivation of microorganisms. Unlike chemical disinfection, UV irradiation is a physical process which inactivates microorganism by damaging their nucleic acid and impeding DNA replication.

Low pressure UV (LP UV) lamps emit a spectral radiation with a maximum peak at 254 nm and several other lesser peaks including one at 185 nm. LP UV lamps have a surface temperature of 90–120 °C and a UVC efficiency of 35% (Schalk et al., 2006). The radiation at 185 nm in combination with oxygen generates ozone which is corrosive, toxic and absorbs UV light. Nevertheless, radiation at 185 nm is used to generate •O or •OH radicals in advanced oxidation processes. Due to this fact, UV lamps can be manufactured as “ozone-free” (reduced the output at 185 nm) or “ozone-generating” lamps (Schalk et al., 2006). LP UV lamps are mainly used for the disinfection of drinking water and surfaces.

Medium pressure UV lamps (MP UV) emit a wide spectrum of UV wavelengths from 200 to 400 nm and require a higher energy input compared to low pressure UV lamps. MP UV lamps also have a higher surface temperature (500–950 °C) and have a lower UVC efficiency (5–15%) compared to LP UV lamps (Schalk et al., 2006). MP UV lamps

are applied in drinking water treatment as advanced oxidation processes (AOPs), usually combined with hydrogen peroxide and ozone (Heering, 2004).

Contaminants in swimming pools and similar environments

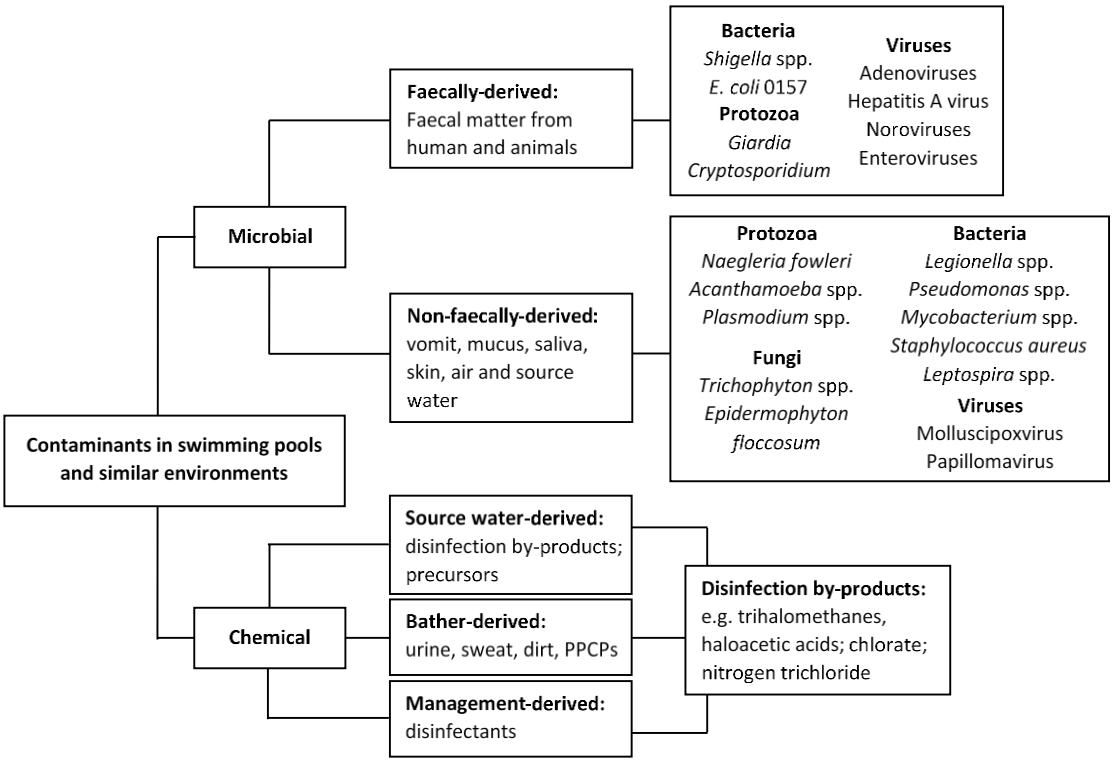

Figure 2. Potential microbial and chemical hazards in swimming pools and similar environments (adapted from WHO (2006))

Microbial hazards

Microbial contaminants in the pool water can be pathogens originating from faecal matter introduced by humans and animals, from human shedding (e.g. vomit, mucus, saliva, and skin) (WHO, 2006) or from source water and air (Figure 2). Many outbreaks associated with pathogens in swimming pools and similar environments have been reported worldwide (Efstratiou et al., 2017; Hlavsa et al., 2018; Leoni et al., 2018). WHO (2006) listed some faecally-derived microorganisms which have been linked to outbreaks: *Shigella* and *Escherichia coli* O157:H7 (bacteria) (Friedman et al., 1999; Keene et al., 1994), *Giardia* and *Cryptosporidium* (protozoa) (Baldursson and Karanis, 2011; Efstratiou et al., 2017; Insulander et al., 2005), and adenovirus, norovirus and hepatitis A virus (Dziuban et al., 2006; Mahoney et al., 1992). Non-faecally-derived pathogens, such as *Legionella pneumophila* and *Pseudomonas aeruginosa* (bacteria) were also reported to cause infections in swimming pools and similar environments (Leoni et al., 2018; Yoder et al., 2004).

The U.S. federal agency Centers for Disease Control and Prevention (CDC) publishes reports on outbreaks in (untreated and treated) recreational waters in the U.S. in Morbidity and Mortality Weekly Report (MMWR). Hlavsa et al. (2015) mentioned that 77% of the outbreaks reported in 2011–2012 in the U.S. occurred in treated recreational water settings. A more recent MMWR stated that most of the reported outbreaks in the U.S. between 2000 and 2014 were associated with swimming pools in hotels (Hlavsa et al., 2018). Within that period, the majority of outbreaks were caused by *Cryptosporidium* (42%), *Legionella* (12%) and *Pseudomonas* (10%). The annual number of *Legionella* and *Cryptosporidium* outbreaks increased by 13% and 25%, respectively, while the annual number of outbreaks caused by *Pseudomonas folliculitis* decreased by 22%.

Due to the threat from microbial contaminants in swimming pools and similar environments, the assessment of the microbial quality of pool water should be performed periodically. As mentioned in WHO (2006), the examined microorganisms in swimming pools preferably include heterotrophic plate count (for general measure of non-specific microbial levels), *E. coli* and thermotolerant coliforms (for faecal indicators). Investigation of the presence of the pathogens *Pseudomonas aeruginosa*, *Legionella* spp. and *Staphylococcus aureus*, which are known to cause outbreaks in swimming pools, is also included in the guideline for assessing microbial quality of swimming pool water. Table 1 shows the guideline values for microorganisms in swimming pool water for water quality assessment in several EU countries.

Table 1. Guideline values for microbial quality in swimming pools and similar environments in different EU countries.

Parameter	Quality requirement			
	Netherlands[a]	Spain[b]	Austria[c]	Italy[d]
Pseudomonas aeruginosa	0 CFU/100 mL	0 CFU/100 mL	0 CFU/100 mL	≤ 1 CFU/100 mL
Enterococcus				0 CFU/100 mL
Staphylococcus aureus				≤ 1 CFU/100 mL
Escherichia coli		0 CFU/100 mL	0 CFU/100 mL	0 CFU/100 mL
Legionella spp.	< 100 CFU/L	< 100 CFU/L	0 CFU/100 mL	
Heterotrophic bacteria at 22 °C				≤ 200 CFU/mL
Heterotrophic bacteria at 37 °C	≤ 100 CFU/mL		≤ 100 CFU/mL	≤ 100 CFU/mL

[a] The Swimming Facilities Hygiene and Safety Decree (2011)

[b] The Spanish national legislation Real Decreto 742/2013 (2013)

[c] Bathing hygiene regulation (2012)

[d] Swimming pools - Requirements for swimming pool water circulation, filtration, disinfection and chemical treatment ("Requisiti degli impianti di circolazione, trattamento, disinfezione e qualità dell'acqua di piscina," 2016)

Despite compliance with microbiological standards for swimming pool water quality, swimming pool related disease outbreaks do occur, even when disinfection is sufficient according to the guidelines. These outbreaks are merely caused by microorganisms that are not covered by the standard microbiological monitoring parameters because they are more resistant to disinfection than the monitoring microorganisms are. Examples are chlorine resistant *Cryptosporidium* or adenoviruses. Another potential public health concern in swimming pool environments may be human pathogenic fungi. WHO (2006) indicated fungal species, e.g. *Trichophyton* spp. and *Epidermophyton floccosum,* as potential microbial hazards in swimming pools since they are known to cause superficial fungal infections among pool users and lifeguards. However, no guideline values or indicator organisms for assessment of fungi were defined.

Some fungal species, filamentous fungi or yeasts, are ubiquitous in swimming pools and have been associated with adverse health effects on humans (Hilmarsdottir et al., 2005; Jankowski et al., 2017; Kamihama et al., 1997). Fungal infections in humans are commonly transmitted by direct contact, person to person contact or contact with contaminated surfaces in swimming pool facilities (e.g. floors). The epidemiology of dermatophytes in swimming pool facilities has been studied since decades (English and Gibson, 1959; Gentles and Evans, 1973). Dermatophytes are commonly causing skin, hair and nail infections (tinea or ringworm). They include members of the genera *Epidermophyton*, *Microsporum* and *Trichophyton*. Dermatophytes and their infections are the focus of most fungal-related publications in swimming pools (Detandt and Nolard, 1988, 1995; Hilmarsdottir et al., 2005; Kamihama et al., 1997; Shemer et al., 2016). In later years, more studies have been done focusing on the occurrence of (pathogenic) fungi in swimming pool facilities, dermatophytes and/or non-dermatophytes (Brandi et al., 2007; Buot et al., 2010; Jankowski et al., 2017; Papadopoulou et al., 2008; Viegas et al., 2011). Fungal species within the genera *Aspergillus, Alternaria, Cladosporium, Penicillium, and Fusarium* were frequently isolated from air, water and surfaces in swimming pool facilities (Ali-Shtayeh et al., 2003; Brandi et al., 2007; Jankowski et al., 2017; Viegas et al., 2011). Similarly, yeasts species, such as members of the genera *Candida*, *Rhodotorula*, and *Trichosporon,* were frequently detected in water and on surfaces in swimming pool facilities.

Similar to other microorganism, some fungal species were found to be more resistant to chlorine disinfection than others. Rosenzweig et al. (1983) showed that spores of four filamentous fungi (*A. fumigatus, A. niger, Cladosporium* sp. and *P. oxalium*) and yeasts (*C. laurentii, R. glutinis, R. rubra*) are more resistant to chlorine than some bacteria (*E. coli, C. freundii,* and *E. cloacae*). Pereira et al. (2013) also mentioned that some fungal species such as *Aspergillus terreus* were more resistant to chlorine inactivation than *E. coli* and poliovirus 1, but less resistant than *Cryptosporidium* oocysts.

Chemical hazards

Disinfection byproducts (DBPs)

Chemicals found in swimming pool water can originate from the source water, chemicals used for treatment (e.g. coagulant, disinfectant), and from bathers (e.g. urine, sweat, lotions) (WHO (2006). The application of chemical disinfectants in swimming pools can lead to the formation of DBPs, produced by the reaction between disinfectants, e.g. chlorine, and natural organic matter or bromide or iodide from the source water used to (re-)fill the pool, and human inputs (e.g. urine, sweat, cosmetics, lotions, skin cells, hair, etc.) (Chowdhury et al., 2014; Kanan and Karanfil, 2011; Kim et al., 2002) (Figure 2). Continuous contaminants and chlorine addition, together with water recirculation with a long replacement time can enhance DBP formation in the pool water (Glauner et al., 2005; Lee et al., 2009).

The main pathways of human DBP exposure in swimming pools are through accidental ingestion of pool water, inhalation and dermal contact. Over 600 DBPs have been identified in chlorinated waters and many of them are mutagenic or carcinogenic, some associated with irritation to skin, throat or eyes, asthma and respiratory illnesses, bladder cancer (Richardson et al., 2010; Richardson et al., 2007; Villanueva et al., 2007) and also suspected to have an adverse effect on the reproductive system (Nieuwenhuijsen et al., 2000). DBPs identified in the swimming pool environment are haloalkanes (e.g. trihalomethanes (THMs)), haloacids (e.g. haloacetic acids (HAAs)), halodiacids, haloaldehydes (e.g. chloral hydrate), halonitriles (HANs), haloketones (HKs), halonitromethanes (HNMs), haloamides, haloalcohols, and chloramines (Richardson et al., 2010). It was also reported that nitrogenous-DBPs are more genotoxic, cytotoxic and carcinogenic than those without nitrogen (i.e. THMs and HAAs) (LaKind et al., 2010; Richardson et al., 2010). HNMs and HANs have higher cytotoxicity and genotoxicity compared to HAAs (Muellner et al., 2007; Teo et al., 2015). N-nitrosamines are one group of nitrogenous-DBPs and most of nitrosamines are carcinogenic. The most frequently studied nitrosamine is N-nitrosodimethylamine (NDMA), which was found abundantly in chlorinated swimming pools and hot tubs with concentrations 500-fold higher than in drinking water (0.7 ng/L) (Walse and Mitch, 2008).

The use of ozone and UV can affect the formation of DBPs. Swimming pool water treated with ozone generally has lower concentrations of DBPs compared to chlorine alone (Carter and Joll, 2017). Nevertheless, in the presence of bromide, ozone can form bromate (Haag and Hoigne, 1983) and brominated DBPs, even without chlorine present (Glaze, 1986). Ozone can also increase the formation of some investigated DBPs (THMs, DCAN, 1,1,1 TCP, TCNM) by causing organic precursors to be more reactive to chlorine (Hansen et al., 2016).

It was suggested that UV disinfection did not significantly alter, form, or affect the rate of DBP formation (U.S. EPA, 1999). Reckhow et al. (2010) concluded that low pressure UV has no effect on the formation of THMs, HAAs, and also HNMs. UV irradiation has also been used for dechloramination (Cimetiere and De Laat, 2014). Soltermann et al. (2013) concluded that UV disinfection can be applied to degrade N-nitrosamine if the water contains a relatively high concentration of N-nitrosamines compared to chloramines and chlorinated secondary amines. However, they also found a substantial risk of UV-induced NDMA formation in swimming pool water with a higher chloramine concentration. Using model organic-N precursors, Weng et al. (2012) found that UV irradiation enhanced the formation of chlorinated nitrogenous-DBPs, such as dichloroacetonitrile and cyanogen chloride, and eliminated inorganic chloramines.

Formation of DBPs can be minimized by applying additional disinfection, such as UV and ozone, together with low concentrations of chlorine (Teo et al., 2015). Walse and Mitch (2008) showed that the application of ozone and UV at a lower chlorine dose resulted in lower levels of NDMA in swimming pool water. Other studies summarized by Carter and Joll (2017) also showed that the concentration of DBPs in the swimming pools applying only chlorine based disinfectants were generally higher compared to swimming pools with ozone and chlorine.

Countries which do not have any guidelines or legislation for DBPs in swimming pools can use the guideline values for allowable DBPs in in the WHO Guidelines for Drinking-water Quality (WHO, 2011) to assess the potential risks of DBP exposure in swimming pools. The Netherlands intends to include regulatory values for some DBPs (total THMs) in their upcoming new legislation for swimming pool water quality (Table 2).

Table 2. Guideline values for DBPs in drinking water and in swimming pool water

Disinfection byproducts	Maximum contaminant level (μg/L)					
	Drinking water				Swimming pool water	
	WHO [a]	U.S. EPA[b]	EU[c]	NL[d]	NL[e]	Austria[f]
Bromoform	100	0	-	-	-	
Dibromochloromethane	100	60	-	-	-	
Bromodichloromethane	60	0	-	15	-	
Chloroform	300	70	-	-	-	
ΣTHM	**	80	100	25	≤ 50***	≤ 20*** not exceed 100
Bromate	10*	10	10	1	≤ 100	
Chlorite	700*	1000	-	-	-	
Chlorate	700*	-	-	-	< 30 mg/L	
Monochloroacetate	20	-	-	-	-	
Dichloroacetate	50*	-	-	-	-	
Trichloroacetate	200	-	-	-	-	
HAA_5	-	60	-	-	-	
Dichloroacetonitrile	20*	-	-	-	-	
Dibromoacetonitrile	70	-	-	-	-	
2,4,6-Trichlorophenol	200	-	-	1 (chlorophenol)	-	
N-Nitrosodimethylamine	0.1	-	-	12 ng/L	-	

*provisional value, **the sum of the ratio of the concentration of each to its respective guideline value should not exceed 1, *** as chloroform

[a] WHO Guidelines for Drinking Water Quality (2011)

[b] U.S. EPA Drinking Water Standards and Health Advisories (2018)

[c] European Drinking Water Directive (1998)

[d] Drinking Water Decree (2011)

[e] Supplementary Decision Swimming and Bathing in Water Basins Environmental Law (2018)

[f] Bathing hygiene regulation (2012)

Pharmaceutical and personal care products and endocrine disrupting compounds

In addition to pathogens and DBPs, pharmaceuticals (i.e. antibiotics, analgesics) and personal care products (PPCPs) (i.e. cosmetics, lotion and suntan oil) are included as chemical hazards in swimming pools and similar environments. Endocrine disrupting compounds (EDCs) are a group of substances found in certain pharmaceuticals, pesticides, industrial chemicals and natural hormones, which are able to disrupt the functions of endocrine system in an organism (Falconer et al., 2006). PPCPs and EDCs in swimming pool water mostly originate from humans. Some pharmaceuticals are not completely metabolized by the human body and excreted through urine, whereas personal care products, such as lotions and sunscreens, are applied externally and

washed off the human body during swimming activity. Personal care products can also contain some pharmaceutical compounds. A trace amount of some PPCPs may already be present in the source water (tap water) (Yang et al., 2017).

PPCPs have been studied in different swimming pool settings (Ekowati et al., 2016; Suppes et al., 2017; Teo et al., 2016; Weng et al., 2014), while EDCs (e.g. estrone, estradiol) have been mainly investigated in environmental waters (Campbell et al., 2006; Li et al., 2015; Petrovic et al., 2004; Yoon et al., 2010). The potential accumulation of PPCPs in pool water is likely to happen as a consequence of water recirculation in swimming pools where the treatment applied is not effective to remove PPCPs. Conventional water treatment processes, such as coagulation-flocculation, are inefficient in removing trace levels of some pharmaceuticals (Stackelberg et al., 2004; Ternes et al., 2002). Moreover, PPCPs are generally non-volatile and their reaction with chlorine is slow, thus they are likely to remain in the swimming pool water (Weng et al., 2014).

Pharmaceuticals and EDCs are of concern due to possible adverse health effects on humans and wildlife (Richardson and Ternes, 2011). The prevalence of PPCPs in the environment and can be toxic to aquatic organisms at different concentrations (Brausch and Rand, 2011; Fabbri and Franzellitti, 2016; Klavarioti et al., 2009). Also, the bioaccumulation of PPCPs in aquaculture organisms has been demonstrated at a concentration which potentially pose a risk to human health (Chen et al., 2015; Grigorakis and Rigos, 2011). Risk assessments of pharmaceuticals in drinking water have demonstrated no adverse health effects from exposure of trace concentrations of pharmaceuticals in drinking water (de Jesus Gaffney et al., 2015; de Jongh et al., 2012; Kumar et al., 2010). However, these risk assessments targeted the effect of each pharmaceutical compound separately and indicated the necessity to study the effects of mixtures of compounds in future studies.

Additionally, PPCPs have the potential to act as DBP precursors and form byproducts (Bottoni et al., 2014; Nakajima et al., 2009; Negreira et al., 2008; Sakkas et al., 2003; Shen and Andrews, 2011). Some pharmaceuticals are susceptible to chlorine (e.g. acetaminophen) (Weng et al., 2014; Westerhoff et al., 2005), but then either they formed byproducts (e.g. 1,4-benzoquinone and *N*-Acetyl-*p*-benzoquinone) (Bedner and MacCrehan, 2006) or they remain unchanged (Glassmeyer and Shoemaker, 2005). Wang *et al.* (2013) demonstrated that some compounds in personal care products (e.g. benzyl alcohol, lecithin, parabens) may serve as halobenzoquinone precursors in chlorinated swimming pools. It was suggested by some authors that the degradation or transformation products of some PPCPs and EDCs (e.g. bisphenol A, acyclovir) are more toxic than the parent compounds (Kockler et al., 2012; Noguera-Oviedo and Aga, 2016; Santos et al., 2012).

Focus of the thesis

Ozone and UV are effective disinfectants and therefore applied in many water treatment systems. Chlorine is needed in swimming pools to provide a residual disinfectant in pool water. The application of a combination of ozone and UV and chlorination in swimming pools has not been much explored yet, with respect to the efficiency to inactivate chlorine resistant microorganisms as well as the effect on DBP formation. It was however suggested that a combination of ozone and UV and a low chlorine dose could minimize DBP formation in swimming pool water.

Fungal species in the dermatophyte group have been considered as potential microbial hazards in swimming pool environments. However, the abundance of human pathogenic fungal species in addition to dermatophytes in swimming pool environments could also pose a threat to human health. Other emerging contaminants in swimming pools, such as PPCPs and DBPs, are concerns related to public health. Applying alternative disinfection technology (ozone and UV) combined with chlorination may improve the effectiveness in inactivation of chlorine-resistant pathogens and may affect the formation of DBPs and degradation of PPCPs.

This research focuses on investigating the occurrence of PPCPs and pathogenic fungal species in public swimming pools, and assesses the performance of alternative disinfection technologies, such as ozone and UV, for swimming pool water disinfection in reducing microbial and chemical hazards.

Outline of the thesis

Chapter 1 describes the general knowledge on swimming pool disinfection, and chemical and microbial threats present in swimming pool environments. The performance of a full scale combined ozone and UV system was assessed in an outdoor swimming pool. The effect of this technology on DBPs (e.g. THMs and HAAs) formation and the removal of selected micropollutants (e.g. pharmaceuticals and EDCs) was studied and is described in Chapter 2. Chapter 3 describes the occurrence of PPCPs in different swimming pool settings and their correlation to pool water treatment. The occurrence of fungi, especially clinically relevant fungi, in an indoor swimming pool facility was studied by determining fungal concentrations and the fungal species present on surfaces and in water (Chapter 4). Since clinically relevant fungi appeared to be ubiquitous in indoor swimming pool facilities, their potential transmission pathways were investigated by means of fungal counting and typing in different areas inside swimming pool facilities (Chapter 5). Chapter 6 summarizes and connects the findings from each of the previous chapters and identifies the knowledge gaps for further research.

References

Ali-Shtayeh, M., Khaleel, T.K.M., Jamous, R.M. (2003). Ecology of dermatophytes and other keratinophilic fungi in swimming pools and polluted and unpolluted streams. *Mycopathologia*, 156(3), 193–205.

Aanvullingsbesluit zwemmen of baden in waterbassins Omgevingswet (2018). Ministry of the Interior and Kingdom Relations, Ministry of Infrastructure and Water Management, https://www.internetconsultatie.nl/aanvullingsbesluit_waterbassins_omgevingswet.

Baldursson, S., Karanis, P. (2011). Waterborne transmission of protozoan parasites: Review of worldwide outbreaks – An update 2004–2010. *Water Research*, 45(20), 6603-6614.

Barbot, E., Moulin, P. (2008). Swimming pool water treatment by ultrafiltration–adsorption process. *Journal of Membrane Science*, 314(1), 50–57.

Bedner, M., MacCrehan, W.A. (2006). Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and *N*-acetyl-*p*-benzoquinone imine. *Environmental Science & Technology*, 40(2), 516–522.

Besluit hygiëne en veiligheid badinrichtingen en zwemgelegenheden (2011). Ministerie van Infrastuctuur en Milieu, the Netherlands.

Bottoni, P., Bonadonna, L., Chirico, M., Caroli, S., Záray, G. (2014). Emerging issues on degradation by-products deriving from personal care products and pharmaceuticals during disinfection processes of water used in swimming pools. *Microchemical Journal*, 112, 13–16.

Brandi, G., Sisti, M., Paparini, A., Gianfranceschi, G., Schiavano, G.F., De Santi, M., Santoni, D., Magini, V., Romano-Spica, V. (2007). Swimming pools and fungi: an environmental epidemiology survey in Italian indoor swimming facilities. *International Journal of Environmental Health Research*, 17(3), 197–206.

Brausch, J.M., Rand, G.M. (2011). A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere*, 82(11), 1518–1532.

Buot, G., Toutous-Trellu, L., Hennequin, C. (2010). Swimming pool deck as environmental reservoir of Fusarium. *Medical Mycology*, 48(5), 780–784.

Camel, V., Bermond, A. (1998). The use of ozone and associated oxidation processes in drinking water treatment. *Water Research*, 32(11), 3208–3222.

Carter, R.A.A., Joll, C.A. (2017). Occurrence and formation of disinfection by-products in the swimming pool environment: A critical review. *Journal of Environmental Sciences*, 58, 19–50.

Chen, H., Liu, S., Xu, X.-R., Liu, S.-S., Zhou, G.-J., Sun, K.-F., Zhao, J.-L., Ying, G.-G. (2015). Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Marine Pollution Bulletin*, 90(1), 181–187.

Chin, A., Bérubé, P. R. (2005). Removal of disinfection by-product precursors with ozone-UV advanced oxidation process. *Water Research*, 39(10), 2136–2144.

Chowdhury, S., Alhooshani, K., Karanfil, T. (2014). Disinfection byproducts in swimming pool: Occurrences, implications and future needs. *Water Research*, 53, 68–109.

Cimetiere, N., De Laat, J. (2014). Effects of UV-dechloramination of swimming pool water on the formation of disinfection by-products: A lab-scale study. *Microchemical Journal*, 112, 34–41.

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption (1998). Official Journal of the European Communities 5.12.98, L330/32–L330/54.

Craun, G.F., Calderon, R.L., Craun, M.F. (2005). Outbreaks associated with recreational water in the United States. *International Journal of Environmental Health Research*, 15(4), 243-262.

de Jesus Gaffney, V., Almeida, C.M.M., Rodrigues, A., Ferreira, E., Benoliel, M.J., Cardoso, V.V. (2015). Occurrence of pharmaceuticals in a water supply system and related human health risk assessment. *Water Research*, 72, 199–208.

de Jongh, C.M., Kooij, P.J.F., de Voogt, P., ter Laak, T.L. (2012). Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water. *Science of the Total Environment*, 427–428, 70–77.

Detandt, M., Nolard, N. (1988). Dermatophytes and swimming pools: seasonal fluctuations. *Mycoses*, 31(10), 495–500.

Detandt, M., Nolard, N. (1995). Fungal contamination of the floors of swimming pools, particularly subtropical swimming paradises. *Mycoses*, 38(11-12), 509–513.

Drinkwaterbesluit (2011). Ministerie van Infrastuctuur en Milieu, the Netherlands.

Dziuban, E.J., Liang, J.L., Craun, G.F., Hill, V., Yu, P.A., Painter, J., Moore, M.R., Calderon, R.L., Roy, S.L., Beach, M.J. (2006). Surveillance for waterborne disease and outbreaks associated with recreational water—United States, 2003–2004. *MMWR Surveillance Summaries*, 55(12), 1–30.

Efstratiou, A., Ongerth, J.E., Karanis, P. (2017). Waterborne transmission of protozoan parasites: Review of worldwide outbreaks - An update 2011–2016. *Water Research*, 114, 14–22.

English, M.P., Gibson, M.D. (1959). Studies in the epidemiology of tinea pedis. II. Dermatophytes on the floors of swimming-baths. *British Medical Journal*, 1(5135), 1446–1448.

Fabbri, E., Franzellitti, S. (2016). Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. *Environmental Toxicology and Chemistry*, 35(4), 799–812.

Falconer, I.R., Chapman, H.F., Moore, M.R., Ranmuthugala, G. (2006). Endocrine-disrupting compounds: A review of their challenge to sustainable and safe water supply and water reuse. *Environmental Toxicology*, 21(2), 181–191.

Florentin, A., Hautemanière, A., Hartemann, P. (2011). Health effects of disinfection by-products in chlorinated swimming pools. *International Journal of Hygiene and Environmental Health*, 214(6), 461–469.

Friedman, M.S., Roels, T., Koehler, J.E., Feldman, L., Bibb, W.F., Blake, P. (1999). *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clinical Infectious Diseases*, 29(2), 298–303.

Gentles, J.C., Evans, E.G.V. (1973). Foot infections in swimming baths. *British Medical Journal*, 3(5874), 260–262.

Glassmeyer, S., Shoemaker, J. (2005). Effects of chlorination on the persistence of pharmaceuticals in the environment. *Bulletin of Environmental Contamination and Toxicology*, 74(1), 24–31.

Glauner, T., Waldmann, P., Frimmel, F.H., Zwiener, C. (2005). Swimming pool water—fractionation and genotoxicological characterization of organic constituents. *Water Research*, 39(18), 4494–4502.

Glaze, W.H. (1986). Reaction products of ozone: a review. *Environmental Health Perspectives*, 69, 151.

Glaze, W.H., Kang, J.-W., Chapin, D.H. (1987). The chemistry of water treatment processes involving ozone, hydrogen peroxide and ultraviolet radiation. *Ozone: Science & Engineering*, 9(4), 335–352.

Gottschalk, C., Libra, J.A., Saupe, A. (2010). Ozonation of water and waste water: A practical guide to understanding ozone and its applications, 2nd ed., John Wiley & Sons.

Grigorakis, K., Rigos, G. (2011). Aquaculture effects on environmental and public welfare – The case of Mediterranean mariculture. *Chemosphere*, 85(6), 899–919.

Haag, W.R., Hoigne, J. (1983). Ozonation of bromide-containing waters: Kinetics of formation of hypobromous acid and bromate. *Environmental Science & Technology*, 17(5), 261–267.

Hansen, K.M.S., Spiliotopoulou, A., Cheema, W.A., Andersen, H.R. (2016). Effect of ozonation of swimming pool water on formation of volatile disinfection by-products – A laboratory study. *Chemical Engineering Journal*, 289, 277–285.

Heering, W. (2004). UV sources-basics, properties and applications. *IUVA news*, 6(4), 7–13.

Hilmarsdottir, I., Haraldsson, H., Sigurdardottir, A., Sigurgeirsson, B. (2005). Dermatophytes in a swimming pool facility: Difference in dermatophyte load in men's and women's dressing rooms. *Acta Dermato-Venereologica*, 1(1), 1–2.

Hlavsa, M.C., Cikesh, B.L., Roberts, V.A., Kahler, A.M., Vigar, M., Hilborn, E.D., Wade, T.J., Roellig, D.M., Murphy, J.L., Xiao, L., Yates, K.M., Kunz, J.M., Arduino, M.J., Reddy, S.C., Fullerton, K.E., Cooley, L.A., Beach, M.J., Hill, V.R., Yoder, J.S. (2018).

Outbreaks associated with treated recreational water — United States, 2000–2014. *MMWR. Morbidity and mortality weekly report*, 67, 547–551.

Hlavsa, M.C., Roberts, V.A., Kahler, A.M., Hilborn, E.D., Mecher, T.R., Beach, M.J., Wade, T.J., Yoder, J.S. (2015). Outbreaks of illness associated with recreational water—United States, 2011–2012. *MMWR. Morbidity and mortality weekly report*, 64(24), 668–672.

Hoigné, J., Bader, H. (1976). The role of hydroxyl radical reactions in ozonation processes in aqueous solutions. *Water Research*, 10(5), 377–386.

Insulander, M., Lebbad, M., Stenström, T.A., Svenungsson, B. (2005). An outbreak of cryptosporidiosis associated with exposure to swimming pool water. *Scandinavian Journal of Infectious Diseases*, 37(5), 354–360.

Jankowski, M., Charemska, A., Czajkowski, R. (2017). Swimming pools and fungi: An epidemiology survey in Polish indoor swimming facilities. *Mycoses*, 60(11), 736–738.

Kamihama, T., Kimura, T., Hosokawa, J., Ueji, M., Takase, T., Tagami, K. (1997). Tinea pedis outbreak in swimming pools in Japan. *Public Health*, 111(4), 249–253.

Kanan, A., Karanfil, T. (2011). Formation of disinfection by-products in indoor swimming pool water: The contribution from filling water natural organic matter and swimmer body fluids. *Water Research*, 45(2), 926–932.

Keene, W.E., McAnulty, J.M., Hoesly, F.C., Williams, L.P., Hedberg, K., Oxman, G.L., Barrett, T.J., Pfaller, M.A., Fleming, D.W. (1994). A Swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New England Journal of Medicine*, 331(9), 579–584.

Kim, H., Shim, J., Lee, S. (2002). Formation of disinfection by-products in chlorinated swimming pool water. *Chemosphere*, 46(1), 123–130.

Klavarioti, M., Mantzavinos, D., Kassinos, D. (2009). Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environment International*, 35(2), 402–417.

Kockler, J., Oelgemöller, M., Robertson, S., Glass, B.D. (2012). Photostability of sunscreens. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, 13(1), 91–110.

Korich, D.G., Mead, J.R., Madore, M.S., Sinclair, N.A., Sterling, C.R. (1990). Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology*, 56(5), 1423–1428.

Kumar, A., Chang, B., Xagoraraki, I. (2010). Human health risk assessment of pharmaceuticals in water: Issues and challenges ahead. *International Journal of Environmental Research and Public Health*, 7(11), 3929.

LaKind, J.S., Richardson, S.D., Blount, B.C. (2010). The good, the bad, and the volatile: can we have both healthy pools and healthy people? *Environmental Science & Technology*, 44(9), 3205–3210.

Lee, J., Ha, K.-T., Zoh, K.-D. (2009). Characteristics of trihalomethane (THM) production and associated health risk assessment in swimming pool waters treated with different disinfection methods. *Science of the Total Environment*, 407(6), 1990–1997.

Leoni, E., Catalani, F., Marini, S., Dallolio, L. (2018). Legionellosis associated with recreational waters: A systematic review of cases and outbreaks in swimming pools, spa pools, and similar environments. *International Journal of Environmental Research and Public Health*, 15(8), 1612.

Mahoney, F.J., Farley, T.A., Kelso, K.Y., Wilson, S.A., Horan, J.M., McFarland, L.M. (1992). An outbreak of Hepatitis A associated with swimming in a public pool. *The Journal of Infectious Diseases*, 165(4), 613–618.

Miettinen, I.T., Vartiainen, T., Nissinen, T., Tuhkanen, T., Martikainen, P.J. (1998). Microbial growth in drinking waters treated with ozone, ozone/hydrogen peroxide or chlorine. *Ozone: Science & Engineering*, 20(4), 303–315.

Morris, J.C., 1966. The acid ionization constant of HOCl from 5 to 35°. *The Journal of Physical Chemistry*, 70(12), 3798–3805.

Muellner, M.G., Wagner, E.D., McCalla, K., Richardson, S.D., Woo, Y.-T., Plewa, M.J. (2007). Haloacetonitriles vs. regulated haloacetic acids: Are nitrogen-containing DBPs more toxic? *Environmental Science & Technology*, 41(2), 645–651.

Nakajima, M., Kawakami, T., Niino, T., Takahashi, Y., Onodera, S. (2009). Aquatic fate of sunscreen agents octyl-4-methoxycinnamate and octyl-4-dimethylaminobenzoate in model swimming pools and the mutagenic assays of their chlorination byproducts. *Journal of Health Science*, 55(3), 363–372.

Negreira, N., Canosa, P., Rodríguez, I., Ramil, M., Rubí, E., Cela, R. (2008). Study of some UV filters stability in chlorinated water and identification of halogenated by-products by gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1178(1–2), 206–214.

Nieuwenhuijsen, M.J., Smith, R., Golfinopoulos, S., Best, N., Bennett, J., Aggazzotti, G., Righi, E., Fantuzzi, G., Bucchini, L., Cordier, S., Villanueva, C. M., Moreno, V., Vecchia, C.L., Bosetti, C., Vartiainen, T., Rautiu, R., Toledano, M., Iszatt, N., Grazuleviciene, R., Kogevinas, M. (2009). Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE. *Journal of Water and Health*, 7(2), 185–207.

Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Fawell, J., Elliott, P. (2000). Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review. *Occupational and Environmental Medicine*, 57(2), 73–85.

Noguera-Oviedo, K., Aga, D.S., 2016. Lessons learned from more than two decades of research on emerging contaminants in the environment. *Journal of Hazardous Materials*, 316, 242–251.

Papadopoulou, C., Economou, V., Sakkas, H., Gousia, P., Giannakopoulos, X., Dontorou, C., Filioussis, G., Gessouli, H., Karanis, P., Leveidiotou, S. (2008). Microbiological

quality of indoor and outdoor swimming pools in Greece: Investigation of the antibiotic resistance of the bacterial isolates. *International Journal of Hygiene and Environmental Health*, 211(3–4), 385–397.

Pereira, V.J., Marques, R., Marques, M., Benoliel, M. J., Barreto Crespo, M.T. (2013). Free chlorine inactivation of fungi in drinking water sources. *Water Research*, 47(2), 517–523.

Real Decreto 742/2013, de 27 de septiembre, por el que se establecen los criterios técnico-sanitarios de las piscinas, vol 244 (2013). Ministerio de Sanidad, Servicios Sociales e Igualdad.

Reckhow, D.A., Linden, K.G., Kim, J., Shemer, H., Makdissy, G. (2010). Effect of UV treatment on DBP formation. *Journal of the American Water Works Association*, 102(6), 100–113.

Requisiti degli impianti di circolazione, trattamento, disinfezione e qualità dell'acqua di piscina (2016). UNI 10637:2016. UNI - Ente Nazionale Italiano di Unificazione, Italy.

Richardson, S.D., DeMarini, D.M., Kogevinas, M., Fernandez, P., Marco, E., Lourencetti, C., Ballesté, C., Heederik, D., Meliefste, K., McKague, A.B., Marcos, R., Font-Ribera, L., Grimalt, J.O., Villanueva, C.M. (2010). What's in the pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. *Environmental Health Perspectives*, 118(11), 1523–1530

Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., DeMarini, D.M. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Research/Reviews in Mutation Research*, 636(1–3), 178–242.

Richardson, S.D., Ternes, T.A. (2011). Water analysis: emerging contaminants and current issues. *Analytical Chemistry*, 83(12), 4614–4648.

Rosenzweig, W., Minnigh, H., Pipes, W. (1983). Chlorine demand and inactivation of fungal propagules. *Applied and Environmental Microbiology*, 45(1), 182–186.

Sakkas, V.A., Giokas, D.L., Lambropoulou, D.A., Albanis, T.A. (2003). Aqueous photolysis of the sunscreen agent octyl-dimethyl-*p*-aminobenzoic acid: Formation of disinfection byproducts in chlorinated swimming pool water. *Journal of Chromatography A*, 1016(2), 211–222.

Santos, A.J.M., Miranda, M.S., Esteves da Silva, J.C.G. (2012). The degradation products of UV filters in aqueous and chlorinated aqueous solutions. *Water Research*, 46(10), 3167–3176.

Schalk, S., Adam, V., Arnold, E., Brieden, K., Voronov, A., Witzke, H. (2006). UV-lamps for disinfection and advanced oxidation–Lamp types, technologies and applications. *IUVA news*, 8(1), 32–37.

Shemer, A., Gupta, A.K., Amichai, B., Baum, S., Barzilai, A., Farhi, R., Kaplan, Y., MacLeod, M.A. (2016). Increased risk of tinea pedis and onychomycosis among swimming pool employees in Netanya Area, Israel. *Mycopathologia*, 181(11), 1–6.

Shen, R., Andrews, S.A. (2011). Demonstration of 20 pharmaceuticals and personal care products (PPCPs) as nitrosamine precursors during chloramine disinfection. *Water Research*, 45(2), 944–952.

Soltermann, F., Lee, M., Canonica, S., von Gunten, U. (2013). Enhanced N-nitrosamine formation in pool water by UV irradiation of chlorinated secondary amines in the presence of monochloramine. Water Research, 47(1), 79–90.

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B. (2004). Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of the Total Environment*, 329(1), 99–113.

Teo, T.L.L., Coleman, H.M., Khan, S.J. (2015). Chemical contaminants in swimming pools: Occurrence, implications and control. *Environment International*, 76(0), 16–31.

Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.-J., Haist-Gulde, B., Preuss, G., Wilme, U., Zulei-Seibert, N. (2002). Removal of pharmaceuticals during drinking water treatment. *Environmental Science & Technology*, 36(17), 3855–3863.

U.S. EPA (1999). Alternative disinfectants and oxidants guidance manual. Washington DC., U.S. Environmental Protection Agency.

U.S. EPA (2018). 2018 Edition of Drinking Water Standards and Health Advisories. Washington, DC, U.S. Environmental Protection Agency.

van der Kooij, D., Hijnen, W.A.M., Kruithof, J.C. (1989). The effects of ozonation, biological filtration and distribution on the concentration of easily Assimilable Organic Carbon (AOC) in drinking water. *Ozone: Science & Engineering*, 11(3), 297–311.

Verordnung des Bundesministers für Gesundheit über Hygiene in Bädern, Warmsprudelwannen (Whirlwannen), Saunaanlagen, Warmluft- und Dampfbädern und Kleinbadeteichen (2012). Bundesministerium für Gesundheit, Austria.

Viegas, C., Alves, C., Carolino, E., Pinheiro, C., Rosado, L., Santos, C.S. (2011). Assessment of fungal contamination in a group of Lisbon's gymnasiums with a swimming pool. *Italian Journal of Occupational and Environmental Hygiene*, 2(1), 15–20.

Villanueva, C.M., Cantor, K.P., Grimalt, J.O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G. (2007). Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *American Journal of Epidemiology*, 165(2), 148–156.

Villanueva, C.M., Cordier, S., Font-Ribera, L., Salas, L.A., Levallois, P. (2015). Overview of disinfection by-products and associated health effects. *Current Environmental Health Reports*, 2(1), 107–115.

Walse, S.S., Mitch, W.A. (2008). Nitrosamine carcinogens also swim in chlorinated pools. *Environmental Science & Technology*, 42(4), 1032–1037.

Wang, W., Qian, Y., Boyd, J.M., Wu, M., Hrudey, S.E., Li, X.-F. (2013). Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environmental Science & Technology*, 47(7), 3275–3282.

Weng, S., Li, J., Blatchley III, E.R. (2012). Effects of UV_{254} irradiation on residual chlorine and DBPs in chlorination of model organic-N precursors in swimming pools. *Water Research*, 46(8), 2674–2682.

Weng, S., Sun, P., Ben, W., Huang, C.-H., Lee, L.T., Blatchley III, E.R. (2014). The presence of pharmaceuticals and personal care products in swimming pools. *Environmental Science & Technology Letters*, 1(12), 495–498.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005). Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environmental Science & Technology*, 39(17), 6649–6663.

WHO (2000). Environmental Health Criteria 216: Disinfectants and disinfectant by-products. Geneva, World Health Organization.

WHO (2006). Guidelines for safe recreational water environments, Vol. 2: Swimming pools and similar environments. Geneva, World Health Organization.

WHO (2011). Guidelines for drinking-water quality. 4th edition. Geneva, World Health Organization.

Yang, Y., Ok, Y.S., Kim, K.-H., Kwon, E.E., Tsang, Y.F. (2017). Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. *Science of the Total Environment*, 596–597, 303–320.

Yoder, J.S., Blackburn, B.G., Craun, G.F., Hill, V., Levy, D.A., Chen, N., Lee, S.H., Calderon, R.L., Beach, M.J. (2004). Surveillance for waterborne-disease outbreaks associated with recreational water—United States, 2001–2002. *MMWR Surveillance Summaries*, 53(8), 1–22.

Yoder, J.S., Hlavsa, M.C., Craun, G.F., Hill, V., Roberts, V., Yu, P.A., Hicks, L.A., Alexander, N.T., Calderon, R.L., Roy, S.L., Beach, M.J. (2008). Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events–United States, 2005–2006. *MMWR Surveillance Summaries*, 57(9), 1–29.

Zwiener, C., Richardson, S.D., De Marini, D.M., Grummt, T., Glauner, T., Frimmel, F.H. (2007). Drowning in disinfection byproducts? Assessing swimming pool water. *Environmental Science & Technology*, 41(2), 363–372.

2

Application of UVOX Redox® for swimming pool water treatment: Microbial inactivation, disinfection byproduct formation and micropollutant removal

Ekowati, Y., Ferrero, G., Farré, M.J., Kennedy, M.D., Buttiglieri, G. (2019). Application of UVOX Redox® for swimming pool water treatment: microbial inactivation, disinfection byproduct formation and micropollutant removal. *Chemosphere*, 220, 176-184.

Abstract

Alternative disinfection technologies may overcome some of the limitations of conventional treatment applied in swimming pools: chlorine-resistant pathogens (e.g. *Cryptosporidium* oocysts and *Giardia* cysts) and the formation of chlorinated disinfection byproducts. In this paper, results of full scale validation of an alternative disinfection technology UVOX Redox® (hereinafter referred to as UVOX) that combines ozonation and UV irradiation are presented. The performance was assessed in terms of microbial inactivation, disinfection byproduct formation and micropollutant removal. UVOX was able to achieve 1.4 – 2.7 log inactivation of *Bacillus subtilis* spores at water flows between 20 to 76 m^3/h. Lower formation of trichloromethane and dichloroacetic acid was observed with UVOX followed by chlorination when compared to chlorination alone. However, due to the use of ozone and the presence of bromide in the pool water, the formation of trihalomethanes and haloacetic acids shifted to more brominated byproducts. Chlorine alone was able to remove the target micropollutants: acetaminophen, atenolol, caffeine, carbamazepine, estrone, estradiol, and venlafaxine (>97% removal) after 24 h, with the exception of ibuprofen (60% removal). The application of UVOX in chlorinated water enhanced the removal of ibuprofen. The application of UVOX could lower the usage of chlorine to the level that provides an adequate residual disinfection effect.

Introduction

The main components of a typical water treatment scheme in swimming pools consist of coagulation-flocculation followed by filtration and disinfection (WHO, 2006). Disinfection is applied to ensure microbial inactivation, and the presence of residual disinfectant provides protection against subsequent microbial contamination. Chlorination is widely used for disinfection in swimming pools due to its efficacy against a wide range of pathogens, its availability and cost effectiveness. Chlorine has strong disinfecting power and provides residual disinfectant effects, but some pathogens have been proven to be resistant to chlorine, e.g. the faecally-derived protozoa, Giardia lamblia cysts and Cryptosporidium parvum oocysts (Jarroll et al., 1981, Korich et al., 1990). Additionally, chlorine reacts with organic and inorganic constituents in water to form disinfection byproducts (DBPs) which are associated with adverse health effects (Florentin et al., 2011, Richardson et al., 2010, Xiao et al., 2012, Vlaanderen et al., 2017).

UV irradiation and ozonation have been utilized for decades in drinking water and wastewater treatment (Betancourt and Rose, 2004, Bolton and Linden, 2003, U.S. EPA, 1999). UV irradiation has proven to be effective against chlorine resistant pathogens (Clancy et al., 2000, Craik et al., 2000, Craik et al., 2001, Shin et al., 2001). Significant inactivation of *Cryptosporidium* oocysts and *Giardia* cysts was observed at UV fluence <20 mJ/cm^2 (Chevrefils et al., 2006, Hijnen et al., 2006). Ozonation is also a stronger disinfectant than chlorine (Korich et al., 1990). Studies have shown that 99% inactivation of *Cryptosporidium* oocysts and *Giardia* cysts can be achieved at CT (concentration x contact time) values <10 mg.min/L and <1 mg.min/L at 20 °C pH 7.0, respectively (Jacangelo et al., 2002, Rennecker et al., 1999). Moreover, ozone improves the inactivation of *Cryptosporidium* oocysts in a sequential use with chlorination (Corona-Vasquez et al., 2002, Driedger et al., 2000, Rennecker et al., 2000). In swimming pool water treatment, ozone and UV could be applied to lower the dependency on chlorination as a primary disinfectant.

The presence of DBPs has been a major concern in disinfected waters. Some DBPs are carcinogenic and mutagenic, and their presence has been associated with asthma, irritation to skin, throat, and eyes (Richardson et al., 2010). The routes of exposure to DBPs are not only through ingestion but also through inhalation and dermal absorption (Villanueva et al., 2007). Sources of DBP precursors in swimming pools are organic materials derived from humans (e.g. urine, sweat) and from the water source itself (Kanan and Karanfil, 2011). Consequently, continuous addition of disinfectant (e.g. chlorine) in the presence of organic loads may lead to high levels of DBPs (Yeh et al., 2014). While more than 700 DBPs have already been identified in disinfected waters, trihalomethanes (THMs) and haloacetic acids (HAAs) are still the most prominent DBPs investigated in swimming pools (Teo et al., 2015). THMs and HAAs are commonly

detected in chlorinated waters (Nieuwenhuijsen et al., 2000) while brominated DBPs are also detected during ozonation (Glaze, 1986).

The effect of ozone and/or UV on DBP formation in swimming pool applications has been demonstrated in many studies (Cheema et al., 2017, Cimetiere and De Laat, 2014, Hansen et al., 2013, 2016, Mao et al., 2014, Spiliotopoulou et al., 2015). Hansen et al. (2016) showed that ozone followed by chlorination resulted in lower THMs formation when compared to chlorination alone. Mao et al. (2014) showed an increase in THM concentrations with the increase in ozone dose from 0 to 2 mg/L during subsequent chlorination. Cimetiere and De Laat (2014) demonstrated an increase in THMs formation and little effect on HAAs by applying high UV doses using low-pressure UV in chlorinated water, while no effect on THMs was observed using medium-pressure UV with or without free chlorine (Spiliotopoulou et al., 2015). Some studies in real swimming pools investigated the effect of UV followed by chlorination, but the results were contradictory; in fact, this resulted in an increase of THMs (Cassan et al., 2006), a decrease of THMs (Beyer et al., 2004), and no effect (Kristensen et al., 2009). Weng et al. (2012) observed higher formation of dichloroacetonitrile and cyanogen chloride by using increasing UV dose, while no change was observed in chloroform levels.

Nevertheless, DBPs are not the only concern in swimming pools. Organic micropollutants (at low concentrations, in the range of µg/L or ng/L) of anthropogenic source, such as pharmaceuticals and endocrine disrupting compounds (EDCs) are of concerns due to adverse health effects to wildlife and humans (Richardson and Ternes, 2011). These compounds can enter swimming pools through human body fluids (e.g. urine and sweat) and/or human skin during swimming (some lotions may contain pharmaceuticals). Several commonly used pharmaceuticals, such as acetaminophen, carbamazepine and ibuprofen, have been detected in different swimming pool settings (Ekowati et al., 2016, Suppes et al., 2017, Teo et al., 2016, Weng et al., 2014), while EDCs (e.g. estrone, estradiol) have been mainly investigated in environmental waters (Campbell et al., 2006, Li et al., 2015, Petrovic et al., 2004, Yoon et al., 2010). The European Commission has placed estrone and estradiol on the watch list of substances for Union-wide monitoring in the field of water policy (European Commission, 2018). Pharmaceuticals, such as carbamazepine and venlafaxine, were included in the 12 indicator substances established by Swiss Federal Office of the Environment (FOEN, 2015) for evaluation of upgrading wastewater treatment plants.

Studies have shown that some micropollutants can be either persistent or susceptible to water treatment. Acetaminophen, estrone and estradiol were easily degraded by chlorination or ozone (Weng et al., 2014, Westerhoff et al., 2005). The application of chlorine and UV separately showed only <10% removal of ibuprofen and carbamazepine while the combination of treatments improved the removal to 98% (Wang et al., 2016, Xiang et al., 2016).

Microbial contamination may continuously occur in swimming pools, hence chlorine is still needed to provide residual disinfection in the pool water despite the formation of chlorine-derived DBPs. The purpose of applying alternative disinfection strategies is to have microbially safe pool water while lowering the chlorine dosage and eventually minimizing the formation of chlorinated DBPs. This study was performed employing a full scale commercial ozone/UV system in an outdoor swimming pool of a large tourist facility; having the opportunity to observe the overall system performance influenced by hydraulic conditions, longer treatment duration, water recirculation, and continuous disinfection. The goal of this research was to assess the efficiency of the ozone/UV technology in inactivating chlorine resistant pathogens. Sequential application of ozone/UV and chlorine was assessed by investigating the effect of their combination on the formation of DBPs and the removal of several organic micropollutants.

Materials and methods

Challenge microorganisms

Bacillus subtilis spores have been used as a surrogate for *Cryptosporidium parvum* oocysts in many disinfection studies (Cho et al., 2006, Choi et al., 2007, Driedger et al., 2001, Jung et al., 2008) because of their similar behaviour with respect to disinfection methods such as chlorination, UV and ozonation. *B. subtilis* spores are used as a biodosimeter (a UV calibrated microorganism) for UV reactor validation (Sommer et al., 2004).

