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Toxicology Classification of Pool Water Quality in Relation to Selected Pollutant Fractions Present in Washings Samples †

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Abstract: The aim of the study is to determine the fractional share of organic pollutants in washing samples collected after pressure filter washings. The evaluation of the physicochemical and toxicological quality of raw washings, fractions <200 kDa, <30 kDa, and <300 Da, has been presented. Separation of selected fractions was carried out with the participation of a multistage pressure membrane system using ultra- and nanofiltration. The physicochemical analysis was conducted based on the total organic carbon (TOC) concentration, dissolved organic carbon, and total carbon. The toxicological classification of isolated fractions was also prepared using the percentage of toxicity effects obtained in commercial bioassays—Microtox® and Artoxkit M. The concentration of TOC in the analyzed samples of the raw washings was ranged from 2.50–11.00 mgC/L. The presented study showed a significant share of the organic pollutants fraction with a molar weight below 300 Da in the examined washings (the TOC was from 0.71 to 1.48 mgC/L). No correlation was observed between the concentration of TOC and the percentage of toxic effect. Screening toxicity tests can be a signal of swimming pool water quality, but they need to be extended with additional test organisms or observations of more morphological parameters of these organisms.

Keywords: swimming pool water; types of pools; total organic carbon (TOC); percentage toxic effect (PE); inhibition of bioluminescence; survival test of individuals; molecular weight fractions; molecular weight cutoff (MWCO); ultrafiltration; nanofiltration

1. Introduction

Organic pollutants introduced by users are the main factor causing significant deterioration of water quality in pool basins. These mainly include products of human metabolism, such as sweat and urine, which are inherently associated with physical activity, but also cosmetics ingredients or metabolites of pharmaceuticals [1–3]. The high reactivity of anthropogenic contamination was observed in the presence of chemical agents used for disinfection. The disinfection byproducts (DBPs) formed as a result of these reactions constitute an increasingly widely studied problem [3–5]. The large interest in the DBPs group is related to their potentially harmful effects on the human body [6,7]. The beds with granular filling currently used in the pool water treatment systems are not completely selective in relation to compounds from the group of DBPs. It is estimated that up to 30%

of organic pollutants are washed out during the filtration process, entering into the purified water supplying the basin [3,8,9].

Most of the DBPs occurring in the pool water environment are characterized by molecular weights below 1000 g/mol [3,7]. The consequence of this is an increased ability of DBPs to penetrate into the organisms of pool users [10,11]. Although the concentrations recorded in the examined pool water samples are often below 1 mg/L, the presence of DBPs is still detrimental to the bathers [12]. The production of enzymes that work in a way that causes oxidative stress in the cells of living organisms by DBPs has been confirmed [6]. Numerous studies in the field of pool water quality assessment using toxicological tests confirmed genotoxic, cytotoxic, and mutagenic properties of DBPs [6,7,12]. Analysis of the contribution of selected pollutant fractions in pool water is particularly important in the light of reports on their genotoxic properties, which increase with decreasing molecular mass [5,7,8]. The highest genotoxicity is observed for compounds with weight below 200 μg/mol [3]. For example, Glauner specifies the share of fraction > 1000 g/mol as 14%, the share of fraction in the range of 200 ÷ 1000 g/mol as 54%, and the share of fraction below 200 g/mol as 32% [7], which proves that a significant share of the smallest fraction exists in the pool water environment. Since genotoxicity and cytotoxicity evaluation methods require access to specialized materials and reagents, their implementation generates high costs [13–15]. It is necessary to attempt to use less sophisticated methods of toxicity evaluation that would be of screening nature and would allow for preliminary assessment of sample quality before proceeding to more advanced analyses. Tools that are commonly used for these purposes are acute toxicity tests for bioluminescence inhibition of the *Aliivibrio fischeri* bacteria or survival of crustaceans—*Artemia franciscana (salina)* or *Daphnia magna* [14,16]. From the point of view of the pool water quality assessment, the concentration of carbon compounds, determined by the concentration of TOC, is particularly important. The TOC parameter shows a high correlation with the concentration of DBPs in samples of water subjected to chlorination processes [3,17]. Therefore, it can indicate information about the share of anthropogenic pollutants in pool water samples.

The aim of the study is to determine the fractional share of organic pollutants in pool water samples taken from the installation of different types of pools of various purposes (a toddler's pool, a swimming pool, and a hot tub). The analyses presented were carried out on samples of waste streams—washings collected after the rinsing of pressure filters. The evaluation of the physicochemical and toxicological quality of raw washings, fractions <200 kDa, <30 kDa, and <300 Da, has been presented. Separation of selected fractions was carried out with the participation of a multistage pressure membrane system using ultra- and nanofiltration. The physicochemical analysis was conducted based on the selected parameters—TOC concentration, dissolved organic carbon (DOC), and total carbon (TC). The toxicological classification of isolated fractions was also prepared using the percentage of toxicity effects obtained with commercial bioassays—Microtox® and Artoxkit M.

2. Methods

2.1. The Subject of the Study

The subject of the study was the washings collected independently of the three water treatment systems—a toddler's pool, a swimming pool, and a hot tub. The washings were selected on the basis of preliminary tests due to the large variation in the quality and quantity of pollutants present in them, which also circulate throughout the pool water treatment systems [18]. The washing samples were collected twice for each of the presented types of pools. Integral elements of the analyzed systems are multilayer pressure filters (quartz sand with different granulation) with activated carbon layers. The filter in the toddler's pool system has a filtration area of 2.01 m^2, and in the hot tub filtration system, the area is 0.65 m^2. In turn, two filters each with filtration areas of 2.54 m^2 are present in the system of the swimming pool. The filtration of water in each filter is carried out at a speed of 30 m/h. The washing samples were collected in batches during the rinsing of the beds (in the evening), through the drain valves that drain the washing water to the overflow channel, and

then to the sanitary sewage system. The washing of the filtration beds (with compressed air and water) depends on the pressure loss during the filtration process every 24 or 48 h. The water for rinsing the beds is drawn from compensating tanks where the pool water losses are supplemented with water from the municipal water supply network.

2.2. Fractionation of Pollutants in a Multistage System

Flat ultra- and nanofiltration membranes from Osmonics Inc. (USA), differing in the scope of the separation, the so-called molecular weight cutoff (MWCO), and the type of membrane-forming material were used for the fractionation of pollutants in the washings. A brief description of the membranes is presented in Table 1.

Table 1. Membrane characteristics.

Process	Membrane Symbol	Membrane Material	MWCO, Da	Operating Pressure TMP, MPa
UF I	YMV53001	Polyvinylidene difluoride (PVDF)	200,000	0.2
UF II	YMV33001	Polyvinylidene difluoride (PVDF)	30,000	0.2
NF	YMHLSP3001	Polyamide—TFC	300–150	1.0

The membranes were placed in a steel filter cell with a volume of 3.80×10^{-4} m^3 where the active filtration surface of the membrane is 38.5×10^{-4} m^2. The filtrations were conducted in a one-way system (dead-end). The fractionation processes were carried out in UF I–UF II–NF multistage systems according to the scheme shown in Figure 1. The feed for the first stage of filtration was made up of raw washings. The permeate obtained after UF I was used as the feed for UF II. The permeate after the second stage of ultrafiltration was used as a feed in the nanofiltration process. As part of the presented work, the focus was only on the physicochemical quality and toxicity classification of protected samples of raw washings and permeates.

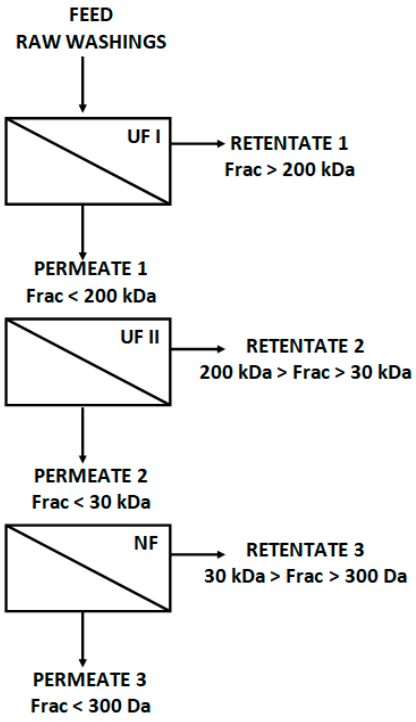

Figure 1. Fractionation (Frac) scheme of washings from pool water installations by membrane filtration.