UV-253.7 nm calibrated *Bacillus subtilis* spores ATCC 6633 was purchased from HAI-SO GmbH, Austria. The inactivation curve of the spores according to Austrian National Standard ÖNORM M 5873-1:2001 and M 5873-2:2003 was provided by the supplier (Annex Figure S1). The freeze dried spores were suspended in sterile deionized water for 24 h prior to use. The enumeration of the spores was done by using the pour plate method with Columbia blood agar base (Oxoid). The plates were incubated at 37 °C for 44 ± 4 h.

Organic carbon content

Body fluid analogue (BFA) was added to pool water to simulate the human body fluid released in swimming pool water. BFA used for the experiments consists of urea (14.80 g/L), creatinine (1.80 g/L), histidine (1.21 g/L), hippuric acid (1.71 g/L), uric acid (0.49 g/L), citric acid 0.64 g/L), sodium phosphate (4.30 g/L) and ammonium chloride (2.00 g/L) (Judd and Bullock, 2003). All compounds were purchased from Sigma-Aldrich. The concentration of total organic carbon (TOC) in water samples was measured using a TOC-VCSH (Shimadzu) instrument.

Micropollutants

All compounds: acetaminophen, atenolol, carbamazepine, ibuprofen, venlafaxine hydrochloride, estrone, estradiol, and caffeine were purchased from Sigma-Aldrich. Isotopically labelled compounds were used as internal standards; acetaminophen-d_4, ibuprofen-d_3, and caffeine-d_3, were purchased from Sigma-Aldrich, atenolol-d_7 from Toronto Research Chemicals, carbamazepine-d_{10}, venlafaxine-d_6, estradiol-17β-2,4-d_2, and estrone-2,4,16,16-d_4 from Cluzeau Info Labo. The details about the compounds are described in Table 1.

Table 1. Selected pharmaceuticals and endocrine disrupting compounds

Compounds	Description	CAS number	Internal standard	Recovery (%)	LOD (ng/L)	LOQ (ng/L)
Acetaminophen	Analgesics/anti-inflammatories	103-90-2	Acetaminophen-d_4	128.0 ± 0.7	5.8	19
Atenolol	β-Blocking agents	29122-68-7	Atenolol-d_7	99.0 ± 2.3	6.6	22
Carbamazepine	Psychiatric drugs	198-46-4	Carbamazepine-d_{10}	83.0 ± 1.3	8.2	27
Ibuprofen	Analgesics/anti-inflammatories	15687-27-1	Ibuprofen-d_3	94.0 ± 26.7	4.3	14
Venlafaxine	Psychiatric drugs	99300-78-4	Venlafaxine-d_6	168.0 ± 14.4	43	142
Caffeine	Endocrine disruptors	58-08-2	Caffeine-d_3	103.9 ± 6.3	0.22	0.73
β-Estradiol	Endocrine disruptors	50-28-2	Estradiol-17β-2,4-d_2	100.7 ± 5.9	2.07	6.09
Estrone	Endocrine disruptors	53-16-7	Estrone-2,4,16,16-d_4	94.5 ± 3.4	0.18	0.61

HPLC-grade methanol, acetonitrile, water (LiChrosolv), ammonium acetate, formic acid and Na_2EDTA were purchased from Merck (Germany). Nitrogen (99.995% purity) for drying was obtained from Abelló Linde (Spain). All the samples collected during the experiment were filtered through 0.45 µm polyvinylidene fluoride membrane filters (Merck Millipore, Germany).

Analysis of pharmaceuticals

In each sample for pharmaceutical analyses, a suitable volume of 0.1 M Na_2EDTA solution was added to achieve a final concentration of 0.1% (g solute/g solution). Samples were pre-concentrated using Oasis HLB cartridges (60 mg, 3 mL) (Waters Corp., Milford, MA, USA) which were conditioned with 5 mL of methanol followed by 5 mL of HPLC grade water. 25 mL of sample was loaded to the cartridge at 1 mL/min. After sample pre-concentration, cartridges were rinsed with 6 mL of HPLC grade water

and were dried with air for 5 min for total water removal. Analytes were eluted with 6 mL of pure methanol. Extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL of methanol. 10 µL of extract were diluted in 1 mL of methanol/water. Finally, 10 µL of an internal standard mixture (at 1 mg/L) was added to the extract. Samples were further analysed using Ultra-PerformanceTM liquid chromatograph system (Waters Corp. Milford, MA, USA) coupled to a quadrupole-linear hybrid ion trap mass spectrometer (5500 QTRAP, Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Chromatographic separation was carried out using Acquity HSS T_3 column (for positive electrospray ionization) and Acquity BEH C_{18} column (for negative electrospray ionization) (Waters Corp. Milford, MA, USA). The detailed procedure of the analysis was as described by Gros et al. (2012). The recovery of the analytes is shown in Table 1.

Analysis of EDCs

All samples were filtered through 0.45 µm polyvinylidene fluoride membrane filters. Samples were extracted using Strata-X cartridges (200 mg, 6 mL) (Phenomenex) which were conditioned with 5 mL of methanol followed by 5 mL of HPLC grade water. Samples (50 mL) were loaded at 1 mL/min. Finally, cartridges were dried under vacuum conditions in order to carry out the elution with a mixture of dichloromethane:methanol (50:50). The extract was reconstituted with 1 mL of methanol:water and 50 µL of internal standard was added. Samples were analysed by an Ultra Performance Liquid Chromatography (Thermo Fisher Scientific) system coupled to a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher Scientific). Chromatographic separation was carried out using a LUNA OMEGA C_{18} 1.6 µm (100 × 2.1 mm) (Phenomenex) column. The detailed procedure of the analysis was described by Gorga et al. (2013) and Kassotaki et al. (2019). The recovery of the analytes is shown in Table 1.

Analysis of DBPs

Water samples were analysed for THMs, HAAs and bromate content. Four THMs (trichloromethane (TCM), dibromochloromethane (DBCM), bromodichloromethane (BDCM), tribromomethane (TBM)) and bromate levels were analysed by Water Technology Center (TZW), Karlsruhe, Germany using headspace GC/MS (DIN EN ISO 10301) and IC-ICP-MS, respectively. Nine HAAs (monochloroacetic (MCAA), dichloroacetic (DCAA), trichloroacetic (TCAA), monobromoacetic (MBAA), dibromoacetic (DBAA), tribromoacetic (TBAA) bromochloroacetic (BCAA), bromodichloroacetic (BDCAA), chlorodibromoacetic (CDBAA) acids) were analysed by LEITAT Technological Center in Barcelona, Spain based on the US EPA Method 552.2 (USEPA, 1995). The relative standard deviation of DBP analytical methods is shown in Table 2.

Table 2. Limit of quantification and relative standard deviation of measurements

Compounds	LOQ (µg/L)	Measurement RSD[a] (%)	Compounds	LOQ (µg/L)	Measurement RSD[b] (%)
TCM	0.3	14.9	MCAA	66	17
BDCM	0.1	14.1	DCAA	3	16
DBCM	0.1	12.1	TCAA	3	11
TBM	0.1	14.9	MBAA	75	12
			DBAA	3	8.8
Bromide	10	7.8	TBAA	75	14
Bromate	1	11.3	BCAA	3	16
			BDCAA	33	8.1
			CDBAA	33	12

[a] Relative standard deviation taken from uncertainty of measurement provided by Water Technology Center (TZW)

[b] Relative standard deviation for replicated samples according to Method 552.2 EPA

Other parameters

Temperature, pH, free and total chlorine were measured on site, each time a sample was collected. Chlorine concentration was measured using Hach cuvette LCK310 and Hach spectrophotometer DR2800 (Hach Lange GmbH, Germany). Temperature and pH were measured using a portable probe (pH meter 3310 from WTW). The ozone concentration was measured using Hach Ozone AccuVac® (MR) ampules and a portable Hach colorimeter DR890.

Study site and equipment

The commercial ozone/UV system was installed in an outdoor swimming pool in a large Mediterranean tourist facility. The swimming pool is composed of two connecting pools, the main pool has a volume of 560 m³ and the children's pool has a volume of 10 m³. The conventional treatment system in the pool consists of two units of rapid sand filtration and chlorination (15% sodium hypochlorite solution is dosed into the recirculated pool water using a membrane dosing pump). The ozone/UV system was placed between the sand filters and the chlorination step (Figure 1).

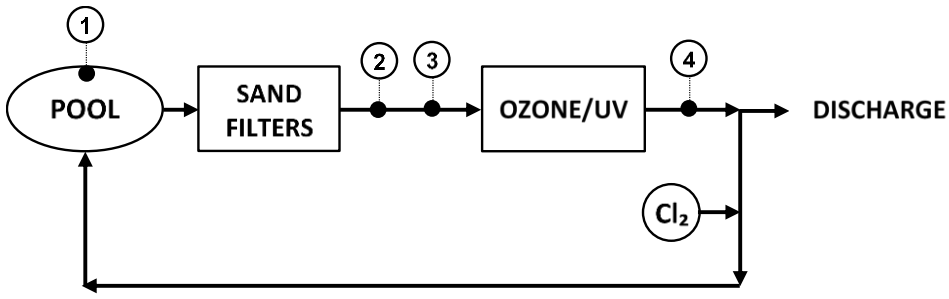

Figure 1. Schematic of pool water treatment. Point 2: dosing point in microbial inactivation experiments; point 4: dosing point in DBP and micropollutant experiments; point 1, 3 and 4: sampling points.

The ozone/UV system was provided by Wapure International GmbH, Germany (UVOX Redox® type UVOX-2000 from here onwards referred to as UVOX). The system is composed of four amalgam low-pressure UV lamps (each 180 W), positioned vertically, each housed in a quartz sleeve located inside a cylindrical reaction chamber (HDPE, dia. 20 cm, net vol. 22 L). The reaction chamber is equipped with a temperature sensor and a UV sensor (D-SiCONORM-LP). The UVOX technology combines ozone generation and UV irradiation in a single unit system. Air suction is created by means of a Venturi and the sucked air travels through the UV reaction chamber (inside the quartz sleeves) where ozone is generated by conversion of oxygen in the air when exposed to UV irradiation at 185 nm. The mixture of water and air flows into the UV reaction chamber, where it is exposed to UV irradiation at 254 nm.

Before starting the experiments, baseline measurements on the UVOX were performed after the pool was filled with municipal tap water. The maximum UV irradiance was 17.47 mW/cm^2. The mixture of ozone and air flow was fixed at 16 L/min in all experiments and the dissolved ozone concentrations in the water before entering the UV chamber varied between 0.01 mg/L and 0.46 mg/L. UV irradiance was monitored during the experiments. The UV doses (IT) reported were calculated as the UV irradiance (I) measured by the UV sensor during the experiment multiplied by the contact time (T).

Experimental design

Microbial inactivation

A stock solution containing *B. subtilis* spores (~10^7 CFU/mL) and BFA was spiked into the water flow using a dosing pump to achieve a final concentration ~10^4 CFU/mL in the feed water to the UVOX system (point 2 in Figure 1). Different concentrations of BFA were added to vary the UV transmittance (UVT) of the model water. Water samples for microbial analysis were collected in 50 mL sterile plastic cups from the inlet (point 3 in

Figure 1) and outlet (point 4 in Figure 1) of the UVOX system. Before starting the experiments, a blank experiment was performed with the UVOX system turned off. The purpose was to observe the effect of hydraulic conditions on the viability of *B. subtilis* spores. At the time of this part of the study, pool water temperature was 12–13 °C and pH was 8.3.

The experiments for microbial inactivation were carried out when the pool was closed to the public. For safety reasons, the experiments comprised one pass through the UVOX system and treated water was discharged into the grey water collection tank, instead of being recirculated into the pool.

No chlorine was present nor was added to the water during the experiments since the primary objective of these experiments was to test the microbial inactivation of UVOX alone.

DBPs and micropollutants

Experiments designed to assess DBP formation and removal of micropollutants were performed in a recirculating system. The swimming pool was filled up to 30% of its total volume (approx. 170 m^3) in order to have a turn over time of 2.8–3 h, following the UVOX manufacturer's recommendation. The flow rate of water in all experiments was 56.7 ± 0.5 m^3/h which was the maximum flow of the water recirculation system. Water temperature ranged from 10.5–12.5 °C and pH 8.0 ± 0.2. Experiments were performed off season (end of January) because carrying out these experiments during peak season with the pool open to the public was not feasible.

Experiments were performed with existing pool water treatment (sand filtration and chlorination) with and without UVOX. Sodium hypochlorite solution (15%) was continuously dosed into the water at a constant rate (1000 mL/h) aiming at initial free chlorine concentration of 0.3 $mgCl_2/L$ (lower limit of free residual chlorine according German standard of water quality in swimming pools (DIN, 2012)). Before starting the experiments with chlorine, some tests were performed (without chlorination) to check the effects of sand filtration alone and sand filtration with UVOX on removal of micropollutants. The experiments were conducted sequentially without pool water renewal.

At the start of each experiment, one litre of BFA stock solution (~170 g/L) was added to the pool water in order to achieve a final concentration of 1 mgC/L of BFA in the pool. A mixture of pharmaceuticals and EDCs (Table 1) in 150 mL of methanol was added to the pool to achieve a final concentration of ~5 µg/L and ~1 µg/L, respectively. Both BFA and micropollutants were added to the pool by injecting concentrated solutions into the circulation pipe (point 4 in Figure 1). After introducing BFA and micropollutants to the pool water, 3 h of mixing was allowed before starting the 24 h experiment. During the

mixing period, the sand filters were bypassed to ensure proper mixing of added compounds. The sand filters were then re-connected before the start of the experiment.

Water samples from the pool (point 1 in Figure 1) were collected in all experiments while samples from the inlet (point 3 in Figure 1) and outlet (point 4 in Figure 1) of the UVOX system were only collected during the experiments with UVOX (with and without chlorination). Hourly samples were collected during mixing, afterwards samples were taken after 3, 6, and 24 h during the main experiments. Samples for micropollutant analysis were taken every 30 min in the first 2 h from the start of the experiment. Water samples for micropollutant analysis were collected using 100 mL amber PET bottles. Immediately after sample collection, the water samples for pharmaceutical analyses were quenched by adding 1 mL ascorbic acid (25 mg/L). Separately, water samples for DBP analysis were collected in 250 mL brown glass bottles with no headspace and were kept at 4 °C. Samples for THM analysis were quenched by adding 1 mL of sodium thiosulfate (20 mg/L) and samples for HAA analyses were quenched by adding 25 mg of NH_4Cl in 250 mL sampling bottles.

Results and discussion

Inactivation of *Bacillus subtilis* spores

The dose response curve (Figure 2) shows the inactivation of *B. subtilis* spores under different conditions; the curves are compared on the basis of the UV dose applied. The UV disinfection curve shows an increase in removal from 0.9 log at IT of 5.8 mJ/cm² up to 1.3 log at 24 mJ/cm², and above this UV dose, the inactivation of *B. subtilis* spores was not optimum. This might be due to the hydraulic conditions as a consequence of lower flow in the reaction chamber. Consequently, the inactivation of *B. subtilis* spores was not optimum at UV doses higher than 24 mJ/cm². The UVOX disinfection curve showed enhanced disinfection of *B. subtilis* spores (39–99%) (Figure 2). The increase of log removal was more substantial at higher UV doses as a consequence of applying a constant flow of ozone and air mixture in the system which leads to a higher ozone dose at lower water flow rates. The experiments using UVOX demonstrated that the addition of ozone significantly enhanced the overall inactivation of *B. subtilis* spores compared to applying low-pressure UV alone. This result is in agreement with previous studies demonstrating synergistic effects of combined UVOX and sequential use of ozone followed by UV on inactivation of microorganisms (*E. coli*, MS2, *B. subtilis* spores, total and faecal coliforms) (Bustos et al., 2010, Fang et al., 2014, Jung et al., 2008, Wu et al., 2015).

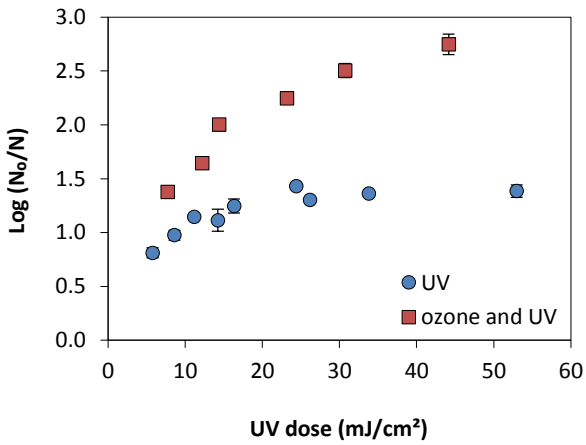

Figure 2. *Bacillus subtilis* spores inactivation by UVOX. UV is UVOX without ozone generation. Error bars indicate the standard deviations of triplicate samples.

Pool Water Treatment and Advisory Group (PWTAG) recommends the UV dose for UV installations in swimming pools that the system should be able to deliver minimum 3 log inactivation in the viability of *Cryptosporidium parvum* oocysts (PWTAG, 2016). The UV dose required for 3 log inactivation of *C. parvum* oocysts is <20 mJ/cm² (Hijnen et al., 2006). According to the biodosimetry test performed (Cabaj et al., 1996), the actual UV dose of 20 mJ/cm² for inactivation *C. parvum* oocysts is equivalent to UV dose of <10 mJ/cm² from UVOX which can be achieved at the maximum water flow rate tested (Annex Figures S2 and S3).

Disinfection byproducts and micropollutants experiments

Chlorine was added at a constant rate during the experiments and BFA was only added at the beginning of each experiment. A gradual increase of free chlorine levels was observed and the increase was more evident between 6 and 24 h of experiment (Figure 3). This increase might be due to the absence of photolysis of chlorine during the night (Nowell and Hoigné, 1992).

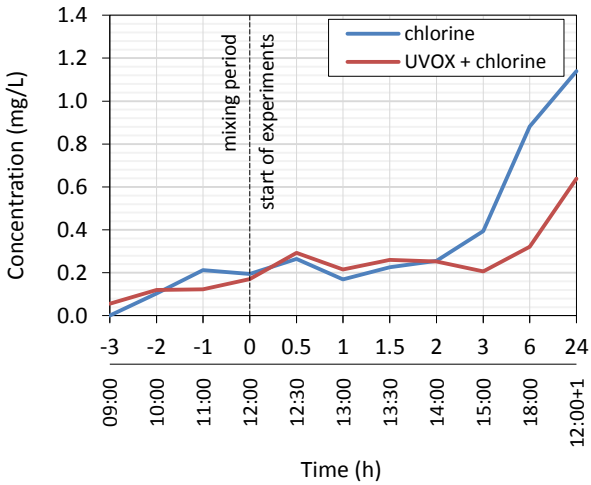

Figure 3. Free chlorine concentrations during the experiments with and without UVOX.

The experiments were conducted in series and the water was reused hence higher organic carbon concentration was observed in the later experiments.

Disinfection byproducts

An increase in THM formation was observed within 24 h in both experiments, applying UVOX followed by chlorination and only chlorination (Figure 4). As expected, the increase of THM concentrations observed followed the order TCM > BDCM > DBCM > TBM as reported in other studies (Cassan et al., 2006, Lourencetti et al., 2012, Panyakapo et al., 2008, Weaver et al., 2009) and total THM (TTHM) was highly dominated by TCM. During UVOX followed by chlorination, TCM, BDCM and DBCM were formed at similar concentrations. The increase of TBM formation was negligible in both experiments.

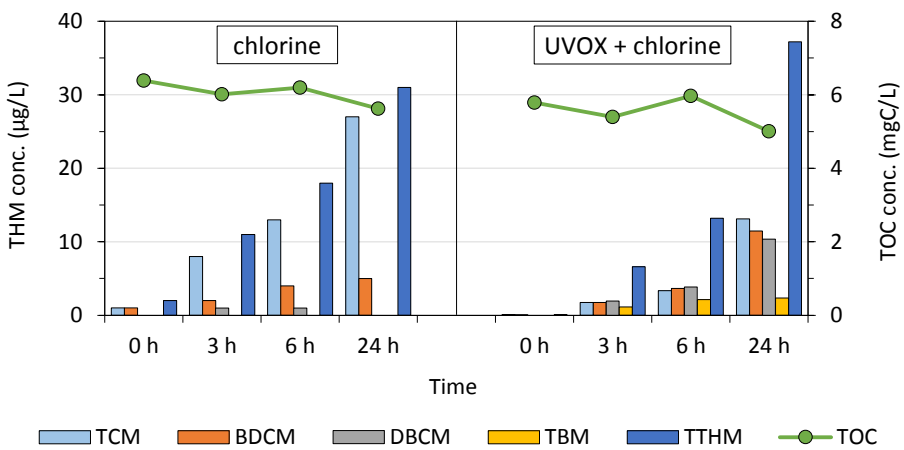

Figure 4. Increase of THMs in pool water treated with chlorination and UVOX followed chlorination.

Bromine incorporation factors (BIF) of THMs at sampling period 0, 3, 6, and 24 h with chlorine only were 0.422, 0.260, 0.246, and 0.260, respectively. Higher BIFs from experiments with UVOX (0.154, 1.098, 1.097, and 0.825, respectively) were observed after 3 h of experiment. Higher BIF values suggest that the formation of DBPs shifted to the brominated species which is attributed to addition of ozone (Mao et al., 2014). In the presence of bromide, ozone and chlorine in the form of $HOCl/OCl^-$ oxidized bromide to aqueous bromine ($HOBr/OBr^-$) (Farkas et al., 1949, Haag and Hoigne, 1983) which react 10 times faster than chlorine with organic matter (Westerhoff et al., 2004). Consequently, the addition of ozone may have shifted the formation of DBPs to more brominated species. The results show that TCM concentrations during experiments with chlorination alone were observed 2–4 times higher than in experiments with UVOX followed by chlorination. On the other hand, higher formations of brominated species (BDCM and DBCM) were observed in experiments with UVOX followed by chlorination.

Additionally, bromide was already present in the filling water for the pool. During the experiment with chlorination only and UVOX followed by chlorination, initial bromide concentrations were 76 µg/L and 160 µg/L, respectively due to decreasing bromide concentration during the period of experiments. As a result, the bromide concentration at the start of the experiment with chlorination alone was half of the initial concentration in the experiment with UVOX. Since an increasing bromide concentration resulted in higher formation of TTHM (Bond et al., 2014, Chowdhury et al., 2010), higher initial concentration of bromide might also contribute in higher THMs formation in experiment with UVOX, particularly for brominated DBPs.

From the nine HAAs analysed, only DCAA, DBAA and BCAA were detected at concentrations above the limit of quantification (Annex Table S1) and the only three HAA species with noticeable changes in concentration during the experiment. Of the chlorinated HAA species, DCAA, was observed as the dominant HAA formed during experiments with chlorination alone while in UVOX followed by chlorination, the detected HAAs species were formed at similar concentrations (Figure 5). The formation of DCAA during experiments with UVOX followed by chlorination was negligible in comparison to the concentrations formed during experiments with chlorination, while the brominated species concentrations were similar in both experiments.

DCAA and TCAA are the most abundant HAAs detected in chlorinated water (Teo et al., 2015). However, in this study, TCAA detected was below the limit of quantification (<3 µg/L). The reason might be that the model precursors in this study have propensity to form dihaloacetic acids rather than trihaloacetic acids. As model precursors, some BFA compounds such as citric acid, have shown to generate higher TCM and DCAA, in comparison to other BFA compounds (Kanan and Karanfil, 2011, Yang et al., 2016). The pH used in this study (pH 8) was also not favourable for TCAA formation. It was suggested that THMs and TCAA have a similar precursor structure and in alkali

conditions, base-catalysed hydrolysis dominates, leading to formation of THMs while TCAA will be formed in acidic conditions (Liang and Singer, 2003).

BIF of HAAs at sampling period 0, 3, 6, and 24 h from experiments with chlorine only were 0.492, 0.246, 0.252, and 0.128, respectively. Higher BIFs from experiments with UVOX (0, 0.995, 0.735, and 1.013, respectively) were observed after 3 h of experiment. Similarly to THMs, BIF values of HAA suggest that the formation of DBPs shifted to brominated species which is attributed to the addition of ozone (Mao et al., 2014).

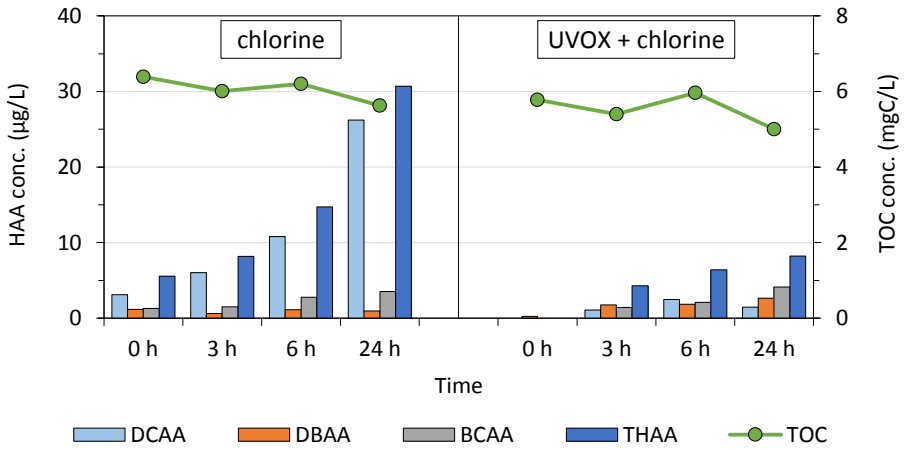

Figure 5. Increase of HAAs in pool water treated with chlorination and UVOX followed by chlorination.

Bromate was also present in the pool water during experiments with chlorination-only and UVOX followed by chlorination at initial concentrations of 91 µg/L and 33 µg/L, respectively which then increased up to 140 µg/L and 82 µg/L. Chlorination, ozonation, and UV/chlorination can form bromate in the presence of bromide (Fang et al., 2017, Margerum and Huff Hartz, 2002, von Gunten and Hoigne, 1994). However, the increase in bromate observed in both experiments after 24 h was in the same order (49 µg/L) regardless of the treatment applied, with or without UVOX. In chlorination experiment using sodium hypochlorite, bromate may be already present as an impurity from commercial sodium hypochlorite solution (Asami et al., 2009, Garcia-Villanova et al., 2010). This suggests that the increase was likely due to continuous chlorine addition rather than bromide in the water.

Pharmaceuticals and EDCs

The concentration profiles of pharmaceuticals and EDCs in water samples collected from the pool in 24 h experiments are shown in Figure 6. In chlorinated pool water, most of the selected pharmaceuticals and EDCs were removed after less than 3 h of chlorination except for caffeine, atenolol and ibuprofen. After 24 h of chlorination, 97% removal was achieved for most of the compounds, while only 60% of ibuprofen was

removed. By adding UVOX to the treatment train, ibuprofen was completely removed after 24 h. It can be concluded that the removal of the target compounds was mostly driven by chlorine except in case of ibuprofen with UVOX enhancing the removal. In both experiments, acetaminophen, venlafaxine, estrone and estradiol were removed faster compared to other compounds (Table 3). Atenolol, carbamazepine, venlafaxine, estrone and caffeine were found to have slower reaction rate when UVOX was used.

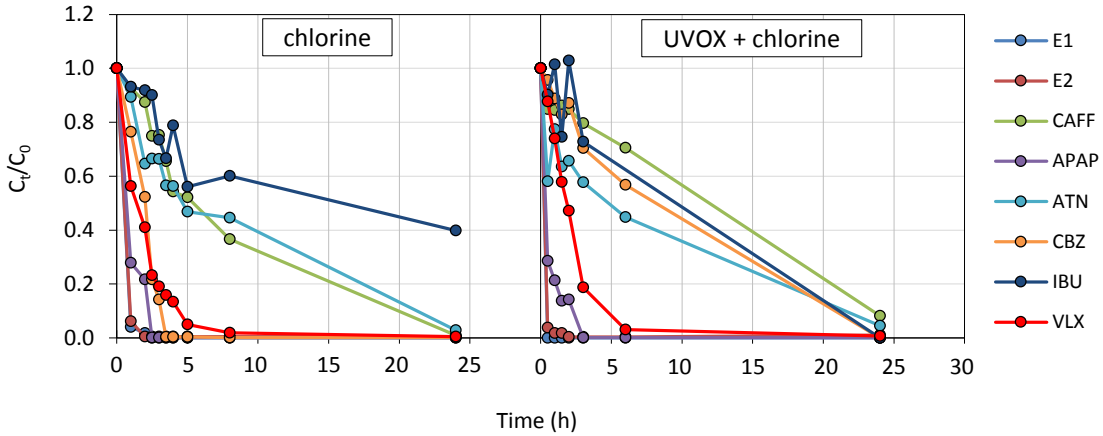

Figure 6. Concentration profiles for pharmaceuticals and EDCs in pool water treated with chlorine and UVOX followed by chlorination. (E1: estrone, E2: estradiol, CAFF: caffeine, APAP: acetaminophen, ATN: atenolol, CBZ: carbamazepine, IBU: ibuprofen, VLX: venlafaxine)

Table 3. Rate constant of pharmaceuticals and EDCs.

Compounds	Chlorination			UVOX + chlorination		
	k (/day)	R^2	t½ (h)	k (/day)	R^2	t½ (h)
Acetaminophen	20.78	0.84	0.80	35.30	0.85	0.47
Atenolol	3.46	0.98	4.81	3.16	0.95	5.26
Carbamazepine	13.33	0.86	1.25	2.35	0.96	7.07
Ibuprofen	1.07	0.88	15.52	2.00	0.32	8.34
Venlafaxine	12.60	0.98	1.32	11.21	0.91	1.48
Estrone	74.69	0.91	0.22	59.88	1.00	0.28
Estradiol	63.75	1.00	0.26	74.29	0.75	0.22
Caffeine	2.99	0.95	5.57	2.43	0.98	6.86

High removal of acetaminophen after 24 h of contact time with chlorine and more recalcitrance of ibuprofen and caffeine to chlorination were also observed in other studies (Weng et al., 2014, Westerhoff et al., 2005). Westerhoff et al. (2005) also demonstrated high removals of estrone, estradiol, and carbamazepine. In a study by Wang et al. (2016), only 5.5% degradation of carbamazepine was observed by chlorination. The differences in results are likely due to the experimental set up (e.g. contact time, pH and dose). The use of UVOX without chlorination has shown partial removal of estrone, estradiol and acetaminophen (<75%) and no effect on other

compounds (data not shown) while combining UVOX and chlorination leads to complete removal of ibuprofen (<LOD). A study by Xiang et al. (2016) showed little or no effect of applying UV and chlorine separately in the degradation of ibuprofen, while the combination of UV and chlorine significantly increased the degradation rate. The presence of bromide in water also increased the degradation rate of caffeine and carbamazepine, but reduced the degradation rate of ibuprofen during UV/chlorine treatment (Cheng et al., 2018). Westerhoff et al. (2005) showed that ozone was able to oxidized >80% of acetaminophen, carbamazepine, ibuprofen, estrone and estradiol. Bromine, chlorine and ozone could effectively oxidized phenolic compounds (Alum et al., 2004, Gallard et al., 2003, Westerhoff et al., 2005). Subsequently, it is expected to observe higher degradation rates of micropollutants, such as acetaminophen, estrone and estradiol. However, it should be noted that most studies on removal of micropollutants use higher doses of UV and ozone (Wang et al., 2016, Xiang et al., 2016). In this study, the use of low concentrations of ozone and the competition with NOM and BFA, limit the oxidization of pharmaceuticals and EDCs by UVOX.

In this study, chlorine was dosed continuously resulting in continuous elimination of micropollutants. However, bromide might also contributed in the process due to formation of $HOBr/OBr^-$ which has higher reaction rate compared to $HOCl/OCl^-$. These micropollutants were continuously exposed to chlorine which provides more time for degradation of compounds with slower reaction rates. Regardless of the effectiveness of the treatment, the degradation of pharmaceuticals and EDCs can generate transformation products which may be equally or more hazardous than the parent compounds (Michael et al., 2014, Noguera-Oviedo and Aga, 2016) and should be considered in future studies.

Conclusions

UVOX treatment is a promising technology for swimming pool water treatment. It has proven to be effective in inactivation of chlorine resistant microorganisms. In chlorinated pool water, inclusion of UVOX reduced the formation of TCM and DCAA, however, as expected, brominated-DBPs (BDCM, DBCM, DBAA and BCAA) were detected at higher concentrations. Consequently, the presence of bromide in water has to be taken into account when using UVOX as pool water treatment, even though low concentrations of ozone were used.

Chlorination was able to remove most of pharmaceuticals tested, however, longer contact time was needed for complete removal of caffeine and atenolol while 40% of ibuprofen was still persistent even after 24 h. In treatment combining UVOX and chlorination, the removal of micropollutants was dominantly influenced by chlorine. It was observed that UVOX enhanced the degradation of ibuprofen, the most resistant pharmaceuticals in chlorinated water in this study.

References

Alum, A., Yoon, Y., Westerhoff, P., Abbaszadegan, M. (2004). Oxidation of bisphenol A, 17β-estradiol, and 17α-ethynyl estradiol and byproduct estrogenicity. *Environmental Toxicology*, 19(3), 257–264.

Asami, M., Kosaka, K., Kunikane, S. (2009). Bromate, chlorate, chlorite and perchlorate in sodium hypochlorite solution used in water supply. *Journal of Water Supply: Research and Technology - Aqua*, 58(2), 107–115.

Betancourt, W.Q., Rose, J.B. (2004). Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. *Veterinary Parasitology*, 126(1), 219–234.

Beyer, A., Worner, H., Van Lierop, R. (2004). The use of UV for destruction of combined chlorine (technical note). Wallace & Tiernan.

Bolton, J.R., Linden, K.G. (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering*, 129(3), 209–215.

Bond, T., Huang, J., Graham, N.J.D., Templeton, M.R. (2014). Examining the interrelationship between DOC, bromide and chlorine dose on DBP formation in drinking water – A case study. *Science of the Total Environment*, 470–471, 469–479.

Bustos, Y.A., Vaca, M., López, R., Torres, L.G. (2010). Disinfection of a wastewater flow treated by advanced primary treatment using O_3, UV and O_3/UV combinations. *Journal of Environmental Science and Health, Part A*, 45(13), 1715–1719.

Cabaj, A., Sommer, R., Schoenen, D. (1996). Biodosimetry: Model calculations for u.v. water disinfection devices with regard to dose distributions. *Water Research*, 30(4), 1003–1009.

Campbell, C.G., Borglin, S.E., Green, F.B., Grayson, A., Wozei, E., Stringfellow, W.T., (2006). Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. *Chemosphere*, 65(8), 1265–1280.

Cassan, D., Mercier, B., Castex, F., Rambaud, A. (2006). Effects of medium-pressure UV lamps radiation on water quality in a chlorinated indoor swimming pool. *Chemosphere*, 62(9), 1507–1513.

Cheema, W.A., Kaarsholm, K.M.S., Andersen, H.R. (2017). Combined UV treatment and ozonation for the removal of by-product precursors in swimming pool water. *Water Research*, 110, 141–149.

Cheng, S., Zhang, X., Yang, X., Shang, C., Song, W., Fang, J., Pan, Y. (2018). The multiple role of bromide ion in PPCPs degradation under UV/chlorine treatment. *Environmental Science & Technology*, 52(4), 1806–1816.

Chevrefils, G., Caron, É., Wright, H., Sakamoto, G., Payment, P., Barbeau, B., Cairns, B. (2006). UV dose required to achieve incremental log inactivation of bacteria, protozoa and viruses. *IUVA News*, 8(1), 38–45.

Cho, M., Kim, J.-H., Yoon, J. (2006). Investigating synergism during sequential inactivation of *Bacillus subtilis* spores with several disinfectants. *Water Research*, 40(15), 2911–2920.

Choi, Y., Cho, M., Lee, Y., Choi, J., Yoon, J. (2007). Inactivation of *Bacillus subtilis* spores during ozonation in water treatment plant: Influence of pre-treatment and consequences for positioning of the ozonation step. *Chemosphere*, 69(5), 675–681.

Chowdhury, S., Champagne, P., James McLellan, P. (2010). Investigating effects of bromide ions on trihalomethanes and developing model for predicting bromodichloromethane in drinking water. *Water Research*, 44(7), 2349–2359.

Cimetiere, N., De Laat, J. (2014). Effects of UV-dechloramination of swimming pool water on the formation of disinfection by-products: A lab-scale study. *Microchemical Journal*, 112, 34–41.

Clancy, J.L., Bukhari, Z., Hargy, T.M., Bolton, J.R., Dussert, B.W., Marshall, M.M. (2000). Using UV to inactivate *Cryptosporidium*, *Journal American Water Works Association*. 92(9), 97–104.

Corona-Vasquez, B., Samuelson, A., Rennecker, J.L., Mariñas, B.J. (2002). Inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. *Water Research*, 36(16), 4053–4063.

Craik, S.A., Finch, G.R., Bolton, J.R., Belosevic, M. (2000). Inactivation of *Giardia muris* cysts using medium-pressure ultraviolet radiation in filtered drinking water. *Water Research*, 34(18), 4325–4332.

Craik, S.A., Weldon, D., Finch, G.R., Bolton, J.R., Belosevic, M. (2001). Inactivation of *Cryptosporidium parvum* oocysts using medium-and low-pressure ultraviolet radiation. *Water Research*, 35(6), 1387–1398.

DIN, 2012. Treatment of the Water of Swimming Pools and Baths - Part 1: General Requirements, Germany Standard DIN 19643-1: 2012-11.

Driedger, A., Staub, E., Pinkernell, U., Mariñas, B., Köster, W., von Gunten, U. (2001). Inactivation of *Bacillus subtilis* spores and formation of bromate during ozonation. *Water Research*, 35(12), 2950-2960.

Driedger, A.M., Rennecker, J.L., Mariñas, B.J. (2000). Sequential inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. *Water Research*, 34(14), 3591–3597.

Ekowati, Y., Buttiglieri, G., Ferrero, G., Valle-Sistac, J., Diaz-Cruz, M.S., Barceló, D., Petrovic, M., Villagrasa, M., Kennedy, M.D., Rodríguez-Roda, I. (2016). Occurrence of pharmaceuticals and UV filters in swimming pools and spas. *Environmental Science and Pollution Research*, 23(14), 14431–14441.

European Commission (2018). Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495 (notified under document C(2018) 3362).

Fang, J., Liu, H., Shang, C., Zeng, M., Ni, M., Liu, W. (2014). *E. coli* and bacteriophage MS2 disinfection by UV, ozone and the combined UV and ozone processes. *Frontiers of Environmental Science & Engineering*, 8(4), 547–552.

Fang, J., Zhao, Q., Fan, C., Shang, C., Fu, Y., Zhang, X. (2017). Bromate formation from the oxidation of bromide in the UV/chlorine process with low pressure and medium pressure UV lamps. *Chemosphere*, 183, 582–588.

Farkas, L., Lewin, M., Bloch, R. (1949). The reaction between hypochlorite and bromides. *Journal of the American Chemical Society*, 71(6), 1988–1991.

Florentin, A., Hautemanière, A., Hartemann, P. (2011). Health effects of disinfection by-products in chlorinated swimming pools. *International Journal of Hygiene and Environmental Health*, 214(6), 461–469.

FOEN (2015). Gewässerqualität: Revision der Gewässerschutzverordnung.

https://www.bafu.admin.ch/bafu/de/home/themen/bildung/medienmitteilungen.msg-id-59323.html, Erläuternder Bericht zur Änderung der Gewässerschutzverordnung. https://www.newsd.admin.ch/newsd/message/attachments/41551.pdf (Accessed 15 November 2018)

Gallard, H., Pellizzari, F., Croué, J.P., Legube, B. (2003). Rate constants of reactions of bromine with phenols in aqueous solution. *Water Research*, 37 (12), 2883–2892.

Garcia-Villanova, R.J., Oliveira Dantas Leite, M.V., Hernández Hierro, J.M., de Castro Alfageme, S., García Hernández, C. (2010). Occurrence of bromate, chlorite and chlorate in drinking waters disinfected with hypochlorite reagents. Tracing their origins. *Science of the Total Environment*, 408(12), 2616–2620.

Glaze, W.H. (1986). Reaction products of ozone: a review. *Environmental Health Perspectives*, 69, 151.

Gorga, M., Petrovic, M., Barceló, D. (2013). Multi-residue analytical method for the determination of endocrine disruptors and related compounds in river and waste water using dual column liquid chromatography switching system coupled to mass spectrometry. *Journal of Chromatography A*, 1295, 57–66.

Gros, M., Rodríguez-Mozaz, S., Barceló, D. (2012). Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *Journal Chromatography A*, 1248, 104–121.

Haag, W.R., Hoigne, J. (1983). Ozonation of bromide-containing waters: Kinetics of formation of hypobromous acid and bromate. *Environmental Science & Technology*, 17(5), 261–267.

Hansen, K.M., Zortea, R., Piketty, A., Vega, S.R., Andersen, H.R. (2013). Photolytic removal of DBPs by medium pressure UV in swimming pool water. *Science of the Total Environment*, 443, 850–856.

Hansen, K.M.S., Spiliotopoulou, A., Cheema, W.A., Andersen, H.R. (2016). Effect of ozonation of swimming pool water on formation of volatile disinfection by-products – A laboratory study. *Chemical Engineering Journal*, 289, 277–285.

Hijnen, W., Beerendonk, E., Medema, G.J., (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research*, 40(1), 3–22.

Jacangelo, J.G., Patania, N.L., Trussed, R.R., Haas, C.N., Gerba, C. (2002). Inactivation of Waterborne Emerging Pathogens by Selected Disinfectants. AWWA Research Foundation and American Water Works Association, Denver, USA.

Jarroll, E.L., Bingham, A.K., Meyer, E.A. (1981). Effect of chlorine on *Giardia lamblia* cyst viability. *Applied and Environmental Microbiology*, 41(2), 483–487.

Judd, S.J., Bullock, G. (2003). The fate of chlorine and organic materials in swimming pools. *Chemosphere*, 51(9), 869-879.

Jung, Y.J., Oh, B.S., Kang, J.-W. (2008). Synergistic effect of sequential or combined use of ozone and UV radiation for the disinfection of *Bacillus subtilis* spores. *Water Research*, 42(6), 1613-1621.

Kanan, A., Karanfil, T. (2011). Formation of disinfection by-products in indoor swimming pool water: The contribution from filling water natural organic matter and swimmer body fluids. *Water Research*, 45 (2), 926–932.

Kassotaki, E., Pijuan, M., Rodriguez-Roda, I., Buttiglieri, G. (2019). Comparative assessment of endocrine disrupting compounds removal in heterotrophic and enriched nitrifying biomass. *Chemosphere*, 217, 659-668.

Korich, D.G., Mead, J.R., Madore, M.S., Sinclair, N.A., Sterling, C.R. (1990). Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology*, 56(5), 1423–1428.

Kristensen, G.H., Klausen, M.M., Andersen, H.R., Erdinger, L., Lauritsen, F., Arvin, E., Albrechtsen, H.J. (2009). Full scale test of UV-based water treatment technologies at Gladsaxe Sport Centre–with and without advanced oxidation mechanisms. The 3rd International Swimming Pool and Spa Conference, London.

Li, Z., Xiang, X., Li, M., Ma, Y., Wang, J., Liu, X. (2015). Occurrence and risk assessment of pharmaceuticals and personal care products and endocrine disrupting chemicals in reclaimed water and receiving groundwater in China. *Ecotoxicology and Environmental Safety*, 119, 74–80.

Liang, L., Singer, P.C. (2003). Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. *Environmental Science & Technology*, 37(13), 2920–2928.

Lourencetti, C., Grimalt, J.O., Marco, E., Fernandez, P., Font-Ribera, L., Villanueva, C.M., Kogevinas, M. (2012). Trihalomethanes in chlorine and bromine disinfected swimming pools: Air-water distributions and human exposure. *Environmental International*, 45(0), 59–67.

Mao, Y., Wang, X., Yang, H., Wang, H., Xie, Y.F. (2014). Effects of ozonation on disinfection byproduct formation and speciation during subsequent chlorination. *Chemosphere*, 117, 515–520.

Margerum, D.W., Huff Hartz, K.E. (2002). Role of halogen(I) cation-transfer mechanisms in water chlorination in the presence of bromide ion. *Journal of Environmental Monitoring*. 4(1), 20–26.

Michael, I., Vasquez, M. I., Hapeshi, E., Haddad, T., Baginska, E., Kümmerer, K., Fatta-Kassinos, D. (2014). Metabolites and Transformation Products of Pharmaceuticals in the Aquatic Environment as Contaminants of Emerging Concern. in: Lambropoulou, D. A. & Nollet, L. M. (Eds.), Transformation Products of Emerging Contaminants in the Environment. John Wiley & Sons Ltd.

Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Fawell, J., Elliott, P. (2000). Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review. *Occupational and Environmental Medicine*, 57(2), 73–85.

Noguera-Oviedo, K., Aga, D. S. (2016). Lessons learned from more than two decades of research on emerging contaminants in the environment. *Journal of Hazardous Materials*, 316, 242-251.

Nowell, L.H., Hoigné, J., 1992. Photolysis of aqueous chlorine at sunlight and ultraviolet wavelengths – II. Hydroxyl radical production. *Water Research*, 26(5), 599–605.

Panyakapo, M., Soontornchai, S., Paopuree, P. (2008). Cancer risk assessment from exposure to trihalomethanes in tap water and swimming pool water. *Journal of Environmental Sciences*, 20(3), 372–378.

Petrovic, M., Eljarrat, E., Lopez de Alda, M.J., Barceló, D. (2004). Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. *Analytical and Bioanalytical Chemistry*, 378(3), 549–562.

PWTAG (2016). 31 Ultraviolet Disinfection: Specification, Maintenance and Validation. Pool Water Treatment Advisory Group.

Rennecker, J.L., Driedger, A.M., Rubin, S.A., Mariñas, B.J. (2000). Synergy in sequential inactivation of *Cryptosporidium parvum* with ozone/free chlorine and ozone/monochloramine. *Water Research*, 34(17), 4121-4130.

Rennecker, J.L., Mariñas, B.J., Owens, J.H., Rice, E.W. (1999). Inactivation of *Cryptosporidium parvum* oocysts with ozone. *Water Research*, 33 (11), 2481–2488.

Richardson, S.D., DeMarini, D.M., Kogevinas, M., Fernandez, P., Marco, E., Lourencetti, C., Ballesté, C., Heederik, D., Meliefste, K., McKague, A.B., Marcos, R., Font-Ribera, L., Grimalt, J.O., Villanueva, C.M. (2010). What's in the Pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. *Environmental Health Perspectives*, 118(11), 1523–1530.

Richardson, S.D., Ternes, T.A. (2011). Water analysis: Emerging contaminants and current issues. *Analytical Chemistry*, 83(12), 4614–4648.

Shin, G.-A., Linden, K.G., Arrowood, M.J., Sobsey, M.D. (2001). Low-pressure UV inactivation and DNA repair potential of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, 67(7), 3029–3032.

Sommer, R., Cabaj, A., Haider, T., Hirschmann, G. (2004). UV drinking water disinfection–requirements, testing and surveillance: Exemplified by the Austrian National Standards M 5873-1 and M 5873-2. *IUVA News*, 6(4), 27–35.

Spiliotopoulou, A., Hansen, K.M.S., Andersen, H.R., 2015. Secondary formation of disinfection by-products by UV treatment of swimming pool water. *Science of the Total Environment*, 520, 96–105.

Suppes, L.M., Huang, C.-H., Lee, W.-N., Brockman, K.J., 2017. Sources of pharmaceuticals and personal care products in swimming pools. *Journal of Water and Health*, 15(5), 829–833.

Teo, T.L.L., Coleman, H.M., Khan, S.J. (2015). Chemical contaminants in swimming pools: Occurrence, implications and control. *Environmental International*, 76(0), 16–31.

Teo, T.L.L., Coleman, H.M., Khan, S.J. (2016). Occurrence and daily variability of pharmaceuticals and personal care products in swimming pools. *Environmental Science and Pollution Research*, 23(7), 6972–6981.

U.S. EPA (1999) Alternative Disinfectants and Oxidants Guidance Manual. U.S. Environmental Protection Agency, Washington DC.

U.S. EPA (1995) Method 552.2 Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid-Liquid Extraction, Derivatization and Gas Chromatography with Electron Capture Detection. National Exposure Research Laboratory Office of Research and Development, Cincinnati, Ohio, USA.

Vlaanderen, J., van Veldhoven, K., Font-Ribera, L., Villanueva, C. M., Chadeau-Hyam, M., Portengen, L., Grimalt, J. O., Zwiener, C., Heederik, D., Zhang, X., Vineis, P., Kogevinas, M., Vermeulen, R. (2017). Acute changes in serum immune markers due to swimming in a chlorinated pool. *Environment International*, 105, 1-11.

Villanueva, C.M., Cantor, K.P., Grimalt, J.O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G. (2007). Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *American Journal of Epidemiology*, 165(2), 148–156.

von Gunten, U., Hoigne, J. (1994). Bromate formation during ozonation of bromide-containing waters: interaction of ozone and hydroxyl radical reactions. *Environmental Science & Technology*, 28(7), 1234-1242.

Wang, W.-L., Wu, Q.-Y., Huang, N., Wang, T., Hu, H.-Y. (2016). Synergistic effect between UV and chlorine (UV/chlorine) on the degradation of carbamazepine: Influence factors and radical species. *Water Research*, 98, 190–198.

Weaver, W.A., Li, J., Wen, Y., Johnston, J., Blatchley, M.R., Blatchley III, E.R. (2009). Volatile disinfection by-product analysis from chlorinated indoor swimming pools. *Water Research*, 43(13), 3308–3318.

Weng, S., Li, J., Blatchley III, E.R. (2012). Effects of UV_{254} irradiation on residual chlorine and DBPs in chlorination of model organic-N precursors in swimming pools. *Water Research*, 46(8), 2674–2682.

Weng, S., Sun, P., Ben, W., Huang, C.-H., Lee, L.T., Blatchley III, E.R. (2014). The presence of pharmaceuticals and personal care products in swimming pools. *Environmental Science & Technology Letters*, 1(12), 495–498.

Westerhoff, P., Chao, P., Mash, H. (2004). Reactivity of natural organic matter with aqueous chlorine and bromine. *Water Research*, 38(6), 1502–1513.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005). Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environmental Science & Technology*, 39(17), 6649–6663.

WHO (2006). Guidelines for Safe Recreational Water Environments Vol. 2: Swimming Pools and Similar Environments. World Health Organization, Geneva.

Wu, D., Lu, G., Zhang, R., You, H., Yan, Z., Li, Y. (2015). Disinfection characteristics of the combined ultraviolet radiation and ozone process using *Escherichia coli* as a probe. *Water Science & Technology: Water Supply*, 16 (1), 163-170.

Xiang, Y., Fang, J., Shang, C. (2016). Kinetics and pathways of ibuprofen degradation by the UV/chlorine advanced oxidation process. *Water Research*, 90, 301–308.

Xiao, F., Zhang, X., Zhai, H., Lo, I. M. C., Tipoe, G. L., Yang, M., Pan, Y., Chen, G. (2012). New halogenated disinfection byproducts in swimming pool water and their permeability across skin. *Environmental Science & Technology*, 46(13), 7112-7119.

Yang, L., Schmalz, C., Zhou, J., Zwiener, C., Chang, V.W.C., Ge, L., Wan, M.P. (2016). An insight of disinfection by-product (DBP) formation by alternative disinfectants for swimming pool disinfection under tropical conditions. *Water Research*, 101, 535–546.

Yeh, R.Y.L., Farré, M.J., Stalter, D., Tang, J.Y.M., Molendijk, J., Escher, B.I. (2014). Bioanalytical and chemical evaluation of disinfection by-products in swimming pool water. *Water Research*, 59, 172–184.

Yoon, Y., Ryu, J., Oh, J., Choi, B.-G., Snyder, S.A. (2010). Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). *Science of the Total Environment*, 408(3), 636–643.

Annex

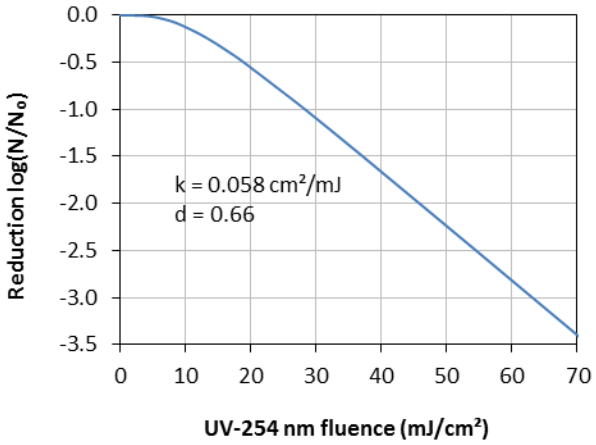

Figure S1. UV-254 nm inactivation curve of biodosimeter according to Austrian National Standard ÖNORM M 5873-1:2001 and M 5873-2:2003 by means of standardized laboratory UV apparatus

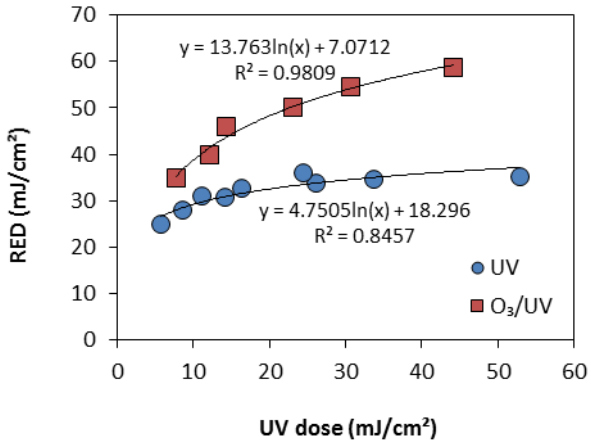

Figure S2. Correlation between the actual UV dose (Reduction Equivalent Dose (RED)) and the calculated UV dose (Intensity x Time).

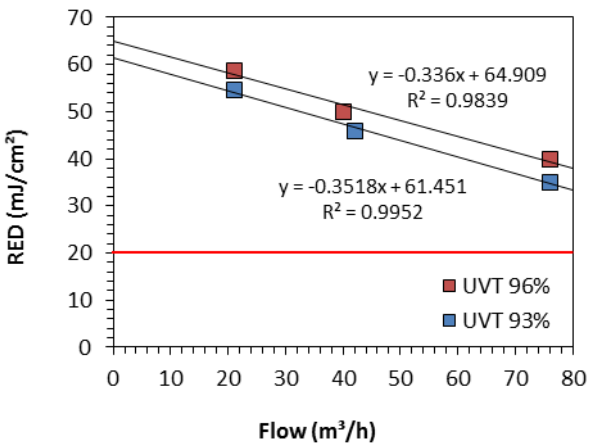

Figure S3. Correlation between log inactivation of *B. subtilis* spores and water flow rate in the system. The red line shows the actual UV dose (RED) of 20 mJ/cm².

3

Occurrence of pharmaceuticals and UV filters in swimming pools and spas

Ekowati, Y., Buttiglieri, G., Ferrero, G., Valle-Sistac, J., Diaz-Cruz, M.S., Barceló, D., Petrovic, M., Villagrasa, M., Kennedy, M.D., Rodríguez-Roda, I. (2016). Occurrence of pharmaceuticals and UV filters in swimming pools and spas. *Environmental Science and Pollution Research*, 23(14), 14431-14441.

Abstract

The occurrence of 32 pharmaceuticals and 14 UV filters in swimming pools and spas was studied. Fifty-one water samples were collected from 17 pools located in sport centres and hotels in Catalonia, Spain. The samples were analysed by liquid chromatography-tandem mass spectrometry. The pharmaceuticals atenolol, carbamazepine, hydrochlorothiazide, metronidazole, ofloxacin, sulfamethoxazole, acetaminophen, ibuprofen, ketoprofen and phenazone were measured in water samples at concentrations higher than their limit of quantification (LOQ). The highest concentration of any individual pharmaceutical was measured for the diuretic hydrochlorothiazide (904 ng/L). The most frequently detected pharmaceutical was carbamazepine, as it was observed in more than half of all the water samples measured (53 %, 27/51). The UV filters at concentrations higher than LOQ in water samples were BP1, BP2, BP3, BP8, THB, 4DHB, 4MBC, OD-PABA, 1HBT, MeBT and DMeBT. The highest concentration of UV filter observed was 4MBC (69.3 ng/L) while the most frequent UV filters in the samples were 1HBT (59 %, 30/51). The results also showed that pharmaceuticals and UV filters were most frequently found in spas. Finally, from a water treatment technology perspective, the lowest occurrence of pharmaceuticals was in the pools applying sand filters followed by disinfection by sodium hypochlorite, while the lowest occurrence of UV filters was in the pools applying coagulation, sand filtration, UV and salt electrolysis.

Introduction

Chemical hazards in swimming pools and similar environments originate from a number of sources, but are mainly attributed to the source water, deliberate addition of chemicals (i.e. disinfectants), or from bathers themselves (i.e. sweat, urine, soap residues, cosmetics and suntan oil) (WHO, 2006).

Pharmaceuticals and personal care products (PPCPs) are part of the bather-derived chemical contaminants and consist of a wide range of chemical compounds, some of which are considered potentially hazardous to the environment and human health. In some studies, bioaccumulation of UV filters was observed in human urine (Felix et al., 1998), human milk (Schlumpf et al., 2010), human semen (León-González et al., 2011) and human placenta (Valle-Sistac et al., 2016). Moreover, PPCPs are designed to be biologically active also at low concentrations and long-term exposure of (mixture) of PPCPs may potentially cause negative effects. Human body fluids, especially urine and sweat, are the vector that introduces PPCPs into swimming pools; some pharmaceuticals are not completely metabolized by humans and are excreted through urine, whereas personal care products such as lotions and sunscreens - which also contain some pharmaceuticals - are applied externally and washed off the human body during swimming. Accumulation of PPCPs in pool water can happen as a consequence of water recirculation and inefficient water treatment; conventional water treatment processes such as coagulation-flocculation have proven to be inefficient in removing trace levels of pharmaceuticals (Stackelberg et al., 2004; Ternes et al., 2002). Moreover, PPCPs are non-volatile and their reaction with chlorine is slow, thus they are likely to remain in swimming pools for quite some time (Weng et al., 2014).

Bather-derived chemicals contain organic and inorganic constituents that could act as disinfection byproduct (DBPs) precursors. The importance of reducing precursors originating from human body fluids (urine and sweat) has been emphasised by different authors (Judd and Black, 2000; Judd and Bullock, 2003; Kanan and Karanfil, 2011; Keuten et al., 2014; Keuten et al., 2012), but importantly PPCPs have been proven to act as DBP precursors (Bottoni et al., 2014; Sakkas et al., 2003; Zhang et al., 2014). Specifically, Shen and Andrews (2011) demonstrated the formation of nitrosamine from 20 PPCPs during disinfection with chloramines where ranitidine rendered the highest conversion to NDMA (N-Nitrosodimethylamine). Some pharmaceuticals are susceptible to chlorine (e.g. acetaminophen) (Weng et al. 2014; Westerhoff et al., 2005) but can either be transformed into new products by chlorination (Bedner and MacCrehan, 2006) or remain unchanged (Glassmeyer and Shoemaker, 2005). Wang et al. (2013) demonstrated that ingredients in lotions and sunscreens may serve as halobenzoquinone precursors in chlorinated swimming pools. UV filters, which are compounds commonly used in sunscreens, when found in aqueous matrices can suffer

degradation due to exposure to sunlight (Kockler et al., 2012) and reaction with disinfectants such as chlorine (Santos et al., 2012). Additionally, some studies suggested that the degradation products of some PPCPs are more toxic than the parent compound (Teo et al., 2015).

PPCPs are ubiquitous in the environment since they are not entirely removed by wastewater treatment processes and are likely to enter the environment in the form of parent compounds, metabolites and byproducts and the effect of unintentional exposure to mixture of PPCPs to non-target organisms cannot be ignored. Numerous publications focused on the occurrence of PPCPs in the environment (Brausch et al., 2012; Brausch and Rand, 2011; Bu et al., 2013; Daughton and Ternes, 1999; Halling-Sørensen et al., 1998; Kasprzyk-Hordern et al., 2008; Peng et al., 2008; Yu and Chu, 2009) as well as their removal by water treatment processes (Kasprzyk-Hordern et al., 2009; Klavarioti et al., 2009; Vieno et al., 2007). Some pharmaceuticals are resistant to biodegradation thus may lead to their accumulation and persistence in the environment (Dalkmann et al., 2012) and their bioaccumulation in aquatic organisms (Li, 2014; Zenker et al., 2014). Previous studies have reported different levels of pharmaceuticals in the environment. For example, a high consumption drug like carbamazepine has been detected in the effluent of drinking water treatment systems at various maximum concentration ranging from below the limit of detection (López-Serna et al., 2010) to 18 ng/L (Benotti et al., 2008) and 258 ng/L (Stackelberg et al., 2004). Similarly, UV filters are known to bioaccumulate in fish (Balmer et al., 2005; Daughton and Ternes, 1999; Gago-Ferrero et al., 2015) and marine mammals (Alonso et al., 2015; Gago-Ferrero et al., 2013a) and the exposure of some UV filters to aquatic environments showed the potential to cause estrogenic effects and effects on reproduction in fish (Fent et al., 2008; Fent et al., 2010; Weisbrod et al., 2007) and to cause skeletal endocrine disruption in corals (Downs et al. 2015). Even though UV filters have not been studied extensively as pharmaceuticals, there is a growing concern regarding their presence in the environment due to increasing usage and their potential negative effects. The highest level of UV filter reported in surface water was 125 ng/L for BP3 (Poiger et al., 2004, Cruz et al., 2008). Residues of UV filters were even reported in drinking water (Díaz-Cruz et al., 2012). Additionally, Teo et al. (2015) reported on studies of UV filters in swimming pools where the highest concentration found was 25 μg/L of octocrylene (Zwiener et al., 2007).

From the above, it emerges a need to further deepen the knowledge on the occurrence of PPCPs in swimming pools. The focus of this work is to highlight any existing patterns on the occurrence of specific types of pharmaceuticals and UV filters and eventually linking their occurrence to the type of pools and the type of water treatment applied. The selected pharmaceuticals and personal care products have a high potential to occur in swimming pool/spa water. They are used in topical formulations applied to body surfaces (skin) or mucous membranes to treat ailments via a large range of classes including but not limited to creams, foams, gels, lotions, and ointments. In addition the

list of target pharmaceuticals includes diuretics and other pharmaceuticals that are excreted significantly in urine.

Pharmaceuticals

Materials

Thirty-two pharmaceuticals compounds are included in this study and their classifications are summarized in Annex Table S1. All pharmaceutical standards used were of high purity grade (>90%). Codein, losartan potassium and naproxen was purchased from Fluka (Seelze, Germany). Irbesartan was from USP reference standard. Acetaminophen, atenolol, azithromycin, bezafibrate, carbamazepine, cefalexin, ciprofloxacin, clarithromycin, dexamethasone, desloratadine, diclofenac sodium salt, erythromycin dihydrate, furosemide, hydrochlorothiazide, ibuprofen, ketoprofen, loratadine, meloxicam sodium salt hydrate, metronidazole, metronidazole-OH, ofloxacin, oxycodone hydrochloride, phenazone, piroxicam, sulfamethoxazole, tenoxicam, tetracycline hydrochloride, torasemide, were from Sigma-Aldrich (Steinheim, Germany).

Isotopically labelled compounds used as internal standards included erithromycin-N,N-dimethyl-$^{13}C_2$, ibuprofen-d_3, meloxicam-d_3, ronidazole-d_3, and ofloxacin-d_3 from Sigma-Aldrich. Dexamethasone-d_4, cimetidine-d_3, bezafibrate-d_6, carbamazepine-d_{10}, atenolol-d_7, hydrochlorothiazide-d_2, and valsartan-d_8 were purchased from CDN isotopes (Quebec, Canada) and azithromycin-d_3, sulfamethoxazole-d_4, acetaminophen-d_4, and furosemide-d_5 and were from Toronto Research Chemicals (Ontario, Canada). Sulfadimethoxine-d_6, sulfadoxine-d_3 and ketoprofen-d_3, which were used as surrogate standards, were purchased from Sigma-Aldrich.

The cartridges used for solid phase extraction were Oasis HLB (60 mg, 3 mL) from Waters Corporation (Milford, MA, USA). Polyvinylidene Difluoride (PVDF) membrane filters (0.45 µm) were purchased from Merck Millipore (Darmstadt, Germany). HPLC-grade methanol, acetonitrile, water (LiChrosolv), ammonium acetate, formic acid 98% and Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) were supplied by Merck (Darmstadt, Germany). Nitrogen for drying 99.995% of purity was from Abelló Linde (Spain).

Solid-phase extraction (SPE)

Swimming pool samples were filtered through 0.45 µm PVDF membrane filters. A suitable volume of a Na_2EDTA solution, having a concentration of 0.1 M, was added to water samples to achieve a final concentration of 0.1% (g solute/g solution) in order to improve the retention of the antibiotics. Some antibiotics have tendency to bind metal ions present in the water and Na_2EDTA acts as a chelating agent, binding the ions and

enhancing the retention of pharmaceuticals in the SPE cartridge. As a control of the SPE procedure, swimming pool samples were spiked with an appropriate volume of a standard mixture containing surrogates standards in order to have a concentration of 50 ng/L.

The samples were processed by vacuum positive pressure using a BAKER spe-12G (J.T. Baker Bond). Samples were extracted using Oasis HLB cartridges (60 mg, 3 mL) and were conditioned with 5 mL of methanol followed by 5 mL of HPLC grade water by gravity. 500 mL of swimming pool sample were loaded into the cartridge at 1 mL/min. After sample pre-concentration, cartridges were rinsed with 6 mL of HPLC grade water by gravity, and were dried for 5 min, under vacuum, to remove excess of water. Finally, analytes were eluted with 6 mL of grade methanol by gravity. Extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL of methanol/water (10:90, v/v). Finally, 10 µL of a 1 ng/µL standard mixture containing all isotopically labelled standards were added to the extract as internal standard.

Liquid chromatography-tandem mass spectrometry

Chromatographic separations were carried out with a Waters Acquity Ultra-Performance™ liquid chromatograph system, equipped with two binary pumps systems (Milford, MA, USA) using an Acquity HSS T_3 column (50 mm × 2.1 mm i.d., 1.8 µm particle size) for the compounds analysed under positive electrospray ionization (PI) and an Acquity BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 µm particle size) for the ones analysed under negative electrospray ionization (NI), both purchased from Waters Corporation. For the analysis in PI mode, the optimized separation conditions were as follows: solvent (A) methanol, solvent (B) 10 mM formic acid/ammonium formate (pH 3.2) at a flow rate of 0.5 mL/min. The gradient elution was: initial conditions 5% A; 0–4.5 min, 5–95% A; 4.5–4.6 min, 100% A; 4.6–6.0 min, 100% A; from 6.0 to 6.1 return to initial conditions; 6.1–6.7, equilibration of the column. The analysis in NI mode was performed by using acetonitrile (A) and 5 mM ammonium acetate/ammonia (pH 8) (B) at a flow rate of 0.6 mL/min. The gradient elution was: 0–1.5 min, 0–60% A; 1.5–2.0 min, 100% A; 2.0–3.0 min, 100% A; 3.20 min return to initial conditions; 3.20–3.70 min, equilibration of the column. The sample volume injected was 5 µL for both modes. The UPLC instrument was coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo ion spray source. The development and optimization of the method can be found in Gros et al., 2012.

UV filters

Materials

The UV filters listed in Annex Table S2 are the target compounds in this study. 2,4-dihydroxybenzophenone (benzophenone-1, BP1), 2,2',4,4'-tetrahydroxybenzophenone (benzophenone-2, BP2), 2-hydroxy-4-methoxybenzophenone (benzophenone-3, BP-3), 2,2'-dihydroxy-4-methoxybenzophenone (benzophenone-8, BP8), 4-hydroxybenzophenone (4HB), 4,4'-dihidroxybenzophenone (4DHB), 2,3,4-trihydroxy benzophenone (THB), 2-ethylhexyl-4-dimethylaminobenzoate (OD-PABA), ethyl-4-aminobenzoate (EtPABA), 1H-benzotriazole (1HBT), and 5,6-dimethyl-1H-benzotriazole monohydrate (DMeBT) were of the highest purity (>99%) and were obtained from Sigma-Aldrich (Steinheim, Germany); 4-methylbenzylidene camphor (4MBC, 99% of purity) was supplied by Dr. Ehrenstorfer (Augsburg, Germany); 5-methyl-1H-benzotriazole (MeBT, purity >99%) and 2-(5-tert-butyl-2-hydroxyphenyl) benzotriazole (TBHPBT, purity >98%) were supplied by TCI (Zwijndrecht, Belgium). Isotopically labelled compounds 2-hydroxy-4-methoxy-benzophenone-2',3',4',5',6'-d5 (BP3-d_5), 3-(4-methylbenzylidene-d_4)camphor (4MBC-d_4), and 1H-benzotriazole-d_4 (BZT-d_4) used as internal standards with 99% purity were purchased to CDN isotopes (Quebec, Canada).

HPLC-grade water, methanol (MeOH), acetone, ethanol (EtOH) and acetonitrile (ACN) were purchased from J.T. Baker (Deventer, The Netherlands). The formic acid (98% purity) was provided by Merck. The N_2 gas (99% purity) was supplied by Air Liquide (Barcelona, Spain), PURADISC syringe filters by Whatman (UK), and the glass fibre filters (1 µm) and nylon membranes (0.45 µm) used in the pre-treatment step by Whatman International Ltd (Maidstone, United Kingdom).

Individual standard stock solutions as well as an isotopically labelled internal standard stock solution were prepared in MeOH at a concentration of 200 mg/L. Standard solutions were stored at -20 °C in the dark. From these solutions, a mixture standard solution of all UV filters was prepared in methanol at a concentration of 20 mg/L. Working solutions were prepared by appropriate dilution of the mixed stock standard solution in MeOH.

On-line trace enrichment

The analysis for UV filters compounds were carried out following a method previously described (Gago-Ferrero et al., 2013b). Briefly, pre-concentration of the samples and its chromatographic separation were performed using an automated on-line SPE–LC instrument Symbiosis™ Pico from Spark Holland (Emmen, The Netherlands). On-line SPE pre-concentration of all samples (previously filtered), aqueous standard solutions, and blanks was performed by loading 5 mL of the corresponding solution at 1 mL/min through a PLRP-s cartridge (Spark Holland) previously conditioned with 1 mL of MeOH,

1 mL of ACN and 1 mL of HPLC-grade water (flow rate 5 mL/min). After sample loading and prior to elution, the cartridges are washed with 0.5 mL of HPLC water at a flow rate of 5 mL/min to ensure complete transfer of the sample and remove interferences such as inorganic salts.

Liquid chromatography-tandem mass spectrometry

Chromatographic separation was performed on a LiChroCART® Purospher® STAR RP-18 ec (125 mm x 2.0 m, 5 µm) from Merck, preceded by a guard column of the same packaging material. The elution of the trapped analytes to the LC system from the automatic SPE system was performed through a chromatographic gradient using ACN (A) and water (B), both HPLC grade and with a 0.1% of formic acid. The adopted elution gradient was: initial conditions 5% A; 0–7 min, 5–75% A; 7–10 min 75–100% A; 10–15 min, 100% A; 15–17 min return to initial conditions and column equilibration. The total run time for each injection was 23 min. The mobile phase flow rate was set to 0.3 mL/min.

MS/MS detection was performed on a 4000 QTRAP™ MS/MS system from Applied Biosystems-Sciex (Foster City, CA, USA), using positive electrospray ionization (ESI+) mode under selected reaction monitoring (SRM). Two major characteristic fragments of the precursor molecular ion ($[M+H]^+$) were monitored per analyte, the most abundant transition was used for quantification, whereas the second most abundant was used for confirmation. Adopted conditions were: capillary voltage, 5000 V; source temperature, 700 °C; curtain gas, 30 psi; ion source gas 1, 50 psi, ion source gas 2, 60 psi; entrance potential 10 V.

Study area and sample collection

The water samples were collected from two hotels and six sport centres located in Catalonia, Spain. Each pool was sampled in three different days, once per day, in September 2014. In total, fifty one water samples were collected from different pools; eight outdoor pools, six indoor pools and three spas. An outdoor jacuzzi in one of the facilities was considered as an outdoor pool because it was directly connected to the outdoor pool (only partially separated from the swimming pool thus the water could flow from the jacuzzi to the swimming pool and vice versa) and the water temperature was also similar to the swimming pool. Details of the location of the facilities, type and characteristic of the pools are described in Table 1.

From the measured parameters showed in Table 1, the measurement of free and total chlorine, pH, and temperature were performed on site. The pH and temperature parameters were measured using a portable probe (pH meter 3310 from WTW). Free and total chlorine were measured using chlorine cuvette test LCK 310 (Hach). The total

organic carbon (TOC) was measured from the collected water samples using TOC-V CSH (Shimadzu) instrument (method adapted from UNE-EN 1484 and Eaton et. al, 2005). The concentration of free and combined chlorine were within the range of allowable values for water quality in swimming pools (0.5–2.0 mg/L for free chlorine and 0.6 mg/L for combined chlorine) mentioned in the Spanish national legislation RD 742/2013, only pool SC2 and SC3 had chlorine concentration >2.0 mg/L while the combined chlorine concentration in all pools was lower than 0.6 mg/L. The TOC concentration exceeded >5 mgC/L in some of the pools, particularly in spas.

Sterile amber plastic bottles, 500 and 1000 mL, were used to collect water samples for UV filters and pharmaceutical analyses, respectively. The samples were quenched by adding 1 mL ascorbic acid (12.5 g/L) to prevent the reaction of target compounds with free chlorine present in the pool water. For UV filter analysis, duplicate samples without acid addition were also collected. Water samples were filtered through 0.45 µm PVDF membrane filters (pharmaceuticals) and nylon membrane filters (UV filters), to remove any solid content in the samples, and then immediately stored in the dark at -20 °C before analysis.

Table 1. Water treatment schemes applied in the swimming pools and water quality parameters measured in the swimming pools studied

Name	Type and location	Treatment			pH	Temperature	Free chlorine	Total chlorine	TOC
		Coagulation	Filtration	Disinfection		(°C)	(mg/L)	(mg/L)	(mg/L)
GP1	indoor	yes	sand filter	UV + electrolysis	7.49 ± 0.07	28.0 ± 1.7	0.95 ± 0.48	1.46 ± 0.44	12.66
GP2	outdoor	yes	sand filter	UV + electrolysis	7.17 ± 0.05	27.4 ± 1.4	0.78 ± 0.40	0.89 ± 0.42	7.62
GN1	outdoor	no	sand filter	NaOCl	7.44 ± 0.09	26.3 ± 0.4	0.42 ± 0.07	0.49 ± 0.07	2.03
GN2	outdoor (children)	no	sand filter	NaOCl	7.12 ± 0.13	26.2 ± 0.6	0.53 ± 0.35	0.73 ± 0.30	4.02
SM1	indoor	yes	sand filter	UV + electrolysis	7.09 ± 0.16	26.8*	0.77 ± 0.08	0.97 ± 0.04	3.92
SM2	outdoor	no	sand filter	NaOCl	7.28 ± 0.19	26.1*	0.88 ± 0.31	1.04 ± 0.30	4.47
BD1	outdoor	n.a.	sand filter	n.a.	7.02 ± 0.14	28.5*	0.59 ± 0.14	0.82 ± 0.27	1.93
BD2	outdoor (jacuzzi)	n.a.	sand filter	n.a.	7.03 ± 0.08	31.8*	0.69 ± 0.34	0.95 ± 0.53	4.11
CB1	outdoor	no	sand filter	NaOCl	7.44 ± 0.18	26.9 ± 0.0	0.99 ± 0.29	1.08 ± 0.31	1.28
CB2	indoor (spa)	no	sand filter	NaOCl	8.32 ± 0.20	33.5 ± 0.0	1.65 ± 1.78	1.91 ± 1.82	7.74
CP1	outdoor	no	sand filter	NaOCl	6.83 ± 0.16	27.3 ± 0.0	1.96 ± 0.05	1.97 ± 0.05	1.10
CP2	indoor (spa)	yes	sand filter	NaBr	6.51 ± 0.22	33.6 ± 0.1			10.81
SC1	indoor	yes	sand filter	NaOCl	7.19 ± 0.06	26.9 ± 0.5	1.96 ± 0.24	2.30 ± 0.21	5.22
SC2	indoor (spa)	yes	sand filter	NaOCl	7.53 ± 0.07	29.7 ± 0.1	2.31 ± 0.26	2.70 ± 0.21	5.76
SC3	indoor	yes	sand filter	NaOCl	7.56 ± 0.06	30.1 ± 0.2	2.47 ± 0.24	2.68 ± 0.19	5.07
BU1	indoor	yes	sand filter	NaOCl	7.55 ± 0.02	27.2 ± 0.1	0.67 ± 0.01	0.93 ± 0.01	5.39
BU2	indoor (children)	yes	sand filter	NaOCl	7.66 ± 0.03	30.2 ± 0.9	0.53 ± 0.11	0.87 ± 0.09	4.45

*only measured once

Results and discussions

Pharmaceuticals

The selected pharmaceutical compounds are commonly found in commercial drugs that are likely to be used by the public making use of the pool. Eleven of the 32 pharmaceuticals analysed were detected above their limit of detection (>LOD) in 96% (49/51) of the water samples analysed. Those compounds mostly belong to the antibiotics and analgesics/anti-inflammatories group (Annex Table S3). Figure 1 summarizes the concentration of pharmaceutical compounds identified at values higher than limit of quantification (LOQ) in 51 water samples. The concentration levels >LOQ was considered as safe margin for discussion. Subsequently, 10 out of 11 pharmaceuticals (atenolol, carbamazepine, hydrochlorothiazide, metronidazole, ofloxacin, sulfamethoxazole, acetaminophen, ibuprofen, ketoprofen and phenazone) were measured at a concentration >LOQ and at least one pharmaceutical was present in 88.2% of the water samples (45/51).

The highest concentration was found in outdoor pool BD2 and it is imputable to hydrochlorothiazide (904 ng/L). However, such compound was found in only one of three samples collected from pool BD2. Anti-inflammatories, such as ketoprofen and ibuprofen, were found at maximum concentrations of 360 ng/L in indoor-children pools BU2 and 171.3 ng/L in spa SC2, respectively, whereas analgesics, such as acetaminophen were found at a concentration of 61.2 ng/L in outdoor children's pool GN2 and the only phenazone detected was at a concentration of 0.8 n/L in spa CB2. Antibiotics, metronidazole, ofloxacin and sulfamethoxazole were detected in the samples, and the highest concentration measured was 3.3 ng/L, 2.1 ng/L and 6.4 ng/L, respectively. The highest concentration of atenolol found in the water samples was 0.6 ng/L while the measured concentration of carbamazepine was 1.4 ng/L.

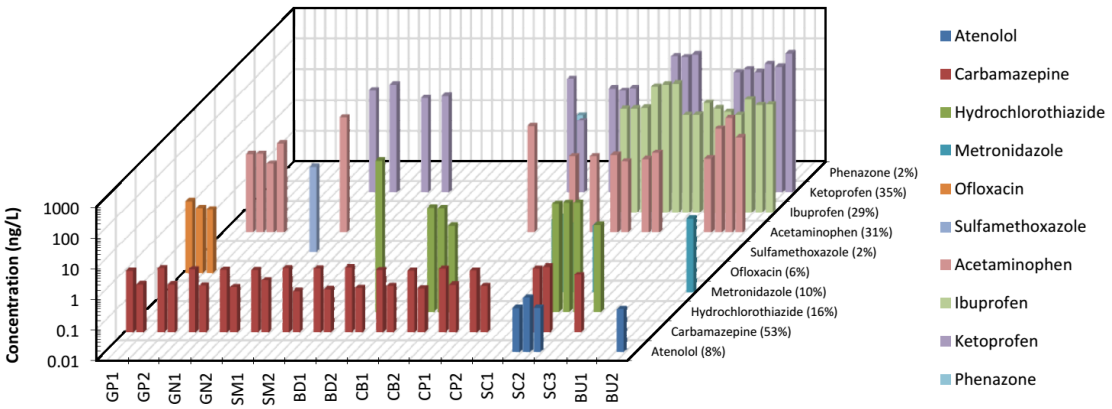

Figure 1. Concentration and occurrence of pharmaceuticals in swimming pools and spas

Figure 1 also shows the frequency of each pharmaceutical detected in the water samples. Carbamazepine was found to be ubiquitous (27 from 51 water samples), as it occurred in more than half of all the samples collected, and was especially prevalent in outdoor pools (67%) and spa (67%). Although high concentrations of some compounds were measured, they did not occur very frequently in the samples investigated in this study. For example, the highest concentration of any single pharmaceutical measured was hydrochlorothiazide but it was only present in eight water samples (16%), of which six were collected from spas. Ibuprofen was detected in 29% of the water samples and was found in samples taken from indoor pools and spas. Ketoprofen was detected in 35% of the samples and was found in every pool types. Finally, sulfamethoxazole was only detected above its detection limit in one outdoor- pool for children. The detailed data analysis on the frequency of detection of each pharmaceutical can be found in Annex Table S6.

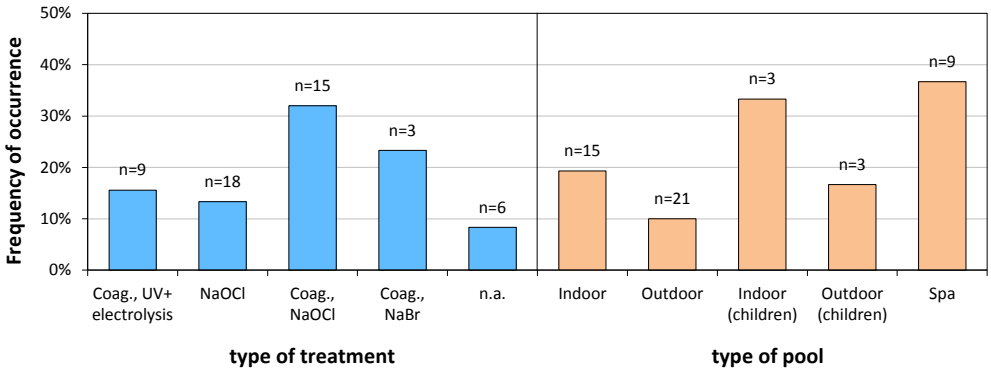

Figure 2. Occurrence of pharmaceuticals categorized by treatment and type of pool (n: number of samples)

The occurrence of ten pharmaceutical compounds (>LOQ) was categorized by treatment and pool type (Figure 2). The number of water samples collected from the swimming pools and spas are shown in the data labels. Ninty-eight positive values (>LOQ) were detected in the 51 samples. The frequency of occurrence presented in Figure 2 was the relative occurrence calculated based on the number of pools in different categories. Since the number of pools assessed in each category was different, the frequency of occurrence was presented as a relative value. For example, considering the pool type, pharmaceuticals were most frequently detected in spas: a total of nine samples were analysed from three different spas, hence the total number of analyses performed can be obtained by multiplying the number of samples (9) for the number of pharmaceuticals (10) and among those 90 analyses, 33 showed values >LOQ which correspond to 36.7% of relative occurrence. The results categorised according to treatment were analysed similarly.

Sand filtration was applied in all pool facilities. Coagulant was used in nine pools while disinfection by sodium hypochlorite was applied in 11 pools. It can be seen from Figure 2

that the pool applying sand filtration and chlorination by sodium hypochlorite showed the lowest occurrence (13.3%) of pharmaceuticals compared to the other treatments. Considering the type of pool, spas showed the highest occurrence of pharmaceuticals (36.7%) compared to the other type of pools. Ketoprofen (78%, 7/9) occurred more frequently than any of the other pharmaceuticals detected in the 3 spas (n = 9) followed by carbamazepine (67%, 6/9) and hydrochlorothiazide (67%, 6/9). More detailed information on the occurrence of pharmaceuticals can be found in Annex Table S5 and S6.

The applied water treatment may not have affected the removal of pharmaceuticals in the pool water. Rapid sand filtration proved to have low efficiency in pharmaceutical removal (Vieno et al., 2007). Some studies on the removal of pharmaceuticals by UV irradiation, applied much higher UV dose compared to typical UV dose required for disinfection (Adams et al., 2002; Kim et al., 2009). High UV dose alone or in combination with hydroxyl radicals are needed for removal of pharmaceuticals.

The routes of administration of some pharmaceuticals can be oral (or parenteral) and/or topical (for dermal use). These pharmaceuticals are introduced into the pool most likely by urine and sweat for oral (or parenteral) and washed from the skin for topical or dermal use. A persistent drug, carbamazepine is consumed orally thus most likely present in urine and can be also present in sweat (Daughton and Ruhoy, 2009). The contribution from sweat compared to urine is still unknown but most likely much lower. Based on the results, carbamazepine was detected in 27 samples from 51 in total water samples. Subsequently, this result indicates that the urination in pools is likely still in practice.

UV filters

Fourteen selected UV filters were analysed from 51 water samples collected from 17 pools in duplicates. Samples quenched or not with acid did not show significant differences except in the case of 1HBT and THB. 1HBT has a pKa value of 8.37 indicating that in some pool waters it could exist in the anionic form. THB is the most affected compound among the hydroxylated UV filters by pH (Tarazona et al. 2010) with best responses obtained under acid pH. For these reasons, and in order to compare with the pharmaceuticals, only the results obtained for UV filters in acid media were considered for further discussion.

Results showed that all samples contained at least one UV filter (>LOD) and that all the 14 UV filters selected were present at least in one sample (Annex Table S4). The three most frequently detected UV filters were Et-PABA (37/51), 1HBT (36/51) and 4HB (36/51), known human metabolite of BP3. Despite it, Et-PABA was always below the limit of quantification. Similarly, OD-PABA was only quantifiable in one sample, and at low concentration. BP8 and 4DHB, another two major human metabolites of BP3, were also

frequently detected, in 29 (57%) and 27 (53%) samples, respectively. Subsequently, this result indicates, as in the case of pharmaceuticals, that the urination in pools appears to be in practice.

4MBC has the highest concentration among the UV filters considered in the study, with a value as high as 69.3 ng/L in an indoor spa water sample (CB2) and a frequency of detection of 35%. In the other two samples collected in the same spa, the concentrations were also relevant (49.7 and 10.9 ng/L). This compound is approved in Canada by Health Canada and in Europe by the European Union's Scientific Committee for Cosmetic Products & Non-Food Products, however its use was banned in some countries, such as Denmark for children use products. It is not approved for use in the USA by the Food and Drug Administration and it is not permitted in Japan because of its potential for endocrine disruption in rat, fish, shellfish and insects. Water samples from spas were those with the highest number of compounds, which may be the result of the beauty and hygiene products used in the body treatments this kind of facilities provide.

Similar to pharmaceuticals, in order to expand the safe margin for discussion on water treatment, only concentrations higher than LOQ were considered in the results analysis, but the complete set of data is listed in Annex Table S4. 11 out of 14 selected UV filters were determined at this level in 94.1% of the water samples (48/51).

Figure 3 shows the concentration of each UV filter measured (>LOQ) in 51 water samples. The highest UV filter concentration was 4MBC found in CB2 (spa) at concentration 69.3 ng/L. In adult-outdoor and indoor pools, the highest UV filter found was THB at concentration of 52 ng/L and 48.4 ng/L, respectively. The maximum concentration of BP1 was 1.2 ng/L, BP2 was 27.2 ng/L, BP3 was 15.2 ng/L, and BP8 was 21.6 ng/L. 4DHB was found at maximum concentration of 30.8 ng/L whereas 1HBT was observed at 18.8 ng/L. The only OD-PABA found in water samples was at a concentration of 2 ng/L in an outdoor pool GN1. MeBt was found at the highest concentration of 3.4 ng/L and DMeBT was found at concentrations up to 3.1 ng/L.

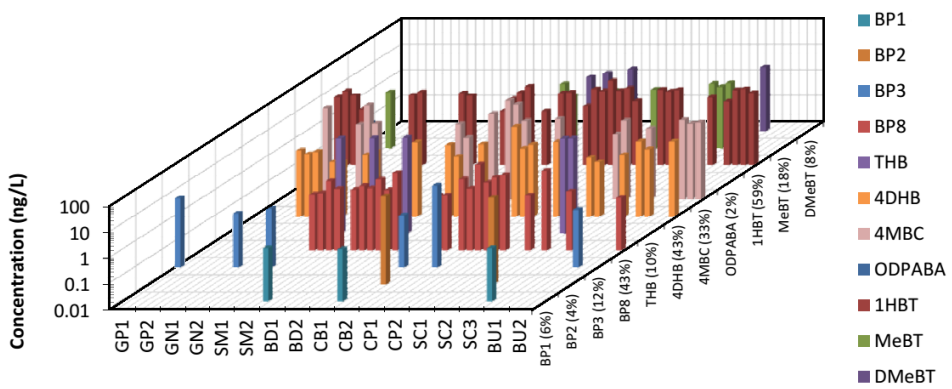

Figure 3. Concentration and occurrence of UV filters in swimming pools and spas

Figure 3 also shows the occurrence frequency of individual UV filters in the water samples from the pools. From the three most frequently detected UV filters (>LOD) mentioned above (Et-PABA, 1HBT and 4HB), only 1HBT had concentration >LOQ and was quantified in 30 out of 51 samples (59%). The detail data analyses on the frequency of detection of each UV filter are listed in Annex Table S8. Comparing between the results of pharmaceuticals and UV filters, the frequency of detection of UV filters was similar to pharmaceuticals (Annex Table S6 and Table S8). Regardless the type of the pool, the highest concentration level of UV filters (69.3 ng/L) found in the samples was 13 times lower than pharmaceuticals (904 ng/L). The low concentration found for UV filters in comparison to pharmaceuticals can be explained by the action of the presence of chlorine in the water. Chlorine derivatives are formed in pools, mainly by reactions between hypochlorous acid (HOCl) and phenol anion. As a result of this process, mono- and dichloro- substitutes, both of the parent compound and its metabolites, are formed (Negreira et al. 2008).

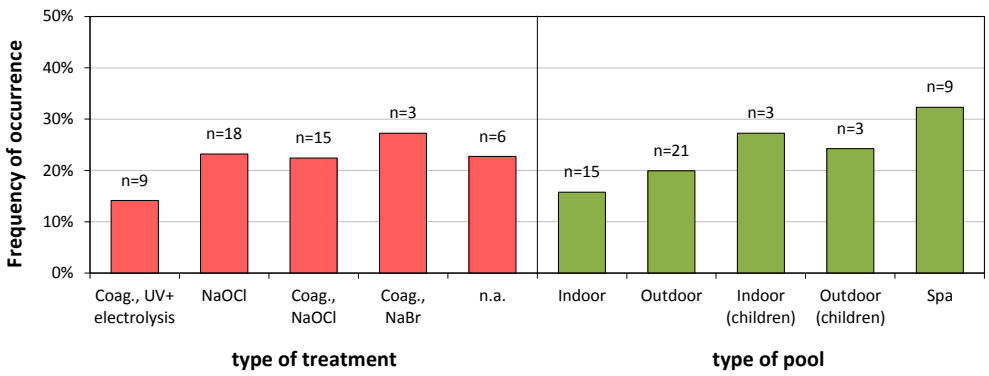

Figure 4. Occurrence of UV filters categorized by treatment and type of pools (n: number of samples)

The occurrence frequency of UV filters in different pools was categorized by treatment and pool type (Figure 4); for this categorization, the 11 UV filters that were found to be present in concentrations >LOQ were considered. Overall 121 positive values were detected in 51 samples. Since a different number of pools of each type were assessed, relative values are presented. For example, UV filters have been detected most frequently in spas: a total of nine samples were analysed from three different spa, hence the total number of analyses performed can be obtained by multiplying the number of samples (9) for the number of UV filters (11); among those 99 analyses, 32 showed values >LOQ, which correspond to 32.3% of relative occurrence. This result is aligned with the results from pharmaceuticals where spa has the highest frequency of detection. Comparing outdoor and indoor pools, a higher occurrence were observed in the outdoor ones. This is an expected finding since more protection is needed where sunlight exists. UV filters were least detected in the pools using (after coagulation and sand filters) UV

followed by salt electrolysis (14.1%). Additional information can be found in Annex Table S7.

Table 2 lists the occurrences of UV filters (BP3, 4MBC, OD-PABA) in swimming pools from previous studies (Teo et al. 2015) and also the results from our study. Only three of the UV filters found in this study were also identified in previous works (Table 2). Most of the studies listed in Table 2 are focused on method development except the study by Zwiener et al. (2007). Furthermore, the method used to quantify the UV filters in this study is more sensitive, with lower limits of detection (Annex Table S2) compared to the other studies.

Table 2. Concentration of UV filters in swimming pools

Reference	Matrix	UV filters concentration (ng/L)			Country
		BP3	4MBC	OD-PABA	
(Lambropoulou et al. 2002)	Swimming pool	2400-3300		<900-2000	Greece
(Giokas et al. 2004)	Swimming pool	4.2-5.7	5.4-6.9		Greece
(Cuderman and Heath 2007)	Swimming pool	103-400	<146-330	<37	Slovenia
(Zwiener et al. 2007)	Baby pool	1200	10000		Germany
	Swimmer pool		600		
	Non-swimmer pool		1400		
(Vidal et al. 2010)	Public pool	<110	<200	<70	Spain
	Private pool	<110	<60	<70	
This study (2015)	Indoor swimming pool	<0.1-4.87	<0.4-5.60	<0.1	Spain
	Outdoor swimming pool	<0.1-15.17	<0.4-35.9	<0.1-2.0	
	Indoor-children	<0.1-1.77	8.6-12.1	<0.1	
	Outdoor-children	<0.1-1.97	8.1-45.4	<0.1	
	Spa	<0.1-1.07	<0.4-69.3	<0.1	

Conclusions

The results show that 88.2% of the water samples (45 out of 51 samples) contained pharmaceuticals while 94.1% contained UV filters (48 out of 51 water samples). From the 32 pharmaceuticals analysed in the water samples, only 10 were detected at a concentration >LOQ; e.g. atenolol, carbamazepine, hydrochlorothiazide, metronidazole, ofloxacin, sulfamethoxazole, acetaminophen, ibuprofen, ketoprofen and phenazone. As for UV filters, 11 out of 14 UV filters analysed were detected at concentrations >LOQ; i.e. BP1, BP2, BP3, BP8, THB, 4DHB, 4MBC, OD-PABA, 1HBT, MeBT, and DMeBT. The most frequently detected pharmaceutical was carbamazepine, and it was found in more than half of the samples collected (53%). The most frequently occurring UV filter was 1HBT which was found in 30 of 51 (59%) water samples collected.

The highest concentration of individual pharmaceuticals and UV filters measured was 904 ng/L and 69.3 ng/L for hydrochlorothiazide and 4MBC, respectively.

To highlight the occurrence of pharmaceuticals and UV filters in the pools based on the type of pools, the highest occurrence of pharmaceuticals and UV filters was in the spas compared to the other type of pools (outdoor and indoor swimming pools). The analysis comparing different type of treatment in pools showed that the lowest frequency of detection of pharmaceuticals was in pool applying sand filters followed by disinfection by sodium hypochlorite, while the lowest frequency of UV filters were found in the pool applying, after coagulation and sand filters, a combination of UV irradiation and salt electrolysis as disinfection.

This study is subjected to some limitations. Firstly, the number of pool users was not documented thus it was not possible to correlate the contribution of pool users to the level of PPCPs found in the pools. Secondly, the effect of different combinations of water treatment is difficult to conclude due to water recirculation and continuous addition of PPCPs from pool users.

PPCPs detected in the water samples of this study are commonly found in the environment as well. Drinking water and wastewater treatment plants have reported experiencing difficulties in the removal or degradation of these compounds, consequently, accumulation in the environment is expected. In swimming pools, the swimmers have direct contact with the analysed compounds and their byproducts that can be present at higher concentration than in the environment in case of ineffective water treatment and recirculation applied. The accumulation of persistent PPCPs is likely to occur in swimming pools where simple sand filtration followed by disinfection (and water recirculation) is the common treatment applied. Therefore, frequent water replacement to dilute the pool water shall be considered as well as water treatment technologies to remove PPCPs effectively (e.g. ozonation). However, further studies are required to establish whether health impacts of PPCPs can justify higher investment and operational costs of water treatment technologies.

References

Adams, C., Wang, Y., Loftin, K., Meyer, M. (2002). Removal of antibiotics from surface and distilled water in conventional water treatment processes. *Journal of Environmental Engineering*, 128(3), 253–260.

Alonso, M.B., Feo, M.L., Corcellas, C., Gago-Ferrero, P., Bertozzi, C.P., Marigo, J., Flach, L., Meirelles, A.C.O., Carvalho, V.L., Azevedo, A.F. (2015). Toxic heritage: Maternal transfer of pyrethroid insecticides and sunscreen agents in dolphins from Brazil. *Environmental Pollution*, 207, 391–402.

Balmer, M.E., Buser, H-R., Müller, M.D., Poiger, T. (2005). Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environmental Science & Technology*, 39(4), 953–962.

Bedner, M., MacCrehan, W.A. (2006). Transformation of acetaminophen by chlorination produces the toxicants 1, 4-benzoquinone and N-acetyl-p-benzoquinone imine. *Environmental Science & Technology*, 40(2), 516–522.

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S.A. (2008). Pharmaceuticals and endocrine disrupting compounds in US drinking water. *Environmental Science & Technology*, 43(3), 597–603.

Bottoni, P., Bonadonna, L., Chirico, M., Caroli, S., Záray, G. (2014). Emerging issues on degradation by-products deriving from personal care products and pharmaceuticals during disinfection processes of water used in swimming pools. *Microchemical Journal*, 112, 13v16.

Brausch, J.M., & Rand, G.M. (2011). A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere*, 82(11), 1518–1532.

Brausch, J.M., Connors, K.A., Brooks, B.W., Rand, G.M. (2012). Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. In: *Reviews of Environmental Contamination and Toxicology*, vol 218, pp 1–99: Springer.

Bu, Q., Wang, B., Huang, J., Deng, S., Yu, G. (2013). Pharmaceuticals and personal care products in the aquatic environment in China: a review. *Journal of Hazardous Materials*, 262:189–211.

Cuderman, P., Heath, E. (2007). Determination of UV filters and antimicrobial agents in environmental water samples. *Analytical and Bioanalytical Chemistry*, 387(4), 1343–1350.

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E., Siemens, J. (2012). Accumulation of pharmaceuticals, Enterococcus, and resistance genes in soils irrigated with wastewater for zero to 100 years in Central Mexico. *PLoS ONE*, 7(9), 10.

Daughton, C.G., Ruhoy, I.S. (2009). Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers. *Environmental Toxicology and Chemistry*, 28(12), 2495–2521.

Daughton, C.G., Ternes, T.A. (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives*, 107(Suppl 6), 907.

Díaz-Cruz, M.S., Llorca, M., Barceló, D. (2008). Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment. *TrAC Trends in Analytical Chemistry*, 27(10), 873–887.

Díaz-Cruz, M.S., Gago-Ferrero, P., Llorca, M., Barceló, D. (2012). Analysis of UV filters in tap water and other clean waters in Spain. *Analytical and Bioanalytical Chemistry*, 402(7), 2325–2333.

Downs, C.A., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Ciner, F., Jeger, R., Lichtenfeld, Y., Woodley, C.M., Pennington, P., Cadenas, K., Kushmaro, A., Loya, Y. (2015). Toxicopathological effects of the sunscreen UV Filter, oxybenzone (benzophenone-3), on coral planulae and cultured primary cells and its environmental contamination in Hawaii and the U.S. Virgin Islands. *Archives of Environmental Contamination and Toxicology*, 1–24.

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Franson, M.A.H. (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington, DC.

Felix, T., Hall, B.J., Brodbelt, J.S. (1998). Determination of benzophenone-3 and metabolites in water and human urine by solid-phase microextraction and quadrupole ion trap GC–MS. *Analytical Chimia Acta*, 371(2–3), 195–203.

Fent, K., Kunz, P.Y., Gomez, E. (2008). UV filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. *CHIMIA International Journal for Chemistry*, 62(5), 368–375.

Fent, K., Kunz, P.Y., Zenker, A., Rapp, M. (2010). A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. *Marine Environmental Research*, 69, Supplement 1, S4–S6.

Gago-Ferrero, P., Alonso, M.B., Bertozzi, C.P., Marigo, J., Barbosa, L., Cremer, M., Secchi, E.R., Domit, C., Azevedo, A., Lailson-Brito, J., Torres, J.P.M., Malm, O., Eljarrat, E., Díaz-Cruz, M.S., Barceló, D. (2013a). First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. *Environmental Science & Technology*, 47(11), 5619–5625.

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D. (2013b). Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1294, 106–116.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D. (2015). UV filters bioaccumulation in fish from Iberian river basins. *Science of the Total Environment*, 518, 518–525.

Giokas, D.L., Sakkas, V.A., Albanis, T.A. (2004). Determination of residues of UV filters in natural waters by solid-phase extraction coupled to liquid chromatography–

photodiode array detection and gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1026(1–2), 289–293.

Glassmeyer. S,, Shoemaker, J. (2005). Effects of chlorination on the persistence of pharmaceuticals in the environment. *Bulletin of Environmental Contamination and Toxicology*, 74(1), 24–31.

Gros, M., Rodríguez-Mozaz, S., Barceló, D. (2012). Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *Journal of Chromatography A*, 1248, 104–121.

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment - A review. *Chemosphere*, 36(2), 357–393.

Judd, S.J., Black, S.H. (2000). Disinfection by-product formation in swimming pool waters: a simple mass balance. *Water Research*, 34(5), 1611–1619.

Judd SJ, Bullock G (2003) The fate of chlorine and organic materials in swimming pools. *Chemosphere*, 51(9), 869–879.

Kanan, A., Karanfil, T. (2011). Formation of disinfection by-products in indoor swimming pool water: The contribution from filling water natural organic matter and swimmer body fluids. *Water Research*, 45(2), 926–932.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J. (2008). The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Research*, 42(13), 3498–3518.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J. (2009). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research*, 439(2), 363–380.

Keuten, M.G.A., Schets, F.M., Schijven, J.F, Verberk, J.Q.J.C., van Dijk, J.C. (2012). Definition and quantification of initial anthropogenic pollutant release in swimming pools. *Water Research*, 46(11), 3682–3692.

Keuten M.G.A, Peters, M.C.F.M., Daanen, H.A.M., de Kreuk, M.K., Rietveld, L.C., van Dijk, J.C. (2014). Quantification of continual anthropogenic pollutants released in swimming pools. *Water Research*, 53, 259–270.

Kim, I., Yamashita, N., Tanaka, H. (2009). Performance of UV and UV/H2O2 processes for the removal of pharmaceuticals detected in secondary effluent of a sewage treatment plant in Japan. *Journal of Hazardous Materials*, 166(2–3), 1134–1140.

Klavarioti, M,, Mantzavinos, D., Kassinos, D. (2009). Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environmental International*, 35(2), 402–417.

Kockler, J., Oelgemöller, M., Robertson, S., Glass, B.D (2012). Photostability of sunscreens. *Journal of Photochemistry and Photobiology C : Photochemistry Reviews*, 13(1), 91–110.

Lambropoulou, D.A., Giokas, D.L., Sakkas, V.A, Albanis, T.A., Karayannis, M.I. (2002). Gas chromatographic determination of 2-hydroxy-4-methoxybenzophenone and octyldimethyl-p-aminobenzoic acid sunscreen agents in swimming pool and bathing waters by solid-phase microextraction. *Journal of Chromatography A*, 967(2), 243–253.

León-González, Z., Ferreiro-Vera, C., Priego-Capote, F., de Castro, M.D.L. (2011). Bioaccumulation assessment of the sunscreen agent 2-ethylhexyl 4-(N, N-dimethylamino) benzoate in human semen by automated online SPE-LC-MS/MS. *Analytical and Bioanalytical Chemistry*, 401(3), 1003–1011.

Li, W.C. (2014) Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environmental Pollution*, 187, 193–201.

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D. (2010). Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry. *Talanta*, 83(2), 410–424.

Negreira, N., Canosa, P., Rodríguez, I., Ramil, M., Rubí, E., Cela, R. (2008). Study of some UV filters stability in chlorinated water and identification of halogenated by-products by gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1178(1–2), 206–214.

Peng, X., Yu, Y., Tang, C., Tan, J., Huang, Q., Wang, Z. (2008). Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. *Science of the Total Environment*, 397(1–3), 158–166.

Poiger, T., Buser, H-R., Balmer, M.E., Bergqvist, P-A., Müller, M.D. (2004). Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. *Chemosphere*, 55(7), 951–963.

Real Decreto 742/2013, de 27 de septiembre, por el que se establecen los criterios técnico-sanitarios de las piscinas, vol 244 (2013). Ministerio de Sanidad, Servicios Sociales e Igualdad.

Sakkas, V.A., Giokas, D.L., Lambropoulou, D.A., Albanis, T.A. (2003). Aqueous photolysis of the sunscreen agent octyl-dimethyl-p-aminobenzoic acid: Formation of disinfection byproducts in chlorinated swimming pool water. *Journal of Chromatography A*, 1016(2), 211–222.

Santos, A.J.M., Miranda, M.S., Esteves da Silva, J.C.G. (2012). The degradation products of UV filters in aqueous and chlorinated aqueous solutions. *Water Research*, 46(10), 3167–3176.

Schlumpf, M., Kypke, K., Wittassek, M., Angerer, J., Mascher, H., Mascher, D., Vökt, C., Birchler, M., Lichtensteiger, W. (2010). Exposure patterns of UV filters, fragrances,

parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: Correlation of UV filters with use of cosmetics. *Chemosphere*, 81(10), 1171–1183.