2.3. Analysis of the Physicochemical Quality of the Separated Pollutant Fractions

The values of selected physicochemical parameters were analyzed in the morning hours for the washings secured in the previous evening. The concentrations of TC, organic carbon (TOC), and dissolved carbon (DOC) were determined in the samples of washings and/or permeate (after 0.45 μm filtration via PVDF syringe filter) using a TOC—L series analyzer using catalytic oxidation with combustion at 680 °C (Shimadzu).

2.4. Classification of the Toxicity of the Obtained Pollutant Fractions

The toxicity classification of the examined samples was based on the average toxicity values obtained in the acute toxicity tests—Microtox® and Artoxkit M. The toxicity analysis using the Microtox® test was carried out according to the MicrotoxOmni screening test procedure in the Microtx Model 500 (Tigret) analyzer, acting as both an incubator and a photometer. Percent inhibition versus the control sample not subjected to the potential toxicant in washings and permeates samples was measured after 5- and 15-minutes of exposure time. The toxicity effect E, % was determined as the percentage of bacterial bioluminescence inhibition.

At the same time, the toxicity of the samples was assessed based on *Artemia salina* crustaceans larvae mortality test in accordance with ASTM E1440—91 (2012) [19]. The number of dead and/or immobile organisms was determined after 24 and 48 h of the test duration. A toxicity effect E, % was calculated for each sample of washings, permeate, and control sample constituting brine solution for crustaceans breeding. The toxicity effect E, % for the presented biotests was determined from the relationship (1):

$$E = \frac{100 \cdot (E_C - E_T)}{E_C}, \% \quad (1)$$

where the observed effect for the control sample was determined as E_C, and E_T is the effect observed for the test sample (washings, permeate).

The system presented in Table 2 [20], which is based on the determination of the so-called PE, is used for toxicity classification. The analyzed value is the average of both bioassays (for the second measurement: 15 min for the Microtox® test and 48 h for the Artoxkit M).

Table 2. The toxicity classification system [20].

PE	Class	Toxicity
≤20%	I	No acute hazard
20 ≤ PE ≤ 50%	II	Slight acute hazard
50 ≤ PE < 100%	III	Acute hazard
PE = 100% [1]	IV	High acute hazard

[1] At least in one bioassay.

Before the bioassays were carried out, the samples of raw washings were dechlorated (free chlorine disappearance in 72 h), and control measurements of chlorine concentration were made, which did not exceed 0.10 $mgCl_2/L$. The statistical elaboration of the results of the toxicological analysis was made on the basis of the data analysis package in Microsoft Excel. The mean and standard deviation values were calculated as shown in Figures 2–4.

3. Results and Discussion

The samples taken from the toddler's pool were characterized by a TC concentration in the range of 11.01–10.76 mgC/L. As a result of the multistage filtration, the TC concentration was lowered to the range from 4.65–1.93 mgC/L (Frac < 300 Da). The concentration of DOC in raw washings was between 1.82 ± 0.18 mgC/L and 4.42 ± 0.19 mgC/L for sampling 1 and 2, respectively. In turn, the concentration of TOC was 6.32 ± 0.07 mgC/L and 5.95 ± 0.08 mgC/L (Figure 2). The TOC values in the successively separated fractions decreased. The concentration of TOC in the permeate after nanofiltration was 1.48 ± 0.05 and 0.71 ± 0.32 mgC/L (Figure 2).

Based on the percentage of toxicity, the samples of raw washings were classified as having a low toxic hazard (PE values of 35.70 ± 4.53% and 31.62 ± 4.80%). The highest PE value was recorded in the fraction below 30 kDa. The samples from the first sampling (Figure 2a) were classified as posing a severe threat to the test organisms (50.65 ± 8.51%). In turn, the samples from the second sampling from the toddler's pool system were characterized by a percentage toxicity effect of 35.70 ± 0.19%. In addition, the first of the separated fractions (Frac < 200 kDa) in the samples from sampling 2 showed a negative PE value which was caused by the strong stimulation of bacterial bioluminescence in the Microtox® test and the low mortality of *A. salina* individuals in contact with this fraction (Figure 2b).