Shen, R., Andrews, S.A. (2011). Demonstration of 20 pharmaceuticals and personal care products (PPCPs) as nitrosamine precursors during chloramine disinfection. *Water Research*, 45(2), 944–952.

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B. (2004). Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of the Total Environment*, 329(1), 99–113.

Tarazona, I., Chisvert, A., León, Z., Salvador, A. (2010). Determination of hydroxylated benzophenone UV filters in sea water samples by dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1217(29), 4771–4778.

Teo, T.L.L., Coleman, H.M., Khan, S.J. (2015). Chemical contaminants in swimming pools: Occurrence, implications and control. *Environment International*, 76, 16–31.

Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F, Brauch, H-J., Haist-Gulde, B., Preuss, G., Wilme, U., Zulei-Seibert, N. (2002). Removal of pharmaceuticals during drinking water treatment. *Environmental Science & Technology*, 36(17), 3855–3863.

UNE-EN 1484: Water analysis. Guidelines for the determination of Total Organic Carbon (TOC) and dissolved organic carbon (DOC). Asociacion Espanola de Normalizacion.

Valle-Sistac, J., Molins-Delgado, D., Díaz, M., Ibáñez, L., Barceló, D., Díaz-Cruz, M.S. (2016). Determination of parabens and benzophenone-type UV filters in human placenta. First description of the existence of benzyl paraben and benzophenone-4. *Environment International*, 88, 243–249.

Vidal, L., Chisvert, A., Canals, A., Salvador, A. (2010). Ionic liquid-based single-drop microextraction followed by liquid chromatography-ultraviolet spectrophotometry detection to determine typical UV filters in surface water samples. *Talanta*, 81(1), 549–555.

Vieno, N.M., Härkki, H., Tuhkanen, T., Kronberg, L. (2007). Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. *Environmental Science & Technology*, 41(14), 5077–5084.

Wang, W., Qian, Y., Boyd, J.M., Wu, M., Hrudey, S.E., Li, X-F. (2013). Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environmental Science & Technology*, 47(7), 3275–3282.

Weisbrod, C.J., Kunz, P.Y., Zenker, A.K., Fent, K. (2007). Effects of the UV filter benzophenone-2 on reproduction in fish. *Toxicology and Applied Pharmacology*, 225(3), 255–266.

Weng, S., Sun, P., Ben, W., Huang, C-H., Lee, L.T., Blatchley III, E.R. (2014). The presence of pharmaceuticals and personal care products in swimming pools. *Environmental Science & Technology Letters*, 1(12), 495–498.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005). Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environmental Science & Technology*, 39(17), 6649–6663.

WHO (2006) Guidelines for safe recreational water environments volume 2 Swimming pools and similar environments. World Health Organization, Geneva.

Yu, C-P., Chu, K-H. (2009). Occurrence of pharmaceuticals and personal care products along the West Prong Little Pigeon River in east Tennessee, USA. *Chemosphere*, 75(10), 1281–1286.

Zenker, A., Cicero, M.R., Prestinaci, F., Bottoni, P., Carere, M. (2014). Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. *Journal of Environmental Management*, 133, 378–387.

Zhang, A., Li, Y., Song, Y., Lv, J., Yang, J. (2014). Characterization of pharmaceuticals and personal care products as N-nitrosodimethylamine precursors during disinfection processes using free chlorine and chlorine dioxide. *Journal of Hazardous Materials*, 276, 499–509.

Zwiener, C., Richardson, S.D., De Marini, D.M., Grummt, T., Glauner, T., Frimmel, F.H. (2007). Drowning in disinfection byproducts? Assessing swimming pool water. *Environmental Science & Technology*, 41(2), 363–372.

Annex

Table S1. Target pharmaceuticals studied

Compound	Precursor ion (m/z)	Internal Standard	Therapeutic groups	CAS number	LOQ (ng/L)	LOD (ng/L)
Compounds analysed under positive mode						
Atenolol	267 $[M+H]^+$	Atenolol- d_7	β-Blocking agents	29122-68-7	0.03	0.01
Azithromycin	749 $[M+H]^+$	Azithromycin-d_3	Antibiotics	83905-01-5	0.96	0.29
Carbamazepine	237 $[M+H]^+$	Carbamazepine-d_{10}	Psychiatric drugs	298-46-4	0.02	0.01
Cefalexin	348 $[M+H]^+$	Sulfamethoxazole- d_5	Antibiotics	15686-71-2	1.16	0.35
Ciprofloxacin	332 $[M+H]^+$	Ofloxacin- d_3	Antibiotics	85721-33-1	37.13	11.40
Clarithromycin	748 $[M+H]^+$	Azithromycin-d_3	Antibiotics	81103-11-9	0.34	0.10
Codeine	300 $[M+H]^+$	Carbamazepine-d_{10}	Analgesics/anti-inflammatories	76-57-3	0.05	0.02
Desloratadine	311 $[M+H]^+$	Cimetidine- d_3	Histamine H_1 and H_2 receptor antagonists	100643-71-8	1.95	0.59
Erythromycin	734 $[M+H]^+$	Erythromycin-N,$N^{13}C_2$	Antibiotics	59319-72-1	1.64	0.49
Loratadine	383 $[M+H]^+$	Cimetidine- d_3	Histamine H_1 and H_2 receptor antagonists	79794-75-5	0.02	0.01
Metronidazole	172 $[M+H]^+$	Ronidazole-d_3	Antibiotics	443-48-1	1.32	0.39
Metronidazole-OH	187 $[M+H]^+$	Ronidazole-d_3	Antibiotics	4812-40-2	0.95	0.28
Ofloxacin	362 $[M+H]^+$	Ofloxacin- d_3	Antibiotics	82419-36-1	0.42	0.13
Oxycodone	316 $[M+H]^+$	Carbamazepine-d_{10}	Analgesics/anti-inflammatories	124-90-3	5.08	1.52
Phenazone	189 $[M+H]^+$	Phenazone-d_3	Analgesics/anti-inflammatories	60-80-0	0.72	0.22
Sulfamethoxazole	254 $[M+H]^+$	Sulfamethoxazole- d_4	Antibiotics	723-46-6	0.42	0.13
Tetracycline	445 $[M+H]^+$	Sulfamethoxazole-d_4	Antibiotics	64-75-5	4.49	1.35

Compound	Precursor ion (m/z)	Internal Standard	Therapeutic groups	CAS number	LOQ (ng/L)	LOD (ng/L)
Compounds analysed under negative mode						
Acetaminophen	150 $[M-H]^-$	Acetaminophen-d_4	Analgesics/anti-inflammatories	103-90-2	1.57	0.47
Bezafibrate	360 $[M-H]^-$	Bezafibrate-d_6	Lipid regulators and cholesterol lowering statin drugs	41859-67-0	0.25	0.07
Dexamethasone	451 $[M-H]^-$	Dexamethasone-d_4	Synthetic glucocorticoid	50-02-2	0.72	0.21
Diclofenac	294 $[M-H]^-$	Ibuprofen-d_3/ Diclofenac	Analgesics/anti-inflammatories	15307-79-6	2.04	0.61
Furosemide	329 $[M-H]^-$	Furosemide-d_5	Diuretic	54-31-9	7.66	2.30
Hydrochloro-thiazide	296 $[M-H]^-$	Hydrochlorothiazide-d_2	Diuretic	58-93-5	2.03	0.61
Ibuprofen	205 $[M-H]^-$	Ibuprofen-d_3	Analgesics/anti-inflammatories	15687-27-1	4.64	1.39
Irbesartan	427 $[M-H]^-$	Valsartan-d_8	Antihypertensives	138402-11-6	0.11	0.03
Ketoprofen	253 $[M-H]^-$	Ibuprofen-d_3	Analgesics/anti-inflammatories	22071-15-4	1.85	0.55
Losartan	421 $[M-H]^-$	Valsartan-d_8	Antihypertensives	124750-99-8	0.42	0.13
Meloxicam	350 $[M-H]^-$	Meloxicam-d_3	Analgesics/anti-inflammatories	71125-39-8	0.34	0.10
Naproxen	229 $[M-H]^-$	Ibuprofen-d_3	Analgesics/anti-inflammatories	22204-53-1	2.32	0.70
Piroxicam	330 $[M-H]^-$	Meloxicam-d_3	Analgesics/anti-inflammatories	36322-90-4	0.28	0.08
Tenoxicam	336 $[M-H]^-$	Meloxicam-d_3	Analgesics/anti-inflammatories	59804-37-4	0.22	0.07
Torasemide	347 $[M-H]^-$	Furosemide-d_5	Diuretic	56211-40-6	n.a.	n.a.

Table S2. UV filters selected for the study

INCI Nomenclature	Abbreviation	Molecular weight	Molecular formula	CAS number	LOQ (ng/L)	LOD (ng/L)
Benzophenone-1	BP1	214.22	$C_{13}H_{10}O_3$	131-56-6	1.0	0.1
Benzophenone-2	BP2	246.22	$C_{13}H_{10}O_5$	131-55-5	1.3	0.4
Benzophenone-3	BP3	228.24	$C_{14}H_{12}O_3$	131-57-7	1.0	0.1
Benzophenone-8	BP8	244.24	$C_{14}H_{12}O_4$	131-53-3	1.0	0.3
Trihydroxybenzophenone	THB	230.22	$C_{13}H_{10}O_4$	1143-72-2	1.0	0.0
4-Hydroxybenzophenone	4HB	198.22	$C_{13}H_{10}O_2$	1137-42-4	1.2	0.4
4-4'-Dihydroxybenzophenone	4DHB	214.22	$C_{13}H_{10}O_3$	611-99-4	1.0	0.1
4-Metylbenzylidene camphor	4MBC	254.37	$C_{18}H_{22}O$	36861-47-9	1.4	0.4
Ethylhexyl dimethyl PABA	ODPABA	277.40	$C_{17}H_{27}NO_2$	21245-02-3	1.0	0.1
Ethyl PABA	EtPABA	165.19	$C_9H_{11}NO_2$	94-09-7	1.0	0.1
Benzotriazole	1HBT	119.12	$C_6H_5N_3$	95-14-7	1.0	0.2
5-Methylbenzotriazole	MeBT	133.15	$C_7H_7N_3$	136-85-6	1.0	0.3
Dimethylbenzotriazole	DMeBT	147.18	$C_8H_9N_3$	4184-79-6	1.0	0.1
2-(5-Tert-Butyl-2-Hydroxyphenyl) Benzotriazole	TBHPBT	267.33	$C_{16}H_{17}N_{3O}$	3147-76-0	11.2	3.3

Table S3 and S4 listed the pharmaceuticals and UV filters found to be present >LOD in at least in one sample from 3 days of sampling in each pools. One water sample per pool per day was collected on 3 different days. The three concentration values per swimming pool/spa in Table S3 and S4 correspond to 3 days of sampling in the same swimming pool/spa (same point).

Table S3. Pharmaceutical compounds present in water samples at a concentration above limits of detection

Compounds	**Concentration in the pools (ng/L)**																	
	GP1			**GP2**			**GN1**			**GN2**			**SM1**			**SM2**		
Atenolol	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
Carbamazepine	1.05	0.37	<LOD	1.26	0.37	<LOQ	1.17	0.33	<LOQ	1.13	0.30	<LOQ	1.12	0.50	<LOD	1.26	0.23	<LOD
Hydrochlorothiazide	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Erythromycin	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD
Metronidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ofloxacin	2.12	1.27	1.15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD
Sulfamethoxazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.42	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acetaminophen	<LOD	<LOD	3.69	3.81	1.81	8.52	<LOD	<LOD	<LOQ	<LOD	<LOD	61.18	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ibuprofen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ketoprofen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	23.10	<LOD	35.93	<LOD	<LOD	12.76	<LOQ	15.11
Phenazone	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD

Compounds	Concentration in the pools (ng/L)																	
	BD1			BD2			CB1			CB2			CP1			CP2		
Atenolol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	1.24	0.26	<LOD	1.35	0.29	<LOD	1.11	0.33	<LOQ	1.07	0.27	<LOQ	1.21	0.36	<LOQ	1.08	0.32	<LOD
Hydrochlorothiazide	<LOD	<LOD	<LOD	<LOD	903.62	<LOD	<LOD	<LOD	<LOD	26.60	25.27	6.71	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Erythromicin	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD
Metronidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ofloxacin	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfamethoxazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acetaminophen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	30.17	<LOD	<LOD	<LOD	3.06	<LOD	3.08
Ibuprofen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ketoprofen	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	53.12	2.26	<LOD	<LOD	26.39	22.27	27.00
Phenazone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	0.78	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ

	SC1			SC2			SC3			BU1			BU2		
Atenolol	<LOQ	<LOD	<LOD	0.28	0.62	0.28	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	0.25	<LOD
Carbamazepine	<LOQ	<LOD	<LOD	1.22	1.42	<LOQ	<LOQ	0.76	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Hydrochlorothiazide	<LOD	<LOD	<LOD	35.01	37.08	37.52	<LOD	7.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Erythromicin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Metronidazole	<LOQ	2.30	3.25	1.75	<LOD	1.51	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.52
Ofloxacin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfamethoxazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acetaminophen	<LOD	3.44	2.09	<LOD	2.51	4.05	<LOD	<LOD	<LOD	<LOD	2.61	25.15	58.93	13.24	<LOD
Ibuprofen	25.68	26.67	27.88	136.06	159.15	171.25	16.31	16.50	39.11	26.86	20.82	16.27	51.62	34.09	35.60
Ketoprofen	<LOD	<LOD	<LOD	292.74	273.94	335.87	<LOD	<LOD	<LOD	83.47	107.76	87.91	158.36	130.67	360.05
Phenazone	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ

Table S4. 14 UV filters present in water samples at a concentration above limits of detection

Compounds	Concentration in the pools (ng/L)																	
	GP1			GP2			GN1			GN2			SM1			SM2		
ACID																		
BP1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	1.20
BP2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP3	4.87	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.27	<LOD	<LOD	<LOD	1.97	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
BP8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	1.50	1.70	5.00	2.50	<LOQ
THB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	48.40	<LOD	<LOD	<LOD	49.20
4HB	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ
4DHB	<LOD	<LOD	<LOD	<LOQ	<LOD	3.67	2.67	3.27	<LOD	1.27	1.27	<LOD	<LOD	2.47	3.27	<LOD	<LOD	<LOD
4MBC	<LOD	<LOD	<LOD	<LOD	<LOD	35.90	<LOD	<LOD	<LOD	8.10	45.40	8.80	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
ODPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EtPABA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOD
IHBT	4.40	7.60	5.10	1.50	<LOQ	<LOD	<LOD	<LOD	<LOQ	5.20	6.90	<LOD	<LOQ	<LOD	<LOD	6.30	4.90	<LOD
MeBT	<LOD	<LOD	<LOD	1.50	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	1.10	<LOD	<LOD	<LOD	<LOD	<LOD
DMeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TBHPBT	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
NO ACID																		
BP1	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	1.70	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	1.10
BP2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.67	<LOD	1.67	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP8	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	38.20	<LOQ	2.20	8.00	2.60	<LOQ
THB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
4HB	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
4DHB	14.37	<LOQ	<LOQ	<LOD	<LOD	1.97	<LOQ	<LOD	<LOD	<LOD	1.67	<LOD	7.37	<LOQ	<LOD	<LOD	1.97	2.27
4MBC	<LOD	<LOD	33.30	<LOD	2.20	<LOD	4.50	<LOD	<LOD	8.00	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	3.60
ODPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EtPABA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOQ
IHBT	8.10	4.60	9.50	7.40	3.40	2.80	<LOD	1.20	1.50	7.00	3.60	2.10	<LOQ	<LOD	1.20	8.90	1.60	1.50
MeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	1.00	<LOD	1.30	<LOD	<LOD	<LOQ
DMeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TBHPBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Compounds	Concentration in the pools (ng/L)																	
	BD1			BD2			CB1			CB2			CP1			CP2		
ACID																		
BP1	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	1.10	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
BP2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	27.20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	1.07	<LOQ	<LOD	<LOD	15.17	<LOD	<LOQ	<LOD	<LOD
BP8	2.30	3.20	2.70	5.70	2.20	10.50	<LOD	<LOQ	<LOQ	<LOD	1.40	1.40	<LOQ	5.80	2.60	21.60	4.30	7.00
THB	<LOD	<LOD	<LOD	52.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
4HB	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD
4DHB	<LOD	7.47	<LOD	<LOQ	<LOQ	5.97	2.07	<LOD	<LOD	6.37	<LOD	<LOQ	<LOQ	30.77	4.37	6.17	<LOD	<LOD
4MBC	<LOD	<LOD	<LOD	8.20	2.40	<LOD	<LOD	21.30	<LOD	69.30	49.70	10.90	<LOD	<LOD	<LOD	13.30	<LOD	<LOD
ODPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EtPABA	<LOQ	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ
1HBT	<LOQ	<LOD	1.30	3.70	6.70	11.50	<LOD	1.30	<LOD	6.30	6.80	<LOQ	2.00	9.10	7.60	18.80	7.80	9.60
MeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.10	1.00	<LOD	<LOD	<LOD	<LOD	1.70	<LOD	<LOQ	<LOD	<LOQ	1.90
DMeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.25	<LOD	1.75	<LOD	<LOD	2.55	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TBHPBT	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
NO ACID																		
BP1	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	24.90
BP3	<LOD	<LOD	2.57	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	1.77	<LOQ	<LOD	<LOD	<LOD	16.57	<LOD	<LOD
BP8	1.60	2.10	6.60	2.80	2.60	4.00	<LOD	<LOD	<LOD	2.80	2.00	2.00	<LOD	<LOQ	1.60	59.10	7.30	18.30
THB	<LOD	<LOD	50.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	49.70
4HB	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOQ
4DHB	1.87	2.57	3.77	<LOD	1.97	<LOD	<LOQ	<LOQ	1.27	2.97	<LOD	<LOD	<LOQ	4.07	1.17	<LOD	<LOD	<LOQ
4MBC	<LOD	4.80	<LOD	7.40	1.40	<LOD	<LOD	<LOD	<LOD	54.30	26.20	28.60	<LOD	<LOD	5.20	<LOQ	<LOD	8.00
ODPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EtPABA	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ
1HBT	4.10	<LOQ	<LOD	14.10	10.10	9.30	<LOD	<LOD	<LOD	3.90	<LOD	6.80	<LOQ	1.30	1.60	12.00	<LOQ	12.00
MeBT	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	2.10
DMeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.95	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TBHPBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Compounds	Concentration in the pools (ng/L)														
	SC1			SC2			SC3			BU1			BU2		
ACID															
BP1	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOD	1.20	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOQ
BP2	<LOD	<LOD	<LOD	<LOD	<LOD	23.70	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP3	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.77	<LOQ	<LOD
BP8	8.50	<LOD	<LOD	1.40	<LOQ	12.70	<LOQ	<LOD	2.00	<LOD	<LOD	<LOD	<LOD	<LOD	1.100
THB	<LOD	<LOD	<LOD	<LOD	47.60	48.40	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
4HB	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD
4DHB	7.77	3.37	<LOD	<LOD	1.87	1.27	<LOD	<LOD	2.47	<LOD	8.17	4.07	<LOD	<LOD	8.07
4MBC	<LOD	<LOD	<LOD	<LOD	3.40	11.20	<LOD	<LOD	5.60	<LOD	<LOD	<LOD	12.10	8.60	9.80
ODPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EtPABA	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ
1HBT	3.10	<LOD	<LOD	8.70	7.30	8.50	<LOD	<LOD	<LOQ	4.30	<LOD	3.00	8.40	9.30	6.60
MeBT	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOD	3.20	2.40	3.40	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
DMeBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.05	<LOD	<LOD	<LOD	<LOD	<LOD
TBHPBT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
NO ACID															
BP1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
BP2	23.60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP3	<LOQ	<LOQ	2.27	<LOD	<LOD	4.67	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP8	5.00	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	1.50	40.90	<LOD	1.20	<LOQ	<LOD	<LOQ	<LOD
THB	<LOD	<LOD	<LOD	<LOD	<LOD	53.80	<LOD	<LOD	<LOD	<LOD	47.90	<LOD	<LOD	<LOD	<LOD
4HB	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
4DHB	2.77	2.37	31.97	<LOD	<LOD	5.97	1.07	<LOD	3.17	<LOD	2.87	2.77	<LOD	9.17	<LOQ
4MBC	<LOD	<LOD	<LOD	<LOD	<LOD	11.70	<LOD	<LOD	<LOD	<LOD	9.00	<LOD	<LOQ	3.80	3.70
ODPABA	<LOD	<LOD	<LOD	<LOD	<LOD	0.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EtPABA	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ
1HBT	3.80	3.60	<LOD	10.30	8.10	5.50	<LOQ	<LOQ	1.40	3.40	4.00	6.90	7.30	6.90	10.50
MeBT	<LOD	1.10	<LOD	<LOD	<LOD	<LOD	3.20	<LOD	2.10	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD
DMeBT	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TBHPBT	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOD

Table S5. Number of samples with detected concentration of pharmaceuticals, classified by different types of pools and treatments

Pools	Number of pools	Number of water samples	Number of pharmaceuticals detected >LOQ	Number of analyses	Occurrence
Based on type of pools					
Indoor adult	5	15	10	150	29 (19.3%)
Outdoor adult	7	21	10	210	21 (10.0%)
Indoor children	1	3	10	30	10 (33.3%)
Outdoor children	1	3	10	30	5 (16.7%)
Spa	3	9	10	90	33 (36.7%)
Total	17	51	10	510	98 (19.2%)
Based on treatments					
Coag., salt electrolysis, UV	3	9	10	90	14 (15.6%)
No coagulation, NaOCl	6	18	10	180	24 (13.3%)
Coagulation, NaOCl	5	15	10	150	48 (32.0%)
Coagulation, NaBr	1	3	10	30	7 (23.3%)
Unknown	2	6	10	60	5 (8.3%)
Total	17	51	10	510	98 (19.2%)

Table S7. Number of samples with detected concentration of UV filters, classified by different type of pools and treatments

Type of pools	Number of pools	Number of water samples	Number of UV filters detected >LOQ	Number of analyses	Occurrence
Based on type of pools					
Indoor adult	5	15	11	165	26 (15.8%)
Outdoor adult	7	21	11	231	46 (19.9%)
Indoor children	1	3	11	33	9 (27.3%)
Outdoor children	1	3	11	33	8 (24.2%)
Spa	3	9	11	99	32 (32.3%)
Total	17	51		561	121 (21.6%)
Based on treatments					
Coag., salt electrolysis, UV	3	9	11	99	14 (14.1%)
No coagulation, NaOCl	6	18	11	198	46 (23.2%)
Coagulation, NaOCl	5	15	11	165	37 (22.4%)
Coagulation, NaBr	1	3	11	33	9 (27.3%)
Unknown	2	6	11	66	15 (22.7%)
Total	17	51		561	121 (21.6%)

Table S6. Occurrence of target pharmaceuticals in different types of pool and treatment scheme (concentrations >LOQ)

Therapeutic group	Pharmaceutical compounds	Frequency of occurrence (%)																					
		All type		Indoor (adult)		Outdoor (adult)		Indoor (children)		Outdoor (children)		Spa		Coag., UV+ electrolysis		NaOCl		Coag., NaOCl		Coag., NaBr		n.a.	
β-Blocking agents	Atenolol	4	8%	0	0%	0	0%	1	33%	0	0%	3	33%	0	0%	0	0%	4	27%	0	0%	0	0%
Psychiatric drugs	Carbamazepine	27	53%	5	33%	14	67%	0	0%	2	67%	6	67%	6	67%	12	67%	3	20%	2	67%	4	67%
Diuretic	Hydrochlorothiazide	8	16%	1	7%	1	5%	0	0%	0	0%	6	67%	0	0%	3	17%	4	27%	0	0%	1	17%
Antibiotics	Metronidazole	5	10%	2	13%	0	0%	1	33%	0	0%	2	22%	0	0%	0	0%	5	33%	0	0%	0	0%
	Ofloxacin	3	6%	3	20%	0	0%	0	0%	0	0%	0	0%	3	33%	0	0%	0	0%	0	0%	0	0%
	Sulfamethoxazole	1	2%	0	0%	0	0%	0	0%	1	33%	0	0%	0	0%	1	6%	0	0%	0	0%	0	0%
Analgesics/anti-inflammatories	Acetaminophen	16	31%	5	33%	3	14%	2	67%	1	33%	5	56%	4	44%	2	11%	8	53%	2	67%	0	0%
	Ibuprofen	15	29%	9	60%	0	0%	3	100%	0	0%	3	33%	0	0%	0	0%	15	100%	0	0%	0	0%
	Ketoprofen	18	35%	4	27%	3	14%	3	100%	1	33%	7	78%	1	11%	5	28%	9	60%	3	100%	0	0%
	Phenazone	1	2%	0	0%	0	0%	0	0%	0	0%	1	11%	0	0%	1	6%	0	0%	0	0%	0	0%

n.a.: not available

Table S8. Occurrence of target UV filters in the swimming pools and spas (samples with ascorbic acid addition) (>LOQ)

UV filters	Frequency of occurrence (%)																					
	All type		Indoor (adult)		Outdoor (adult)		Indoor (children)		Outdoor (children)		Spa		Coag., UV+ electrolysis		NaOCl		Coag., NaOCl		Coag., NaBr		n.a.	
BP1	3	6%	1	7%	2	10%	0	0%	0	0%	0	0%	0	0%	2	11%	1	7%	0	0%	0	0%
BP2	2	4%	0	0%	0	0%	0	0%	0	0%	2	22%	0	0%	1	6%	1	7%	0	0%	0	0%
BP3	6	12%	1	7%	2	10%	1	33%	1	33%	1	11%	1	11%	4	22%	1	7%	0	0%	0	0%
BP8	22	43%	4	27%	10	48%	1	33%	0	0%	7	78%	2	22%	6	33%	5	33%	3	100%	6	100%
THB	5	10%	1	7%	2	10%	0	0%	0	0%	2	22%	1	11%	1	6%	2	13%	0	0%	1	17%
4DHB	22	43%	7	47%	8	38%	1	33%	2	67%	4	44%	3	33%	8	44%	8	53%	1	33%	2	33%
4MBC	17	33%	1	7%	4	19%	3	100%	3	100%	6	67%	1	11%	7	39%	6	40%	1	33%	2	33%
ODPABA	1	2%	0	0%	1	5%	0	0%	0	0%	0	0%	0	0%	1	6%	0	0%	0	0%	0	0%
1HBT	30	59%	6	40%	11	52%	3	100%	2	67%	8	89%	4	44%	10	56%	9	60%	3	100%	4	67%
MeBT	9	18%	4	27%	4	19%	0	0%	0	0%	1	11%	2	22%	3	17%	3	20%	1	33%	0	0%
DMeBT	4	8%	1	7%	2	10%	0	0%	0	0%	1	11%	0	0%	3	17%	1	7%	0	0%	0	0%

n.a.: not available

4

Clinically relevant fungi in water and on surfaces in an indoor swimming pool facility

Ekowati, Y., van Diepeningen, A.D., Ferrero, G., Kennedy, M.D., de Roda Husman, A.M., Schets, F.M. (2017). Clinically relevant fungi in water and on surfaces in an indoor swimming pool facility. *International Journal of Hygiene and Environmental Health*, 220(7), 1152-1160.

Abstract

The density of fungal contamination and the fungal diversity in an indoor swimming pool facility were assessed. A total of 16 surface samples and 6 water samples were analysed by using a combination of different (semi-) selective culture media. Isolated fungal colonies were identified to the genus or species level by sequencing of the internal transcribed spacer (ITS). The highest fungal counts in water and on surfaces were in the recreational pool (17 CFU/100 mL) and on a flexibeam (5.8 CFU/cm²), respectively as compared with low counts (<0.1 CFU/cm²) on the diving platform, bench tops and walls. The 357 obtained isolates belonged to 79 species and species complexes, 42 of which known as clinically relevant. *Phialophora oxyspora* (13.7%) and *Phoma* spp. (12.3%) were the most frequently identified groups. We demonstrated that despite chlorine treatment and regular cleaning of surfaces both water and surfaces were commonly infested with fungi, including many clinically relevant species.

Introduction

Both filamentous and yeast-like fungi are ubiquitous in natural and man-made environments and some fungal species are known to cause diseases in plants, animals, and/or humans (Fisher et al., 2012; San-Blas & Calderone, 2008). Of all the estimated millions of fungal species, approximately 600 species are known as obligate or opportunistic pathogens (de Hoog et al., 2015). Human mycoses vary from relatively innocent, superficial infections in otherwise healthy people (e.g. ringworm and onychomycosis) to life-threatening invasive deep and disseminated infections (e.g. candidiasis, aspergillosis) especially in immunocompromised individuals. Most human mycoses are caused by opportunistic pathogens with a common occurrence in the environment (e.g. *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus* spp.).

Fungal infections in humans appear to be increasing worldwide (Brandt & Park, 2013; Brown et al., 2012; Dufresne et al., 2017). This may be partially due to better detection methods, but the increasing number of immunocompromised patients can also play a role (Low & Rotstein, 2011). In addition, there are concerns about the effects of climate change on pathogenic fungi: rising temperatures may increase the growth of waterborne pathogens, including fungi (de Roda Husman & Schets, 2010; van der Wielen et al., 2014). Other concerns are about the emergence of resistance to antifungal agents in pathogenic fungi over the past decades (Alcazar-Fuoli & Mellado, 2014; Gago et al., 2016).

In public facilities like swimming pools, people can get exposed to pathogens. The risk of acquiring infections in swimming pools is often associated with microbial contamination of the water due to faecal matter, non-faecal human shedding, and inadequate disinfection. Direct contact with contaminated surfaces and inhalation of air are also potential routes of exposure to pathogens. Studies on fungal contamination in swimming pool environments (water, surface and air) by Brandi et al. (2007) and Viegas et al. (2011) demonstrated the common occurrence of clinically relevant fungi of the genera *Cladosporium, Aspergillus*, *Fusarium* and *Trichophyton*. The species within these genera are known to be associated with human fungal infections (de Hoog et al., 2015). Although it is clear that fungi can affect human health, guidelines to prevent fungal contamination in swimming pool environments are limited. The dermatophytic, *Trichophyton* spp. and *Epidermophyton floccosum*, which are known to cause athlete's foot, are the only fungal species considered as potential microbial hazards in the WHO guidelines for safe recreational water (WHO, 2006).

To date, most studies in swimming pool facilities focused on specific fungal species (e.g. *Candida albicans* (Sato et al., 1995)), fungal genera (e.g. *Fusarium* (Buot et al., 2010)), fungal groups (e.g. dermatophytes (Ali-Shtayeh et al., 2003; Detandt & Nolard, 1995; Hilmarsdottir et al., 2005)) or specific fungal infections (e.g. tinea pedis (Kamihama et

al., 1997; Shemer et al., 2016)). Some studies assessed the total fungal contamination in swimming pool environments from different samples collected at different sites throughout swimming pools (Brandi et al., 2007; Viegas et al., 2011). However, fungal identification in most of these studies only went as far as the genus level and was based on morphologic characteristics.

In this study, the occurrence of different fungal species in water and on surfaces in an indoor swimming pool facility was investigated, focusing on clinically relevant fungal species. We defined clinically relevant fungal species as fungal species which are known to have caused infection and/or disease in humans. The purpose of this study was to broaden the knowledge on the fungal community in the swimming pool environment, in relation to a better understanding of the exposure routes of fungal infections in swimming pools. The diversity of fungal species was assessed by applying different sampling methods and culture media. Fungal identification to the genus level and where possible to the species level, was based on ITS sequencing.

Materials and methods

Sampling locations and water quality parameters

The sampling was carried out in one swimming pool facility in the Netherlands. The facility comprised of six separate pools with different sizes: pool A with a pool size of 375 m^2, pool B with a pool size of 50 m^2, pool C and D: separate pools with a total pool size of 300 m^2, pool E with a pool size of 135 m^2, and pool F with a pool size of 4.5 m^2. The sampling locations were chosen such that they represented the sites where exposure to the possibly present fungi would be most likely through direct contact with surfaces or water (Table 1).

Water quality parameters were measured in water samples collected from each pool. The pH was measured using a portable probe (pH meter 3310, WTW, Germany). Free chlorine levels were measured using DPD free chlorine powder pillows (Hach, USA). Total organic carbon (TOC) was measured using a TOC-L analyser (Shimadzu, Japan).

Table 1. Sampling locations

Location	Location code	Sample code	Sample type
Competition pool	A		
Pool		A	water
Diving platform		A-DP	surface
Top step of the ladder (outside the water)		A-TL	surface
Floors next to the pool		A-PS	surface
Preferential pathway (floors)		A-PP	surface
Bench top		A-BT	surface
Swimming starting platform		A-SP	surface
Wall in main pool area		A-WA	surface
Children's pool	B		
Pool		B	water
Pool with water slide	C		
Pool		C	water
Floors next to the pool		C-PS	surface
Recreational pool	D		
Pool		D	water
Floors next to the pool		D-PS	surface
Pool for special groups	E		
Pool		E	water
Floors next to the pool		E-PS	surface
Whirlpool	F		
Pool		F	water
Floors next to the pool		F-PS	surface
Dressing room	DR		
Preferential pathway (floors)		DR-PP	surface
Bench top		DR-BT	surface
Floors by the lockers		DR-LF	surface
Shower floors		DR-SF	surface
Others			
Flexibeam (foam)		FB	surface

Sample collection and preparation

Water samples were collected from the six pools using plastic containers with a volume of 20 L, which were previously cleaned using chlorine tablets (Suma Tab D4 Tab, Diversey, the Netherlands) according to the manufacturer's recommendation. The volume collected from each pool was 20 L and in each container sodium thiosulfate (final concentration 0.2 mM) was added to quench residual chlorine. The water samples were transported to the laboratory at ambient temperature and subsequently stored at 4 °C until further analysis. Sample volumes of 2 L, 1 L and 2x0.5 L of pool water from each sampling location were filtered through 0.45 µm pore size membrane filters (Millipore, no. EZHAWG474, the Netherlands). Membrane filters were placed on different (semi-)selective culture media (see Sample cultivation).

Each surface sample was taken by applying Replicate Organism Detection and Counting (RODAC) plates and Enviro swabs (3M, the Netherlands) on adjacent surfaces. RODAC plates with different kinds of culture media (see Sample cultivation) were applied by pressing the plates gently on the surface for 10 seconds (Nivens et al., 2009). Swab samples were collected by swabbing the surface inside a sterile sampling template 10x10 cm^2 (Copan Diagnostics, USA) by moving the swab back and forth across the surface with horizontal and vertical strokes (8–10 strokes per direction) covering the entire surface. Swabs were washed with 20 mL of 0.1% peptone saline, which was added to the tubes with the swabs followed by vigorous mixing by vortexing for 1 minute. Subsequently, the swabs were pressed firmly against the wall of the tube and removed from the solution. The extracted solution in the tubes was homogenized by gentle shaking by hand for 5–10 sec. Sample volumes of 0.1 mL, 0.5 mL and 2x1 mL were spread over the surface of different (semi-) selective media (see Sample cultivation).

Sample cultivation

For all samples, the following culture media were used:

a. Malt Extract Agar (MEA) (Oxoid, Thermo Fisher Scientific, UK) with penicillin and streptomycin, which allows general fungal growth. MEA was prepared by dissolving 50 g malt extract agar (Oxoid) in 1 L distilled water. The pH was set at 5.4 ± 0.2 and the medium was sterilized at 115 °C for 10 min. Penicillin and streptomycin were added to a final concentration of 50 mg/L after cooling to 55–60 °C.

b. Pentachloronitrobenzene medium (PCNB) with rose Bengal (van Wyk et al., 1986), streptomycin and chloramphenicol was used to select for *Fusarium* and other PCNB tolerant species. PCNB was prepared by dissolving 25 g agar, 15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g PCNB, 0.05 g Rose Bengal, and 0.05 g chloramphenicol in 1 L of distilled water and the mixture was sterilized at 121 °C for 20 min. Streptomycin was added to a final concentration of 50 mg/L after cooling to 55–60 °C.

c. Erythritol-chloramphenicol agar (ECA) with streptomycin was used to select for black yeasts (de Hoog & Haase, 1993). ECA was prepared by dissolving 25 g agar, 6.7 g yeast nitrogen base, 10 g meso-erythritol and 0.05 g chloramphenicol in 1 L of distilled water and the mixture was sterilized at 121 °C for 20 min. Streptomycin was added to a final concentration of 50 mg/L after cooling to 55–60 °C.

d. Sabouraud dextrose agar (SDA) (Oxoid, Thermo Fisher Scientific, UK) with cycloheximide and chloramphenicol (Kamihama et al., 1997) was used to select for dermatophytes and other pathogenic fungi. SDA was prepared by dissolving 65 g Sabouraud dextrose agar (Oxoid) in 1 L of distilled water and the pH was set at 5.6 ± 0.2. A 500 mg/L of cycloheximide and 50 mg/L of chloramphenicol were added before sterilization at 121 °C for 15 min.

All plates were incubated in the dark: ECA, PCNB, and SDA plates at 24 °C and MEA plates both at 24 °C and at 40 °C (MEA40). MEA40 was used to select for thermophilic fungi like the human pathogen *Aspergillus fumigatus*. After 7 days of incubation, the number of colonies was counted and was expressed as the number of colony forming units (CFU)/100 mL for water samples and CFU/cm^2 for surface samples. In some cases where overgrowth or too many colonies were observed after 7 days of incubation, the plates were not counted and were not included in the calculation.

Calculation and data analysis

The fungal concentration in water samples was expressed as colony forming units (CFU)/100 mL which was calculated based on the average number of colonies in each filtered sample volume. Equation 1 was used to calculate the fungal concentration where CFU_n is the number of colonies on a plate, V_n is the filtered volume (L), n is the number of counted plates.

$$\frac{CFU}{100\ mL} = \frac{\left(\frac{CFU_1}{V_1}+\frac{CFU_2}{V_2}+\cdots+\frac{CFU_n}{V_n}\right)}{n \times 10} \tag{1}$$

The fungal concentration on surfaces from RODAC plates was calculated based on the effective area of the RODAC plate. Equation 2 was used to calculate the fungal concentration where CFU_n is the number of colonies on a plate, A is RODAC plate's effective area (25 cm^2).

$$\frac{CFU}{cm^2} = \frac{CFU_n}{A} \tag{2}$$

The fungal concentration on surfaces from swabs was calculated based on the area represented by the sample volume. Equation 3 was used to calculate the fungal concentration where CFU_n is the number of colonies on a plate, V_n is the inoculated sample volume, n is the number of counted plates, A is the swabbed area (100 cm^2), V is the total sample volume (20 mL).

$$\frac{CFU}{cm^2} = \frac{\left(\frac{CFU_1}{V_1}+\frac{CFU_2}{V_2}+\cdots+\frac{CFU_n}{V_n}\right)}{n \times \frac{A}{V}} \tag{3}$$

Data analyses for comparison of fungal counts obtained from swabs and RODAC plates was carried out using Kruskal-Wallis ANOVA ($\alpha = 0.05$). The number of references on fungal pathogenicity was based on the Atlas of Clinical Fungi (de Hoog et al., 2015) and was used to describe the degree of clinical relevance of a certain fungal species. Furthermore, the species richness was calculated using Menhinick's index and the diversity of fungal species was calculated using the Simpson's diversity index (1-D) (Magurran, 2004). Species richness and diversity index are used as a measure of fungal

biodiversity and complexity of structure in an ecological community (Zak & Willig, 2004). The richness index and diversity index were used to quantitatively compare different culture media and sampling sites.

Selection of fungi

From every culture medium of each sample, up to five colonies were selected for isolation and identification. The selected colonies were the ones closest to the centre of the plates or membrane filters and preferably loose individual colonies.

The colonies were isolated and grown on oat meal agar (OA) slants at 24 °C for 3 days and subsequently stored at 10 °C. OA was prepared by simmering 20 g oatmeal in 1 L distilled water for 20 minutes, after which 15 g agar, 1 mg of $FeSO_4.7H_2O$, $MnCl_2.4H_2O$, and 1 mg of $ZnSO_4.7H_2O$ were added. The mixture was subsequently sterilized at 121 °C for 20 min.

DNA extraction

Fungal DNA from the isolated colonies that were grown on OA as described above was extracted using Chelex® 100 (Bio-Rad Laboratories, USA). The extraction was done by harvesting 5–10 mm^3 of mycelium using an inoculation needle and subsequently putting the mycelium in sterile reaction tubes together with 5–10 acid-washed glass beads (1.5–2 mm). The tubes with fungal material were frozen in liquid nitrogen for 3–5 minutes after which a bead beater was used to disrupt the cells for 10 seconds at 30 beats/sec. Both freezing and bead beating were repeated 2 times. Afterwards, 100 µL of 5% Chelex resin and 10 µL Proteinase K (20 mg/mL) were added to the lysed samples. The tubes were incubated for 30 min at 56 °C followed by 10 min at 95 °C using dry block heaters and then centrifuged for 1 min at 12,000 rpm to settle the beads and resin. The supernatant was transferred to a new reaction tube. The centrifugation was repeated 2–3 times until all resin was removed. The extracted DNA was stored at -20 °C until further analysis.

DNA identification

Amplification of the internal transcribed spacer (ITS) gene followed by sequencing was performed to identify the fungal genus and/or species. Primers ITS1 or ITS5 and ITS4 were used to amplify the DNA (White et al., 1990). The total volume of 12.5 µL PCR mixture contained 1.25 µL PCR buffer (10xNH_4^+), 0.5 µL $MgCl_2$ (50 mM), 0.75 µL dNTP (10 µM), 0.38 µL of each primer (10 µM), 0.5 µL BSA, 1 µL Bioline Taq polymerase (0.5U/µL) (Bioline, UK), 6.74 µL sterile demineralized water, and 1 µL DNA template. The PCR protocol consisted of 5 min pre-denaturation at 95 °C, 30 cycles of 95 °C for 45 sec, 48 °C for 30 sec, and 72 °C for 1 min, followed by 6 min of post-elongation at 72 °C. Prior to sequencing, the DNA fragments in PCR products were confirmed using

1% agarose gel electrophoresis. PCR products were sequenced in both directions using the same primers as in the PCR, and the Applied Biosystems BigDye® terminator cycle sequencing kit (Thermo Fisher Scientific Inc., USA). The cycle sequencing reaction mixture with a total volume of 10 µL contained 3 µL buffer, 0.7 µL BigDye reagent, 4.3 µL sterile demineralized water, 1 µL primer (10 µM) and 1 µL PCR product. Sequencing products were purified using D-Pure™ Dye terminator clean-up magnetic beads (NimaGen, the Netherlands) on a fully automated Hamilton's Microlab® STARlet instruments (NimaGen, the Netherlands) and analysed on an Applied Biosystems 3730xl DNA analyser (Thermo Fischer Scientific, USA). The forward and reverse sequences were assembled using SeqMan Pro from Lasergene software (DNASTAR, USA) and checked against the GenBank database. The fungal species were identified based on the closest results obtained with the BLAST algorithm (BLASTN 2.5.1) with a similarity of at least 98%.

Results

Water quality parameters and area cleaning regime

Water quality parameters for each pool are summarized in Table 2. The pH values were within the range of legal requirements (6.8 ≤ pH ≤ 7.8 (Bhvbz, 2011)), and so were the free chlorine levels in most pools (0.5–1.5 $mgCl_2/L$ for indoor swimming pools (Bhvbz, 2011)). The free chlorine levels in water from the children's pool and the pool for special groups were however below the regulated value.

Table 2. Water quality parameters

Sample name	Location	Temp. (°C)	pH	TOC (mgC/L)	Free chlorine (mg/L)
A	Competition pool	28.8	7.10	3.52	0.85
B	Children's pool	28.1	7.25	3.38	0.22
C	Pool with water slide	32.5	7.40	3.12	1.47
D	Recreational pool	31.8	7.25	4.60	1.32
E	Pool for special groups	32.8	7.05	3.38	0.35
F	Whirlpool	32.5	7.30	4.18	0.66

Information regarding the cleaning regime in the swimming pool facility was provided by the pool manager. In general, the floors are cleaned twice a day by the pool personnel, before opening and after closing hours, with tap water and twice a week by cleaning company with a biodegradable cleaning agent. The benches are cleaned on daily basis in the evening and the walls are cleaned twice a week. The flexibeams are replaced every 3 months.

Fungal concentrations in water samples

Fungi were detected in the pool water of all the investigated pools. The numbers of fungal colonies per 100 mL sample ranged between 0 and 17 CFU (Figure 1, Annex Table S1). When comparing the different culture media used, the lowest fungal counts were observed on MEA40. When comparing the fungal counts from different pools on MEA, the highest number of colonies was found in water from the pool for special groups (E) while the lowest number of colonies was observed in water from the recreational pool (D). It was also observed that one sample from the recreational pool on SDA contained many yeast/yeast-like colonies; this was the sample with the highest fungal count (17 CFU/100 mL).

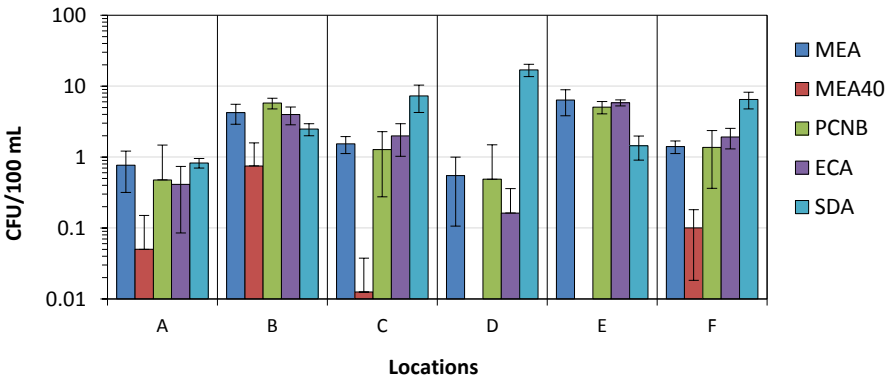

Figure 1. Fungal colony counts in water from different pools, cultured on different culture media. A: competition pool; B: children's pool; C: pool with water slide; D: recreational pool; E: pool for special groups; F: whirlpool.

Fungal concentrations in surface samples

The number of colonies on RODAC plates varied between 0–2.5 CFUs/cm² while the numbers from surface swabs varied between 0–5.8 CFUs/cm² (Figure 2, Annex Table S2). Based on statistical analysis, the fungal counts from RODAC plates and swabs did not significantly differ ($p > 0.05$) either calculated per medium or for all media together. High fungal counts were also observed on the floors near the pools and preferential pathways. The highest counts were observed on the surface of the flexibeam. Additionally, only one colony could be grown from wall surface samples and no fungi were detected in the samples from the floor of the shower area sampled either with RODAC plates or swabs.

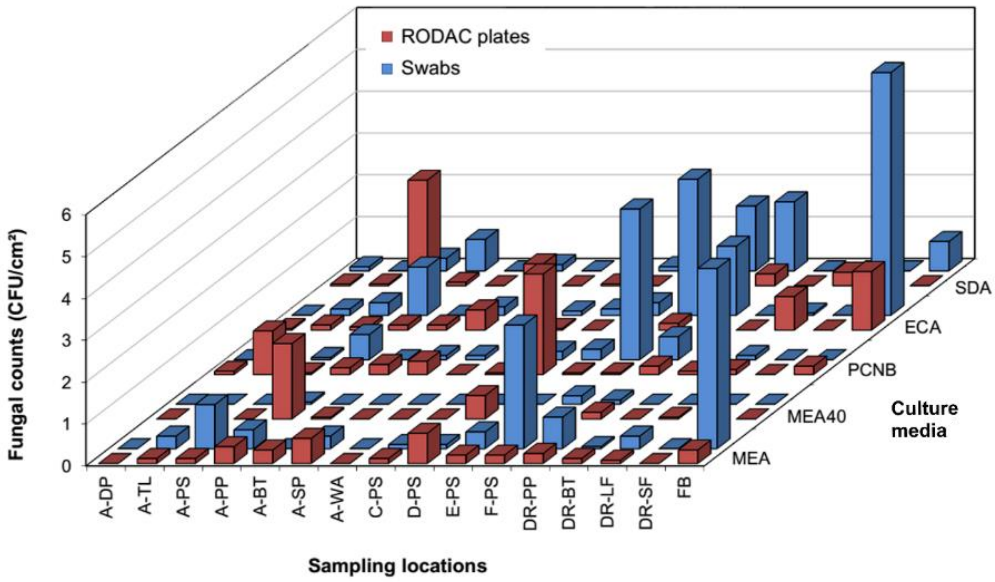

Figure 2. Fungal counts on surfaces in the swimming pool environment in different culture media, obtained by using RODAC plates and swabs for sampling. A: competition pool; B: children's pool; C: pool with water slide; D: recreational pool; E: pool for special groups; F: whirlpool; DR: dressing room. DP: diving platform; TL: top step of ladder; PS: floors next to the pool; PP: preferential pathway; BT: bench top; SP: swimming platform; WA: wall in main pool area; LF: floors by the lockers; SF: shower floors; FB: flexibeam.

Identification of fungal species

Fungal species isolated on different culture media

MEA and ECA gave the highest numbers of different fungal species, and also gave the highest numbers of unique species while MEA40 gave the lowest numbers (Table 3). Although only low numbers of fungal species were isolated from SDA and MEA40, more than 75% of the species isolated from SDA (18/24) and MEA40 (7/9) were clinically relevant fungal species.

Menhinick's species richness index was the highest on ECA, directly followed by MEA and PCNB. Simpson's diversity index of isolates from MEA, PCNB and ECA was comparably high while a lower diversity was observed among isolates from MEA40 and SDA (Table 3).

Table 3. Number of fungal species isolated and identified from water and surfaces grown on different culture media

Number of identified species*	**Culture medium**					
	Total	**MEA**	**MEA40**	**PCNB**	**ECA**	**SDA**
Total	79	41	9	37	45	24
From water samples	45	22	5	22	22	15
From surface samples	53	27	5	25	28	12
From a specific medium		13	2	6	15	6
Total clinically relevant species	42	22	7	23	27	18
Clinically relevant species from water samples	25	11	4	14	15	9
Clinically relevant species from surface samples	30	15	3	14	17	12
Clinically relevant species from a specific medium		3	2	1	7	5
Menhinick's species richness index**		4.23	1.64	3.92	4.91	3.10
Simpson's diversity index**		0.95	0.78	0.96	0.96	0.85

* the identified species are listed in Annex Table S3

** based on all identified species

Isolated species

In total, 357 isolates were isolated and identified based on their ITS sequences: 236 isolates from surfaces (66%) and 121 isolates from water (34%). The ITS sequences resulted in 79 different species or species groups (Annex Table S3); 34 species were isolated from surfaces only and 26 species were isolated from pool water only. Nineteen species were isolated from both surfaces and pool water. The most commonly isolated species were *Phialophora oxyspora* (13.7%), *Trichosporon dohaense* (6.7%) and species belonging to the genus *Phoma* (12.3%) and the *Cladosporium cladosporioides* complex (4.5%). These species were all isolated from pool water as well as from surfaces. Of the species that were isolated from pool water and surfaces, some species were clearly more frequently isolated from surface samples than from water (e.g. *Phialophora oxyspora, Sporobolomyces roseus,* etc.) whilst other species were more frequently isolated from water than from surfaces (e.g. *Aspergillus niger* complex, *Cladosporium cladosporioides* complex, etc.) (Annex Table S3).

The ITS region is often used as a barcode for species-specific fungal identification (Iwen et al., 2002; Nilsson et al., 2009; Sparagano & Foggett, 2009). However, some fungal genera, e.g. *Fusarium*, *Aspergillus*, *Penicillium*, and *Cladosporium* have proven to be hard to identify to the species level using ITS sequences (Schoch et al., 2012). Consequently, some fungi isolated in this study, were identified only up to the genus level, species complex or section.

Clinically relevant species

Almost 70% of the total of isolated fungal colonies (n = 357) belonged to clinically relevant species (n = 247). More than 50% of the identified fungal species (n = 79) were clinically relevant species (n = 42). Similar portions of clinically relevant species were isolated from water (55.6%, n = 25/45) and from surfaces (56.6%, n = 30/53). Higher numbers of clinically relevant species were observed among isolates from surfaces (71.4%, n = 30/42) compared to isolates from pool water (59.5%, 25/42). *Phialophora oxyspora* (13.7%), *Phoma* spp. (12.3%) and *Cladosporium cladosporioides* complex (4.5%) were the most frequently isolated clinically relevant fungi. Respectively 5.0%, 4.2% and 3.4% of the total number of isolates belonged to the clinically relevant genera *Aspergillus*, *Penicillium*, and *Trichoderma*. The isolates that belonged to the genera *Aspergillus* and *Penicillium* were more often isolated from water than from surfaces, which is also true for *Cladosporium cladosporioides* and *Fusarium solani* species complex (Annex Table S3). *Phialophora oxyspora* was more frequently isolated from surfaces than from pool water while *Phoma* spp. were equally frequently detected in pool water and on surfaces. Some species in RG-2 level which were rarely identified, *Scedospodium boydii*, was present in pool water (0.6%), while *Trematosphaeria grisea* (0.3%) and *Candida tropicalis* (0.3%) were isolated from surfaces. The only dermatophyte identified was *Trichophyton interdigitale* and it was isolated from pool water.

Health risk levels

Seventy-four percent of the clinically relevant species (Annex Table S4) are classified at biosafety level 1 and are risk group 1 organisms. Biosafety levels and risk groups are used to characterize the occupational health risks and intrinsic virulence associated with pathogenicity of fungal species (de Hoog, 1996; de Hoog et al., 2015). In this study, the number of publications on pathogenicity based on cases in humans (inclusion as references in the Atlas of Clinical Fungi (de Hoog et al., 2015)) was also used to estimate the degree of clinical relevance. A higher number of publications on human cases or infections indicated a higher relevance of a fungal species as an infectious agents or pathogen. The highest number of such publications was on *Aspergillus fumigatus*, which was implicated in 91 publications, including different cases of aspergillosis followed by *Fusarium solani* complex (63 publications), *Purpureocillium lilacinum* (47 publications), *Candida parapsilosis* (43 publications) and *Scedosporium boydii* (43 publications) supporting that these fungi are the most relevant related to human infection (Annex Table S4).

Spatial distribution of fungi in swimming pool environments

The diversity of fungal species at different sampling locations is shown in Table 4. The diversity of fungal species was higher in pool water than on surfaces. Of the surfaces, the diving platform, the ladder and the bench top in the competition pool area, and the floor near the lockers in the dressing room area showed a high diversity of fungal species (Simpson's diversity index: 0.90–0.94) in combination with lower fungal counts (0.03–0.18 CFU/cm²). On the contrary, the floor next to the competition pool and the recreational pool showed relatively low diversity (Simpson's diversity index: 0.63, 0.74) in combination with higher fungal counts (0.45, 0.42 CFU/cm²). More than half of the species isolated from water and surfaces were clinically relevant fungal species, except the ones isolated from the pool ladder.

Table 4. Fungal distribution in different places in the swimming pool environment

Sample code	Location*	Fungal counts**	Numbers of			Diversity index
			Isolates	Species	Clinically relevant species	
SURFACE						
A-DP	diving platform	0.03	5	4	3	0.90
A-TL	top step ladder	0.18	16	11	3	0.94
A-PS	floors next to the pool	0.45	15	5	3	0.63
A-PP	preferential pathway	0.56	25	9	5	0.84
A-BT	bench top	0.07	13	8	6	0.90
A-SP	starting platform	0.27	20	8	5	0.77
A-WA	wall in main pool area	0.01	1	1	0	0.00
C-PS	floors next to the pool	0.06	13	6	5	0.83
D-PS	floors next to the pool	0.42	19	6	3	0.74
E-PS	floors next to the pool	0.20	16	9	6	0.88
F-PS	floors next to the pool	1.21	22	9	6	0.86
DR-PP	preferential pathway	0.59	25	14	11	0.84
DR-BT	bench top	0.03	7	3	3	0.67
DR-LF	floors by the lockers	0.18	21	12	9	0.91
DR-SF	shower floors	0.00	0	0	0	0.00
FB	flexibeam	1.27	18	6	5	0.84
WATER						
A	competition pool	0.51	20	12	8	0.94
B	children's pool	3.44	23	14	8	0.95
C	pool with water slide	2.33	20	12	9	0.94
D	recreational pool	3.64	15	12	6	0.97
E	pool for special groups	3.74	19	13	8	0.96
F	whirlpool	2.25	24	16	9	0.96

* A: competition pool; B: children's pool; C: pool with water slide; D: recreational pool; E: pool for special groups; F: whirlpool; DR: dressing room.

** Fungal counts on surfaces in CFU/cm² (average number of colonies on 5 media on RODAC plates and swabs), fungal counts in water in CFU/100 mL.

Regarding the spatial distribution of fungi in the swimming pool facility, higher fungal counts were detected next to the competition pool, the recreational pool and the whirlpool. Eight samples were collected from the surroundings of the competition pool, which consisted of seven surface samples and one water sample. Fungal counts on the floor next to the pool and on the preferential pathway were higher than at any other locations surrounding the competition pool, such as diving platform, bench top and wall.

Discussion

Occurrence of fungi in the swimming pool environment

The presence of fungi on surfaces and in water in the swimming pool environment indicates a potential exposure of pool visitors to fungi, including clinically relevant species. Despite regular cleaning of floors in the facility, fungi were detected on surfaces where barefooted visitors may come into direct contact with fungi, such as the preferential pathways next to the pools and in the dressing room. The presence of fungi in chlorinated swimming pool water suggests that some fungal species may be improperly removed or inactivated by the pool water treatment.

The fungi in the pool water most likely originate from the pool users but may also originate from the tap water that is used as filling and replacement water. Half of the species detected in the pool water in this study have also been reported to occur in the drinking water distribution system in the Netherlands (A.D. van Diepeningen, personal communication, 2016). Drinking water companies in the Netherlands do not apply chlorination in their treatment process, neither for primary disinfection nor to maintain residual disinfectant in the distribution network. Although the susceptibility of pathogenic fungal species to disinfection has not been explored as much as it has been done for protozoa, bacteria and viruses, some studies in drinking water demonstrated that certain fungal species were resistant to commonly applied levels of chlorination (Hageskal et al., 2012; Pereira et al., 2013; Rosenzweig et al., 1983). Swimming pools apply a higher concentration of residual free chlorine (0.5–1.5 mg/L (Bhvbz, 2011)) compared to drinking water (e.g. 0.2 mg/L (WHO, 2011)) but this seems to be insufficient to inactivate certain species.

Methods for the detection of fungi in the swimming pool environment

The samples collected from surfaces in the swimming pool facility using both RODAC plates and swabs did not yield significantly different fungal counts. Hence, both methods can be considered suitable for sampling, however, RODAC plates are preferable because of their ease of use during sampling and in sample processing.

Different culture media were used with the purpose to select specific fungal species and groups. However, both overlapping and unique fungal species were isolated from each

of the different culture media. ECA was included to isolate black yeasts, however from nine black yeast species identified overall, only five species were from ECA and none was exclusively isolated from ECA. PCNB was included to isolate *Fusarium* and other PCNB tolerant species; but only one of the 23 (4%) clinically relevant species isolated from PCNB was not isolated by using other media. A higher number of unique clinically relevant species (5/18, 28%) was identified on SDA, a medium commonly used in hospitals for the isolation of dermatophytes, other fungi and yeasts. Moreover, the fungal species isolated from ECA, PCNB and SDA also included species which were not part of the fungal groups intended for these media. All different media used in this study detected some unique fungal species which together gave an advantage for demonstrating the diversity of fungal populations in the swimming pool environment. MEA, a general purpose culture medium, seems to be a medium of choice in larger sampling campaigns. Depending on the purpose of the study, a combination of MEA with one or two of the (semi-)selective media, such as ECA, PCNB, or SDA, would be beneficial.

Clinical relevance of fungi in the swimming pool environment

Of the 79 identified species isolated from various sites in the swimming pool facility, 42 species are known to have been involved in human infections. The identified clinically relevant fungi are opportunists which cause infections in immunocompromised patients, but some can also affect otherwise healthy people. Clinically relevant species from different genera like *Aspergillus*, *Cladosporium*, *Fusarium, Penicillium, Phialophora*, and *Phoma*, were also found in other studies on the swimming pool environment (Brandi et al., 2007; Papadopoulou et al., 2008; Viegas et al., 2011) as well as in drinking water (Arvanitidou et al., 1999; Göttlich et al., 2002; Grabińska-Łoniewska et al., 2007; Hageskal et al., 2006).

The most frequently isolated species in the current study was *Phialophora oxyspora*, a black yeast-like fungi, which has previously been isolated from human cutaneous samples (Saunte et al., 2012). Another frequently identified black yeast-like species, *Aureobasidium pullulans*, has been implicated in systemic infections (Hawkes et al., 2005). Other group of fungal species frequently isolated in this study were the *Phoma* spp., which are ubiquitous in nature, present in soil and plants, but cases of human infection are actually rare (de Hoog et al., 2015). *Cladosporium cladosporioides* was reported to be involved in pulmonary, cutaneous, and subcutaneous infections in humans (de Hoog et al., 2015). *Aspergillus fumigatus* is known as the causative agent of aspergillosis, mainly in immunocompromised patients (Latgé, 1999) although some cases in healthy people have been reported (Cavling Arendrup et al., 2006; Yasokawa et al., 2003). In the group of *Aspergillus niger* complex, *A. niger* is the common causative agent of otomycosis (Yehia et al., 1990), and has also been reported to be implicated in pulmonary cases and other cases of human infections (de Hoog et al., 2015) and

A. tubingensis was reported in more recent cases of osteomyelitis, keratitis, and pulmonary disorders (de Hoog et al., 2015).

Red yeast-like species, *Rhodotorula mucilaginosa* and *R. glutinis* were isolated from water and surfaces, both are clinically relevant species involved in cases of fungemia, endocarditis and meningitis (de Hoog et al., 2015). *Ochroconis musae* was isolated from surfaces at four locations; it is primarily waterborne, and was reported to have been involved in cutaneous and subcutaneous infections. All water samples contained fungal species from the genus *Penicillium*. Infections caused by *Penicillium spp.* other than *P. marneffei* are rare, however species such as *P. brevicompacta*, *P. citrinum*, *P. spinulosum*, *P. expansum* and *P. chrysogenum* are known to have been involved in various types of infection and disease (de Hoog et al., 2015; Garg et al., 2016; Lyratzopoulos et al., 2002). Most of the studies that examined the swimming pool environment, focused on the occurrence of dermatophytes, which cause typical swimming pool diseases such as tinea pedis. In this study, dermatophytes were hardly detected; the only dermatophyte identified was *Trichophyton interdigitale*. A longer incubation period (2–3 weeks) may be needed to produce growth of dermatophytes.

Epicoccum nigrum, *Fusicolla aquaeductuum*, and *Sporobolomyces roseus* were isolated from water and surface samples from at least four different locations in the swimming pool facility. However, they seem to be not clinically relevant as no case report on human infections has been published thus far. Although *Epicoccum nigrum* is not considered as a clinically relevant species, it has been recognized to cause allergic fungal sinusitis (Noble et al., 1997).

It is important to remark that one of the frequently occurring species, *Trichosporon dohaense*, which is not incorporated yet as clinically relevant in the Atlas of Clinical Fungi, was identified in five surface samples and four water samples. This species was isolated from patients with onychomycosis, tinea pedis and a catheter site infection in Qatar (Taj-Aldeen et al., 2009) and recently isolated from the fingernail of patients with onychomycosis in Egypt (Abdel-Sater et al., 2016).

The presence of clinically relevant fungi in the swimming pool environment, particularly at locations where people walk bare feet, suggests a possible risk of infection. Lian and de Hoog (2010) suggested that softened human skin due to bathing might be more susceptible to fungal infections, Gentles and Evans (1973) and Gentles et al. (1974) demonstrated the swimming pool environment as an exposure site of tinea pedis, and Morales-Cardona et al. (2014) proved that walking barefoot and the use of swimming pools were infection risk factors. Precautionary steps could be recommended to the pool manager, such as frequent cleaning of surfaces using cleaning products that are known to be effective against fungi and fungal spores, to suggest the pool users to wear appropriate footwear and adequate water treatment, are needed to limit direct contact to clinically relevant fungi in swimming pool environments. From the results of this

study, future work should target a larger number of swimming pools to prove the fungal contamination in different environments, with particular focus on the clinically relevant species, at specific exposure-relevant sites in the swimming pool environments. Furthermore, preventive measures aimed at reducing the clinically relevant fungal contamination should be investigated, encompassing surface cleaning regimes and water treatment strategies.

Conclusions

- Fungi, including clinically relevant species, occur widely in the swimming pool environment. Both water and surfaces are commonly infested with fungi despite chlorine treatment and regular cleaning warranting further studies into chlorine resistance of clinically relevant fungi.
- The differences in fungal counts obtained with RODAC plates and swabs were not significant, however RODAC plates are preferred for practical reasons.
- The use of different culture media allowed the detection of different groups of clinically relevant fungi and contributed to insight in the diversity of fungal populations in the swimming pools.
- The presence of clinically relevant fungi in swimming pools, particularly at sites where people walk bare feet, suggests a possible risk of infection and warrants further research into preventive measures and disinfection needs to reduce fungal contamination and limit the infection risk.

References

Abdel-Sater, M.A., Moubasher, A.A.H., Soliman, Z. (2016). Identification of three yeast species using the conventional and internal transcribed spacer region sequencing methods as first or second global record from human superficial infections. *Mycoses*, 59(10), 652–661.

Alcazar-Fuoli, L., Mellado, E. (2014). Current status of antifungal resistance and its impact on clinical practice. *British Journal of Haematology*, 166(4), 471-484.

Ali-Shtayeh, M., Khaleel, T.K.M., Jamous, R.M. (2003). Ecology of dermatophytes and other keratinophilic fungi in swimming pools and polluted and unpolluted streams. *Mycopathologia*, 156(3), 193–205.

Arvanitidou, M., Kanellou, K., Constantinides, T.C., Katsouyannopoulos, V. (1999). The occurrence of fungi in hospital and community potable waters. *Letters in Applied Microbiology*, 29(2), 81–84.

Besluit hygiëne en veiligheid badinrichtingen en zwemgelegenheden (2011). Available at http://wetten.overheid.nl/BWBR0003716/2011-07-01/

Brandi, G., Sisti, M., Paparini, A., Gianfranceschi, G., Schiavano, G.F., De Santi, M., Santoni, D., Magini, V., Romano-Spica, V. (2007). Swimming pools and fungi: An environmental epidemiology survey in Italian indoor swimming facilities. *International Journal of Environmental Health Research*, 17(3), 197–206.

Brandt, M.E., Park, B.J. (2013). Think fungus - Prevention and control of fungal infections. *Emerging Infectious Diseases*, 19(10), 1688–1689.

Brown, G.D., Denning, D.W., Levitz, S.M. (2012). Tackling human fungal infections. *Science*, 336(6082), 647–647.

Buot, G., Toutous-Trellu, L., Hennequin, C. (2010). Swimming pool deck as environmental reservoir of *Fusarium*. *Medical Mycology*, 48(5), 780–784.

Cavling Arendrup, M., Ronan O'Driscoll, B., Petersen, E., Denning, D.W. (2006). Acute pulmonary aspergillosis in immunocompetent subjects after exposure to bark chippings. *Scandinavian Journal of Infectious Diseases*, 38(10), 945–949.

de Hoog, G.S. (1996). Risk assessment of fungi reported from humans and animals. *Mycoses*, 39(11-12), 407–417.

de Hoog, G.S., Guarro, J., Gené, J., Figueras, M. (Eds.). (2015). Atlas of Clinical Fungi, fourth ed. CBS-KNAW Fungal Biodiversity Centre, Utrecht.

de Hoog, G.S., Haase, G. (1993). Nutritional physiology and selective isolation of *Exophiala dermatitidis*. *Antonie Van Leeuwenhoek*, 64(1), 17–26.

de Roda Husman, A., Schets, F.M. (2010). Climate change and recreational water-related infectious diseases. RIVM Report 330400002/2010. National Institute for Public Health and the Environment. http://www.rivm.nl/bibliotheek/rapporten/330400002.pdf

Detandt, M., Nolard, N. (1995). Fungal contamination of the floors of swimming pools, particularly subtropical swimming paradises. *Mycoses*, 38(11-12), 509–513.

Dufresne, S., Cole, D., Denning, D., Sheppard, D. (2017). Serious fungal infections in Canada. *European Journal of Clinical Microbiology and Infectious Diseases*, 1–6.

Fisher, M.C., Henk, D.A., Briggs, C. J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, 484(7393), 186–194.

Gago, S., Serrano, C., Alastruey-Izquierdo, A., Cuesta, I., Martín-Mazuelos, E., Aller, A.I., Gómez-López, A., Mellado, E. (2016). Molecular identification, antifungal resistance and virulence of *Cryptococcus neoformans* and *Cryptococcus deneoformans* isolated in Seville, Spain. *Mycoses*, 60, 40–50.

Garg, A., Stuart, A., Fajgenbaum, M., Laidlaw, D. A., Stanford, M. (2016). Chronic postoperative fungal endophthalmitis caused by *Penicillium citrinum* after cataract surgery. *Journal of Cataract & Refractive Surgery*, 42(9), 1380–1382.

Gentles, J., Evans, E., Jones, G. (1974). Control of tinea pedis in a swimming bath. *British Medical Journal*, 2(5919), 577–580.

Gentles, J.C., Evans, E.G.V. (1973). Foot infections in swimming baths. *British Medical Journal*, 3(5874), 260–262.

Göttlich, E., van der Lubbe, W., Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., Flemming, H-C., de Hoog, G.S. (2002). Fungal flora in groundwater-derived public drinking water. *International Journal of Hygiene and Environmental Health*, 205(4), 269–279.

Grabińska-Łoniewska, A., Koniłłowicz-Kowalska, T., Wardzyńska, G., Boryn, K. (2007). Occurrence of fungi in water distribution system. *Polish Journal of Environmental Studies*, 16(4), 539–547.

Hageskal, G., Knutsen, A.K., Gaustad, P., de Hoog, G.S., Skaar, I. (2006). Diversity and significance of mold species in Norwegian drinking water. *Applied and Environmental Microbiology*, 72(12), 7586–7593.

Hageskal, G., Tryland, I., Liltved, H., Skaar, I. (2012). No simple solution to waterborne fungi: various responses to water disinfection methods. *Water Science & Technology: Water Supply*, 12(2), 220–226.

Hawkes, M., Rennie, R., Sand, C., Vaudry, W. (2005). *Aureobasidium pullulans* infection: fungemia in an infant and a review of human cases. *Diagnostic Microbiology and Infectious Disease*, 51(3), 209–213.

Hilmarsdottir, I., Haraldsson, H., Sigurdardottir, A., Sigurgeirsson, B. (2005). Dermatophytes in a swimming pool facility: difference in dermatophyte load in men's and women's dressing rooms. *Acta Dermato-Venereologica*, 1(1), 1–2.

Iwen, P.C., Hinrichs, S.H., Rupp, M.E., 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. *Medical Mycology*, 40(1), 87–109.

Kamihama, T., Kimura, T., Hosokawa, J., Ueji, M., Takase, T., Tagami, K. (1997). Tinea pedis outbreak in swimming pools in Japan. *Public Health*, 111(4), 249–253.

Latgé, J-P. (1999). *Aspergillus fumigatus* and aspergillosis. *Clinical Microbiology Reviews*, 12(2), 310–350.

Lian, X., de Hoog, G.S. (2010). Indoor wet cells harbour melanized agents of cutaneous infection. *Medical Mycology*, 48(4), 622–628.

Low, C.Y., Rotstein, C. (2011). Emerging fungal infections in immunocompromised patients. *F1000 Medicine Reports*, 3, 14.

Lyratzopoulos, G., Ellis, M., Nerringer, R., Denning, D.W. (2002). Invasive Infection due to *Penicillium* species other than *P. marneffei*. *Journal of Infection*, 45(3), 184–195.

Magurran, A.E. (2004). Measuring Biological Diversity. Blackwell Publishing, United Kingdom.

Morales-Cardona, C.A., Valbuena-Mesa, M.C., Alvarado, Z., Solorzano-Amador, A., (2014). Non-dermatophyte mould onychomycosis: A clinical and epidemiological study at a dermatology referral centre in Bogota, Colombia. *Mycoses*, 57(5), 284–293.

Nilsson, R.H., Ryberg, M., Abarenkov, K., Sjökvist, E., Kristiansson, E. (2009). The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters*, 296(1), 97–101.

Nivens, D.E., Co, B.M., Franklin, M.J. (2009). Appendix: Sampling and quantification of biofilms in food processing and other environments. In: Fratamico, P.M., Annous, B.A., Guenther, N. (Eds.), Biofilms in the Food and Beverage Industries. Woodhead Publishing Limited and CRC Press LLC, pp. 539–568.

Noble, J., Crow, S., Ahearn, D., Kuhn, F. (1997). Allergic fungal sinusitis in the southeastern USA: involvement of a new agent *Epicoccum nigrum* Ehrenb. ex Schlecht. 1824. *Journal of Medical and Veterinary Mycology*, 35(6), 405–409.

Papadopoulou, C., Economou, V., Sakkas, H., Gousia, P., Giannakopoulos, X., Dontorou, C., Filioussis, G., Gessouli, H., Karanis, P., Leveidiotou, S. (2008). Microbiological quality of indoor and outdoor swimming pools in Greece: Investigation of the antibiotic resistance of the bacterial isolates. *International Journal of Hygiene and Environmental Health*, 211(3–4), 385–397.

Pereira, V.J., Marques, R., Marques, M., Benoliel, M.J., Barreto Crespo, M.T. (2013). Free chlorine inactivation of fungi in drinking water sources. *Water Research*, 47(2), 517-523.

Rosenzweig, W., Minnigh, H., Pipes, W. (1983). Chlorine demand and inactivation of fungal propagules. *Applied & Environmental Microbiology*, 45(1), 182–186.

San-Blas, G., Calderone, R.A. (2008). Pathogenic Fungi: Insights in Molecular Biology. Caister Academic Press, Norfolk.

Sato, M., Sanchez, P., Alves, M., Stoppe, N., Martins, M. (1995). Evaluation of culture media for *Candida albicans* and *Staphylococcus aureus* recovery in swimming pools. *Water Research*, 29(10), 2412–2416.

Saunte, D.M., Tarazooie, B., Arendrup, M.C., de Hoog, G.S. (2012). Black yeast-like fungi in skin and nail: it probably matters. *Mycoses*, 55(2), 161–167.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Consortium, F.B. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceeding of the National Academy of Sciences USA*, 109(16), 6241–6246.

Shemer, A., Gupta, A.K., Amichai, B., Baum, S., Barzilai, A., Farhi, R., Kaplan, Y., MacLeod, M.A. (2016). Increased risk of tinea pedis and onychomycosis among swimming pool employees in Netanya area, Israel. *Mycopathologia*, 181(11), 1–6.

Sparagano, O., Foggett, S. (2009). Chapter 2 Diagnosis of clinically relevant fungi in medicine and veterinary sciences. *Advances in Applied Microbiology*, 66, 29–52.

Taj-Aldeen, S.J., Al-Ansari, N., El Shafei, S., Meis, J.F., Curfs-Breuker, I., Theelen, B., Boekhout, T. (2009). Molecular identification and susceptibility of *Trichosporon* species isolated from clinical specimens in Qatar: Isolation of *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout sp. nov. *Journal of Clinical Microbiology*, 47(6), 1791–1799.

van der Wielen, P., Italiaander, R., Wullings, B., Heijnen, L., van der Kooij, D. (2014). Opportunistic pathogens in drinking water in the Netherlands. In: van der Kooij, D., van der Wielen, P. (Eds.), Microbial Growth in Drinking Water Supplies: Problems, Causes, Control and Research Needs. IWA Publishing, London, pp. 177–205.

van Wyk, P., Scholtz, D., Los, O. (1986). A selective medium for the isolation of Fusarium spp. from soil debris. *Phytophylactica*, 18(2), 67–69.

Viegas, C., Alves, C., Carolino, E., Pinheiro, C., Rosado, L., Santos, C.S. (2011). Assessment of fungal contamination in a group of Lisbon's gymnasiums with a swimming pool. *Italian Journal of Occupational and Environmental Hygiene*, 2(1), 15–20.

White, T.J., Bruns, T., Lee, S.,Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York, pp. 315–322.

WHO (2006). Guidelines for safe recreational water environments volume 2 Swimming pools and similar environments. World Health Organization, Geneva.

WHO (2011). Guidelines for drinking-water quality. World Health Organization, Geneva.

Yasokawa, Y., Yano, H., Murase, S., Shinoda, J., Sakai, N. (2003). Invasive cerebral aspergillosis in an immunocompetent patient: a case report. *Brain and Nerve*, 55(2), 127–131.

Yehia, M.M., Al-Habib, H.M., Shehab, N.M. (1990). Otomycosis: A common problem in North Iraq. *The Journal of Laryngology & Otology*, 104(5), 387-389.

Zak, J.C., Willig, M.R. (2004). Fungal biodiversity patterns. In: Mueller, G.M, Bills, G.F., Foster, M.S (Eds.), Biodiversity of fungi: Inventory and Monitoring Methods. Elsevier Academic Press, Amsterdam, pp. 59–75.

Annex

Table S1. Average fungal counts from water samples

Sample name	Locations	Fungal counts (CFU/100 mL)				
		MEA	MEA40	PCNB	ECA	SDA
A	Competition pool	0.8 ± 0.5	0.1 ± 0.1	0.5 ± 0.2	0.4 ± 0.3	0.8 ± 0.1
B	Children's pool	4.2 ± 1.3	0.7 ± 0.8	5.8 ± 0.7	4.0 ± 1.1	2.5 ± 0.5
C	Pool with water slide	1.5 ± 0.4	0.0 ± 0.0	1.3 ± 0.7	2.0 ± 1.0	7.3 ± 3.1
D	Recreational pool	0.6 ± 0.4	0.0	0.5 ± 0.5	0.2 ± 0.2	17.0 ± 3.4
E	Pool for special groups	6.4 ± 2.6	0.0	5.1 ± 0.9	5.8 ± 0.6	1.4 ± 0.5
F	Whirlpool	1.4 ± 0.3	0.1 ± 0.1	1.4 ± 0.5	1.9 ± 0.6	6.5 ± 1.7

Table S2. Average fungal count from surface samples collected using RODAC plates and swabs

Sample name	Locations	RODAC plate fungal counts (CFU/cm²)					Swab fungal counts (CFU/cm²)				
		MEA	MEA40	PCNB	ECA	SDA	MEA	MEA40	PCNB	ECA	SDA
A-DP	Diving platform	0.00	0.00	0.08	0.04	0.04	0.00	0.00	0.00	0.00	0.10
A-TL	Top step of the ladder	0.12	0.00	1.04	0.12	0.04	0.30	0.00	0.00	0.15	0.00
A-PS	Floors next to the pool	0.12	0.00	0.04	0.08	2.52	1.05	0.00	0.05	0.30	0.30
A-PP	Preferential pathway	0.40	1.80	0.16	0.12	0.08	0.45	0.05	0.60	1.15	0.75
A-BT	Bench top	0.32	0.04	0.24	0.12	0.00	0.00	0.00	0.00	0.00	0.00
A-SP	Swimming starting platform	0.60	0.00	0.32	0.48	0.52	0.30	0.00	0.10	0.20	0.15
A-WA	Wall in main pool area	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
C-PS	Floors next to the pool	0.12	0.00	0.04	0.04	0.04	0.10	0.00	0.10	0.10	0.05
D-PS	Floors next to the pool	0.72	0.56	2.40	0.00	0.00	0.10	0.00	0.20	0.15	0.10
E-PS	Floors next to the pool	0.20	0.00	0.04	0.76	0.00	0.40	0.00	0.25	0.30	0.05
F-PS	Floors next to the pool	0.20	0.00	0.04	0.16	0.12	2.95	0.20	3.60	3.25	1.55
DR-PP	Preferential pathway	0.24	0.16	0.20	0.36	0.28	0.75	0.10	0.55	1.65	1.65
DR-BT	Bench top	0.12	0.00	0.08	0.00	0.00	0.05	0.00	0.05	0.00	0.00
DR-LF	Floors by the lockers	0.08	0.04	0.12	0.80	0.32	0.30	0.00	0.10	0.05	0.00
DR-SF	Shower floors	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FB	Flexibeam	0.32	0.00	0.20	1.40	0.00	4.30	0.00	0.00	5.80	0.70

Table S3. Fungal species isolated from surface and water samples in different culture media

No.	Species	No. of positive samples* (n=22)	% positive samples		Number of isolates per medium					Number of isolates	Occurrence (n=357) (%)
			Surface (n=16)	Water (n=6)	MEA	MEA40	PCNB	ECA	SDA		
WATER											
1	***Aspergillus flavus* complex**	1		16.7		1				1	0.3
2	***Aspergillus fumigatus***	4		66.7	1	5	2			8	2.2
3	*Bionectria* sp.	1		16.7				1		1	0.3
4	*Coniochaeta* sp.	1		16.7					1	1	0.3
5	***Cryptococcus magnus***	1		16.7			1	1		2	0.6
6	***Cutaneotrichosporon cutaneum***	1		16.7					1	1	0.3
7	*Cutaneotrichosporon jirovecii*	1		16.7	1					1	0.3
8	***Debaryomyces hansenii***	1		16.7	1		1	1		3	0.8
9	*Gliomastix polychroma*	1		16.7	1					1	0.3
10	***Meyerozyma guilliermondii***	1		16.7		1				1	0.3
11	*Paraphaeosphaeria neglecta*	1		16.7				1		1	0.3
12	***Penicillium* section *Brevicompacta***	2		33.3	1		1	2		4	1.1
13	***Penicillium* section *Citrina***	1		16.7			1	1		2	0.6
14	*Penicillium* section *Digitata*	1		16.7	1					1	0.3
15	*Penicillium* section *Exilicaulis*	3		50			1	1	3	5	1.4
16	*Penicillium* section *Fasciculata*	1		16.7			1		1	2	0.6
17	*Penicillium* section *Ramosa*	1		16.7	1					1	0.3
18	*Pestalotiopsis* sp.	1		16.7				1		1	0.3
19	***Scedosporium boydii***	1		16.7		1	1			2	0.6
20	*Sporobolomyces symmetricus*	1		16.7	1					1	0.3
21	***Trichocladium* sp.**	1		16.7				1		1	0.3
22	***Trichoderma harzianum* complex**	1		16.7					2	2	0.6
23	*Trichoderma pleuroticola*	1		16.7			2		1	3	0.8
24	***Trichophyton interdigitale***	2		33.3	1		1		2	4	1.1
25	*Ustilago filiformis*	1		16.7				1		1	0.3
26	*Vishniacozyma carnescens*	1		16.7	1					1	0.3

SURFACE											
1	***Alternaria alternata* complex**	1	6.3		2					2	0.6
2	***Aspergillus versicolor* complex**	1	6.3				1			1	0.3
3	***Candida tropicalis***	1	6.3		1					1	0.3
4	***Coniosporium epidermidis***	1	6.3						1	1	0.3
5	*Cutaneotrichosporon debeurmannianum*	2	12.5		4			1		5	1.4
6	*Cyphellophora guyanensis*	1	6.3				1			1	0.3
7	*Cystobasidium benthicum*	1	6.3				1			1	0.3
8	*Diaporthe viticola*	2	12.5				2			2	0.6
9	*Eutypa lata*	1	6.3				1			1	0.3
10	*Eutypella scoparia*	2	12.5				3	1		4	1.1
11	***Exophiala cancerae***	2	12.5		1			1	2	4	1.1
12	***Exophiala lecanii-corni***	1	6.3						1	1	0.3
13	*Gloeophyllum trabeum*	1	6.3		1					1	0.3
14	*Gnomoniopsis idaeicola*	1	6.3					1		1	0.3
15	***Hortaea werneckii***	1	6.3		2					2	0.6
16	*Lophiostoma fuckelii*	1	6.3		1					1	0.3
17	*Massarina* sp.	1	6.3		1			1		2	0.6
18	*Naganishia liquefaciens*	1	6.3				1			1	0.3
19	***Ochrochonis musae***	4	25				1	3	2	6	1.7
20	***Penicillium* section *Aspergilloides***	1	6.3					1		1	0.3
21	***Penicillium* section *Penicillium***	1	6.3					1		1	0.3
22	*Peniophora quercina*	1	6.3					1		1	0.3
23	*Phaeosphaeria avenaria*	1	6.3		1					1	0.3
24	*Phaeosphaeria phragmiticola*	1	6.3					1		1	0.3
25	***Purpureocillium lilacinum***	1	6.3					2		2	0.6
26	***Rhinocladiella similis***	1	6.3		4		2	1	2	9	2.5
27	*Rhodotorula marina*	1	6.3		1					1	0.3
28	***Roussoella* sp.**	1	6.3					1		1	0.3
29	*Sphaerulina amelanchier*	2	12.5					2		2	0.6
30	***Talaromyces rugulosus***	1	6.3						1	1	0.3

31	***Trematosphaeria grisea***	1	6.3					1		1	0.3
32	***Trichoderma atroviride***	1	6.3		3	3	2	2		10	2.8
33	*Ustilago sparsa*	1	6.3		2			3		5	1.4
34	***Zygoascus hellenicus***	1	6.3					1		1	0.3
WATER AND SURFACE											
1	***Aspergillus niger* complex**	6	12.5	66.7	2	2		2	2	8	2.2
2	***Aureobasidium pullulans***	7	25	50	4		4	1		9	2.5
3	***Candida parapsilosis***	3	12.5	16.7	1		1		1	3	0.8
4	***Cladosporium cladosporioides* complex**	11	37.5	83.3	3		7	5	1	16	4.5
5	***Cladosporium herbarum* complex**	5	25	16.7	1		3	1	1	6	1.7
6	***Cladosporium sphaerospermum* complex**	5	25	16.7	1		3	1	4	9	2.5
7	*Epicoccum nigrum*	5	6.3	66.7	3		3	1		7	2.0
8	***Exophiala oligosperma***	2	6.3	16.7			1	1		2	0.6
9	***Fusarium solani* complex**	3	6.3	33.3	1	3	1	2		7	2.0
10	*Fusicolla aquaeductuum*	7	25	50	3	1	4	2		10	2.8
11	***Penicillium* section *Chrysogena***	6	6.3	83.3	3		1	2	1	7	2.0
12	*Peniophora cinerea*	3	12.5	16.7	2		3			5	1.4
13	***Phialophora oxyspora***	14	75	33.3	10		9	7	23	49	13.7
14	***Phoma* spp.**	15	68.8	66.7	18		8	14	4	44	12.3
15	***Rhodotorula glutinis***	2	6.3	16.7	1		2	2		5	1.4
16	***Rhodotorula mucilaginosa***	4	18.8	16.7	1		2	2	1	6	1.7
17	*Sporobolomyces roseus*	5	25	16.7	2		4	1	1	8	2.2
18	*Talaromyces minioluteus*	3	6.3	33.3	2			2		4	1.1
19	*Trichosporon dohaense*	9	31.3	66.7	2	13	6	2	1	24	6.7
Total no. of isolates					94	30	89	84	60	357	100
Isolates from water samples					30	12	28	30	21	121	34
Isolates from surface samples					64	18	61	54	39	236	66
Total clinically relevant species isolates					63	16	56	60	52	247	69
Clinically relevant species isolates from water samples					16	8	16	22	13	75	
Clinically relevant species isolates from surface samples					47	8	40	38	39	172	

Note: clinically relevant species is written in bold

* number of locations where the fungi species were isolated

Table S4. Pathogenicity of clinically relevant fungal species identified in this study

No.	Species	Risk level*		Publication on pathogenicity (in humans)**
WATER				
1	*Aspergillus flavus* complex	BSL-1	RG-2	*A. flavus*: 29, *A. oryzae*: 8
2	*Aspergillus fumigatus*	BSL-2	RG-2	91
3	*Cryptococcus magnus*	BSL-1	RG-1	1
4	*Cutaneotrichosporon cutaneum*	BSL-2	RG-1	***
5	*Debaryomyces hansenii*	BSL-1	RG-1	7
6	*Meyerozyma guilliermondii*	BSL-1	RG-1	12
7	*Penicillium* section *Brevicompacta*	BSL-1	RG-1	*P. brevicompatum*: 1
8	*Penicillium* section *Citrina*	BSL-1	RG-1	*P. citrinum*: 6
9	*Scedosporium boydii*	BSL-2	RG-2	43
10	*Trichocladium* sp.	BSL-1	RG-1	*T. asperum*: 2
11	*Trichoderma harzianum* complex	BSL-1	RG-1	4
12	*Trichophyton interdigitale*	BSL-2	RG-1	3
SURFACE				
1	*Alternaria alternata* complex	BSL-1	RG-1	*A. tenuissima*: 9, *A. alternata*: 24
2	*Aspergillus versicolor* complex	BSL-1	RG-1	*A. versicolor*: 4, *A. sydowii*: 10
3	*Candida tropicalis*	BSL-2	RG-2	24
4	*Coniosporium epidermidis*	BSL-1	RG-1	1
5	*Exophiala cancerae*	BSL-1	RG-1	1
6	*Exophiala lecanii-corni*	BSL-2	RG-1	1
7	*Hortaea werneckii*	BSL-1	RG-1	4
8	*Ochroconis musae*	BSL-1	RG-1	2
9	*Penicillium* section *Aspergilloides*	BSL-1	RG-1	*P. spinulosum*: 3
10	*Penicillium* section *Penicillium*	BSL-1	RG-1	*P. expansum*: 1
11	*Purpureocillium lilacinum*	BSL-1	RG-1	47
12	*Rhinocladiella similis*	BSL-2	RG-2	2
13	*Roussoella* sp.	BSL-1	RG-1	*R. percutanea*: 1
14	*Talaromyces rugulosus*	BSL-1	RG-1	2
15	*Trematosphaeria grisea*	BSL-1	RG-2	5
16	*Trichoderma atroviride*	BSL-1	RG-1	1
17	*Zygoascus hellenicus*	BSL-1	RG-1	1

No.	Species	Risk level*		Publication on pathogenicity (in humans)**
WATER AND SURFACE				
1	*Aspergillus niger* complex	BSL-1	RG-1	*A. niger*: 28, *A. tubingensis*: 3
2	*Aureobasidium pullulans*	BSL-1	RG-1	21
3	*Candida parapsilosis*	BSL-1	RG-1	43
4	*Cladosporium cladosporioides* complex	BSL-1	RG-1	16
5	*Cladosporium herbarum* complex	BSL-1	RG-1	3
6	*Cladosporium sphaerospermum* complex	BSL-1	RG-1	3
7	*Exophiala oligosperma*	BSL-2	RG-1	10
8	*Fusarium solani* complex	BSL-2	RG-1	*F. solani*: 45, *F. keratoplasticum*: 2, *F. falsiforme*: 16
9	*Penicillium* section *Chrysogena*	BSL-1	RG-1	*P. chrysogenum*: 9
10	*Phialophora oxyspora*	BSL-1	RG-1	1
11	*Phoma* spp.	BSL-1	RG-1	*P. glomerata*: 1, *P. herbarum*: 1
12	*Rhodotorula glutinis*	BSL-1	RG-1	13
13	*Rhodotorula mucilaginosa*	BSL-1	RG-1	22

* BSL: Biosafety Level, RG: Risk Group

** Number of publications in the Atlas of Clinical Fungi (de Hoog et al., 2015)

*** Reported under different taxa

5

Potential transmission pathways of clinically relevant fungi in indoor swimming pool facilities

Ekowati, Y., Ferrero, G., Kennedy, M.D., de Roda Husman, A.M., Schets, F.M. (2018). Potential transmission pathways of clinically relevant fungi in indoor swimming pool facilities. *International Journal of Hygiene and Environmental Health*, 221(8), 1107–1115.

Abstract

Possible transmission pathways of fungi in indoor swimming pool facilities were assessed through fungal counting in different areas of the facilities and typing of the collected fungal isolates. Air, water and surface samples were collected from seven different indoor swimming pool facilities. Fungal species were identified based on their internal transcribed spacer (ITS) sequences. Maximum fungal concentrations of 6.2 CFU/cm², 1.39 CFU/100 mL, and 202 CFU/m³ were found on surfaces, in water and air, respectively. In total, 458 isolates were obtained, belonging to 111 fungal species, of which 50 species were clinically relevant. *Phialophora oxyspora* (13.3%) and *Trichosporon dohaense* (5.0%) were the most frequently isolated species and were merely detected on floors, as were the dermatophytes, *Trichophyton interdigitale* and *T. rubrum*. *Penicillium* spp. and *Aspergillus* spp. were the dominant fungi in water and air. No typical patterns of fungal concentrations along the preferential pathways of pool visitors were observed, however, sites where pool visitors converge while moving from one room (e.g. dressing room) to another (e.g. shower room) and walking barefoot displayed the highest fungal concentrations thus posing the highest risk of contamination. The dispersal of fungi on floors is most likely facilitated by the pool visitors and cleaning tools. Clinically relevant fungi, including the ones rarely identified in nature, were widely detected on floors, in water and in air, as well as on cleaning tools and flexibeams. Preventive measures such as cleaning should minimize the prevalence of clinically relevant fungi in swimming pool facilities since these potentially pose health risks to those vulnerable for infections.

Introduction

Studies have shown that swimming pool facilities have contributed to the spread of fungal infections (Gentles and Evans, 1973; Seebacher et al., 2008). Epidemiological studies of fungal infections and/or diseases related to swimming pools have been conducted for decades, mostly focusing on tinea pedis and onychomycosis caused by dermatophytes (Ali-Shtayeh et al., 2003; Detandt and Nolard, 1988, 1995; English and Gibson, 1959; Gentles and Evans, 1973; Gudnadóttir et al., 1999). Fungal skin and nail infections are mainly caused by dermatophytes although some cases caused by non-dermatophytes have also been reported (Ellabib et al., 2002; Lateur et al., 2003; Morales-Cardona et al., 2014; Sharma and Sharma, 2012; Thomas et al., 2010). Besides dermatophytes, other clinically relevant fungi such as *Fusarium* spp., *Aspergillus* spp., and *Candida* spp., were detected on surfaces, in water and in air inside swimming pool facilities (Aho and Hirn, 1980; Brandi et al., 2007; Buot et al., 2010; Jankowski et al., 2017; Maghazy et al., 1989; Viegas et al., 2011). Clinically relevant fungal species include fungal species which have been implicated in human cases of superficial (e.g piedra, otitis externa), cutaneous (e.g. ringworm, onychomycosis), and subcutaneous (e.g. mycetoma) mycoses, as well as deep mycoses (e.g. fungemia). The immune system in healthy humans is generally able to resist infections from exposure to most fungi, however, patients with immunodeficiency are more susceptible to fungal infections (Brown et al., 2012).

In swimming pool facilities, fungal skin and nail infections (e.g. tinea pedis, onychomycosis) are prevalent among pool visitors (Detandt and Nolard, 1995; Kamihama et al., 1997; Shemer et al., 2016). Surfaces in swimming pool facilities may contain skin fragments from infected persons and thus the infection may spread when infected skin fragments adhere to e.g. the feet of thus far uninfected individuals while walking over contaminated surfaces (English and Gibson, 1959). Our previous study (Ekowati et al., 2017) demonstrated that fungi were ubiquitously present on surfaces and in water in an indoor swimming pool facility, with higher fungal counts on floors where people walked barefoot compared to other surfaces such as benches and diving platforms. Particularly floors near pools appeared to be prone to fungal contamination, with clinically relevant fungi being present, including *Aspergillus fumigatus*, *Fusarium solani* complex, *Purpureocillium lilacinum*, and *Candida parapsilosis*.

The focus of this study was to identify possible transmission pathways in seven different indoor swimming pool facilities by comparing the fungal populations and concentrations on floors where people walk barefoot, in pool water and in air in the facilities. The presence of fungi on cleaning equipment and teaching aids was also investigated in order to observe their role in facilitating the dispersal of fungi in indoor swimming pool facilities.

Materials and methods

Sampling locations

Sampling was carried out in seven swimming pool facilities (A-G) within the same province in the Netherlands. In total, 59 samples were collected from floors in swimming pool facilities, whereas seven water samples and seven air samples were taken. In each facility, six samples were taken from floor surfaces in different areas in the facility, following the visitors' pathway from the dressing rooms to the swimming hall, one water sample was taken from the pool closest to the shower room and one air sample was taken in the swimming hall closest to the sampled pool (Table 1). In four of the pool facilities (A, B, C, D), two swimming pools were located in the same swimming hall hence one additional sample was taken from the floor close to the second swimming pool. One sample was collected from the surface of one of the flexibeams (foam teaching aids) in each swimming pool facility. When the equipment was available, samples were taken from the cleaning tools (mop, scrubber and wiper) used to clean the floors in the facility.

Water quality parameters were measured in water samples collected from the examined pools. Temperature and pH were measured using a portable probe (pH meter 3310, WTW, Germany). Free chlorine levels were measured using DPD free chlorine reagent powder pillows and a portable colorimeter DR890 (Hach, USA). Total organic carbon (TOC) was measured using a TOC-L analyser (Shimadzu, Japan).

Table 1. Sampling sites in each of the studied swimming pool facilities

Pool facility	Site number	Sampling site	Description of sampling site	Sample matrix	Sample code
A, B, C, D, E, F, G	1	Dressing room (pathway)	Floor	Surface	DR
	2	Entrance from dressing room to shower room	Floor	Surface	DR-SH
	3	Shower room	Floor	Surface	SH
	4	Entrance from shower room to swimming hall	Floor	Surface	SH-P
	5	Swimming hall (in front of pool ladder)	Floor	Surface	P-LA
	6	Swimming hall (preferential pathway)	Floor	Surface	P-PP
		Flexibeam	Foam teaching aid	Surface	FB
		Swimming pool	Water	Water	Water
		Swimming hall	Air	Air	Air
A, B, C, D	7	Swimming hall (pathway close to the 2nd pool)	Floor	Surface	EXT
A, C, E		Mop/scrubber	Cleaning tool	Surface	CT-MS
C, D, E		Wiper	Cleaning tool	Surface	CT-W

Sample collection and processing

Ten litres of water were collected from the pools using plastic containers, which were previously cleaned using chlorine tablets (Suma Tab D4 Tab, Diversey, the Netherlands) according to the manufacturer's recommendation, and subsequently thoroughly rinsed with demineralized water. Sodium thiosulfate (final concentration 0.2 mM) was added to the water samples to quench residual chlorine. The water samples were transported to the laboratory at ambient temperature and subsequently stored at 4 °C until further analysis. Sample volumes of 2 L, 1 L and 2 × 0.5 L of pool water from each sampling location were filtered through 0.45 µm pore size membrane filters (Millipore, no. EZHAWG474, the Netherlands). Membrane filters were placed on MEA (Malt Extract Agar) and SDA (Sabouraud Dextrose Agar) plates, which were prepared as described by Ekowati et al. (2017).

Each surface sample was taken by applying Replicate Organism Detection and Counting (RODAC) plates filled with MEA and SDA. RODAC plates were applied by pressing the plates gently on the surface for 10 seconds.

Air samples were collected using two simultaneously running AirPort MD8 (Sartorius Stedim Biotech, Germany) air samplers, each sampling for culture either on MEA or on SDA. The sample volume was 500 L at a sampling speed of 40 L/min. The air was filtered through 8 µm pore size cellulose nitrate filters (Sartorius Stedim Biotech no. 11301--80-

---ALN). Immediately after air sample collection, the filters were placed on MEA and SDA plates.

The limit of detection for the culture methods is 1 CFU per analysed volume (for water and air samples) or analysed area (for surface samples).

Cultivation and isolation of fungi

MEA and SDA plates were incubated in the dark at 24 °C. After 7 days of incubation, the number of colonies was counted and was expressed as the number of colony forming units (CFU)/100mL for water samples, CFU/m³ for air samples and CFU/cm² for surface samples. In some cases where overgrowth or too many colonies were observed after 7 days of incubation, the plates were not counted and were not included in the calculation of concentrations, however, some loose colonies were picked for sub-culturing and typing.

From each sample, up to five colonies from both MEA and SDA were selected for isolation and identification. The selected colonies were the ones closest to the centre of the plates or membrane filters and loose individual colonies. The colonies were picked and directly cultured on MEA slants at 24 °C for 3 days and subsequently stored at 10 °C.

DNA extraction and identification

DNA was extracted following the Quick CTAB extraction as described by Zhou et al. (2014). DNA samples were stored at -20 °C until further use.

Amplification of the internal transcribed spacer (ITS) followed by sequencing was performed to identify fungal species. Primers ITS1 or ITS5 and ITS4 were used to amplify DNA (White et al., 1990). The total volume of 25 µL PCR mixture contained 2.5 µL PCR buffer (10xNH_4^+), 1 µL $MgCl_2$ (50 mM), 2.5 µL dNTP (10 µM), 1 µL of each primer (10 µM), 1.5 µL DMSO, 1 µL Bioline Taq polymerase (0.5U/µL) (Bioline, UK), 13.5 µL sterile demineralized water, and 1 µL DNA template. Amplification of fungal DNA and sequencing of PCR products were done as described by Ekowati et al. (2017).

The forward and reverse sequences were assembled using SeqMan Pro from Lasergene software (DNASTAR, USA) and checked against the GenBank database. The fungal species were identified based on the closest results obtained with the BLAST algorithm (BLASTN 2.7.0) with a similarity of at least 98%. In some cases, with results of less than 98% similarity, the genus, family or order name was assigned to the isolates concerned, based on the highest similarity found in BLASTN.

Phylogenetic tree

All sequences were aligned using online version multiple sequence alignment program, MAFFT version 7 (Katoh et al., 2017; Katoh and Standley, 2013). Phylogenetic analyses

were conducted using MEGA version 7 (Kumar et al., 2016) with Maximum Likelihood method using 1,000 bootstrap replications.

Statistical analysis

Statistical analysis to compare the fungal concentrations was carried out by using a non-parametric Kruskal-Wallis test ($\alpha = 0.01$) followed by post hoc Dunn's multiple comparison test. The comparisons were done for the different sampling sites in the swimming pool facilities (Table 1) and for the different pool facilities (A–G).

Results

Water quality parameters and cleaning regimes

The measured water quality parameters are shown in Table 2. Free chlorine and pH levels were within the range of standard requirements for swimming pool water quality in the Netherlands: $6.8 \leq pH \leq 7.8$ and free chlorine 0.5–1.5 $mgCl_2/L$ (Bhvbz, 2011).

Table 2. Water quality in different swimming pools

Pool facility	pH	Temperature (°C)	Free chlorine (mg/L)	TOC (mg/L)
A	7.43	28.0	1.30	2.91
B	7.41	28.0	0.98	3.11
C	7.10	30.0	1.04	3.42
D	7.07	29.0	0.98	2.75
E	7.33	27.5	0.75	2.51
F	7.40	28.0	0.96	1.52
G	7.31	30.4	1.03	2.80

A questionnaire regarding the cleaning regime in swimming pool facilities was filled in by the pool managers. The floors inside all the facilities were cleaned at least once a day using a floor scrubbing machine, usually after closing hours. Some areas in some facilities were cleaned more frequently: pool facility D cleaned the shower room and the floors in the dressing/locker rooms about 3–4 times per day, pool facility C cleaned the dressing/locker room floors twice a day, and pool facility F cleaned the shower room twice a day. The products used for cleaning or disinfecting purposes in each facility varied in their chemical content, such as phosphoric acid, sodium hydroxide, hydrogen peroxide, and a mixture of alkali substances.

Fungal concentrations in water, air and surface samples

Fungi were detected in water from all the sampled swimming pools. Overall, fungal concentrations in water samples from the different pools cultured on MEA and SDA ranged between 0–1.53 CFU/100 mL (Figure 1A, Annex Table S1). The highest concentration was detected in the water of pool D. The fungal counts in air samples cultured on MEA and SDA ranged between 0–202 CFU/m³ with the highest count in air in pool facility E (Figure 1B, Annex Table S1).

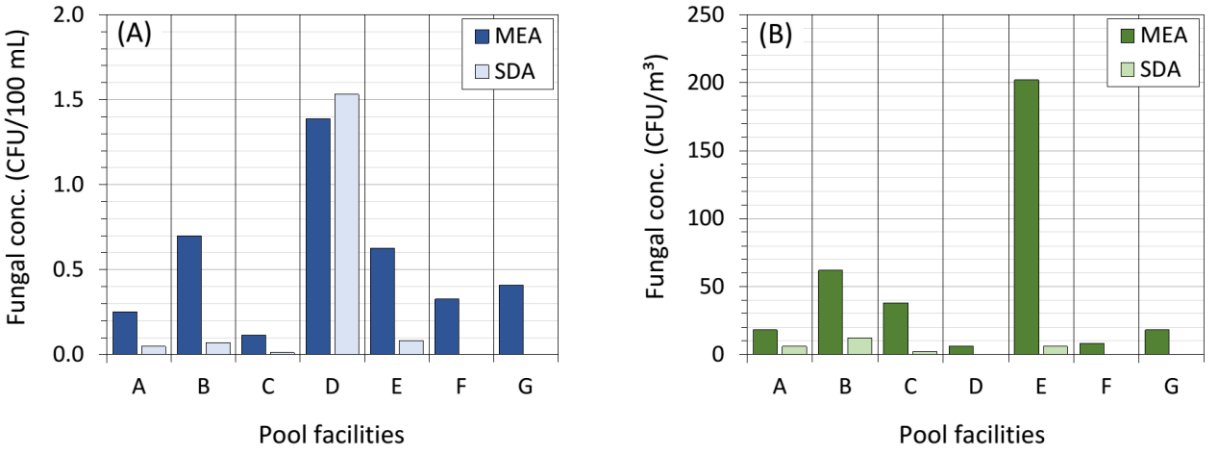

Figure 1. Fungal concentrations in water (A) and air (B) samples.