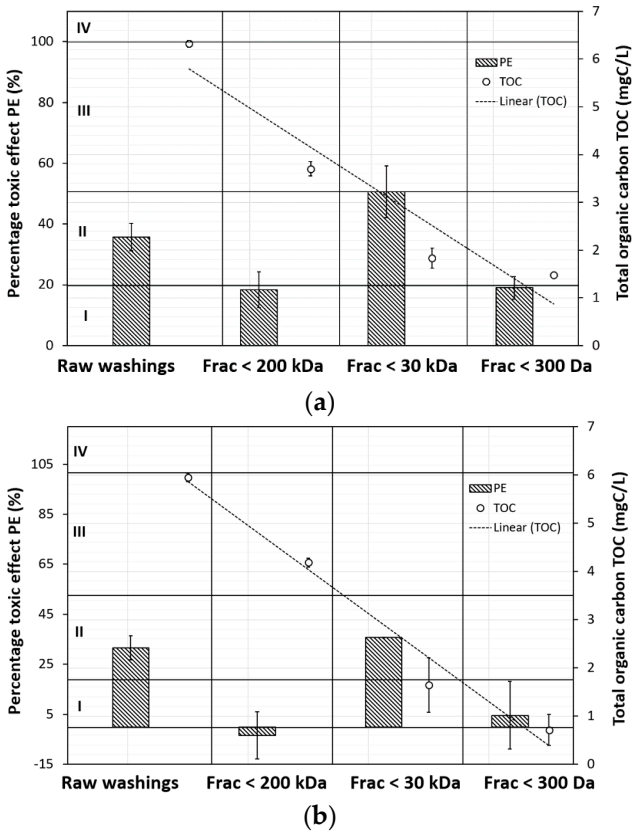

Figure 2. Values of total organic carbon (TOC) and percentage toxic effect (PE) in raw washings and separated fractions. Samples were taken from (**a**) the toddler's pool—sampling 1; (**b**) the toddler's pool—sampling 2.

Higher contents of organic pollutants were noted in the samples of washings collected from the swimming pool installation. The TC concentration was in the range of 17.41–6.99 mgC/L (for sampling 1) and from 15.54–4.46 mgC/L (for sampling 2). However, the content of DOC for the samples of raw washings in samplings 1 and 2 was 9.22 ± 0.26 mgC/L and 7.82 ± 0.12 mgC/L, respectively. Similarly, as was the case in the samples of the washings from the toddler's pool installation, there was a clear decrease in the TOC concentration in the successively separated fractions (Figure 3). The TOC concentration ranged from 10.99–1.00 mgC/L (Figure 3a) and from 8.15 –0.93 mg/L (Figure 3b).

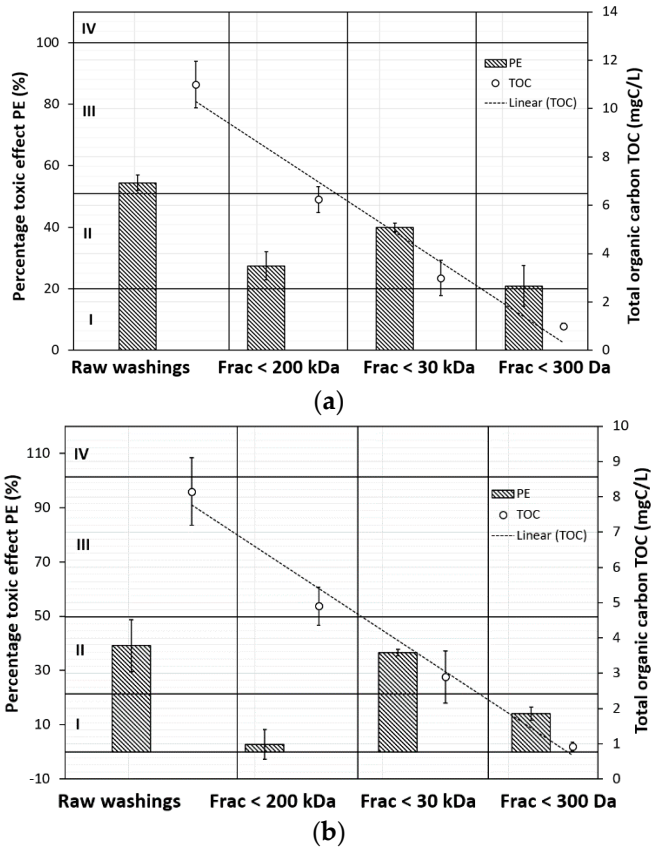

Figure 3. Values of TOC and PE in raw washings and separated fractions. Samples were taken from (**a**) the swimming pool—sampling 1; (**b**) the swimming pool—sampling 2.