For comparison of fungal concentrations on floors at different sampling sites in seven pool facilities, concentrations on MEA are displayed in Figure 2 (count data are available in Annex Table S1). Higher fungal concentrations were observed on floors at the sites where people leave the dressing rooms and enter the shower room (DR-SH; (2)), as well as the sites where people leave the shower room and enter the swimming hall (SH-P; (4)) (Figure 2 and 3). Almost no fungi were observed on the shower floors except in pool facility A. Fungal concentrations on the surfaces of flexibeams ranged between 0 and 0.20 CFU/cm² (Figure 2). Fungal concentrations in the additional samples from the floors in the swim halls of facility A–D ranged between 0 and 3.56 CFU/cm² and on the surfaces of cleaning tools were between 0.32 and 2.96 CFU/cm² (Figure 2).

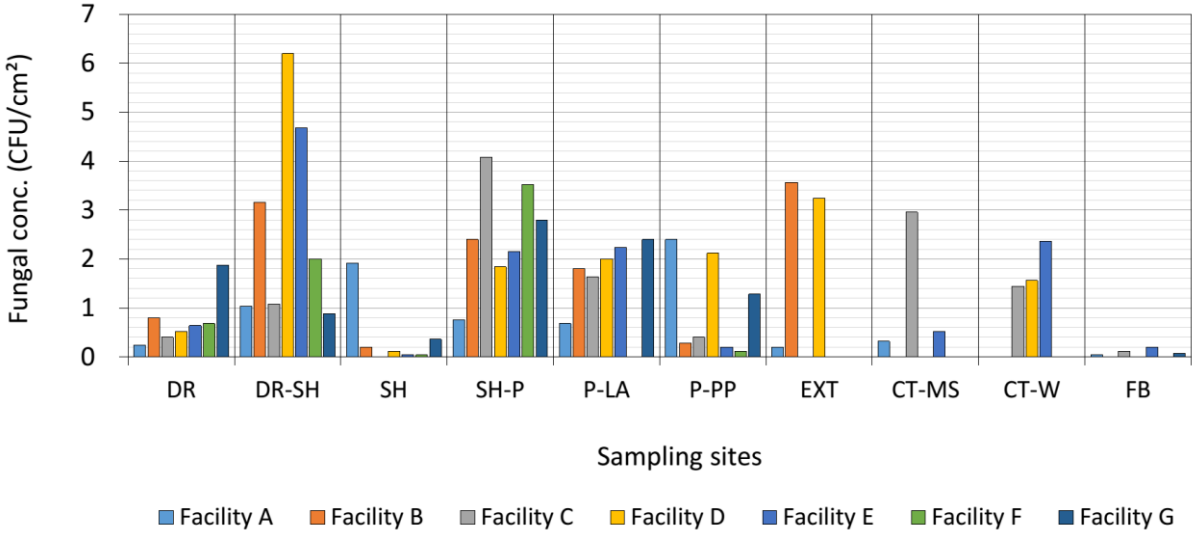

Figure 2. Fungal concentrations on surfaces, in samples cultured on MEA. DR: dressing room, DR-SH: dressing room to shower room, SH: shower room, SH-P: shower room to swimming hall, P-LA: pool ladder, P-PP: preferential pathway, FB: flexibeam, EXT: second pool pathway, CT-MS: mop/scrubber, CT-W: wiper.

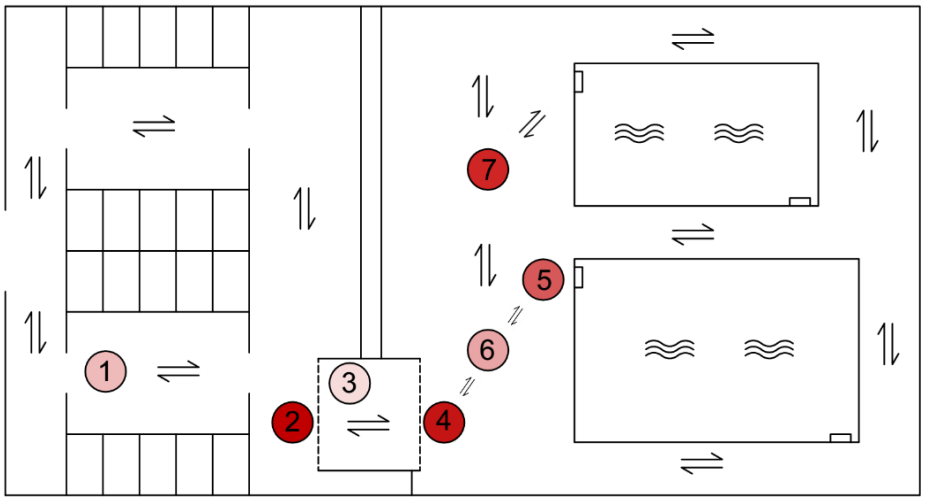

Figure 3. Fungal concentrations on floors in samples collected from seven swimming pool facilities, cultured on MEA based on mean values and shown in gradient colour from white to red displaying the lowest to the highest concentration. ①: dressing room (DR), ②: dressing room to shower room (DR-SH), ③: shower room (SH), ④: shower room to swimming hall (SH-P), ⑤: pool ladder (P-LA), ⑥: preferential pathway (P-PP), ⑦: second pool pathway (EXT).

Statistical comparison was performed on fungal concentrations on MEA only since this medium is a general purpose culture medium and thus yielded more representative fungal counts than SDA, a (semi-)selective medium. No significant differences ($H(6) = 1.5960$, $p = 0.95$) were observed in the fungal concentrations between pool facilities

(comparing only floor samples, except the floor samples taken near the second pool in facility A–D designated EXT), However, there were significant differences (H(5) = 21.1123, p = 0.0008) when comparing the fungal concentrations on floors between the different sampling sites. Further analysis using the post hoc Dunn's multiple comparison test revealed that the fungal concentrations on shower floors (SH) were significantly lower than the concentrations found on the floors at the exit of the dressing rooms leading to the shower room (DR-SH) and the exit of the shower room leading to the swimming hall (SH-P), while the rest of pairwise comparisons did not show significant differences.

Isolated fungal species

A total of 458 isolates obtained from the seven pool facilities, were identified to the species level based on their ITS sequences. Some fungal species were difficult to identify to the species level based on their ITS sequences, and were therefore identified up to the genus, species complex or section level.

The isolates comprised of 46 isolates from water, 40 isolates from air, and 372 isolates from surfaces. Fungal DNA identification resulted in 111 fungal species: 73 species were detected only on MEA and 18 species were from SDA, while 20 species were isolated from both media. Based on sampling sites, 27 species were isolated from water, 21 from air and 88 from surfaces. Among the identified species, 11, 10 and 68 fungal species, respectively, originated from water, air and surfaces only and 22 species were recovered from at least two different environmental matrices. The number of fungal species and isolates in samples from the studied swimming pool facilities can be found in Annex Table S2.

Phialophora oxyspora was the most frequently isolated fungal species (13.3%, 61/458). It was detected mostly on surfaces, rarely in water and was never detected in air samples in any of the pool facilities. Another frequently isolated fungal species, *Trichosporon dohaense* (5.0%, 23/458) was detected only in samples from floors, whereas fungal species in the *Penicillium* section *Chrysogena* (4.6%, 21/458) were isolated from water, air and floors. Fungal species isolated in this study are listed in Annex Table S3.

Clinically relevant fungal species

Fifty of the isolated fungal species (45%, 50/111) were considered clinically relevant. Of the total number of 458 isolates, 316 isolates (69%) belonged to clinically relevant species (35 isolates from water, 29 isolates from air and 252 isolates from surfaces). Based on the number of fungal species, 59% (16/27), 67% (14/21) and 46% (41/88) of the species from water, air and surfaces, respectively, were clinically relevant.

Phialophora oxyspora was not only the most frequently isolated fungal species, but also the most commonly isolated clinically relevant fungal species (Annex Table S3).

Additionally, clinically relevant fungal species in the genera *Penicillium* and *Aspergillus* were also frequently detected on surfaces, in water and in air with overall occurrences of 12% (53/458) and 9% (42/458), respectively. Five percent of the isolates (22/458) belonged to five clinically relevant species within the genus *Exophiala* which were detected on surfaces.

Although rarely isolated, some fungal species belonging to risk group 2, were detected at different sampling sites in different pool facilities. *Candida auris* was detected in water samples from pool facilities A and D, *Scedosporium boydii* which was isolated from water samples in pool facilities A and G, *Rhinocladiella similis* was isolated from a cleaning tool in pool facility D and *Exophiala dermatitidis* was isolated once from pool facility A.

Two dermatophyte species were isolated from both floors and water. *Trichophyton interdigitale* (1.3%, 6/458) was isolated from the floors in the dressing rooms in facility F, the dressing room exit leading to shower room in facility A and the swimming hall in facilities A and F, as well as from pool water in facility B. *Trichophyton rubrum* (0.4%, 2/458) was isolated from the floors in the shower room in facility B and the floors in the swimming hall of facility F.

Distribution of fungi in swimming pool facilities

Regardless of the sampling sites, 40 fungal species were isolated from at least two different pool facilities. *Phialophora oxyspora* was detected in all pool facilities except pool facility A. *Trichosporon dohaense*, *Rhodotorula mucilaginosa* and *Purpureocillium lilacinum* were present in five pool facilities. *Penicillium*, *Aspergillus*, *Cutaneotrichosporon*, *Fusarium*, and *Exophiala* were the most frequent fungal genera observed in the different pool facilities.

Phylogenetic trees were constructed based on the fungal species detected in each pool facility (Figure 4, Annex Figure S1-S6). The markers in different colours represent different sampling sites (floor, water, air, teaching aid and cleaning tool). If the same fungal species was isolated more than once in one sampling site, only one sequence was used to construct the phylogenetic tree. Figure 4 shows the phylogenetic tree of the fungal species isolated from pool facility E representing that which displayed most species diversity. From a total of 74 isolates and 37 fungal species, sections and species complexes found in this pool facility, six fungal species/sections/complexes were isolated from samples taken from two different sampling sites. *Cladosporium cladosporioides* complex was detected on floors, in water and in air. *Cladosporium herbarum* complex was isolated from floors and a teaching aid. *Phoma* spp. were detected in air, on floors and on a cleaning tool. *Alternaria infectoria* were isolated from water, floors, and a teaching aid. *Penicillium* section *Chrysogena* was isolated from water and floors and *Aureobasidium pullulans* was isolated from water and a teaching aid.

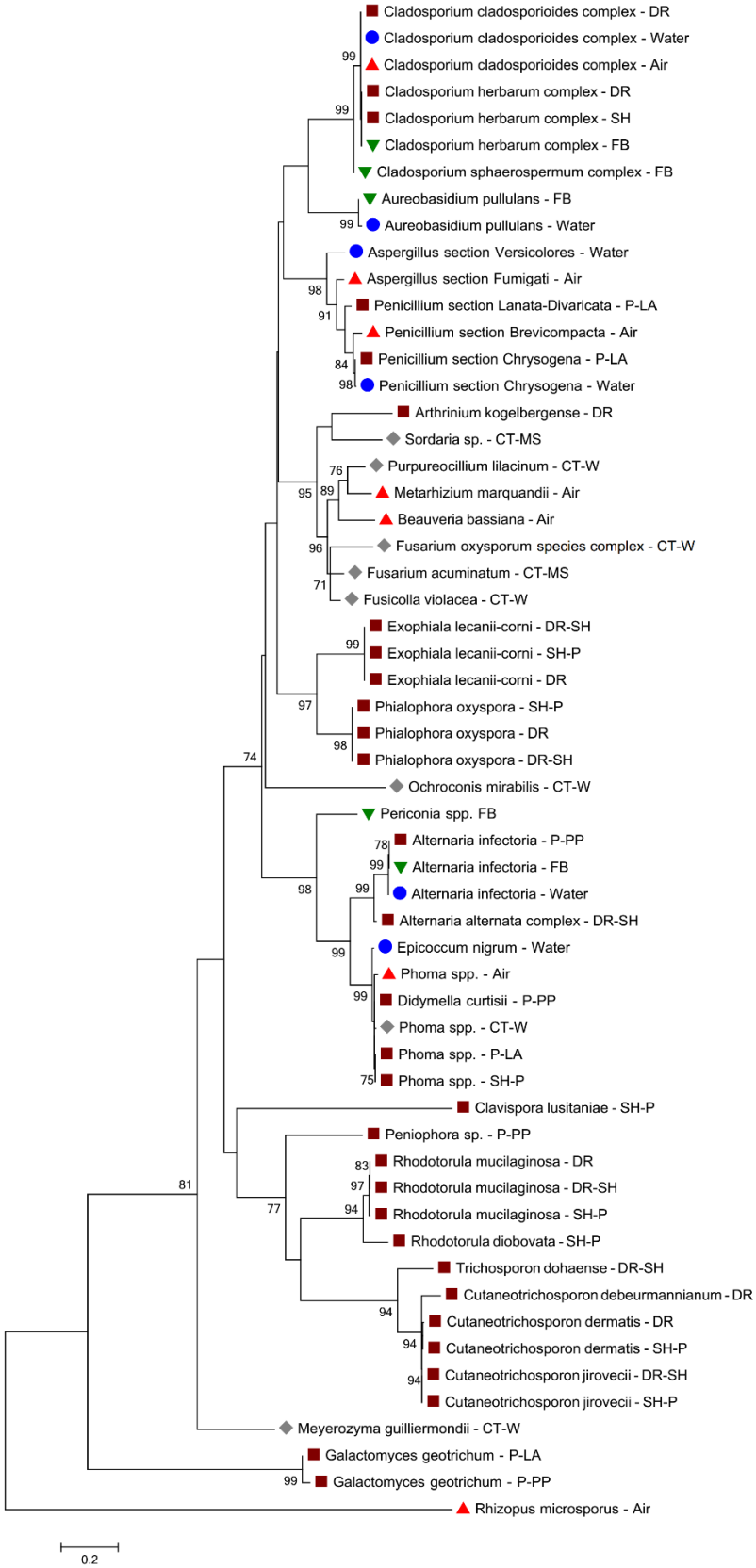

Figure 4. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility E. (■ : floor, ▲ : air, ● : water, ▼ : teaching aid, ◆ : cleaning tool)

Examining the phylogenetic trees from all the pool facilities (data not shown except for pool facility E) revealed that in total, 12 fungal species (and sections and species complexes) were detected in the combination of water, air and/or surfaces (either floor, teaching aid or cleaning tool). In the studied pool facilities, some corresponding fungal species (and sections and species complexes) were detected in water, in air and on surfaces except in pool A and D (Table 3). While most of the corresponding species (and sections and species complexes) were isolated from two different environments, only a species belonging to *Cladosporium cladosporioides* complex was detected in air, in water and on surfaces (in pool facility E).

Table 3. Fungal species detected in or on at least two environments: water, air, floor, teaching aid, and cleaning tool in the same facility

Pool facility	**Fungal species/species complex/section**	**Environmental matrices**				
		Air	**Water**	**Surface**		
				Floor	**Teaching aid**	**Cleaning tool**
A	*Cutaneotrichosporon dermatis*			X		X
	Penicillium section *Fasciculata*			X		X
	Rhodotorula mucilaginosa			X		X
B	*Aspergillus* section *Versicolores*	X	X			
	Penicillium section *Chrysogena*	X	X			n/a
	Trichophyton interdigitale		X	X		
C	*Aspergillus* section *Nigri*			X	X	
	Cyphellophora sp.			X		X
	Exophiala oligosperma			X		X
	Penicillium section *Brevicompacta*	X	X			
	Phialophora oxyspora		X	X	X	
D	*Fusarium oxysporum* species complex			X		X
	Phialophora oxyspora			X	X	
	Phoma spp.			X		X
E	*Cladosporium cladosporioides* complex	X	X	X		
	Cladosporium herbarum complex			X	X	
	Phoma spp.	X		X		X
	Alternaria infectoria		X	X	X	
	Penicillium section Chrysogena		X	X		
	Aureobasidium pullulans		X		X	
F	*Alternaria alternata* complex		X	X		n/a
G	*Cladosporium herbarum* complex	X		X		
	Rhodotorula mucilaginosa	X		X		n/a
	Penicillium section Chrysogena		X	X		

From the ten fungal species detected on the surfaces of the sampled teaching aids (i.e. flexibeams), seven species were clinically relevant and six species were also detected either in the water or on the floors in the corresponding facility.

Samples collected from six cleaning tools (mops, scrubbers and wipers) used in four different facilities resulted in 21 fungal species; 15 of them were clinically relevant species. Seven fungal species isolated from the cleaning tools were also isolated from floor surfaces. At least one fungal species isolated from cleaning tools in each facility was also found on floor samples in that facility (Table 3).

Discussion

Clinically relevant fungi in swimming pool environments

Phialophora oxyspora, the most frequently isolated fungal species in this study, was isolated from six out of seven pool facilities studied. The pathogenicity of this species is considered low since it was only occasionally isolated from cutaneous samples (de Hoog et al., 2015). Fungal species belonging to the genera *Penicillium* (7/7) and *Aspergillus* (6/7) dominated the fungal species isolated from air samples. The presence of these fungi in indoor air samples from swimming pool facilities was also demonstrated by Brandi et al. (2007) and Viegas et al. (2011). *Aspergillus* spp., especially *A. fumigatus*, are known to cause deep mycoses in immunocompromised humans while cases of human infections by *Penicillium* spp. other than *P. marnefeii*, are rare (de Hoog et al., 2015). Clinically relevant species belonging to the genus *Exophiala* were detected in five pool facilities. *Exophiala oligosperma* and *Exophiala dermatitidis* were previously detected in swimming pools and saunas and were associated with systemic mycoses (de Hoog et al., 2000; de Hoog et al., 2003; Matos et al., 2002; Zeng et al., 2007). Other clinically relevant fungal species which were isolated in five pool facilities were *Rhodotorula mucilaginosa* and *Purpureocillium lilacinum*. Both were reported to be involved in cases of catheter-related fungemia and cutaneous infections (de Hoog et al., 2015). *Candida auris* was unexpectedly isolated from water samples in two different facilities. In recent years, *Candida auris* has been recognised as fungal pathogen associated with invasive infections, causing outbreaks in healthcare settings around the world (Chowdhary et al., 2016; ECDC, 2018). *Trichophyton interdigitale* (3/7) and *T. rubrum* (2/7), causative agents of human nail and skin infections (Weitzman and Summerbell, 1995), were rarely isolated in the current study.

Although most of the isolated clinically relevant fungi are opportunistic pathogens, it is important to be aware of their presence, especially in public facilities such as swimming pools, where many people interact and use the same space and equipment. The presence of clinically relevant fungal species in the studied swimming pool facilities suggests a

possible health risk for immunocompromised persons such as children, pregnant women, and the elderly or with otherwise suppressed immune systems.

Transmission pathways

The spread of fungal skin and nail infections is facilitated by direct contact with infected skin fragments (e.g. on surfaces and in water) or skin-to-skin contact (English and Gibson, 1959). Deep fungal infections in immunocompromised patients are acquired through the respiratory tract, the gastrointestinal tract, or intravascular devices (de Hoog et al., 2015; Walsh and Dixon, 1996). In swimming pool facilities, contaminated surfaces, water, and air may contribute to the transmission of fungal infections to susceptible visitors.

Floors

In none of the studied pool facilities, consistent patterns were observed for fungal concentrations on the floor surfaces of the visitors' pathways from the dressing rooms up to the swimming hall. However, the results showed that the fungal concentrations on the floors in the shower room were the lowest in all facilities except pool facility A. In pool facility A, the shower room samples were taken from the floor in the middle of the shower room, for logistic reasons, whereas in the other pool facilities the samples were taken from floors under the shower heads. The deviating result in facility A indicates that the flow of water from the showers in the other facilities most likely flushed away the fungal material and resulted in lower fungal counts. The observed higher fungal concentrations on floors where people move from one part of the facility to another, like from the dressing room to the shower room and from the shower room to the swimming hall, can be explained by people converging at narrower exits and entrances.

The presence of *Trichophyton interdigitale* and *Trichophyton rubrum* on floors from the dressing rooms leading to the swimming hall, demonstrated the spread of dermatophytes which can potentially cause fungal skin infections. Moreover, the abundance of species like *Phialophora oxyspora*, *Trichosporon dohaense*, and *Cutaneotrichosporon debeurmannianum* on floors in different areas in the pool facilities suggests that pool visitors facilitated the dispersal of these fungi inside the facility.

Cleaning tools

Fungi were also detected on the mops, scrubbers and wipers used to clean the floors in the facilities. Some of the fungi isolated from the cleaning tools were also isolated from the floors. The tools may have become contaminated with these species while being used for the cleaning of contaminated floors, which subsequently gave the possibility that the use of these contaminated cleaning tools resulted in (re)contamination of floors and

dissemination of fungi to other areas inside the pool facilities. Using a single-use cleaning tool or using a properly cleaned or disinfected tool or using several type of disinfectants could be good options to avoid recontamination of floors, especially when the pool staff uses a mop or a wiper to remove water from the floors without using cleaning solutions. However, further study is needed to investigate the effectiveness of different interventions. As considerations, some fungi could survive after cleaning due to ineffective disinfectants (Bobichon et al., 1993; Pap et al., 2006) and cleaning or disinfecting a large area using a single mop could even facilitate the dissemination of pathogens (Exner et al., 2004).

Teaching aids

Both in our previous study (Ekowati et al., 2017) and in the current study, fungi were detected on the surface of flexibeams from different swimming pool facilities. While in use, flexibeams are in contact with water and swimmers for at least 30 min up to 2 h (information from the questionnaire, Schets et al. (2014)). Since fungal contamination of the surface of flexibeams may occur, proper drying and cleaning after use of these and other foam teaching aids are necessary, to avoid possible infection of the users and contamination of the pool water. It is also recommended to avoid the growth of pathogenic bacteria such as *Pseudomonas aeruginosa* (Schets et al., 2014).

Indoor air

Many studies have been done on the presence of fungi in indoor air in residential settings but only limited data can be found for swimming pool facilities. A study by Brandi et al. (2007) obtained 32.7 CFU/m^3 and 48.2 CFU/m^3 of fungal loads in air samples in swimming halls and in locker rooms, respectively. The fungal concentrations in this study were in a similar range as in Brandi et al. (2007). However, one air sample from facility E showed a much higher concentration compared to other air samples (202 CFU/m^3) for which we have no explanation based on the available characteristics and information on facility E. Rao et al. (1996) summarized quantitative standards and guidelines for fungal concentrations in indoor air from different institutions and research studies. WHO (1988) and Health Canada (1995) mentioned that fungal concentrations up to 150 CFU/m^3 in mixture species as acceptable, while some research mentioned in Rao et al. (1996) proposed higher values. In a more recent document, the Portuguese legislation on indoor air quality (Decree Law no. 79/2006, April 4), the maximum limit for fungal contamination is set at 500 CFU/m^3. Although different methods were used to quantify the fungal contamination in the guidelines summarized by Rao et al. (1996), such as different culture media and sampling methods, based on the guidelines values mentioned, the fungal concentrations in the air in the here studied swimming pool facilities can be considered as acceptable. For carrying out a health assessment on indoor air, a more extensive sampling campaign is required, taking into

account not only fungal concentrations, but also the fungal species present. The influence of outdoor air should not be overlooked.

Pool water

Direct contact with contaminated pool water is unlikely to cause skin infections since the infected skin fragments in pool water may not be able to attach firmly enough to the swimmers' skin (English and Gibson, 1959). Ear infections, such as otitis externa, are commonly contracted by swimmers (Bernius and Perlin, 2006). Some cases of otitis externa caused by *Aspergillus* spp. and *Candida* spp. associated with swimming activities have been reported (Dorko et al., 2004; Ozcan et al., 2003). Cases of fungal infections due to ingestion of contaminated water have not been reported. However, some cases of aspergillosis in the gastrointestinal tract (Eggimann et al., 2006; Myoken et al., 2001; Prescott et al., 1992) and a case of mucormycosis due to ingestion of naturopathic medicine (Oliver et al., 1996) have been described, which suggests that ingestion may be a potential route of transmission. In a study of fungi in drinking water, Hageskal et al. (2009) mentioned that consumption of fungi-contaminated drinking water has thus far not led to acute disease.

Reoccurring fungal species in an indoor swimming pool facility

More than half of the fungal species detected in pool facility D in this study, were the same species as detected in the previous study (Ekowati et al., 2017), and nearly 80% of these reoccurring species were clinically relevant fungal species. Frequently isolated fungal species in both studies; *Phialophora oxyspora* was previously isolated from plant materials and human skin (Feng et al., 2014); *Trichosporon dohaense* and *Cutaneotrichosporon debeurmannianum* were previously isolated from clinical samples (Abdel-Sater et al., 2016; Nath et al., 2017); *Phoma* spp. are common soil fungi; and *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., and *Alternaria* spp. are commonly found in outdoor and indoor air (Flannigan, 1997) and were also detected on different sites of the skin of healthy humans (Findley et al., 2013). The occurrence of these fungi suggests that the fungal population in pool facility D is influenced by the outdoor environment and pool visitors.

Preventive measures

The fungal concentrations observed at different sites in the studied swimming pool facilities only suggest that there is a possibility of infections. The existing guidelines recommend the maximum limits of fungal concentrations, but there are no guideline or limit values that indicate a specific concentration which can pose a risk of fungal infections. In general, the guidelines for indoor environments provide suggestions for prevention and remediation of fungal contamination.

Indoor fungal growth is associated with humidity of indoor air which can be controlled by ventilation and air conditioning (WHO, 2009). Ventilation plays a major role in removing and reducing fungal materials in indoor air. However, if it is not well maintained, ventilation could be the source of fungal contamination. The ventilation in indoor swimming pool facilities should be adequate to remove and dilute the pollutants in indoor air.

In general, swimming pools have to apply disinfection to prevent the microbial contamination of pool water. Chlorination is the most commonly used disinfectant in swimming pool practices. However, some fungal spores have a higher resistance to chlorine compared to indicator microorganisms, such as *Escherichia coli* (Ma and Bibby, 2017; Pereira et al., 2013). The application of alternative disinfection technologies which are able to inactivate chlorine resistant pathogens, such as UV and ozone, could be valuable in reducing fungal contamination in pool water.

The spread of fungal infections through direct contact with contaminated surfaces in swimming pool facilities can be avoided by taking some preventive measures. In this study, the information about the cleaning regime in each facility obtained from the pool managers was not sufficient enough to assess the effect of different cleaning regimes on the fungal concentration on surfaces. Watanabe et al. (2000) showed that washing feet with soap and subsequent wiping dry with a towel significantly reduced skin contamination. Gentles et al. (1974) demonstrated that by applying an intervention such as supplying and encouraging pool visitors to use foot powder could reduce the incidence of tinea pedis. The WHO Guideline for Safe Recreational Water Environments volume 2 (WHO, 2006) emphasises public education about fungal disease (e.g. tinea pedis), the importance of limiting contact to infected people and medical treatment. Other preventive measures were mentioned by Al-Doory and Ramsey (1987) such as pre-swim showers, wearing sandals and frequent cleaning of surfaces. The latter indicates the importance of having a regular check and thorough cleaning of the swimming pool environments, especially in favourable places that support fungal growth while being aware of possible fungal spread by contaminated cleaning tools.

Conclusions

In this study, possible transmission pathways in swimming pool facilities were identified from the exposure sites by means of fungal counts and typing. Although, there were no typical patterns of fungal concentrations along the preferential pathways, it can be concluded that sites where all pool visitors converge because they have to walk from one room (e.g. dressing room) to another (e.g. shower room) posed the highest risk of contamination, indicated by higher concentrations of fungi at these particular sites. The fungal species identified along the most likely transmission pathways, suggest that the dispersal of fungi on floors is most likely facilitated by the pool visitors and cleaning

tools. Clinically relevant fungi, including the ones rarely identified in nature, were widely present on floors, in water and in air in the studied swimming pool facilities. Their presence potentially poses a health risk to those vulnerable, and thus their prevalence should be minimized by implementing preventive measures.

References

Abdel-Sater, M.A., Moubasher, A.A.H., Soliman, Z. (2016). Identification of three yeast species using the conventional and internal transcribed spacer region sequencing methods as first or second global record from human superficial infections. *Mycoses*, 59(10), 652-661.

Aho, R., Hirn, J. (1980). A survey of fungi and some indicator bacteria in chlorinated water of indoor public swimming pools. *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene. 1. Abt. Originale B, Hygiene*, 173(3-4), 242-249.

Al-Doory, Y., Ramsey, S. (1987). Cutaneous mycotic diseases. *Moulds and health: Who is at risk*, 61-68.

Ali-Shtayeh, M., Khaleel, T.K.M., Jamous, R.M. (2003). Ecology of dermatophytes and other keratinophilic fungi in swimming pools and polluted and unpolluted streams. *Mycopathologia*, 156(3), 193–205.

Bernius, M., Perlin, D. (2006). Pediatric Ear, Nose, and Throat Emergencies. *Pediatric Clinics of North America*, 53(2), 195–214.

Besluit hygiëne en veiligheid badinrichtingen en zwemgelegenheden (2011). Ministerie van Infrastuctuur en Milieu, the Netherlands.

Bobichon, H., Dufour-Morfaux, F., Pitort, V. (1993). In vitro susceptibility of public indoor swimming pool fungi to three disinfectants. *Mycoses*, 36(9-10), 305–311.

Brandi, G., Sisti, M., Paparini, A., Gianfranceschi, G., Schiavano, G.F., De Santi, M., Santoni, D., Magini, V., Romano-Spica, V. (2007). Swimming pools and fungi: An environmental epidemiology survey in Italian indoor swimming facilities. *International Journal of Environmental Health Research*, 17(3), 197–206.

Brown, G.D., Denning, D.W., Gow, N.A., Levitz, S.M., Netea, M.G., White, T.C. (2012). Hidden killers: Human fungal infections. *Science translational medicine*, 4(165), 165rv113–165rv113.

Buot, G., Toutous-Trellu, L., Hennequin, C. (2010). Swimming pool deck as environmental reservoir of *Fusarium*. *Medical Mycology*, 48(5), 780-784.

Chowdhary, A., Voss, A., Meis, J.F. (2016). Multidrug-resistant *Candida auris*: 'new kid on the block' in hospital-associated infections? *Journal of Hospital Infection*, 94(3), 209–212.

de Hoog, G., Guarro, J., Gené, J., Figueras, M. (2015). Atlas of Clinical Fungi, 4th ed. Utrecht, the Netherlands, CBS-KNAW Fungal Biodiversity Centre.

de Hoog, G., Queiroz-Telles, F., Haase, G., Fernandez-Zeppenfeldt, G., Angelis, D.A., Gerrits van den Ende, A.H.G., Matos, T., Peltroche-Llacsahuanga, H., Pizzirani-Kleiner, A., Rainer, J. (2000). Black fungi: Clinical and pathogenic approaches. *Medical Mycology*, 38(sup1), 243–250.

de Hoog, G., Vicente, V., Caligiorne, R., Kantarcioglu, S., Tintelnot, K., van den Ende, A.G., Haase, G. (2003). Species diversity and polymorphism in the *Exophiala*

spinifera clade containing opportunistic black yeast-like fungi. *Journal of Clinical Microbiology*, 41(10), 4767–4778.

Decree-Law No. 79/2006 of April 4th. Diário da República no. 67/06—I Série-A. (2006). Ministry of Public Works, Transport and Communications, Lisbon, Portugal.

Detandt, M., Nolard, N. (1988). Dermatophytes and swimming pools: seasonal fluctuations. *Mycoses*, 31(10), 495–500.

Detandt, M., Nolard, N. (1995). Fungal contamination of the floors of swimming pools, particularly subtropical swimming paradises. *Mycoses*, 38(11-12), 509–513.

Dorko, E., Jenča, A., Orenčák, M., Virágová, S., Pilipčinec, E. (2004). Otomycoses of candidal origin in eastern Slovakia. *Folia Microbiologica*, 49(5), 601–604.

ECDC (2018). *Candida auris* in healthcare settings – Europe – first update, 23 April 2018. Stockholm, European Centre for Disease Prevention and Control.

Eggimann, P., Chevrolet, J.C., Starobinski, M., Majno, P., Totsch, M., Chapuis, B., Pittet, D. (2006). Primary invasive aspergillosis of the digestive tract: report of two cases and review of the literature. *Infection*, 34(6), 333–338.

Ekowati, Y., van Diepeningen, A.D., Ferrero, G., Kennedy, M.D., de Roda Husman, A.M., Schets, F.M. (2017). Clinically relevant fungi in water and on surfaces in an indoor swimming pool facility. *International Journal of Hygiene and Environmental Health*, 220(7), 1152–1160.

Ellabib, M., Khalifa, Z., Kavanagh, K. (2002). Dermatophytes and other fungi associated with skin mycoses in Tripoli, Libya. *Mycoses*, 45(3-4), 101–104.

English, M.P., Gibson, M.D. (1959). Studies in the epidemiology of tinea pedis. II. Dermatophytes on the floors of swimming-baths. *British Medical Journal*, 1(5135), 1446–1448.

Exner, M., Vacata, V., Hornei, B., Dietlein, E., Gebel, J. (2004). Household cleaning and surface disinfection: new insights and strategies. *Journal of Hospital Infection*, 56, 70–75.

Feng, P., Lu, Q., Najafzadeh, M. J., Gerrits van den Ende, A.H.G., Sun, J., Li, R., Xi, L., Vicente, V. A., Lai, W., Lu, C., de Hoog, G. S. (2014). *Cyphellophora* and its relatives in *Phialophora*: biodiversity and possible role in human infection. *Fungal Diversity*, 65(1), 17–45.

Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J. A., Schoenfeld, D., Nomicos, E., Park, M., Kong, H. H., Segre, J. A. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature*, 498(7454), 367–370.

Flannigan, B. (1997). Air sampling for fungi in indoor environments. *Journal of Aerosol Science*, 28(3), 381-392.

Gentles, J., Evans, E., Jones, G. (1974). Control of tinea pedis in a swimming bath. *British Medical Journal*, 2(5919), 577–580.

Gentles, J. C., Evans, E. G. V. (1973). Foot infections in swimming baths. *British Medical Journal*, 3(5874), 260–262.

Gudnadóttir, G., Hilmarsdóttir, I., Sigurgeirsson, B. (1999). Onychomycosis in Icelandic swimmers. *Acta Dermato-Venereologica*, 79(5), 376.

Hageskal, G., Lima, N., Skaar, I. (2009). The study of fungi in drinking water. *Mycological Research*, 113(2), 165–172.

Health Canada (1995). Indoor air quality in office buildings: a technical guide (93-EHD-166). A report of the Federal-Provincial Advisory Committee on Environmental and Occupational Health. Ottawa, Canada.

Jankowski, M., Charemska, A., Czajkowski, R. (2017). Swimming pools and fungi: An epidemiology survey in Polish indoor swimming facilities. *Mycoses*, 60(11), 736–738.

Kamihama, T., Kimura, T., Hosokawa, J., Ueji, M., Takase, T., Tagami, K. (1997). Tinea pedis outbreak in swimming pools in Japan. *Public Health*, 111(4), 249–253.

Katoh, K., Rozewicki, J., Yamada, K.D. (2017). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, bbx108–bbx108.

Katoh, K., Standley, D.M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780.

Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874.

Lateur, N., Mortaki, A., André, J. (2003). Two hundred ninety-six cases of onychomycosis in children and teenagers: A 10-Year Laboratory Survey. *Pediatric Dermatology*, 20(5), 385–388.

Ma, X., Bibby, K. (2017). Free chlorine and monochloramine inactivation kinetics of *Aspergillus* and *Penicillium* in drinking water. *Water Research*, 120, 265–271.

Maghazy, S.M.N., Abdel-Mallek, A.Y., Bagy, M.M.K. (1989). Fungi in two swimming pools in Assiut Town, Egypt. *Zentralblatt für Mikrobiologie*, 144(3), 213–216.

Matos, T., Hoog, G.S.D., Boer, A.G.D., Crom, I.D., Haase, G. (2002). High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. *Mycoses*, 45(9-10), 373–377.

Morales-Cardona, C.A., Valbuena-Mesa, M.C., Alvarado, Z., Solorzano-Amador, A. (2014). Non-dermatophyte mould onychomycosis: A clinical and epidemiological study at a dermatology referral centre in Bogota, Colombia. *Mycoses*, 57(5), 284–293.

Myoken, Y., Sugata, T., Kyo, T.-i., Fujihara, M., Kohara, T., Katsu, M., Tamura, M., Mikami, Y. (2001). Invasive *Aspergillus stomatitis* in patients with acute leukemia: Report of 12 cases. *Clinical Infectious Diseases*, 33(12), 1975–1980.

Nath, R., Sargiary, P., Borkakoty, B., Parida, P. (2017). *Cutaneotrichosporon* (*Trichosporon*) *debeurmannianum*: A rare yeast isolated from blood and urine samples. *Mycopathologia*. 183(3), 585-590.

Oliver, M.R., Van Voorhis, W.C., Boeckh, M., Mattson, D., Bowden, R.A. (1996). Hepatic mucormycosis in a bone marrow transplant recipient who ingested naturopathic medicine. *Clinical Infectious Diseases*, 22(3), 521–524.

Ozcan, K.M., Ozcan, M., Karaarslan, A., Karaarslan, F. (2003). Otomycosis in Turkey: predisposing factors, aetiology and therapy. *The Journal of Laryngology & Otology*, 117(1), 39–42.

Pap, K., Szilli, M., Kiskó, G. (2006). Testing antimicrobial efficiency of seven disinfectants against bacteria and fungi with surface test. *Acta Alimentaria*, 35(2), 163–170.

Pereira, V. J., Marques, R., Marques, M., Benoliel, M. J., Barreto Crespo, M. T. (2013). Free chlorine inactivation of fungi in drinking water sources. *Water Research*, 47(2), 517–523.

Prescott, R.J., Harris, M., Banerjee, S.S. (1992). Fungal infections of the small and large intestine. *Journal of Clinical Pathology*, 45(9), 806–811.

Rao, C.Y., Burge, H.A., Chang, J.C. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association*, 46(9), 899–908.

Schets, F., van den Berg, H., Baan, R., Lynch, G., de Roda Husman, A. (2014). *Pseudomonas aeruginosa* on vinyl-canvas inflatables and foam teaching aids in swimming pools. *Journal of Water and Health*, 12(4), 772–781.

Seebacher, C., Bouchara, J.-P., Mignon, B. (2008). Updates on the epidemiology of dermatophyte infections. *Mycopathologia*, 166(5-6), 335–352.

Sharma, M., Sharma, R. (2012). Profile of dermatophytic and other fungal infections in Jaipur. *Indian Journal of Microbiology*, 52(2), 270–274.

Shemer, A., Gupta, A.K., Amichai, B., Baum, S., Barzilai, A., Farhi, R., Kaplan, Y., MacLeod, M.A. (2016). Increased risk of tinea pedis and onychomycosis among swimming pool employees in Netanya Area, Israel. *Mycopathologia*, 181(11), 1–6.

Thomas, J., Jacobson, G., Narkowicz, C., Peterson, G., Burnet, H., Sharpe, C. (2010). Toenail onychomycosis: An important global disease burden. *Journal of Clinical Pharmacy and Therapeutics*, 35(5), 497–519.

Viegas, C., Alves, C., Carolino, E., Pinheiro, C., Rosado, L., Santos, C.S. (2011). Assessment of fungal contamination in a group of Lisbon's gymnasiums with a swimming pool. *Italian Journal of Occupational and Environmental Hygiene*, 2(1), 15–20.

Walsh, T. J., Dixon, D. M. (1996). Spectrum of Mycoses. In: Baron, S. (Ed.), *Medical Microbiology* (4th ed.). University of Texas Medical Branch at Galveston, Galveston (TX), USA.

Watanabe, K., Taniguchi, H., Katoh, T. (2000). Adhesion of dermatophytes to healthy feet and its simple treatment. Dermatophytenbefall gesunder Fsse und dessen einfache Behandlung. *Mycoses*, 43(1/2), 45–50.

Weitzman, I., Summerbell, R.C. (1995). The dermatophytes. *Clinical Microbiology Reviews*, 8(2), 240–259.

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., & White, T. J. (Eds.), *PCR protocols: a guide to methods and applications* (Vol. 18). Academic Press, Inc., New York, pp. 315–322.

WHO (1988). WHO Regional Publication European Series, No. 31: Indoor Air Quality: Biological Contaminants; Report on a WHO meeting, Copenhagen, Denmark, WHO Regional Office for Europe.

WHO (2006). Guidelines for safe recreational water environments, Vol. 2: Swimming pools and similar environments. Geneva, World Health Organization.

WHO (2009). WHO guidelines for indoor air quality: dampness and mould. Copenhagen, Denmark, WHO Regional Office for Europe.

Zeng, J., Sutton, D., Fothergill, A., Rinaldi, M., Harrak, M., de Hoog, G. (2007). Spectrum of clinically relevant *Exophiala* species in the United States. *Journal of Clinical Microbiology*, 45(11), 3713–3720.

Zhou, X., Rodrigues, A.M., Feng, P., de Hoog, G.S. (2014). Global ITS diversity in the *Sporothrix schenckii* complex. *Fungal Diversity*, 66(1), 153–165.

Annex

Table S1. Fungal concentrations in seven swimming pool facilities (A–G)

Sample code	Sample matrix	Description of sampling site	Fungal counts on MEA*						
			A	B	C	D	E	F	G
DR	Floor	Dressing room pathway	0.24	0.80	0.40	0.52	0.64	0.68	1.88
DR-SH	Floor	Entrance from dressing room to shower room	1.04	3.16	1.08	6.20	4.68	2.00	0.88
SH	Floor	Shower room	1.92	0.20	0.00	0.12	0.04	0.04	0.36
P-SH	Floor	Entrance from shower room to swimming hall	0.76	2.40	4.08	1.84	2.16	3.52	2.80
P-LA	Floor	Swimming hall (in front of swim ladder)	0.68	1.80	1.64	2.00	2.24	TNTC	2.40
P-PP	Floor	Swimming hall (preferential pathway)	2.40	0.28	0.40	2.12	0.20	0.12	1.28
FB	Teaching aid	Flexibeam	0.04	0.00	0.12	0.00	0.20	0.00	0.08
Water	Water	Swimming pool	0.25	0.70	0.11	1.39	0.63	0.33	0.41
Air	Air	Swimming hall	18	62	38	6	202	8	18
EXT	Floor	Swimming hall (preferential pathway)	0.20	3.56	TNTC	3.24	n.a.	n.a.	n.a.
CT-MS	Cleaning tool	Mop/scrubber	0.32	n.a.	2.96	n.a.	0.52	n.a.	n.a.
CT-W	Cleaning tool	Wiper	n.a.	n.a.	1.44	1.56	2.36	n.a.	n.a.

* Surface sample in CFU/cm², water sample in CFU/100 mL, air sample in CFU/m³

Sample code	Sample matrix	Description of sampling site	Fungal counts on SDA*						
			A	B	C	D	E	F	G
DR	Floor	Dressing room pathway	0.14	0.00	0.20	0.00	0.26	0.80	2.37
DR-SH	Floor	Entrance from dressing room to shower room	0.00	0.48	0.24	0.83	0.50	0.27	1.92
SH	Floor	Shower room	0.22	0.00	0.00	0.04	0.00	0.00	0.12
P-SH	Floor	Entrance from shower room to swimming hall	0.61	0.27	6.16	3.76	2.92	0.00	1.79
P-LA	Floor	Swimming hall (in front of swim ladder)	0.68	0.60	TNTC	1.61	TNTC	0.42	0.00
P-PP	Floor	Swimming hall (preferential pathway)	1.82	0.00	3.76	2.11	0.06	0.06	0.00
FB	Teaching aid	Flexibeam	0.00	0.04	0.04	0.35	0.00	0.00	0.00
Water	Water	Swimming pool	0.05	0.07	0.01	1.53	0.08	0.00	0.00
Air	Air	Swimming hall	6	12	2	0	6	0	0.00
EXT	Floor	Swimming hall (preferential pathway)	0.00	0.90	TNTC	TNTC	n.a.	n.a.	n.a.
CT-MS	Cleaning tool	Mop/scrubber	0.00	n.a.	TNTC	n.a.	TNTC	n.a.	n.a.
CT-W	Cleaning tool	Wiper	n.a.	n.a.	2.76	TNTC	0.14	n.a.	n.a.

* Surface sample in CFU/cm², water sample in CFU/100 mL, air sample in CFU/m³

Table S2. Number of fungal species and isolates in samples from seven indoor swimming pool facilities

Number of identified species	**All**	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Total number of identified species	111 (458)	35 (64)	21 (63)	31 (89)	27 (78)	37 (74)	25 (44)	22 (46)
Water samples	27 (46)	6 (6)	4 (9)	3 (5)	8 (9)	6 (8)	5 (5)	4 (4)
Air samples	21 (40)	1 (4)	2 (10)	5 (6)	2 (3)	7 (8)	4 (4)	5 (5)
Surface samples (floors, teaching aid and cleaning tools)	88 (372)	28 (54)	18 (44)	25 (78)	17 (66)	31 (58)	17 (35)	16 (37)
Floors	75 (319)	25 (48)	18 (44)	19 (59)	16 (58)	20 (40)	17 (35)	14 (35)
Teaching aids	10 (13)	1 (1)	0 (0)	2 (4)	1 (1)	5 (5)	0 (0)	2 (2)
Cleaning tools	21 (40)	5 (5)	-	8 (15)	3 (7)	8 (13)	-	-
Total number of clinically relevant species	50 (316)	21 (43)	11 (41)	19 (66)	15 (60)	22 (52)	11 (15)	16 (39)
Clinically relevant species in water samples	16 (35)	4 (4)	3 (8)	3 (5)	4 (5)	5 (7)	3 (3)	3 (3)
Clinically relevant species in air samples	14 (29)	0 (0)	2 (10)	4 (5)	1 (1)	6 (7)	2 (2)	4 (4)
Clinically relevant species in surface samples	41 (252)	17 (39)	9 (23)	14 (56)	10 (54)	17 (38)	7 (10)	12 (32)
Clinically relevant species on floors	34 (212)	17 (33)	9 (23)	14 (41)	10 (46)	17 (27)	7 (10)	12 (32)
Clinically relevant species on teaching aids	7 (10)	1 (1)	0 (0)	2 (4)	1 (1)	4 (4)	0 (0)	0 (0)
Clinically relevant species on cleaning tools	15 (30)	5 (5)	-	5 (11)	3 (7)	5 (7)	-	-

*Note: the number of isolates is indicated in parentheses

Table S3. Fungal species isolated from different sites in swimming pool facilities (A–G)

No.	Species	DR	DR-SH	SH	SH-P	P-LA	P-PP	FB	Water	Air	EXT	CT-MS	CT-W	Total	Freq.	No. of facilities
1	*Abortiporus biennis*									1 F				1	0.2%	1
2	*Acremonium persicinum*								1 D					1	0.2%	1
3	*Acremonium sclerotigenum*					1 A								1	0.2%	1
4	***Alternaria alternata* complex**		1 E		1 F		3 D, G		1 F					6	1.3%	4
5	***Alternaria infectoria***						2 E	1 E	1 E					4	0.9%	1
6	*Arthrinium arundinis*							1 G						1	0.2%	1
7	*Arthrinium kogelbergense*	2 E, G	1 G											3	0.7%	2
8	*Arthrinium rasikravindrae*	1 A												1	0.2%	1
9	*Arthrinium saccharicola*	1 C												1	0.2%	1
10	***Aspergillus* section *Aspergillus***									1 F				1	0.2%	1
11	***Aspergillus* section *Flavi***				3 G	3 C, G	4 C, G							10	2.2%	2
12	***Aspergillus* section *Fumigati***	2 C							1 F	1 E				4	0.9%	3
13	***Aspergillus* section *Nigri***					2 C	3 C	3 C			2 D			10	2.2%	2
14	***Aspergillus* section *Versicolores***	1 C							7 B, E, G	9 B, D				17	3.7%	5
15	***Aureobasidium pullulans***	1 G						1 E	1 E					3	0.7%	2
16	***Beauveria bassiana***									2 E				2	0.4%	1
17	*Botrytis cinerea*				2 D									2	0.4%	1
18	***Candida auris***								3 A, D					3	0.7%	2
19	***Candida parapsilosis***											1 A		1	0.2%	1
20	*Candida sojae*											1 C		1	0.2%	1
21	*Chaetomium erectum*								1 D					1	0.2%	1
22	***Chaetomium globosum***							1 A						1	0.2%	1
23	*Chaetomium subaffine*								1 D					1	0.2%	1
24	*Chaetothyriales* sp.				1 A									1	0.2%	1
25	*Cladophialophora chaetospira*	2 F	1 F											3	0.7%	1

No.	Species	DR	DR-SH	SH	SH-P	P-LA	P-PP	FB	Water	Air	EXT	CT-MS	CT-W	Total	Freq.	No. of facilities
26	***Cladosporium cladosporioides* complex**	1 E							1 E	2 E, F		1 A		5	1.1%	3
27	***Cladosporium herbarum* complex**	1 E	1 F	1 E			2 G	1 E		1 G				7	1.5%	3
28	***Cladosporium sphaerospermum* complex**							1 E	1 D					2	0.4%	2
29	***Clavispora lusitaniae***				1 E									1	0.2%	1
30	*Coprinellus domesticus*								1 F					1	0.2%	1
31	*Cutaneotrichosporon debeurmannianum*	2 E, F	2 F	1 F	2 F	1 F								8	1.7%	2
32	***Cutaneotrichosporon dermatis***	3 B, E	1 A, C		2 A, E	1 B						1 A		8	1.7%	3
33	*Cutaneotrichosporon jirovecii*		3 E	1 F	3 C, E, F	4 B, D, F					1 B			12	2.6%	5
34	***Cyphellophora pluriseptata***												1 C	1	0.2%	1
35	*Cyphellophora* spp.					1 C					3 C		1 C	5	1.1%	1
36	*Cystobasidium benthicum*					1 F								1	0.2%	1
37	*Daedaleopsis confragosa*										1 A			1	0.2%	1
38	*Daldinia* sp.								1 G					1	0.2%	1
39	*Didymella americana*	3 D												3	0.7%	1
40	*Didymella curtisii*		2 C, G		2 C	1 F	1 E				1 C			7	1.5%	4
41	*Didymella microchlamydospora*										1 C			1	0.2%	1
42	*Dinemasporium sasae*		1 F											1	0.2%	1
43	*Engyodontium album*	1 A												1	0.2%	1
44	*Epicoccum nigrum*	2 A, B	1 F						1 E					4	0.9%	4
45	*Erythrobasidium hasegawianum*	1 C												1	0.2%	1
46	***Exophiala cancerae***	1 A				5 B								6	1.3%	2
47	***Exophiala dermatitidis***										1 A			1	0.2%	1
48	***Exophiala equina***					1 A								1	0.2%	1
49	***Exophiala lecanii-corni***	1 E	2 E, G	1 G	2 E									6	1.3%	2
50	***Exophiala oligosperma***	2 C	1 C									1 C	4 C	8	1.7%	1

No.	Species	DR	DR-SH	SH	SH-P	P-LA	P-PP	FB	Water	Air	EXT	CT-MS	CT-W	Total	Freq.	No. of facilities
51	*Filobasidium uniguttulatum*								1 F					1	0.2%	1
52	*Fusarium acuminatum*											2 E		2	0.4%	1
53	***Fusarium oxysporum* species complex**	2 G	6 B, D		1 A								2 D, E	11	2.4%	5
54	***Fusarium solani* species complex**					2 C								2	0.4%	1
55	*Fusarium* sp.										2 A			2	0.4%	1
56	*Fusicolla aquaeductuum*						1 A							1	0.2%	1
57	*Fusicolla violacea*										1 C		1 E	2	0.4%	2
58	*Galactomyces geotrichum*					1 E	1 E							2	0.4%	1
59	*Gliomastix murorum*								1 D					1	0.2%	1
60	*Hypocreales* sp.					2 A	2 A							4	0.9%	1
61	*Isaria fumosorosea*				1 A									1	0.2%	1
62	*Metarhizium marquandii*									1 E				1	0.2%	1
63	*Meyerozyma caribbica*											1 C	1 C	2	0.4%	1
64	***Meyerozyma guilliermondii***	3 A, F											1 E	4	0.9%	3
65	***Mucor circinelloides***											1 C		1	0.2%	1
66	***Mucor hiemalis***											1 C		1	0.2%	1
67	*Naganishia diffluens*			1 B						1 F				2	0.4%	2
68	***Ochroconis musae***			2 G			1 F						2 E	5	1.1%	3
69	*Paracremonium inflatum*						1 B							1	0.2%	1
70	*Paraphaeosphaeria neglecta*	1 B												1	0.2%	1
71	***Penicillium* section *Aspergilloides***									2 C, G				2	0.4%	2
72	***Penicillium* section *Brevicompacta***								5 A, C, D, F	3 C, E				8	1.7%	5
73	*Penicillium* section *Charlesia*				1 D									1	0.2%	1
74	***Penicillium* section *Chrysogena***		3 A, G	2 A	1 G	5 E, G			6 B, E, G	3 B, C	1 A			21	4.6%	5
75	***Penicillium* section *Citrina***	1 C		3 D			1 C		1 A					6	1.3%	3

No.	Species	DR	DR-SH	SH	SH-P	P-LA	P-PP	FB	Water	Air	EXT	CT-MS	CT-W	Total	Freq.	No. of facilities
76	*Penicillium* section *Exilicaulis*									2 D				2	0.4%	1
77	***Penicillium* section *Fasciculata***	2 A, D	2 D		4 A, D	1 D	2 A		1 C			1 A		13	2.8%	3
78	*Penicillium* section *Lanata-Divaricata*	1 B				1 E				1 C				3	0.7%	3
79	***Penicillium* section *Penicillium***						2 B			1 C				3	0.7%	2
80	*Peniophora* sp.						2 E							2	0.4%	1
81	*Periconia* spp.	1 A						1 E						2	0.4%	2
82	*Pestalotiopsis* sp.	1 F												1	0.2%	1
83	***Phialophora oxyspora***	1 E	5 C, D, E	4 D, F, G	13 B, C, D, E	10 B, C, D, F	7 C, D	2 C, D	2 C		17 B, C, D			61	13.3%	6
84	***Phoma* spp.**	2 C, G			1 E	5 A, E				1 E	3 D		6 D, E	18	3.9%	5
85	*Pleosporales* sp.								1 A					1	0.2%	1
86	*Pochonia chlamydosporia*			1 B										1	0.2%	1
87	*Pseudocosmospora vilior*						1 B							1	0.2%	1
88	***Purpureocillium lilacinum***		2 C, D	3 G	2 G	3 A, C	2 D						1 E	13	2.8%	5
89	***Pyrenochaeta keratinophila***										2 B			2	0.4%	1
90	***Rhinocladiella similis***												2 D	2	0.4%	1
91	***Rhizopus 138icrospores***									1 E				1	0.2%	1
92	*Rhodotorula diobovata*				1 E									1	0.2%	1
93	*Rhodotorula graminis*		1 F											1	0.2%	1
94	***Rhodotorula mucilaginosa***	2 B, E	3 A, E, G		3 A, E, G	1 A				1 G		1 A	3 C	14	3.1%	5
95	***Sarocladium strictum***									1 G				1	0.2%	1
96	***Scedosporium boydii***								2 A, G					2	0.4%	2
97	*Simplicillium* sp.	1 C												1	0.2%	1
98	*Simplicillium sympodiophorum*									4 A				4	0.9%	1
99	*Sordaria* sp.											3 E		3	0.7%	1
100	*Talaromyces radicus*									1 G				1	0.2%	1

No.	Species	DR	DR-SH	SH	SH-P	P-LA	P-PP	FB	Water	Air	EXT	CT-MS	CT-W	Total	Freq.	No. of facilities
101	*Talaromyces verruculosus*							1 G	1 B					2	0.4%	2
102	***Tintelnotia destructans***								1 D					1	0.2%	1
103	*Torula herbarum*								1 A					1	0.2%	1
104	***Trichoderma atroviride***			5 A										5	1.1%	1
105	***Trichophyton interdigitale***	1 F	1 A		1 B	1 F	1 A		1 B					6	1.3%	3
106	***Trichophyton rubrum***			1 B			1 F							2	0.4%	2
107	***Trichosporon asahii***						2 D							2	0.4%	1
108	*Trichosporon dohaense*	2 F	6 B, C, E	3 B, F	8 B, D, F	1 F	2 B, F				1 B			23	5.0%	5
109	*Trichothecium roseum*	1 D												1	0.2%	1
110	*Ustilago sparsa*					2 D								2	0.4%	1
111	***Yarrowia lipolytica***			1 A										1	0.2%	1
Total number of isolates		50	46	30	56	56	44	13	46	40	37	15	25	458		
Total number of clinically relevant isolates		27	28	23	35	40	33	10	35	29	26	8	22	316		
Total number of species		33	21	15	22	24	22	10	27	21	14	12	12	111		
Total number of clinically relevant species		17	12	10	13	13	14	7	16	14	6	8	9	50		

Note: DR: dressing room, DR-SH: dressing room to shower room, SH: shower room, SH-P: shower room to swimming hall, P-LA: pool ladder, P-PP: pathway, FB: flexibeam, EXT: second pool pathway, CT-MS: mop/scrubber, CT-W: wiper.

POOL FACILITY A

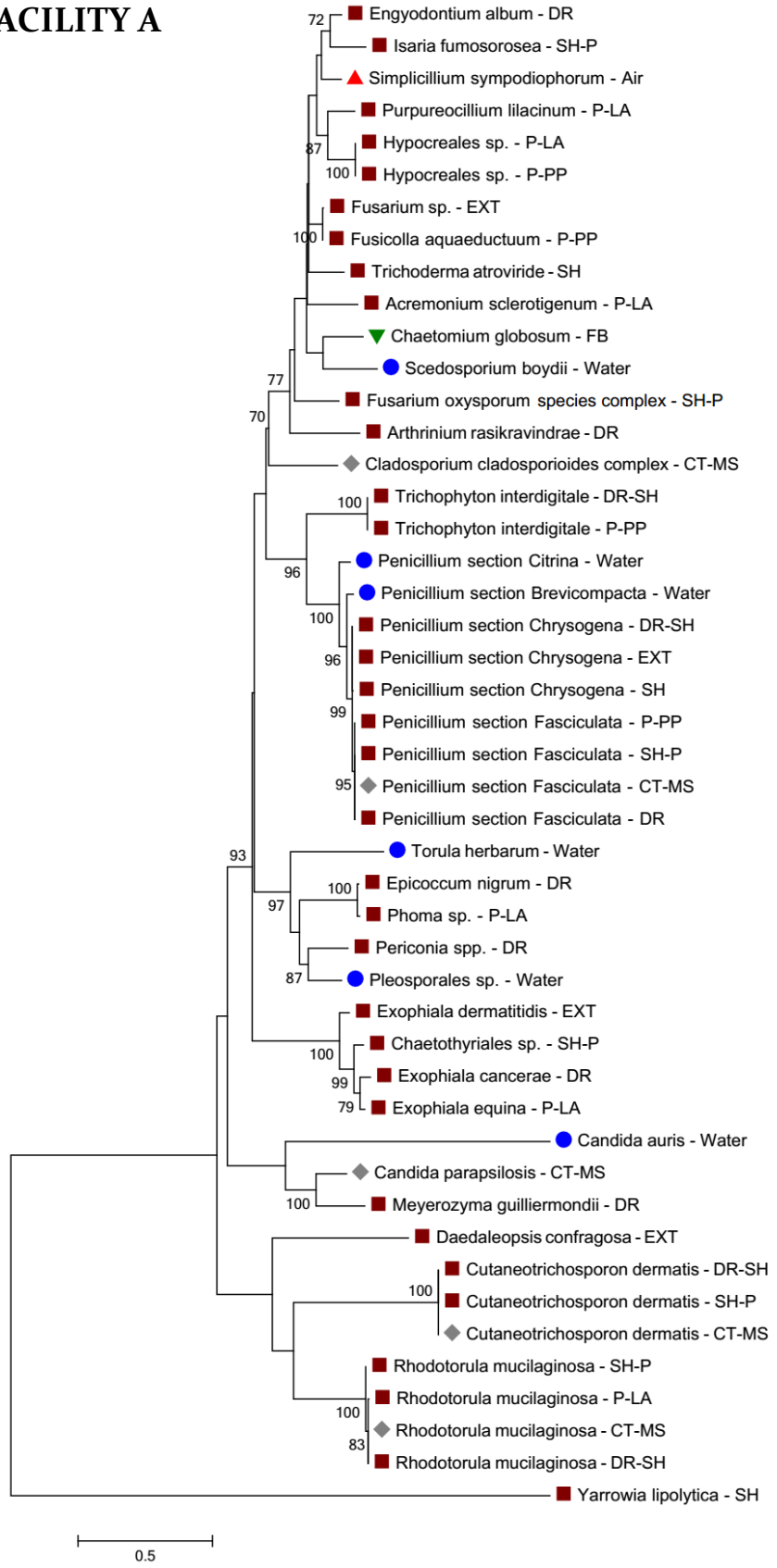

Figure S1. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility A. (■ : floor, ▲ : air, ● : water, ▼ : teaching aid, ◆ : cleaning tool)

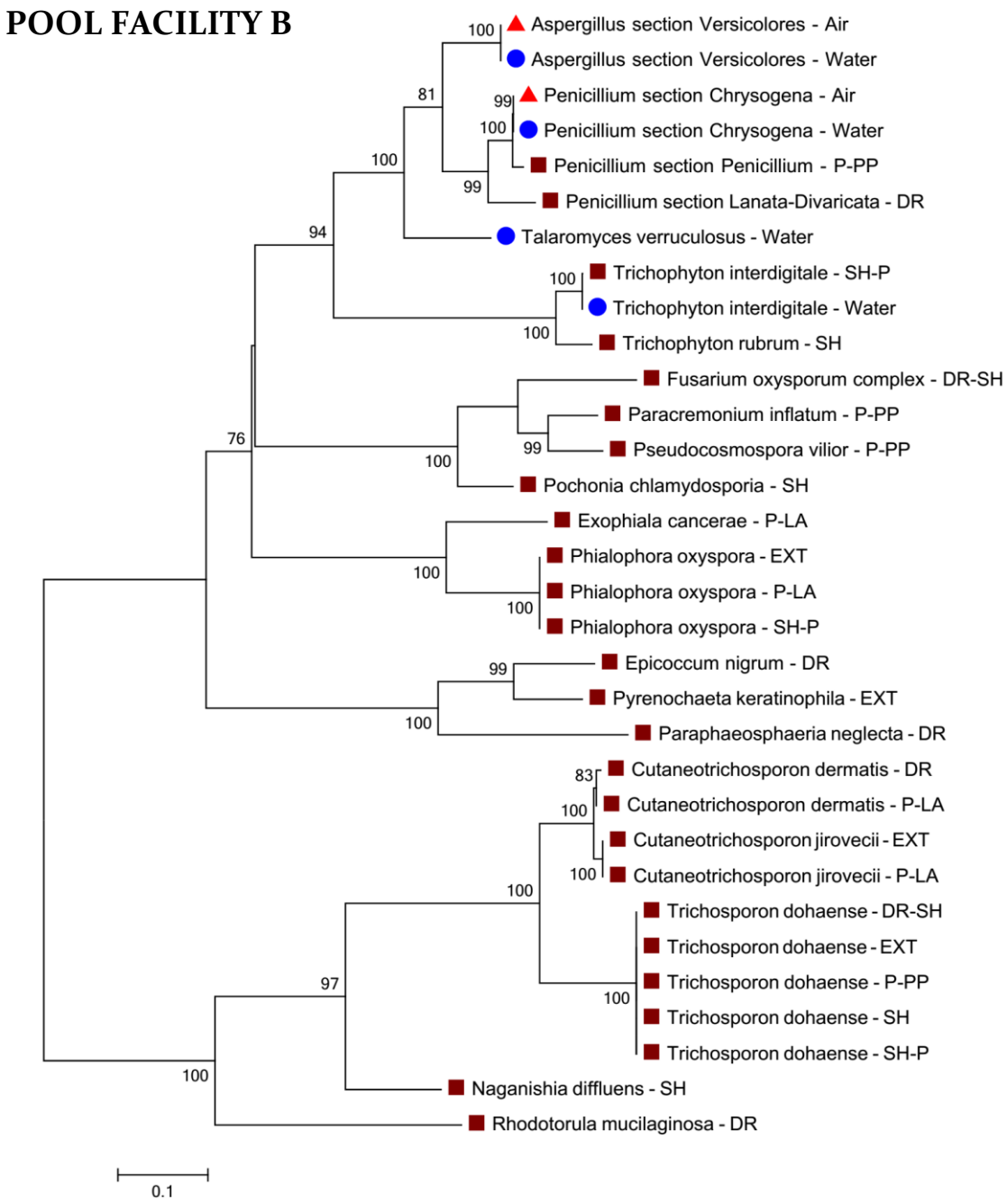

Figure S2. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility B. (■ : floor, ▲ : air, ● : water)

POOL FACILITY C

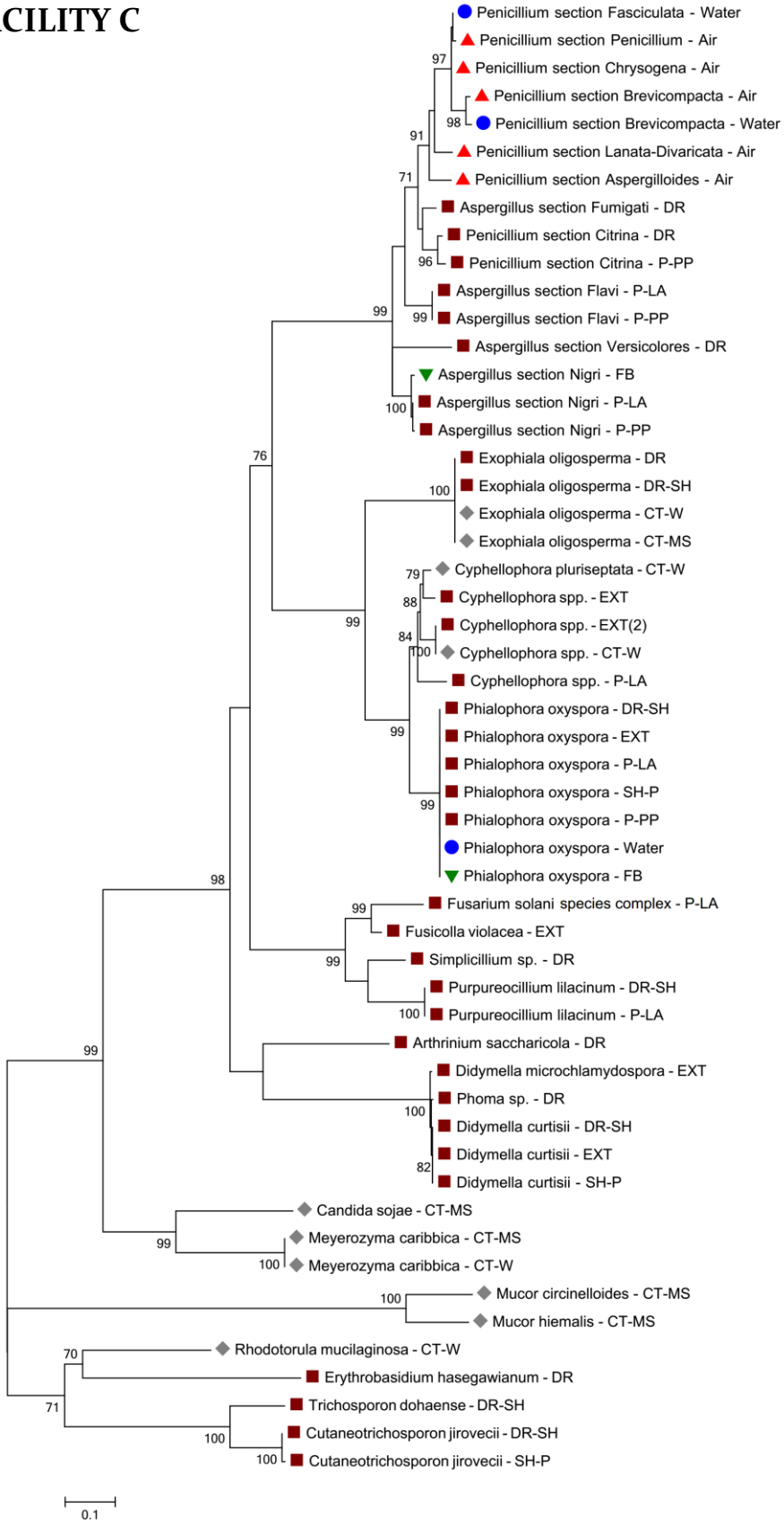

Figure S3. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility C. (■ : floor, ▲ : air, ● : water, ▼ : teaching aid, ◆ : cleaning tool)

POOL FACILITY D

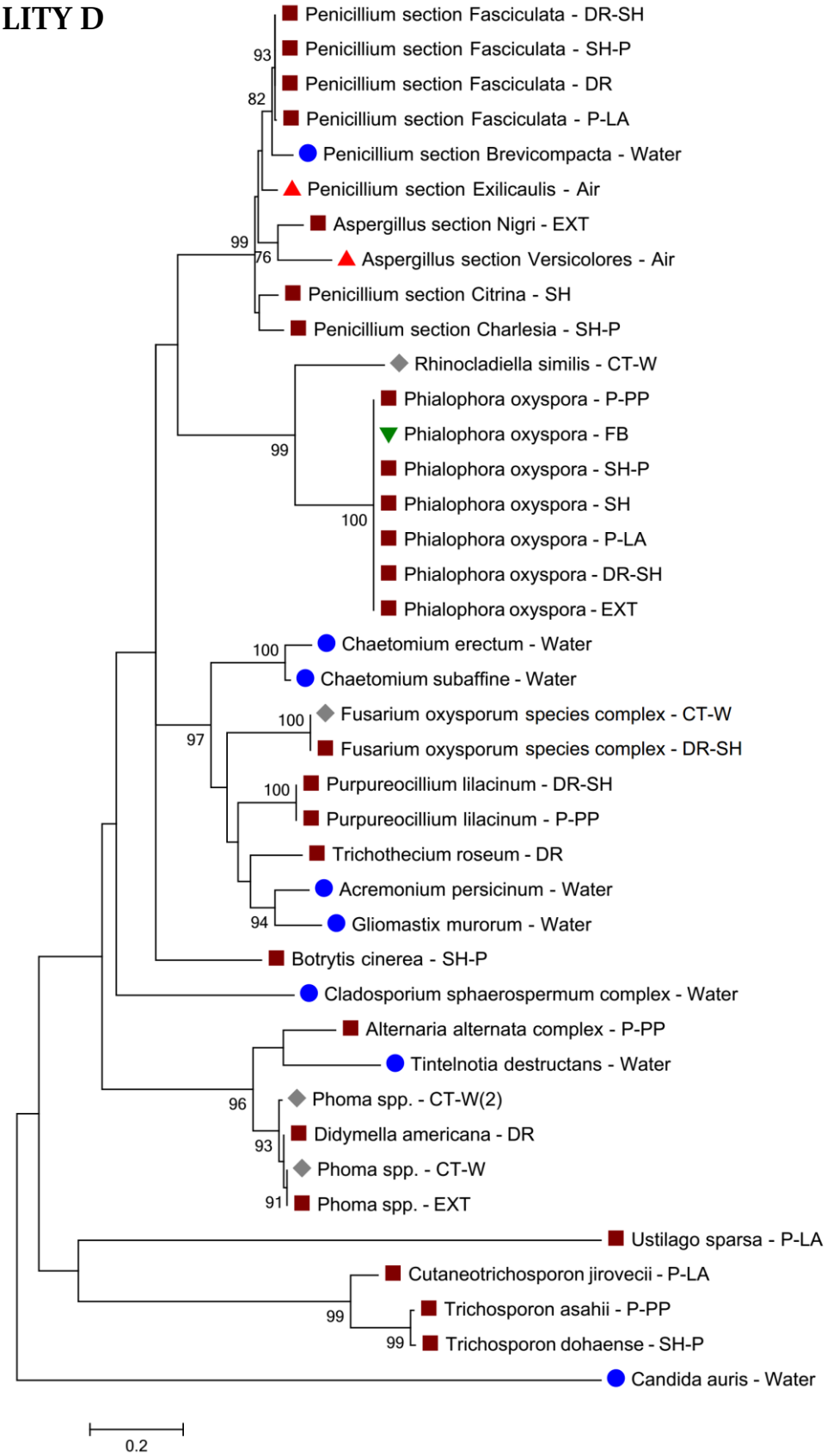

Figure S4. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility D. (■ : floor, ▲ : air, ● : water, ▼ : teaching aid, ◆ : cleaning tool)

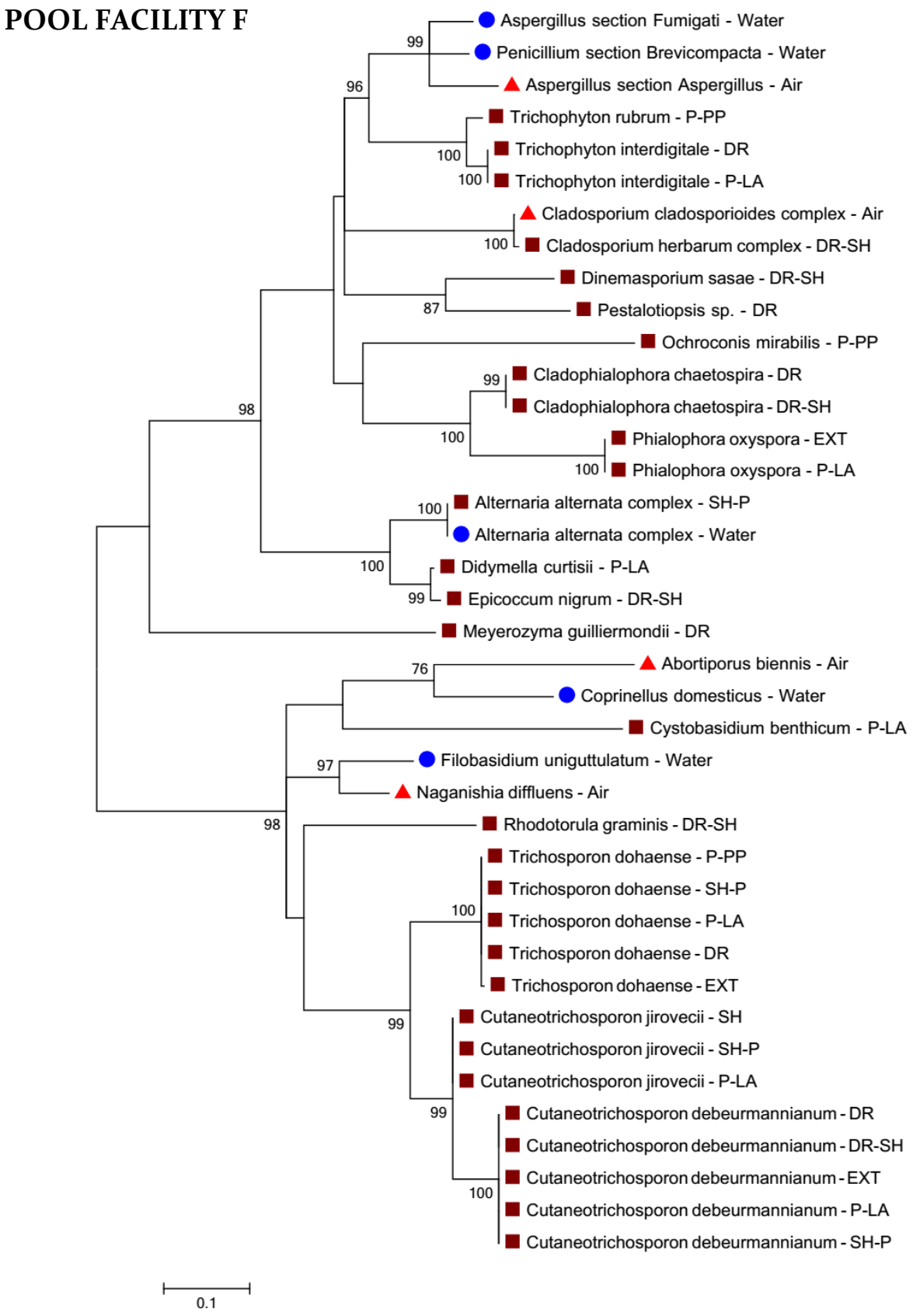

Figure S5. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility F. (■ : floor, ▲ : air, ● : water)

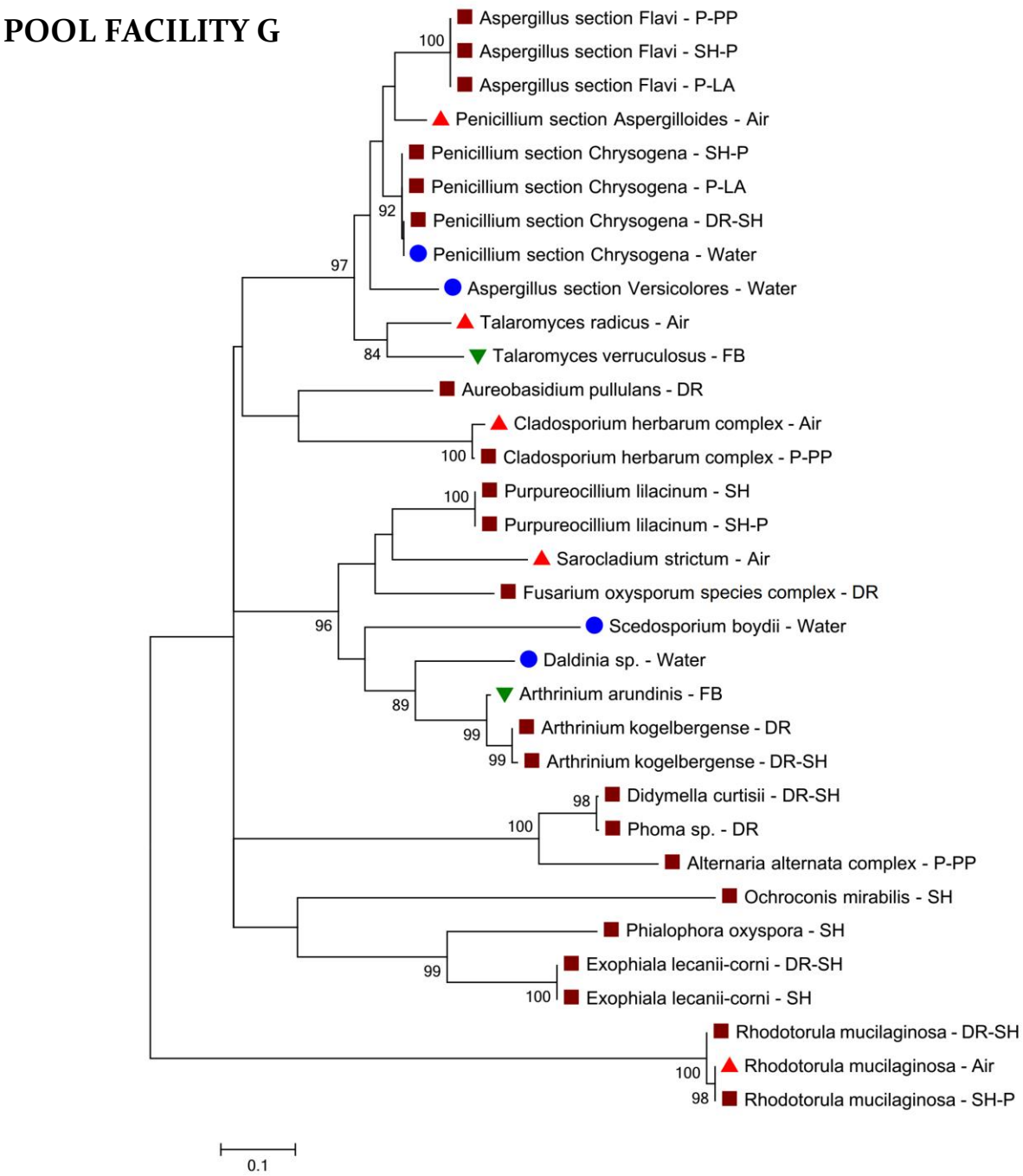

Figure S6. Maximum likelihood tree based on ITS sequences of fungal isolates from pool facility G. (■ : floor, ▲ : air, ● : water, ▼ : teaching aid)

6

General discussion

This thesis describes the occurrence of microbial and chemical contaminants in swimming pool environments and the assessment of alternative disinfection technologies that may reduce reliance on chlorine and eventually reduce the formation of chlorinated disinfection byproducts (DBPs) in swimming pools. This chapter includes a summary of the obtained results and discussion on contaminants in swimming pool environments (§ 1), discussion on human exposure and health risks (§ 2), preventive measures (§ 3), and the application of an alternative disinfection technology in pool water treatment (§ 4). Finally, possible guidance for contaminants in swimming pools are discussed (§ 5) and an overall conclusion is presented (§ 6).

Contaminants in swimming pools

Microbial contaminants in swimming pools can originate from faecal (e.g. faecal releases by bathers or animal contamination) and non-faecal (e.g. vomit, mucus, saliva and skin) matter (WHO, 2006). Chemical contaminants in swimming pool water may also be introduced by the bathers (e.g. urine, sweat, lotion etc.), through the pool water treatment (e.g. disinfectants) or may be already present in the source water. These sources of contaminants may carry pathogenic organisms, such as the protozoan parasites *Cryptoporidium* and *Giardia*, viruses such as adenoviruses, bacteria such as *Legionella* and *Pseudomonas aeruginosa*, and fungi such as *Trichophyton* and *Epidermophyton*, and chemicals such as precursors of disinfection byproducts, pharmaceuticals and personal care products, that can be harmful to human health. Pool visitors can be exposed in a number of ways to microbial and chemical contaminants in water, on surfaces and in air.

Fungi

Fungi are ubiquitous in the environment, including indoor environments, thus it is not surprising to find them in indoor swimming pool facilities. The WHO Guidelines for safe recreational water (WHO, 2006) mentioned fungi, especially the dermatophytes group, as potential microbial hazards in swimming pools. However, other pathogenic fungal species may have been overlooked or considered not relevant.

Some new and unexpected fungal species were detected (**Chapter 5**). New species of fungi belonging to the genus *Cyphellophora* were isolated and are being identified further (not included in this study). *Cyphellophora* is a genus of black yeast-like fungi in which some of the species are frequently isolated from clinical samples (Feng et al., 2014). Three isolates identified as *Candida auris* were isolated from pool water samples collected from two different facilities (**Chapter 5**). *Trichosporon dohaense* was frequently isolated from surface samples. The ecological niches of these species are not known yet. So far, both *Trichosporon dohaense* and *Candida auris* have only been isolated from clinical samples.

Disinfection byproducts

Chlorine-based disinfectants are commonly used in swimming pools to inactivate pathogens. However, chlorine-based disinfectants also generate harmful DBPs such as THMs and HAAs through the reaction with organic and inorganic matter. The formation of THMs and HAAs was used to study disinfection technologies by comparing the DBPs formed in chlorinated pool water, with and without an additional alternative disinfection (**Chapter 2**). Other researchers have shown the formation of DBPs other than THMs and HAAs, such as bromate, which is typically formed in bromide containing water treated with ozone, and nitrogenous DBPs (N-DBPs) which are formed by reaction of organic nitrogen and disinfectants. N-DBPs detected in swimming pools include halonitromethanes (HNMs), haloacetonitriles (HANs), and N-nitrosamines. Some N-DBPs may be more genotoxic, cytotoxic and carcinogenic than carbonaceous DBPs such as THMs and HAAs (Muellner et al., 2007; Plewa et al., 2009).

Pharmaceuticals and Personal Care Products (PPCPs)

PPCPs are ubiquitous in the aquatic environment and mainly originate from wastewater treatment plants (Focazio et al., 2008). Although most of these compounds can be effectively removed by water treatment processes, such as ozone, some of them were still detected in trace concentrations in drinking water (Benotti et al., 2008; de Jongh et al., 2012; Huerta-Fontela et al., 2011; Loraine and Pettigrove, 2006; Ternes et al., 2002; Yang et al., 2017). PPCPs are mostly present in swimming pool water due to the continuous bather load. Body substances from the bathers, such as urine and sweat, and personal care products used by bathers, such as lotions and sunscreens, are the source of PPCPs. Additionally, the filling water which commonly is local tap water, already contains some PPCPs that remain in the swimming pool water (Suppes et al., 2017).

Chapter 3 shows that at least one pharmaceutical of a set of ten pharmaceuticals (atenolol, carbamazepine, hydrochlorothiazide, metronidazole, ofloxacin, sulfamethoxazole, acetaminophen, ibuprofen, ketoprofen and phenazone) investigated in this study, was present in 96% of the water samples. As for UV filters, all water samples contained at least one out of the 14 UV filters measured. The highest concentration of PPCPs was detected in spas. Spa pools are smaller and have a higher relative number of users, compared to other types of pools, which likely resulted in the higher concentration of PPCPs.

Human exposure to contaminants and health risks

There are at least three possible routes of exposure to microbial and chemical contaminants in the swimming pool environment: inhalation, skin contact and ingestion. *Cryptosporidium*, *Legionella*, and *Pseudomonas* are pathogens that are known

as the cause of outbreaks in treated recreational water (Hlavsa et al., 2018). *Cryptosporidium* is transmitted by ingestion of contaminated water, *Legionella* is transmitted by inhalation of the aerosolized water droplets containing this bacterium, and *Pseudomonas* is transmitted by skin contact with contaminated water. Exposure to fungi in the swimming pool environment occurs through direct contact to skin fragments infected with fungi on surfaces and in water, or skin-to-skin contact with an infected person, inhalation of fungal spores, or ingestion of fungal-contaminated pool water. As for exposure to DBPs, previous studies have shown that besides through water ingestion, exposure also occurs through dermal absorption and inhalation (Fantuzzi et al., 2001; Gordon et al., 1998; Villanueva et al., 2007; Xu et al., 2002; Xu and Weisel, 2004).

Some fungi detected in swimming pool environments have been involved in many cases of human mycoses (e.g. *Aspergillus* spp., *Fusarium* spp, *Candida* spp.), ranging from superficial infections to deep infections. Dermatophytes infections are most likely related to exposure by direct contact with contaminated surfaces (Detandt and Nolard, 1988; English and Gibson, 1959; Hilmarsdottir et al., 2005). Inhalation is known to be the main portal of entry of fungal spores, however, a well maintained building with a good ventilation system should be able to limit fungal contamination in indoor air (Eduard, 2009). While the risk of fungal skin infections from pool water exposure is low, since fungi in pool water are unlikely to attach firmly enough to the human skin to cause infections (English and Gibson, 1959), swimmers are more likely at higher risk of contracting otomycosis or fungal ear infections (Vennewald and Klemm, 2010).

Pool visitors are most likely responsible for the dispersal of fungi inside swimming pool facilities (**Chapter 5**). Higher fungal concentrations were detected on floors at the entrance of shower rooms from the dressing rooms, and floors from shower rooms leading to swimming halls. These sites could be the ones which highly contribute to the spread of fungi inside a swimming pool facility. The identification of fungi detected at different sites, on different objects, and in different environmental compartments, such as floors, air, water, teaching aids and cleaning tools, demonstrated the presence of some overlapping fungal species (e.g. the same species were detected on floors and on cleaning tools). This suggests the possibility of interaction between sites, objects and environments leading to (re)contamination.

Fungal infections may not be an imminent danger to otherwise healthy people, however, the increasing number of immunocompromised people and the occurrence of environmental azole-resistant fungi may lead to an increasing risk of fungal infections. Previous studies have shown the relation between the use of azole and the development of medical azole-resistant *Aspergillus fumigatus*, *Candida glabrata*, and *Candida parasilopsis* complex (Brilhante et al., 2019; Faria-Ramos et al., 2014; Snelders et al., 2012) which may lead to the presence of medical azole-resistant fungi in the

environment (Snelders et al., 2009). Azole is used in many products related to plant protection, and in coatings, paints, and wall paper pastes for material preservation.

Aspergillus fumigatus, a common fungal species in the environment, was found in the studied swimming pool facilities (**Chapter 4**). This is also the fungal species implicated in the highest number of publications on human infections. Emerging pathogenic fungi, such as *Candida auris*, have become a concern due to their multi-antifungal resistance (ECDC, 2018; Larkin et al., 2017). *Candida auris* is known to be associated with invasive infections and high mortality rates in health-care settings (Chowdhary et al., 2016) and this species were detected at our study sites (**Chapter 5**).

Many health concerns (e.g. carcinogenicity, asthma, irritation to skin, throat, and eyes) arose regarding DBPs (Jacobs et al., 2007; Richardson et al., 2007; Villanueva et al., 2007). For instance, long term exposure to THMs may double the risk of bladder cancer (Villanueva et al., 2007). Some studies assessed the cancer risk from exposure to THMs in swimming pools (Chen et al., 2011; Lee et al., 2009; Panyakapo et al., 2008) and demonstrated that the estimated lifetime cancer risks were generally higher than 10^{-6}, the regulatory limit defined by U.S. EPA (2006a). This indicates that the health risk posed by DBPs in swimming pools should not be neglected.

Exposure to PPCPs in the aquatic environment has been associated with adverse health effects to aquatic organisms (Brausch et al., 2012; Brausch and Rand, 2011; Dhillon et al., 2015; Sharma et al., 2019). Although the presence of PPCPs in drinking water has been demonstrated in many studies (Benotti et al., 2008; Huerta-Fontela et al., 2011; Stackelberg et al., 2004), health risk assessments from the UK, the USA and Australia, and other research studies, indicated that no adverse health effects are to be expected from exposure to pharmaceuticals in drinking water (de Jesus Gaffney et al., 2015; de Jongh et al., 2012; Houtman et al., 2014; Schriks et al., 2010; Touraud et al., 2011; WHO, 2011b). A recent publication by Fantuzzi et al. (2018) stated that the conducted health risk assessment of ingestion of pharmaceuticals in pool water showed negligible risks for swimmers. Similarly, the health risk posed by exposure to substances in personal care products such as triclosan and DEET in water is insignificant to humans (Blanset et al., 2007; Lu et al., 2017; Merel and Snyder, 2016; Yost et al., 2017). The concentration of PPCPs in pool water may be higher than in drinking water due to the applied water treatment, water recirculation and continuous addition of PPCPs. However, swimming activities result in less frequent exposure and water ingestion compared to the drinking of tap water. The presence of PPCPs in treated water, however, does warrant the necessity of further studies on the effects of long term exposure to low levels of PPCPs. Synergistic effects of mixtures of compounds should also be studied, not only focusing on the parent compounds, but also on their transformation products (Boxall et al., 2012; Ebele et al., 2017; Fantuzzi et al., 2018).

Preventive measures

Every treatment technology has its limitations and thus it is unlikely to find a disinfectant which is able to solve the problems of chlorine resistant microorganisms, the formation of DBPs and the elimination of PPCPs all together. However, besides improving water treatment systems for swimming pools, preventive measures that can limit the contaminants that enter the swimming pool water may also largely contribute to the improvement of swimming pool water quality, such as pre-swim showers, wearing sandals and cleaning of surfaces.

Hygiene-related behaviour

Hygiene-related behaviour in swimming pools is important to reduce health risks from microbial and chemical contaminants (Pasquarella et al., 2014). So far, hygiene-related behaviour in swimming pools has not been widely discussed. In the last decade, several studies, mostly from Italy, investigated hygiene-related behaviour of pool users by conducting surveys among swimmers in order to collect data (Amodio et al., 2014; Bonini et al., 2011; Gallè et al., 2016; Liguori et al., 2007; Pasquarella et al., 2013; Pasquarella et al., 2014; Wiant, 2012). These studies revealed that some pool users were urinating while bathing, not showering before entering the pool, not wearing footwear, and not using a footbath and were even swimming when having skin wounds, infectious skin diseases and diarrhoea.

Chapters 4 and 5 show that the fungal concentrations in the swimming halls of the studied swimming pools were comparable to the concentrations on floors before the shower rooms, hence wearing sandals in swimming pools could be recommended to avoid direct contact with fungi on floors. Keuten et al. (2012) demonstrated that initial anthropogenic pollutants release, which consists of sweat, microorganisms and cosmetics, can be reduced by pre-swim showering. **Chapter 3** shows that the PPCPs present in the studied pool water were likely introduced by the bathers, and thus can be reduced by pre-swim showering. The reduction of anthropogenic pollutants (e.g. sweat and cosmetics) may lead to reduction of DBP formation since these pollutants can act as DBP precursors (Li and Blatchley, 2007; Wang et al., 2013). Keuten et al. (2012) also showed that the amount of anthropogenic pollutants washed from the body of participants who took a shower after swimming was comparable to the amount of anthropogenic pollutants washed of the ones who took a shower before swimming. It indicates that showering after swimming is of equal importance to pre-swim showering. Short messages and posters in swimming pool facilities can be implemented to draw attention to and actively encourage pre-swim showering among the swimmers (Ribbers et al., 2017; Stronks and Keuten, 2016). Additionally, knowledge of rules or regulations and of infections and/or diseases transmitted in swimming pools could promote the practice of hygienic behaviour.

Cleanliness of surfaces in swimming pool facilities

The outdoor environment is an important factor that can influence the number and the diversity of indoor fungi. In fact, the occurrence of fungi in indoor environments is mainly dominated by the outdoor environment, rather than by indoor growth (Nevalainen et al., 2015). However, in swimming pools, growth may play a bigger role because of the elevated relative humidity and temperature which could support fungal growth. Adan et al. (2011) mentioned that fungal growth on surfaces can be avoided by limiting the relative humidity to below 80%, although a study by Johansson et al. (2012) showed that common fungal species (*Cladosporium*, *Penicillium*, and *Aspergillus*) can grow on building materials at a relative humidity lower than 80%. Nevertheless, ventilation and cleaning are important in limiting growth of fungi in the indoor environment.

Surfaces in swimming pool facilities can be an important transmission pathway for fungi and may also be a transmission pathway for other pathogenic microorganisms (Flores et al., 2011; Kramer et al., 2006; Reynolds et al., 2005). The research described in **Chapters 4 and 5** showed that despite of regular cleaning in swimming pool facilities, clinically relevant fungi were present on the surfaces in the investigated swimming pool facilities, especially on floors. The disinfectants used to clean the floors in swimming pool facilities may have different effects on fungal spores. A study by Bobichon et al. (1993) on the efficiency of disinfectants commonly used to clean swimming pool floors concluded that the use of several disinfectants of different composition would limit fungal contamination. However, performing surface cleaning alone is ineffective since there is continuous re-contamination by the pool visitors and from the outdoor environment. In the studies presented in **Chapters 4 and 5**, no obvious relation between cleaning regimes and fungal concentrations in each of the studied swimming pool facilities was observed. However, the information collected on cleaning regimes was not complete, especially the details on the type of cleaning solution and cleaning tools were lacking. Moreover, samples were collected once per day in each facility and the sampling was done at a different time of the day in the different facilities. For these reasons, this sampling approach may not have been representative of the general conditions in the pool facilities. Future studies shall investigate the effectiveness of different types of cleaning tools and cleaning solutions on surface cleaning. However, since surface contamination occurs continuously, it is necessary to investigate the effect of cleaning regimes on fungal concentrations on surfaces, especially floors, by performing a sampling campaign during a longer period. Nevertheless, maintaining cleanliness of the surfaces, adequate disinfection and regular checks at the sites where fungi or other pathogenic microorganisms can grow or accumulate in swimming pool facilities (e.g. teaching aids, tiles inside the pool, air ventilation system) can contribute to the prevention of dispersal of fungi and other pathogens.

The effects of disinfection in microbial inactivation, DBP formation and removal of PPCPs

Alternative water disinfection for swimming pool applications

Chlorine is commonly used as swimming pool water disinfectant. However, there are some challenges that need to be faced when using chlorine: chlorine-resistant pathogens and DBP formation. In practice, it is difficult to avoid using chlorine in swimming pools since chlorine provides residual disinfection needed to protect bathers against microbial contamination during swimming. One of the objectives of this research was to apply an alternative disinfection technology which is effective against microbial contamination, and thus can reduce the amount of chlorine needed to a level that provides an adequate residual disinfection capacity in combination with a lower level of DBP formation.

The study described in **Chapter 2** assessed the full scale application of a novel commercial disinfection technology that combines ozone and UV (UVOX Redox®). The results show that UVOX is effective in inactivating chlorine resistant *Bacillus subtilis* spores, which were used as a surrogate for chlorine resistant pathogens such as *Cryptosporidium*. The use of low concentrations of ozone enabled a two-fold increase in the inactivation of *B. subtilis* spores compared to when only UV was applied. Since the UV dose depends on UV transmission and flow rate of the water, those two factors have to be well-controlled so that UV can deliver an adequate dose. The microbial inactivation experiments with UVOX were only performed under conditions without chlorine in the water. Laboratory scale experiments (not described in the chapters) on the other hand demonstrated that a relatively small synergistic effect (16%) resulted from a combination of ozone and UV followed by chlorination. To be noted, the ozone concentrations used in the laboratory experiments were higher and the contact time was longer than in the full scale UVOX study, also, a saturated ozone solution was used. Cho et al. (2006) observed no synergism in sequential use of UV and chlorine, and although they showed that there was synergistic inactivation of *B. subtilis* spores in sequential use of ozone and chlorine, they applied a higher initial ozone concentration (2 mg/L) than in the study described in **Chapter 2**. Based on the results of Cho et al. (2006), it is likely that the contributing factor to synergism with chlorine in inactivation of *B. subtilis* spores is the ozone concentration.

Disinfection of fungi in swimming pool water

The wide presence of clinically relevant fungi in swimming pool environments (**Chapters 4 and 5**) may raise some concerns. Although fungal infections through skin attachment or ingestion of fungi in swimming pool water have not been demonstrated yet, and disinfection aimed at inactivation of fungi in pool water is not mandatory or regulated, it is valuable to understand the susceptibility of fungi to common

disinfectants in swimming pools. The susceptibility of clinically relevant fungal species to disinfection has not been explored as much as for protozoa, bacteria and viruses, but data do exist. Fungal species such as *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp. appeared to be more resistant to chlorine-based disinfectants such as sodium hypochlorite, chlorine dioxide, and monochloramine, than *Escherichia coli* (Ma and Bibby, 2017; Pereira et al., 2013; Rosenzweig et al., 1983; Wen et al., 2017a) and have a comparable resistance to free chlorine as *Legionella pneumophila*, *Mycobacterium* spp. and *Giardia* cysts (Ma and Bibby, 2017; Pereira et al., 2013). The application of alternative disinfectants such as UV irradiation and ozone is promising. A 4 log reduction of *Aspergillus* spp. can be achieved by applying a UV dose of 20.75 mJ/cm² (Nourmoradi et al., 2012). However, different fungal species require a different UV dose. The fungal species in the dermatophyte group, *Epidermophyton floccosum*, *Trichophyton violaceum*, *Trichophyton schoenleinii*, and *Trichophyton mentagrophytes* are more susceptible to UV than other dermatophytes such as *Trichophyton tonsurans* and *Trichophyton rubrum* (Sisti et al., 2014). A UV dose of 48 mJ/cm² was required to achieve 1.71 and 2.4 log removal of *Trichophyton tonsurans* and *Trichophyton rubrum*, respectively. Higher UV resistance of fungal spores was observed in a study by Wen et al. (2017b) which used single strains of *Trichoderma* sp., *Acremonium* sp., *Penicillium* sp., and *Cladosporium* sp., in their inactivation experiments (2 log removal at a UV dose of 45, 50, 65, 130 mJ/cm², respectively). For comparison, 2 log removal of *Cryptosporidium* oocysts and *Giardia* cysts can be achieved at a UV dose of 5.8 mJ/cm² and 5.2 mJ/cm² (U.S. EPA, 2006b), respectively.

The use of UVOX for fungal inactivation in swimming pool water treatment has not been examined yet. However, from the results described in **Chapter 2**, it is clear that the UVOX system has an inactivation capacity equivalent to an actual UV dose higher than 30 mJ/cm² at the maximum water flow rate in the studied pool, which suggests that UVOX could be able to at least inactivate some of the fungi that were detected in the investigated swimming pools.

Removal of PPCPs by pool water treatment

Some pharmaceuticals and UV filters appeared to be present in different types of swimming pools in Spain (**Chapter 3**). Of these different types of pools (outdoor, indoor and spa), the occurrence of PPCPs was highest in spas. When comparing different treatments applied in the studied pools, the occurrence of UV filters was lowest in pools applying treatment stages of coagulation, sand filters, followed by UV irradiation and salt electrolysis, while pools applying sand filters followed by sodium hypochlorite showed the lowest occurrence of pharmaceuticals. However, it is not possible to draw the conclusion that these pool water treatment processes are more effective than others, since other possibly contributing factors, such as pool size and number of visitors, were not considered. Future studies should therefore include a longer sampling duration

period during the peak season and include swimming pools with similar characteristics, such as venue settings, pool size, and number of visitors.

Some of the pharmaceuticals observed frequently in the study described in **Chapter 3**, such as acetaminophen, ibuprofen, atenolol, and carbamazepine, and some endocrine disruptors which can be commonly found in the environment, such as caffeine, estrone, and estradiol, were selected to investigate the effect of UVOX on the removal of micropollutants (**Chapter 2**). More than 97% removal of micropollutants was observed after 24 h under sand filtration and chlorination, except for ibuprofen, of which 40% remained present. The addition of UVOX to the swimming pool water treatment had little or no effect on the removal of most of the selected micropollutants, but it enabled the complete removal of ibuprofen. Estrone, estradiol, and acetaminophen were partially removed (>60%) by UVOX without chlorination after 24 h, however, the removal of these compounds was dominated by chlorine whenever it was present in the water. Based on the reaction rate constants of the micropollutants in chlorinated water, the reaction rate of some compounds was observed to be up to 20% lower after UVOX inclusion. The most noticeable change was observed for carbamazepine, with an 80% lower reaction rate when UVOX was applied. The explanation for these reduced reaction rates may be reaction competition between compounds and oxidants or chlorine photolysis by UV irradiation, but this hypothesis has to be proven in further research.

The formation of DBPs in chlorinated swimming pools

The formation of DBPs by applying UVOX as an additional disinfection technology was investigated (**Chapter 2**). Lower formation of THMs and HAAs was observed when UVOX was applied, however, the presence of bromide in the pool source water resulted in higher formation of brominated DBPs. Although bromate formation did not occur, the application of UVOX shifted the formation of DBPs towards more brominated DBPs. Thus, it is important to take the composition of the source water into consideration when designing swimming pool water treatment. In the presence of bromide, it may be better to select UV treatment without ozone to avoid brominated DBPs and/or bromate formation.

Full scale testing of UVOX (**Chapter 2**) was crucial for understanding the performance of this technology under specific hydraulic conditions and coupled with other treatment steps like sand filtration. However, there may be some limitations, such as a low flexibility of the system to vary the water flow, or risks for public health associated with spiking challenge microorganisms, or external factors such as the ambient temperature. It is complex to carry out fundamental scientific experiments that could help elucidate the mechanisms that take place during the treatment processes, such as formation of hydroxyl radicals, competition between different compounds, and formation and/or removal of substances. These limitations can affect the results in a full scale setting and

need to be considered when explaining the results. A bench scale laboratory set-up or a smaller swimming pool set-up, where parameters can be controlled, is recommended to investigate the reaction mechanisms in more detail.

Guidelines for microbial and chemical contaminants in swimming pools

The existing guidelines and regulations for swimming pools include Legionella spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus*, *Escherichia coli* and thermotolerant coliforms for assessment of microbial quality of swimming pool water (Bhvbz, 2011; BHygV, 2012; Real Decreto, 2013; WHO, 2006). As for DBPs, only THM (as chloroform) have been regulated in European countries (ANSES, 2012; DIN, 2012; Simard et al., 2013).

Exposure to fungi can cause allergic, toxic and irritant symptoms and diseases, thus having allowable safe levels of fungal contamination on surfaces, in water and air in swimming pools should get more attention. Despite the fact that fungi can cause adverse health effects, the relationship between fungal concentrations and human disease has not been established. The most common standards and/or guidelines for fungal contamination are intended for indoor air, especially for residential and office buildings. The summaries by Rao et al. (1996) and updated by Gots et al. (2003), showed a wide range of allowable limits of fungal concentrations in indoor air. The standards, guidelines and research studies mentioned in those publications applied different sampling strategies, culturing methods and identification approaches (Baxi et al., 2016; Verhoeff and Burge, 1997) which does not support proper comparison of fungal concentrations to derive guideline values for swimming pools. Health risk assessments are also impractical to establish guideline values because of the lack of definitive measures for the quantitative assessment of exposure (WHO, 2009). The WHO Guidelines for safe recreational water vol. 2 have acknowledged some fungal species, DBPs and PPCPs as contaminants of relevance in swimming pools, but quantitative limits of these contaminants are still lacking (WHO, 2006). This WHO guideline also mentioned the spread of pathogenic fungi through surfaces in swimming pools and recommended thorough frequent cleaning and disinfection, but did not suggest periodic assessment or monitoring.

The guidelines for DBPs in swimming pool water are not as comprehensive as the guidelines for drinking water. The WHO Guideline for drinking water quality (2011a) established guideline values for 14 DBPs and acknowledged another 13 DBPs with no guideline values, but with an indication of health significance. For DBPs in swimming pool water, the WHO Guideline for safe recreational water environments vol. 2 (2006) suggested to use the values from the WHO Guideline for drinking water for assessment purposes, while making appropriate assumptions considering less volume of water

ingested, shorter exposure periods and non-ingested exposure. A provisional guideline value for trichloramine, which is known to cause eye and respiratory irritation, was added in the WHO Guideline for safe recreational water vol. 2. The upcoming new legislation for swimming pool water quality in the Netherlands (Anonymous, 2018) will include some guideline values for the DBPs THMs, bromate, chlorate and trichloramine.