In addition, the samples of raw washings collected in the first sampling were characterized by an acute toxic hazard for the test organisms (PE equal to 54.45 ± 2.48%). The PE value recorded for the second sampling was 39.09 ± 9.53% (class II: Small acute threat). The percentage effect values were lower in the separated fractions, but again, the highest value was observed for the <30 kDa fraction, with the PE for the 1 and 2 samplings equal to 39.92 ± 1.37% and 36.63 ± 1.23% respectively (Figure 3).The washings from the hot tube installation were characterized by a varied content of carbon compounds depending on the sampling. In the first analysis, the TC concentration ranged from 13.84–4.02 mgC/L, and in the second, from 4.67–2.21 mgC/L. In addition, DOC values of 5.94 ± 0.21 mgC/L and 1.57 ± 0.09 mgC/L were recorded for sampling 1 and 2, respectively.

The concentration of TOC in the samples of the washings from the first sampling was in the range of 7.27–0.97 mgC/L. In turn, in the successively collected washings, it ranged from 2.55–0.80 mgC/L (Figure 4). Most of the analyzed samples were classified as posing a low toxic hazard to the test organisms. Again, the fraction <30 kDa was distinguished as the one with the highest percentage of toxic effect (Figure 4).

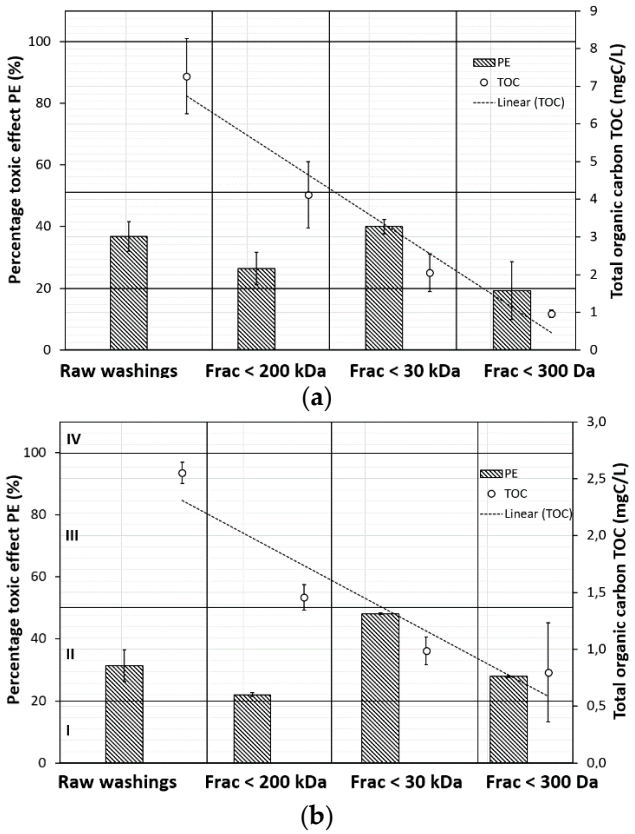

Figure 4 Values of TOC and PE in raw washings and separated fractions. Samples were taken from (**a**) the hot tub—sampling 1; (**b**) the hot tub—sampling 2.

4. Conclusions

The use of a multistage membrane system allowed for the extended physicochemical and toxicological analysis of pool water quality. The analyzed fractions of pollutants with molecular weights below 200 kDa, 30 kDa, and 300 Da, were characterized by diverse properties:

- The presented study showed a significant share of the organic pollutants fraction with a molar weight below 300 Da in the examined washings. The quality of washings (the value of selected physicochemical parameters) varied depending on the type of pool from which the samples were taken.
- There was a clear reduction in the concentration of organic pollutants along with the subsequent processes.
- The values of TOC and DOC concentrations are commonly analyzed parameters of pool water quality. The TOC values presented in the literature show large variations and depending on a load of objects and the applied water treatment purification technology, they range from 0.70–85 mgC/L [3,21–23]. It can be assumed that all values obtained in this study did not differ from the literature data (ranging from 0.71–11.00 mgC/L).
- The analysis of the toxicity of the separated fractions did not show the tendency presented in the studies of other authors [7]. The highest toxicity in the samples tested was observed for fractions below 30 kDa. In addition, none of the samples were classified as being highly toxic to the test organisms.
- There was no correlation between the concentration of TOC and the percentage of toxic effect.
- Screening toxicity tests can be a signal of the quality of environmental samples, including swimming pool water. However, analyses of this type require the extension of additional test organisms or observations of a larger number of morphological parameters of these organisms.

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