So far, there is no guideline or legislation for PPCPs in swimming pools. While for drinking water, some of the human pharmaceuticals (e.g. estrone, 17α-ethinyl estradiol, diclofenac, erythromycin) have been included in the Contaminants Candidate List 4 (CCL 4) (U.S. EPA, 2016). The contaminants listed in CCL 4 are substances anticipated to be present in drinking water, but they are not subject to the EPA drinking water regulations. Moreover, some PCP substances have been restricted (e.g. 4-methylidene camphor (UV filters)) or even prohibited (e.g. benzylparaben (preservatives)) for use in cosmetics in the European Union (European Commission, 2009).

Although, for DBPs and PPCPs, quantitative limits from drinking water guidelines can be applied for indication, it would be advised to draft guideline values specific for swimming pools, since the dominant exposure pathway in drinking water and swimming pool water is different. In public settings like swimming pools, quantitative standards together with mandatory periodic sampling could support better preventive actions required by the swimming pool facilities to protect the pool visitors from microbial and chemical contamination.

General conclusion

Exposure of swimmers and employees to various microbial and chemical threats occurs in swimming pool environments, including exposure to the fungi *Trichosporon dohaense* and *Candida auris*, which were isolated from pool water for the first time. Swimming pools were also found to harbour potentially harmful DBPs and PPCPs. These contaminants have not been regulated yet and are not part of operational monitoring programmes. Preventive measures could help minimizing the risks. Moreover, UVOX is a promising technology to minimize microbial and chemical threats. UVOX is effective in inactivation of chlorine resistant *Bacillus subtilis* spores and its application potentially limits DBP formation. However, for effective UVOX treatment, the source water characteristics should be carefully considered, as the presence of bromide could impact the formation of brominated DBPs. This thesis also demonstrates that the combination of low chlorine dosage and UVOX is effective in removing all selected micropollutants. The removal of clinically relevant fungi by applying this treatment needs to be shown, but based on other studies and our studies with microbial indicators, this seems probable. Future swimming pool water guidance should include awareness raising among swimmers, pool operators and managers on hygiene-related behaviour, and opportunities for hygiene measures and alternative disinfection.

References

Adan, O.C.G., Huinink, H.P., Bekker, M. (2011). Water relations of fungi in indoor environments. In: Adan, O. C. & Samson, R. A. (Eds.), Fundamentals of mold growth in indoor environments and strategies for healthy living. Wageningen Academic Publishers, Wageningen, the Netherlands.

Amodio, E., Costantino, C., Asciutto, R., Dino, C., Bianco, A., Maringhini, G., Mammina, C., Calamusa, G. (2014). Knowledge, risk perception and behaviours in swimming pool users of Palermo city, Sicily. *European Journal of Sport Science*, 14(supl), S51–S56.

Aanvullingsbesluit zwemmen of baden in waterbassins Omgevingswet (2018). Ministry of the Interior and Kingdom Relations, Ministry of Infrastructure and Water Management, https://www.internetconsultatie.nl/aanvullingsbesluit_water bassins_omgevingswet.

ANSES (2012). Health Risk Assessment in Swimming Pools. Part 1: Regulated Pools.

Baxi, S.N., Portnoy, J.M., Larenas-Linnemann, D., Phipatanakul, W., Barnes, C., Baxi, S., Grimes, C., Horner, W.E., Kennedy, K., Larenas-Linnemann, D., Levetin, E., Miller, J.D., Phipatanakul, W., Portnoy, J.M., Scott, J., Williams, P.B., 2016. Exposure and health effects of fungi on humans. *The Journal of Allergy and Clinical Immunology: In Practice*, 4(3), 396–404.

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S.A. (2008). Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environmental Science & Technology*, 43(3), 597–603.

Besluit hygiëne en veiligheid badinrichtingen en zwemgelegenheden (2011). Ministerie van Infrastuctuur en Milieu, the Netherlands.

Verordnung des Bundesministers für Gesundheit über Hygiene in Bädern, Warmsprudelwannen (Whirlwannen), Saunaanlagen, Warmluft- und Dampfbädern und Kleinbadeteichen, 2012. Bundesministerium für Gesundheit, Austria.

Blanset, D.L., Zhang, J., Robson, M.G. (2007). Probabilistic estimates of lifetime daily doses from consumption of drinking water containing trace levels of N,N-diethyl-meta-toluamide (DEET), triclosan, or acetaminophen and the associated risk to human health. *Human and Ecological Risk Assessment: An International Journal*, 13(3), 615–631.

Bobichon, H., Dufour-Morfaux, F., Pitort, V. (1993). In vitro susceptibility of public indoor swimming pool fungi to three disinfectants. *Mycoses*, 36(9-10), 305–311.

Bonini, M., Bodina, A., Bonali, D., Bascucci, B., Pellino, P., Castaldi, S. (2011). Investigation and comparison of behaviours of adults and children in swimming pools. *Annali di igiene: medicina preventiva e di comunita*, 23(4), 319–328.

Boxall, A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T. (2012). Pharmaceuticals and personal care

products in the environment: What are the big questions? *Environmental Health Perspectives*, 120(9), 1221–1229.

Brausch, J.M., Connors, K.A., Brooks, B.W., Rand, G.M. (2012). Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. in: Whitacre, D. M. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 218. Springer US, Boston, MA, pp. 1–99.

Brausch, J.M., Rand, G.M. (2011). A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *Chemosphere*, 82(11), 1518–1532.

Brilhante, R.S.N., de Alencar, L.P., Bandeira, S.P., Sales, J.A., de Jesus Evangelista, A.J., Serpa, R., de Aguiar Cordeiro, R., de Aquino Pereira-Neto, W., Sidrim, J. J. C., de Souza Collares Maia Castelo-Branco, D., Rocha, M.F.G. (2019). Exposure of *Candida parapsilosis* complex to agricultural azoles: An overview of the role of environmental determinants for the development of resistance. *Science of the Total Environment*, 650, 1231–1238.

Chen, M.-J., Lin, C.-H., Duh, J.-M., Chou, W.-S., Hsu, H.-T. (2011). Development of a multi-pathway probabilistic health risk assessment model for swimmers exposed to chloroform in indoor swimming pools. *Journal of Hazardous Materials*, 185(2), 1037–1044.

Cho, M., Kim, J.-H., Yoon, J. (2006). Investigating synergism during sequential inactivation of *Bacillus subtilis* spores with several disinfectants. *Water Research*, 40(15), 2911–2920.

Chowdhary, A., Voss, A., Meis, J.F. (2016). Multidrug-resistant *Candida auris*: 'new kid on the block' in hospital-associated infections? *Journal of Hospital Infection*, 94(3), 209–212.

de Jesus Gaffney, V., Almeida, C. M. M., Rodrigues, A., Ferreira, E., Benoliel, M. J., Cardoso, V. V., 2015. Occurrence of pharmaceuticals in a water supply system and related human health risk assessment. *Water Research*, 72, 199–208.

de Jongh, C.M., Kooij, P.J.F., de Voogt, P., ter Laak, T.L. (2012). Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water. *Science of the Total Environment*, 427–428, 70–77.

Detandt, M., Nolard, N. (1988). Dermatophytes and swimming pools: seasonal fluctuations. *Mycoses*, 31(10), 495–500.

Dhillon, G., Kaur, S., Pulicharla, R., Brar, S., Cledón, M., Verma, M., Surampalli, R. (2015). Triclosan: Current status, occurrence, environmental risks and bioaccumulation potential. *International Journal of Environmental Research and Public Health*, 12(5), 5657.

DIN (2012). Treatment of the Water of Swimming Pools and Baths - Part 1: General Requirements, Germany Standard DIN 19643-1: 2012-11.

Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3(1), 1–16.

ECDC (2018). *Candida auris* in healthcare settings – Europe – first update, 23 April 2018. Stockholm, European Centre for Disease Prevention and Control.

Eduard, W. (2009). Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. *Critical Reviews in Toxicology*, 39(10), 799–864.

English, M.P., Gibson, M.D. (1959). Studies in the epidemiology of tinea pedis. II. Dermatophytes on the floors of swimming-baths. *British Medical Journal*, 1(5135), 1446–1448.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (Text with EEA relevance), 2009.

Fantuzzi, G., Aggazzotti, G., Righi, E., Predieri, G., Castiglioni, S., Riva, F., Zuccato, E. (2018). Illicit drugs and pharmaceuticals in swimming pool waters. *Science of the Total Environment*, 635, 956–963.

Fantuzzi, G., Righi, E., Predieri, G., Ceppelli, G., Gobba, F., Aggazzotti, G. (2001). Occupational exposure to trihalomethanes in indoor swimming pools. *Science of the Total Environment*, 264(3), 257–265.

Faria-Ramos, I., Tavares, P.R., Farinha, S., Neves-Maia, J., Miranda, I.M., Silva, R.M., Estevinho, L.M., Pina-Vaz, C., Rodrigues, A.G. (2014). Environmental azole fungicide, prochloraz, can induce cross-resistance to medical triazoles in *Candida glabrata*. *FEMS Yeast Research*, 14(7), 1119–1123.

Feng, P., Lu, Q., Najafzadeh, M.J., Gerrits van den Ende, A.H.G., Sun, J., Li, R., Xi, L., Vicente, V.A., Lai, W., Lu, C., de Hoog, G.S. (2014). *Cyphellophora* and its relatives in *Phialophora*: biodiversity and possible role in human infection. *Fungal Diversity*, 65(1), 17–45.

Flores, G.E., Bates, S.T., Knights, D., Lauber, C.L., Stombaugh, J., Knight, R., Fierer, N. (2011). Microbial biogeography of public restroom surfaces. PLoS One, 6(11), e28132.

Focazio, M.J., Kolpin, D.W., Barnes, K.K., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Barber, L.B., Thurman, M.E. (2008). A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States — II) Untreated drinking water sources. *Science of the Total Environment*, 402(2), 201–216.

Gallè, F., Dallolio, L., Marotta, M., Raggi, A., Di Onofrio, V., Liguori, G., Toni, F., Leoni, E. (2016). Health-related behaviors in swimming pool users: Influence of knowledge of regulations and awareness of health risks. *International Journal of Environmental Research and Public Health*, 13(5), 513.

Gordon, S.M., Wallace, L.A., Callahan, P.J., Kenny, D.V., Brinkman, M.C. (1998). Effect of water temperature on dermal exposure to chloroform. *Environmental Health Perspectives*, 106(6), 337–345.

Gots, R.E., Layton, N.J., Pirages, S.W. (2003). Indoor health: Background levels of fungi. *AIHA Journal*, 64(4), 427–438.

Hilmarsdottir, I., Haraldsson, H., Sigurdardottir, A., Sigurgeirsson, B. (2005). Dermatophytes in a swimming pool facility: difference in dermatophyte load in men's and women's dressing rooms. *Acta Dermato-Venereologica*, 1(1), 1–2.

Hlavsa, M.C., Cikesh, B.L., Roberts, V.A., Kahler, A.M., Vigar, M., Hilborn, E.D., Wade, T.J., Roellig, D.M., Murphy, J.L., Xiao, L., Yates, K.M., Kunz, J.M., Arduino, M.J., Reddy, S.C., Fullerton, K.E., Cooley, L.A., Beach, M.J., Hill, V.R., Yoder, J.S. (2018). Outbreaks associated with treated recreational water — United States, 2000–2014. *MMWR. Morbidity and mortality weekly report*, 67, 547–551.

Houtman, C.J., Kroesbergen, J., Lekkerkerker-Teunissen, K., van der Hoek, J.P. (2014). Human health risk assessment of the mixture of pharmaceuticals in Dutch drinking water and its sources based on frequent monitoring data. *Science of the Total Environment*, 496, 54–62.

Huerta-Fontela, M., Galceran, M. T., Ventura, F. (2011). Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. *Water Research*, 45(3), 1432–1442.

Jacobs, J., Spaan, S., Van Rooy, G., Meliefste, C., Zaat, V., Rooyackers, J., Heederik, D. (2007). Exposure to trichloramine and respiratory symptoms in indoor swimming pool workers. *European Respiratory Journal*, 29(4), 690–698.

Johansson, P., Ekstrand-Tobin, A., Svensson, T., Bok, G. (2012). Laboratory study to determine the critical moisture level for mould growth on building materials. *International Biodeterioration & Biodegradation*, 73, 23–32.

Keuten, M.G.A., Schets, F.M., Schijven, J.F., Verberk, J.Q.J.C., van Dijk, J.C. (2012). Definition and quantification of initial anthropogenic pollutant release in swimming pools. *Water Research*, 46(11), 3682–3692.

Kramer, A., Schwebke, I., Kampf, G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC infectious diseases*, 6(1), 130.

Larkin, E., Hager, C., Chandra, J., Mukherjee, P. K., Retuerto, M., Salem, I., Long, L., Isham, N., Kovanda, L., Borroto-Esoda, K. (2017). The emerging pathogen *Candida auris*: Growth phenotype, virulence factors, activity of antifungals, and effect of SCY-078, a novel glucan synthesis inhibitor, on growth morphology and biofilm formation. *Antimicrobial agents and chemotherapy*, 61(5), e02396–02316.

Lee, J., Ha, K.-T., Zoh, K.-D. (2009). Characteristics of trihalomethane (THM) production and associated health risk assessment in swimming pool waters treated with different disinfection methods. *Science of the Total Environment*, 407(6), 1990–1997.

Li, J., Blatchley, E.R. (2007). Volatile disinfection by-product formation resulting from chlorination of organic–nitrogen precursors in swimming pools. *Environmental Science & Technology*, 41(19), 6732–6739.

Liguori, G., Castaldi, S., Signorelli, C., Auxilia, F., Alfano, V., Saccani, E., Visciano, A., Fanti, M., Spinelli, A., Pasquarella, C. (2007). Hygienic risks in swimming pool: Knowledge and behaviours of consumers of three structures in Crema, Parma and Naples. *Annali di igiene: medicina preventiva e di comunita*, 19(4), 325–335.

Loraine, G.A., Pettigrove, M.E. (2006). Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. *Environmental Science & Technology*, 40(3), 687–695.

Lu, J., Mao, H., Li, H., Wang, Q., Yang, Z. (2017). Occurrence of and human exposure to parabens, benzophenones, benzotriazoles, triclosan and triclocarban in outdoor swimming pool water in Changsha, China. *Science of the Total Environment*, 605–606, 1064–1069.

Ma, X., Bibby, K. (2017). Free chlorine and monochloramine inactivation kinetics of *Aspergillus* and *Penicillium* in drinking water. *Water Research*, 120, 265–271.

Merel, S., Snyder, S.A. (2016). Critical assessment of the ubiquitous occurrence and fate of the insect repellent N,N-diethyl-m-toluamide in water. *Environment International*, 96, 98–117.

Muellner, M.G., Wagner, E.D., McCalla, K., Richardson, S.D., Woo, Y.-T., Plewa, M.J. (2007). Haloacetonitriles vs. regulated haloacetic acids: Are nitrogen-containing DBPs more toxic? *Environmental Science & Technology*, 41(2), 645–651.

Nevalainen, A., Täubel, M., Hyvärinen, A. (2015). Indoor fungi: Companions and contaminants. *Indoor air*, 25(2), 125–156.

Nourmoradi, H., Nikaeen, M., Stensvold, C.R., Mirhendi, H. (2012). Ultraviolet irradiation: An effective inactivation method of *Aspergillus* spp. in water for the control of waterborne nosocomial aspergillosis. *Water Research*, 46(18), 5935–5940.

Panyakapo, M., Soontornchai, S., Paopuree, P. (2008). Cancer risk assessment from exposure to trihalomethanes in tap water and swimming pool water. *Journal of Environmental Sciences*, 20(3), 372–378.

Pasquarella, C., Veronesi, L., Napoli, C., Castaldi, S., Pasquarella, M. L., Saccani, E., Colucci, M. E., Auxilia, F., Gallè, F., Di Onofrio, V., Tafuri, S., Signorelli, C., Liguori, G. (2013). Swimming pools and health-related behaviours: Results of an Italian multicentre study on showering habits among pool users. *Public Health*, 127(7), 614–619.

Pasquarella, C., Veronesi, L., Napoli, C., Castaldi, S., Pasquarella, M. L., Saccani, E., Colucci, M. E., Auxilia, F., Gallè, F., Di Onofrio, V., Tafuri, S., Signorelli, C., Liguori, G. (2014). What about behaviours in swimming pools? Results of an Italian multicentre study. *Microchemical Journal*, 112, 190–195.

Pereira, V.J., Marques, R., Marques, M., Benoliel, M.J., Barreto Crespo, M.T. (2013). Free chlorine inactivation of fungi in drinking water sources. *Water Research*, 47(2), 517–523.

Plewa, M.J., Wagner, E.D., Muellner, M.G., Hsu, K.-M., Richardson, S.D. (2009). Comparative mammalian cell toxicity of N-DBPs and C-DBPs. In: Karanfil, T., Krasner, S. W., Westerhoff, P., & Xie, Y. (Eds.), Disinfection By-products in Drinking Water: Occurrence, Formation, Health Effects and Control, ACS Symposium Series, vol. 995. American Chemical Society, Washington DC, pp. 36–50.

Rao, C. Y., Burge, H. A., Chang, J. C. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association*, 46(9), 899–908.

Real Decreto 742/2013, de 27 de septiembre, por el que se establecen los criterios técnico-sanitarios de las piscinas, 2013. Ministerio de Sanidad, Servicios Sociales e Igualdad.

Reynolds, K.A., Watt, P.M., Boone, S.A., Gerba, C.P. (2005). Occurrence of bacteria and biochemical markers on public surfaces. *International Journal of Environmental Health Research*, 15(3), 225–234.

Ribbers, J., Keuten, M., van Rompay, T. (2017). I spy, I spy with my little eye. Abstract from 7th International Conference 2017 on Swimming Pool and Spa Waters, Kos Island, Greece.

Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., DeMarini, D.M. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Research/Reviews in Mutation Research*, 636(1–3), 178–242.

Rosenzweig, W., Minnigh, H., Pipes, W. (1983). Chlorine demand and inactivation of fungal propagules. *Applied and Environmental Microbiology*, 45(1), 182–186.

Schriks, M., Heringa, M.B., van der Kooi, M.M.E., de Voogt, P., van Wezel, A.P. (2010). Toxicological relevance of emerging contaminants for drinking water quality. *Water Research*, 44(2), 461–476.

Sharma, B.M., Bečanová, J., Scheringer, M., Sharma, A., Bharat, G.K., Whitehead, P.G., Klánová, J., Nizzetto, L. (2019). Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India. *Science of the Total Environment*, 646, 1459–1467.

Simard, S., Tardif, R., Rodriguez, M.J. (2013). Variability of chlorination by-product occurrence in water of indoor and outdoor swimming pools. *Water Research*, 47(5), 1763–1772.

Sisti, M., Pieretti, B., De Santi, M., Brandi, G. (2014). Inactivation of pathogenic dermatophytes by ultraviolet irradiation in swimming pool thermal water. *International Journal of Environmental Health Research*, 24(5), 412–417.

Snelders, E., Camps, S.M.T., Karawajczyk, A., Schaftenaar, G., Kema, G.H.J., van der Lee, H.A., Klaassen, C.H., Melchers, W.J.G., Verweij, P.E. (2012). Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. PLoS One, 7(3), e31801.

Snelders, E., Huis in 't Veld, R.A.G., Rijs, A.J.M.M., Kema, G.H.J., Melchers, W.J.G., Verweij, P.E. (2009). Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. *Applied and Environmental Microbiology*, 75(12), 4053–4057.

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B. (2004). Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking water treatment plant. *Science of the Total Environment*, 329(1), 99–113.

Stronks, I., Keuten, M. (2016). How to improve hygienic behaviour in holiday park swimming pools. Abstract from Symposium on Improving Pool Water Quality, Zell am See, Austria.

Suppes, L. M., Huang, C.-H., Lee, W.-N., Brockman, K. J. (2017). Sources of pharmaceuticals and personal care products in swimming pools. *Journal of Water and Health*, 15(5), 829–833.

Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.-J., Haist-Gulde, B., Preuss, G., Wilme, U., Zulei-Seibert, N. (2002). Removal of pharmaceuticals during drinking water treatment. *Environmental Science & Technology*, 36(17), 3855–3863.

Touraud, E., Roig, B., Sumpter, J.P., Coetsier, C. (2011). Drug residues and endocrine disruptors in drinking water: Risk for humans? *International Journal of Hygiene and Environmental Health*, 214(6), 437–441.

U.S. EPA (2006a). National primary drinking water regulations: stage 2 disinfectants and disinfection by products rule: final rule. Federal Register Vol. 71, No. 2.

U.S. EPA (2006b). Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule. Washington, DC., U.S. Environmental Protection Agency.

U.S. EPA (2016). Contaminant Candidate List 4 - CCL-4. U.S. Environmental Protection Agency, Available at http://www2.epa.gov/ccl/chemical-contaminants-ccl-4.

Vennewald, I., Klemm, E. (2010). Otomycosis: Diagnosis and treatment. *Clinics in Dermatology*, 28(2), 202–211.

Verhoeff, A.P., Burge, H.A. (1997). Health risk assessment of fungi in home environments. *Annals of Allergy, Asthma & Immunology*, 78(6), 544–556.

Villanueva, C.M., Cantor, K.P., Grimalt, J.O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G. (2007). Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *American Journal of Epidemiology*, 165(2), 148–156.

Wang, W., Qian, Y., Boyd, J.M., Wu, M., Hrudey, S.E., Li, X.-F. (2013). Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environmental Science & Technology*, 47(7), 3275–3282.

Wen, G., Xu, X., Huang, T., Zhu, H., Ma, J. (2017a). Inactivation of three genera of dominant fungal spores in groundwater using chlorine dioxide: Effectiveness, influencing factors, and mechanisms. *Water Research*, 125, 132–140.

Wen, G., Xu, X., Zhu, H., Huang, T., Ma, J., (2017b). Inactivation of four genera of dominant fungal spores in groundwater using UV and UV/PMS: Efficiency and mechanisms. *Chemical Engineering Journal*, 328, 619–628.

WHO (2006). Guidelines for safe recreational water environments, Vol. 2: Swimming pools and similar environments. Geneva, World Health Organization.

WHO (2009). WHO guidelines for indoor air quality: Dampness and mould. Copenhagen, Denmark, WHO Regional Office for Europe.

WHO (2011a). Guidelines for drinking-water quality. 4th edition. Geneva, World Health Organization.

WHO (2011b). Pharmaceuticals in drinking-water. Geneva, World Health Organization.

Wiant, C. (2012). New public survey reveals swimmer hygiene attitudes and practices. *International Journal of Aquatic Research and Education*, 6(3), 4.

Xu, X., Mariano, T.M., Laskin, J.D., Weisel, C.P. (2002). Percutaneous absorption of trihalomethanes, haloacetic acids, and haloketones. *Toxicology and Applied Pharmacology*, 184(1), 19–26.

Xu, X., Weisel, C.P. (2004). Dermal uptake of chloroform and haloketones during bathing. *Journal of Exposure Analysis and Environmental Epidemiology*, 15, 289.

Yang, Y., Ok, Y. S., Kim, K.-H., Kwon, E.E., Tsang, Y.F. (2017). Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. *Science of the Total Environment*, 596–597, 303–320.

Yost, L.J., Barber, T.R., Gentry, P.R., Bock, M.J., Lyndall, J.L., Capdevielle, M.C., Slezak, B.P. (2017). Evaluation of triclosan in Minnesota lakes and rivers: Part II - human health risk assessment. *Ecotoxicology and Environmental Safety*, 142, 588–596.

Summary

Swimming pools have been used for bathing and recreational purposes for over 5,000 years. However, disinfection of swimming pool water was introduced only in the early 1900s and since then pool water treatment has improved yielding better pool water quality. Poor water quality, chemically or microbiologically, may pose a threat to human health. Guidelines and legislation on swimming pool water quality were established to ensure the safety of bathers. Water quality monitoring programmes followed by interventions when indicated are put in place to ensure chemically and microbiologically safe swimming pool water at all times. However, some microbial and chemical contaminants are not covered by current swimming pool water quality standards and legislation, such as protozoan parasites, viruses and fungi, as well as a great majority of disinfection byproducts (DBPs) and chemical micropollutants such as pharmaceuticals and personal care products (PPCPs). This thesis explored such microbial and chemical contaminants in swimming pool environments and assessed an alternative disinfection technology for swimming pool water treatment.

Disinfection is crucial in reducing the risk of infections by pathogens, such as *Cryptosporidium*, *Giardia*, *Legionella* and *Pseudomonas aeruginosa* which are known to cause outbreaks in swimming pools. However, there are health concerns, such as asthma, irritation of the skin, throat or eyes that arise from DBPs formed by the reaction of disinfectants and organic and inorganic constituents in swimming pool water. Commonly used disinfectants in swimming pools, such as chlorine-based disinfectants, can react with natural organic matter, bromide or iodide, and anthropogenic compounds such as urine, sweat, cosmetics, skin cells and hair, thus forming DBPs. Consequently, in order to protect the health of the bathers, DBP formation should be minimized while at the same time maintaining the microbial safety of swimming pool water.

One of the solutions to minimize DBP formation is to apply alternative disinfection technologies. In **Chapter 2**, a novel alternative disinfection technology for swimming pool water, UVOX Redox® (referred to as UVOX) was assessed in a full scale swimming pool. UVOX combines ozonation and UV irradiation in a single system. The assessment covered the inactivation of chlorine resistant microorganisms, represented by *Bacillus subtilis* spores, formation of DBPs, and removal of micropollutants. The inactivation experiments showed that UVOX was able to achieve up to 2.7 log reduction of *Bacillus subtilis* spores. The application of UVOX generated lower concentrations of chlorinated DBPs compared to chlorination alone. However, the presence of bromide in the water also has to be taken into consideration when using UVOX in pool water treatment, because the ozone in the UVOX system can cause the formation of brominated DBPs. In combination with chlorination, UVOX enhanced the removal of micropollutants

(such as ibuprofen). It can be concluded that UVOX was effective in microbial inactivation and its application could lower the usage of chlorine to a level that still provides an adequate residual disinfection effect in combination with lower DBP formation.

Chemical contaminants, such as PPCPs are of concern in swimming pool water due to their potential adverse human health effects. Furthermore, PPCPs can act as DBP precursors. The occurrence of pharmaceuticals and UV filters (personal care products) in 17 pools in Catalonia, Spain was demonstrated in **Chapter 3**. In total, 51 water samples were analysed for the presence of 32 different pharmaceuticals and 14 UV filters. Ten pharmaceuticals were detected at concentrations above their limit of quantifications (0.02–4.64 ng/L). Carbamazepine was the most frequently detected pharmaceutical, while hydrochlorothiazide was detected at the highest concentration. Eleven UV filters were detected at concentrations higher than their limit of quantifications (1.0–1.4 ng/L). The most frequently detected UV filter was 1H-benzotriazole, while 4-methylbenzylidene camphor was detected at the highest concentration. It was shown that in the 17 different pools investigated, including indoor, outdoor, and spa pools, PPCPs were most frequently detected in spas. Moreover, based on the water treatment applied, the lowest frequency of detection of pharmaceuticals was in swimming pools applying sand filtration followed by chlorination with sodium hypochlorite while the lowest frequency of detection of UV filters was in swimming pools applying coagulation, sand filtration, UV and salt electrolysis. This study showed that regardless of the type of swimming pool and the applied pool water treatment, pharmaceuticals and UV filters occurred widely, and that their presence was closely related to the continuous contamination by bathers.

Microbial contaminants including fungi, are commonly found in outdoor and indoor environments. The research described in **Chapter 4** demonstrated that a wide range of fungal species, such as *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp., and clinically relevant species such as *Trichophyton* spp. and *Candida* spp., were prevalent in a swimming pool facility, both on surfaces and in the pool water. The highest fungal concentrations were found on floors in the swimming hall. The results from a wider follow-up study in seven swimming pool facilities (**Chapter 5**) concurred with the results of **Chapter 4**, demonstrating the prevalence of clinically relevant fungal species in all of seven studied swimming pool facilities. Additionally, **Chapter 5** describes potential transmission pathways of clinically relevant fungi inside swimming pool facilities. Samples were collected from floors, water, and air in indoor swimming pool facilities. Floors at the sites where the pool visitors converge, such as the exit of the dressing rooms leading to the shower room, and the exit of the shower room leading to the swimming hall, showed the highest fungal concentrations. Some fungal species were isolated at many of the sampling sites, indicating that pool visitors may facilitate the dispersal of fungi. The distribution of fungi in the swimming pool facilities demonstrated

that clinically relevant fungi were present on floors where pool visitors walk barefoot, which highlights a possible transmission pathway and suggests a risk of contracting fungal infections in swimming pool facilities.

The research presented in this thesis showed that clinically relevant fungal species were ubiquitous in the swimming pool environment, and that DBPs and PPCPs were frequently present. Other studies have shown that exposure to these contaminants through inhalation, skin contact and ingestion may cause adverse health effects to humans. Since there are no guidelines for these contaminants in swimming pool environments, hygiene-related behaviour, such as pre-swim showering, wearing sandals, not urinating while swimming, not swimming when having skin wounds or infectious skin diseases can be put into practice to reduce anthropogenic contamination. Frequent cleaning can also be applied to maintain the cleanliness inside a swimming pool facility, especially at the sites where pathogenic microorganisms can grow or accumulate. As for swimming pool water, UVOX is effective in inactivation of chlorine resistant *Bacillus subtilis* spores and its application potentially limits DBP formation. Previous studies have shown that some fungal species belonging to the genera *Aspergillus*, *Cladosporium*, and *Penicillium* appeared to be resistant to chlorine-based disinfectants. Based on the results in this and other studies, the inactivation of these pathogenic fungi probably can be done effectively by UVOX. Another advantage of UVOX is that it enhanced the removal of micropollutants in chlorinated water. Future swimming pool water guidance should include raising awareness among swimmers, pool operators and managers about hygienic behaviour and opportunities for better hygiene measures and cleaning, and application of alternative disinfection technologies such as UVOX to reduce anthropogenic contamination.

Samenvatting

Zwembaden worden al meer dan 5000 jaar gebruikt voor zwem- en recreatiedoeleinden. Desinfectie van zwembadwater werd echter pas vanaf 1900 geïntroduceerd. Sindsdien is de zwembadwaterbehandeling verder verbeterd, waardoor ook het zwembadwater van betere kwaliteit is geworden. Een slechte waterkwaliteit, zowel chemisch als microbiologisch, kan een bedreiging voor de volksgezondheid vormen. Om de veiligheid en gezondheid van zwemmers te waarborgen, zijn richtlijnen en wetgeving voor de kwaliteit van zwembadwater opgesteld. Er zijn monitoringsprogramma's opgezet om de waterkwaliteit te bewaken, zo nodig gevolgd door interventies, om te allen tijde chemisch en microbiologisch veilig zwembadwater te garanderen. Sommige microbiële en chemische verontreinigingen vallen echter buiten de huidige zwemwaterkwaliteitsnormen en wetgeving voor zwembadwater, zoals protozoaire parasieten, virussen en schimmels, maar ook het merendeel van de desinfectiebijproducten (DBPs) en chemische microverontreinigingen zoals geneesmiddelen en persoonlijke verzorgingsproducten (PPCPs). Dit proefschrift onderzocht dergelijke microbiële en chemische verontreinigingen in het zwembadmilieu en bestudeerde een alternatieve desinfectietechnologie voor de behandeling van zwembadwater.

Desinfectie is van cruciaal belang om het risico van infecties veroorzaakt door ziekteverwekkers zoals *Cryptosporidium*, *Giardia*, *Legionella* en *Pseudomonas aeruginosa* te reduceren. Van deze ziekteverwekkers is bekend dat ze ziekte uitbraken in zwembaden veroorzaken. Door de reactie van desinfectiemiddelen en organische en anorganische bestanddelen in het zwembadwater doen zich echter ook gezondheidsproblemen voor, zoals astma en irritatie van de huid, keel of ogen. Vaak gebruikte desinfectiemiddelen in zwembaden, zoals desinfectiemiddelen op basis van chloor, kunnen reageren met natuurlijk organisch materiaal, bromide of jodide, en antropogene verbindingen zoals urine, zweet, cosmetica, huidcellen en haar en vormen zo DBPs. Om de gezondheid van de zwemmers te beschermen, moet daarom de vorming van DBPs geminimaliseerd worden, terwijl tegelijkertijd de microbiële veiligheid van het zwembadwater behouden wordt.

Een van de oplossingen om DBP-vorming tot een minimum te beperken is het toepassen van alternatieve desinfectietechnologieën. In **Hoofdstuk 2**, werd een nieuwe alternatieve desinfectietechnologie voor zwembadwater, UVOX Redox® (verder aangeduid als UVOX) onderzocht in een op ware grootte aangelegd test-zwembad. UVOX combineert ozonisatie en UV-straling in één systeem. Het onderzoek had betrekking op de inactivering van chloor-resistente micro-organismen, vertegenwoordigd door sporen van *Bacillus subtilis*, de vorming van DBPs en de verwijdering van microverontreinigingen. De inactivatie-experimenten met *Bacillus*

subtilis sporen toonden aan dat UVOX in staat was om tot 2,7 log reductie van deze sporen te bewerkstelligen. De toepassing van UVOX genereerde lagere concentraties gechloreerde DBPs in vergelijking met de toepassing van alleen chloor. Bij het gebruik van UVOX voor de behandeling van zwembadwater moet echter ook rekening worden gehouden met de aanwezigheid van broom in het water, omdat het ozon in het UVOX-systeem de vorming van gebromeerde DBPs kan veroorzaken. In combinatie met chloor zorgt UVOX voor een betere verwijdering van microverontreinigingen, zoals ibuprofen. Er kan geconcludeerd worden dat microbiële inactivatie door UVOX effectief was en dat toepassing van UVOX het gebruik van chloor zou kunnen verlagen tot een niveau dat nog steeds voldoende residuele desinfectie biedt, in combinatie met een geringere vorming van DBPs.

Chemische verontreinigingen, zoals PPCPs in het zwembadwater, zijn een punt van zorg vanwege hun mogelijke schadelijke gevolgen voor de volksgezondheid. Bovendien kunnen PPCPs als precursor voor DBPs fungeren. In **Hoofdstuk 3** is aangetoond dat in de 17 onderzochte zwembaden in Catalonië, Spanje, geneesmiddelen en UV-filters (producten voor persoonlijke verzorging) voorkwamen. In totaal werden 51 watermonsters geanalyseerd op de aanwezigheid van 32 verschillende geneesmiddelen en 14 UV-filters. Tien geneesmiddelen werden gedetecteerd in concentraties boven hun kwantificatielimiet (0,02–4,64 ng/L). Carbamazepine was het meest gedetecteerde geneesmiddel, terwijl hydrochloorthiazide in de hoogste concentratie werd gedetecteerd. Elf UV-filters werden gedetecteerd in een concentratie hoger dan hun kwantificatielimiet (1,0–1,4 ng/L). Het meest gedetecteerde UV-filter was 1H-benzotriazool, terwijl 4-methylbenzylideenkamfer in de hoogste concentratie werd gedetecteerd. In de 17 verschillende onderzochte zwembaden, waaronder binnen-, buiten- en spabaden, werden PPCPs het vaakst gedetecteerd in spabaden. Kijkend naar de toegepaste manier van waterbehandeling, werden geneesmiddelen in de laagste frequentie gedetecteerd in zwembaden met zandfiltratie gevolgd door chloorbehandeling met natriumhypochloriet, terwijl de laagste detectiefrequentie van UV-filters werd gezien in zwembaden met coagulatie, zandfiltratie, UV- en zoutelektrolyse. Deze studie toonde aan dat ongeacht het type zwembad en de toegepaste zwembadwaterbehandeling, geneesmiddelen en UV-filters op grote schaal voorkwamen en dat hun aanwezigheid nauw verband hield met de continue vervuiling door baders.

Microbiële verontreinigingen, waaronder die door schimmels, zijn algemeen in het buiten- en het binnenmilieu. Het in **Hoofdstuk 4** beschreven onderzoek toonde aan dat een breed spectrum van verschillende schimmels, zoals *Aspergillus* spp., *Cladosporium* spp. en *Penicillium* spp., en klinisch relevante soorten zoals *Trichophyton* spp. en *Candida* spp., in een zwembadcomplex aanwezig waren, zowel op oppervlakken als in het zwembadwater. De hoogste schimmelconcentraties werden aangetroffen op vloeren in de zwemzaal. De resultaten van een breder vervolgonderzoek in zeven

zwembadcomplexen (**Hoofdstuk 5**) kwamen overeen met de resultaten in **Hoofdstuk 4**, en toonden het voorkomen van klinisch relevante schimmelsoorten in alle zeven onderzochte zwembadcomplexen aan. In **Hoofdstuk 5** worden daarnaast mogelijke transmissieroutes van klinisch relevante schimmels in zwembadcomplexen beschreven. Er werden monsters genomen van vloeren, water en lucht in binnenzwembaden. Vloeren op de plaatsen waar de bezoekers van het zwembad samenkomen, zoals de uitgang van de kleedkamers naar de doucheruimte en de uitgang van de doucheruimte naar de zwemzaal, vertoonden de hoogste schimmelconcentraties. Sommige van deze schimmels werden op veel van de bemonsteringsplaatsen geïsoleerd, wat erop wijst dat de bezoekers van de zwembadcomplexen de verspreiding van schimmels mogelijk maken. De verdeling van de schimmels binnen de zwembadcomplexen toonde aan dat er klinisch relevante schimmels aanwezig waren op vloeren waar bezoekers op blote voeten lopen. Dit wijst op een mogelijke transmissieroute en suggereert een risico op schimmelinfecties in zwembadcomplexen.

Het onderzoek in dit proefschrift toonde aan dat klinisch relevante schimmelsoorten alomtegenwoordig waren in het zwembadmilieu en dat DBPs en PPCPs vaak aanwezig waren. Andere studies hebben aangetoond dat blootstelling aan deze verontreinigingen door inademing, huidcontact en inslikken nadelige gevolgen kunnen hebben voor de volksgezondheid. Aangezien er geen richtlijnen voor deze contaminanten in het zwembadmilieu bestaan, kan hygiënisch gedrag, zoals douchen vóór het zwemmen, het dragen van sandalen, niet plassen tijdens het zwemmen, niet zwemmen met huidwonden of bij infectieziekten, in de praktijk worden gebracht om antropogene besmetting te verminderen. Ook frequente reiniging kan ervoor zorgen dat een zwembadcomplex schoon blijft, vooral op de plaatsen waar pathogene micro-organismen kunnen groeien of zich kunnen ophopen. In zwembadwater kan UVOX effectief chloor-resistente sporen van *Bacillus subtilis* inactiveren en de toepassing van UVOX beperkt mogelijk ook de vorming van DBPs. Eerdere studies hebben aangetoond dat sommige schimmelsoorten uit de geslachten *Aspergillus*, *Cladosporium* en *Penicillium* resistent zijn tegen desinfectiemiddelen op basis van chloor. Op basis van de resultaten van de hier gepresenteerde en overige studies zou de inactivering van deze pathogene schimmels effectief kunnen worden uitgevoerd met UVOX. Een ander voordeel van de toepassing van UVOX is dat het de verwijdering van microverontreinigingen in gechloreerd water bevorderd. Toekomstige richtlijnen voor zwembadwater moeten zwemmers, zwembadbeheerders en -managers bewust maken van hygiënisch gedrag en mogelijkheden bieden voor betere hygiënemaatregelen, betere schoonmaak en de toepassing van alternatieve desinfectietechnologieën zoals UVOX om antropogene vervuiling te reduceren.

Acknowledgement

My PhD journey finally has come to an end. The completion of this thesis would not be possible without the support from many people who helped along the way. I am lucky to have the chance to work with them and I owe a debt of gratitude to each one of them.

First and foremost, I would like to thank my promotors and co-promotors. Prof. Ana Maria de Roda Husman, thank you for your guidance, enthusiasm and also your confidence in me. I am grateful that you always manage to have time for me, even when you were busy with meetings. Prof. Maria Kennedy, thank you for your guidance since I started my education in IHE Delft. I am grateful for the opportunities to work in different research projects which motivated me to continue working in research. My co-promotors, Ciska Schets and Giuliana Ferrero, thank you for being patience in supervising me all these years. Both of you are surely the greatest mentors one can have. Ciska, I really appreciate your endless effort to help me to finish. You taught me to think critically, and helped me to structure my work. I am very grateful for your detailed reviews and comments on my writing, for your time to read and to correct it, even during your weekends and holidays. Giuliana, I can't thank you enough for giving me the chance to pursue a PhD. Your critical comments helped me shape my writing. Thank you for always being there to listen and to give advice. I am truly grateful for your encouragement and how you never stop reminding me to think positive and to be more optimistic.

My research led me to work with many people from different institutions. I would like to acknowledge ICRA for accommodating me during my research in Girona, Spain. Particularly, I would like to thank Maria José Farré and Ignasi Rodriguez Roda. My gratitude to Gianluigi Buttiglieri, for your many kinds of support from collecting samples to writing publications. Thank you Gigi, my work would not have been completed without your help. I also would like to acknowledge Westerdijk Fungal Biodiversity Institute. I would like to express my gratitude to Anne van Diepeningen for sharing your expertise. My thanks also goes to Kimberly Westland who gave such a huge help in sample processing. Many thanks to the people in Medical Mycology Group who were very welcoming, especially Sybren de Hoog, Bert Gerrits van den Ende, Abdullah Al-Hatmi, Sarah Abdallah, and Meizhu Wang. I am also grateful for the support from RIVM, especially Gretta Lynch and Claudia Coipan. Thank you to Barbara Berson (Wapure International GmbH) for providing both a laboratory and a full scale UVOX. I am grateful for the support from the IHE laboratory staff, Fred Kruis, Frank Wiegman, Ferdi Battes, Peter Heerings, Lyzette Robbemont and Berend Lolkema.

I would like to thank the members of the assessment committee for evaluating this thesis. I am also grateful to all co-authors of my manuscripts for your valuable comments and suggestions.

Living far away from home, I was lucky to be surrounded by wonderful friends. I deeply value our friendship and your supports help me to overcome challenges in my life. I would like to express my sincere gratitude to my friends and colleagues, Nirajan and Anita, Nikola, Kalmah, Anne, Yos, Andre, Pedro, Clara, Mohan, Isnaeni, Anuar, Leo, Dikman, Selvi, Mita, Motasem, Mary, Mohanad, Mohaned, Ahmed, Javier, and Laurens. My special thanks to Fiona, Sony, Shah, Linh, Tarn and Gladys. I will surely miss our joyful time in many trips, dinner parties, culinary tours and board-game nights. Many thanks to Cisca, and Stevie, Pungky and Senot, my family in Delft who always welcome me in your house. Pak Suryadi, I really appreciate your kind support. Loreen and Helga, thank you for being my mentor and friend, I learned a lot from both of you.

Finally, my appreciation to my parents, who always support me, even when I decided to travel far away from them. My brother and sister, Ari and Sharah, thank you for your patience in taking care of our father and mother, especially in their difficult times. And to my auntie, Lestari, thank you for always giving me motivation and support that I need.

List of publications

Peer-reviewed publications

Villacorte, L.O., Ekowati, Y., Winters, H., Amy, G.L., Schippers, J.C., Kennedy, M.D. (2013). Characterisation of transparent exopolymer particles (TEP) produced during algal bloom: a membrane treatment perspective. *Desalination & Water Treatment*, 51 (4-6), 1021-1033.

Ekowati, Y., Msuya, M., Salinas-Rodriguez, S.G., Veenendaal, G., Schippers, J.C., Kennedy, M.D. (2014). Synthetic organic polymer fouling in municipal wastewater reuse reverse osmosis. *Journal of Water Reuse and Desalination*, 4, 125-136.

Villacorte, L.O., Ekowati, Y., Neu, T.R., Kleijn, J.M., Winters, H., Amy, G.L., Schippers, J.C., Kennedy, M.D. (2015). Characterisation of algal organic matter produced by bloom-forming marine and freshwater algae. *Water Research*, 73, 216-230.

Villacorte L.O., Ekowati Y., Winters H., Amy G.L., Schippers J.C., Kennedy M.D. (2015). MF/UF rejection and fouling potential of algal organic matter from bloom-forming marine and freshwater algae. *Desalination*, 367, 1-10.

Villacorte, L.O., Ekowati, Y., Calix-Ponce, H.N., Schippers, J.C., Amy, G.L., Kennedy, M.D. (2015). Improved method for measuring transparent exopolymer particles (TEP) and their precursors in fresh and saline water. *Water Research*, 70, 300-312.

Li, S., Winters, H., Villacorte, L.O., Ekowati, Y., Emwas, A.-H., Kennedy, M.D., Amy, G.L. (2015). Compositional similarities and differences between transparent exopolymer particles (TEPs) from two marine bacteria and two marine algae: Significance to surface biofouling. *Marine Chemistry*, 174, 131-140.

Ekowati, Y., Buttiglieri, G., Ferrero, G., Valle-Sistac, J., Diaz-Cruz, M.S., Barceló, D., Petrovic, M., Villagrasa, M., Kennedy, M.D., Rodríguez-Roda, I. (2016). Occurrence of pharmaceuticals and UV filters in swimming pools and spas. *Environmental Science and Pollution Research*, 23(14), 14431-14441.

Villacorte, L.O., Ekowati, Y., Calix-Ponce, H.N., Kisielius, V., Kleijn, J.M., Vrouwenvelder, J.S., Schippers, J.C.,Kennedy, M.D. (2017). Biofouling in capillary and spiral wound membranes facilitated by marine algal bloom. *Desalination*, 424, 74-84.

Ekowati, Y., van Diepeningen, A.D., Ferrero, G., Kennedy, M.D., de Roda Husman, A.M., Schets, F.M. (2017). Clinically relevant fungi in water and on surfaces in an indoor swimming pool facility. *International Journal of Hygiene and Environmental Health*, 220(7), 1152-1160.

Ekowati, Y., Ferrero, G., Kennedy, M.D., de Roda Husman, A.M., Schets, F.M. (2018). Potential transmission pathways of clinically relevant fungi in indoor swimming pool facilities. *International Journal of Hygiene and Environmental Health*, 221(8), 1107-1115.

Ekowati, Y., Ferrero, G., Farré, M.J., Kennedy, M.D., Buttiglieri, G. (2018). Application of UVOX Redox® for swimming pool water treatment: microbial inactivation, disinfection byproduct formation and micropollutant removal. *Chemosphere*, 220, 176-184.

Oral presentation at international conferences

Ekowati, Y., Villacorte, L.O., Winters, H., Schippers, J.C., Amy, G., and Kennedy, M.D. (2012). Characterisation, rejection and fouling potential of algal organic matter from 'red tide' algae. EDS Conference on Membranes in Drinking and Industrial Water Production, Leeuwarden, Netherlands, 10-12 September 2012.

Ekowati, Y., Msuya, M., Salinas Rodriguez, S.G., Veenendaal, G., Schippers, J.C., Kennedy, M.D. (2013). Synthetic organic polymer fouling in water reuse reverse osmosis system. 7th IWA Specialised Membrane Technology Conference and Exhibition for Water and Wastewater Treatment and Reuse, Toronto, Canada, 25-29 August 2013.

Ekowati, Y., van Diepeningen, A.D., Ferrero, G., Kennedy, M.D., de Roda Husman, A.M., Schets, F.M. (2017). Clinically relevant fungi on surfaces and in water in an indoor swimming pool facility. 7th International Conference on Swimming Pool and Spa, Kos Island, Greece, 2-5 May 2017.

Ekowati Y., Buttiglieri G., Ferrero G., Valle-Sistac J., Diaz-Cruz M.S., Petrovic M., Villagrasa M., Kennedy M.D., Rodríguez-Roda I. (2017). Occurrence of pharmaceuticals and UV filters in swimming pools and spas. 7th International Conference on Swimming Pool and Spa, Kos Island, Greece, 2-5 May 2017.

Ekowati, Y., Ferrero, G., Slokar, Y.M, Kruithof, J.C., Kennedy, M.D. (2017). An innovative application of ozone-UV treatment for swimming pool water disinfection. 7th International Conference on Swimming Pool and Spa, Kos Island, Greece, 2-5 May 2017.

About the author

Yuli Ekowati was born on July 8th, 1978 in Pati, Indonesia. She obtained her bachelor degree in Civil Engineering at Diponegoro University, Semarang in 2001. Shortly after, she worked as project estimator at a contractor company in Jakarta.

In 2009, she was granted a Nuffic fellowship to pursue a master's degree in IHE Delft, the Netherlands. In 2011, she graduated from Water Supply Engineering program and became a research fellow in the Department of Environmental Engineering and Water Technology in IHE Delft. She worked in a joint project between IHE Delft and the King Abdullah University of Science and Technology (KAUST) on characterization of algal organic matter for two years. During that time, she was involved in a master student thesis on fouling due to polymers and cleaning in RO membranes sponsored by WMD Water. Afterwards, she worked in a joint project between IHE Delft and OASEN on the application of ion exchange as post treatment for RO permeate and the assessment of biological stability of RO permeate and re-mineralized water.

In 2014, she had the opportunity to continue as a PhD candidate in IHE Delft. Her PhD research focused on microbial and chemical contaminants in swimming pool environments and assessment of an alternative disinfection technology for swimming pools. The research was conducted in collaboration with Catalan Water Research Institute (ICRA), Westerdijk Institute and National Institute for Public Health and Environmental Protection (RIVM).

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

D I P L O M A

For specialised PhD training

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Yuli Ekowati

born on 08 July 1978 in Pati, Indonesia

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Utrecht, 11 March 2019

On behalf of the SENSE board	the SENSE Director of Education
Prof. dr. Martin Wassen	Dr. Ad van Dommelen

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)

K O N I N K L I J K E N E D E R L A N D S E
A K A D E M I E V A N W E T E N S C H A P P E N

The SENSE Research School declares that **Yuli Ekowati** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 37.1 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2014)
- Research in context activity: 'Co-organizing 5th demEAUmed Project meeting in IHE Delft (16-17 June 2016)'

Other PhD and Advanced MSc Courses

- Safe microbiological techniques, University of Applied Sciences Leiden (2016)
- Mycology, University of Applied Sciences Leiden(2016)
- Bacteriology, University of Applied Sciences Leiden (2016)
- Molecular Biology, University of Applied Sciences Leiden (2016)
- Good Manufacturing Practices (GMP): the Basics, University of Applied Sciences Leiden (2016)

External training at a foreign research institute

- Molecular biology techniques for fungi, Westerdijk institute, The Netherlands

Management and Didactic Skills Training

- Co-supervising three MSc students with thesis entitled 'Disinfection of recreational water with UVOX and chlorine' (2015), 'Disinfection of recreational water with UVOX and chlorine' (2016) and 'Effect of operational parameters on formation of disinfection by- products in recreational water' (2016)

Oral Presentations

- Clinically-relevant fungi on surfaces and in water in an indoor swimming pool facility. 7th International conference of swimming pool and spa, 2-5 May 2017, Kos, Greece
- An innovative application of ozone-UV treatment for swimming pool water disinfection. 7th International conference of swimming pool and spa, 2-5 May 2017, Kos, Greece
- *Occurrence of pharmaceuticals and UV filters in swimming pools and spas.* 7th International conference of swimming pool and spa, 2-5 May 2017, Kos, Greece

SENSE Coordinator PhD Education

Dr. Peter Vermeulen

This thesis describes the occurrence of microbial and chemical contaminants in swimming pools and the investigation of an alternative disinfection technology, UVOX Redox® that could reduce reliance on chlorine and the formation of chlorinated disinfection byproducts (DBPs) in swimming pools. This technology was effective in inactivation of chlorine resistant microorganisms, represented by *Bacillus subtilis* spores, and in combination with chlorine generated lower concentrations of chlorinated DBPs compared to chlorination alone. It enhanced the removal of pharmaceuticals and personal care products (PPCPs), which were frequently present in indoor, outdoor and spa pools. Carbamazepine and 1H-benzotriazole were the most frequently detected PPCPs, while hydrochlorothiazide and 4-methylbenzylidene camphor were detected at the highest concentration. An investigation of seven different swimming pool facilities showed that clinically relevant fungi were omnipresent. Floors at the sites where the pool visitors converge, such as the exit leading to shower rooms, showed the highest fungal concentrations. The distribution of fungi inside the swimming pool facilities highlighted potential transmission pathways and a possible risk of fungal infections. Future swimming pool water guidance should include raising awareness among swimmers, pool operators and managers about hygienic behaviour and better hygiene measures, and application of alternative disinfection technologies such as UVOX.

ISBN 978-0-367-25135-2

9 780367 251352

an informa business

This book is printed on paper from sustainably managed forests and controlled sources