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FEASIBILITY STUDY OF USING COMPLEX OF HYDROGEN PEROXIDE AND SILVER FOR DISINFECTING SWIMMING POOL WATER AND ITS ENVIRONMENT

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ABSTRACT

In this research the application of Nanosil containing hydrogen peroxide and silver was studied in disinfecting swimming pool water and it's Environment. The effect of the disinfectant on *Candida albicans* (ATCC No. 10231), *Aspergillus niger* (ATCC No. 16404), *Pseudomonas aeruginosa* (ATCC No. 9027), *Serratia marcescens* (PTCC No. 1111), *Klebsiella pneumoniae* (ATCC No. 10031) and *Staphylococcus aureus* (ATCC No. 29737)) was evaluated. The main objective of this experiment was to determine the effective dose of Nanosil which could be used for disinfecting the environment of swimming pools and other surface area. Then, the effectiveness of Nanosil was studied in two private and one public swimming pools. Heterotrophic plate count, thermotolerant coliforms, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were monitored as the target microorganisms in disinfection practice According to the result of this study, it is recommended to apply the Nanosil with the concentration of ⩾3% (30000 mg/L) for contact time of 30 min or more for practical disinfection in swimming pools environment. The application of Nanosil in real conditions of two private and one public swimming pools indicated that the acceptable microbial quality is also achievable if the disinfectant residual concentration would be as high as 20 mg/L in swimming pool water.

Key words: Disinfection, swimming pool, hydrogen peroxide, silver

INTRODUCTION

It is globally accepted that a wide variety of microorganisms can be present in swimming pools and other water recreational based environments. Microorganisms of water and the environment of pools, contribute to various types of infections. According to the recent publication of World Health Organization, the risk of illness or infection would be associated to either faecal contamination or non faecal sources such as vomit, mucus, saliva or skin (WHO, 2006). Water and environment of pools are supposed to be contaminated by infected users. These infected media (pool water and its environment) then play important roles in infecting the healthy users. Accordingly, it is accepted that many of outbreaks related to swimming pools could be prevented or reduced if a reliable disinfection is practiced.

It is known that chlorine and other chlorine-based products have been widely used for disinfecting of swimming pools water and its environment. The wide application of chlorine and other chlorine-compounds was mainly because of their simple application and economical point of view. But it is emphasized in many recent publications that chlorination of swimming pool water can lead to formation of specific hazardous by-products like trihalomethanes. Data from 114 residential pools in the USA were examined by Sandel (1990) which reported average concentrations of chloroform of 67.1 µg/L with a maximum value of

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313 µg/L. It was estimated that the average amount of water swallowed by non-adults and adults was 37 mL and 16 mL, respectively (Dufour *et al*., 2006). Therefore, the intake of disinfection by-products seems to be considerable for people who are frequently swimming in pools.

The respiratory heath consequences of chlorine in indoor swimming pools are investigated by Nemery *et al*., (2003). Acute hazards related to the chlorination of swimming pools, occupational exposure to swimming-pool air, and health of swimmers are mentioned as the main issues in swimming pools which are using chlorine compounds (Nemery *et al*., 2003). It has been recently shown by Kohlhammer *et al*., (2006) that "impaired integrity of the lung epithelial by exposure to chlorination by-products might facilitate a closer contact to allergens and therefore could result in higher rates of hay fever". Direct ingestion of water, inhalation of volatile compounds and aerosol, and absorption through the skin are the main routes of exposure to theses by-products which increasingly threat the human health in recreational water environments which are disinfected by chlorine-based chemicals.

Therefore, the level of disinfection by-products in swimming pool water is limited and specific measures is required to be set up to minimize their formation. As a solution, there is increasing interest in alternative chemicals for disinfecting swimming pool waters and their environments without by-product formation, such as hydrogen peroxide. The interaction of silver ions and hydrogen peroxide in the inactivation of *E.coli* in water was studied by Pedahzur *et al*., (1995). Low toxicity, long lasting residual effects and low disinfection by-product formation are mentioned as the advantages of this type of disinfectant. One hour exposure of 30 mg/L silver, 30 mg/L hydrogen peroxide and their combination yielded 2.87, 0.65, and 5 logs of inactivation respectively (Pedahzur *et al*., 1995).

In another study, the synergistic effect of silver and hydrogen peroxide was studied on the viability of *E.coli* K-12 and up to 3 logs of reduction was obtained (Pedahzur *et al*., 1997). There is a not much publication available on the practical application of hydrogen peroxide and silver in swimming pools. The main objectives of this study were to examine the effect of hydrogen peroxide and silver on the indicator microorganisms in swimming pools and the feasibility of its application for the disinfection of pools area.

MATERIALS AND METHODS

Preparation of hydrogen peroxide and silver solutions

In this study, Nanosil was used as the source of hydrogen peroxide and silver. The Hydrogen peroxide and silver content of Nanosil were 50% and 0.05%, respectively. Basically, the oxidizing agent of Nanosil is hydrogen peroxide, which is bonded with stabilizing agents to form a complex solution. Silver would ensure a long-lasting effect and acts as a catalyst in trace amounts. Required concentrations were prepared by diluting the Nanosil stock solution.

Evaluation disinfection effect of Nanosil on different microorganisms

As previously mentioned, many kinds of microbial infections may affect the heath of swimmers through the environment of water recreation area. So the effect of Nanosil on different microorganisms such as (*Candida albicans* (ATCC No. 10231), *Aspergillus niger* (ATCC No. 16404), *Pseudomonas aeruginosa* (ATCC No. 9027), *Serratia marcescens* (PTCC No. 1111), Klebsiella *pneumoniae* (ATCC No. 10031) and *Staphylococcus aureus* (ATCC No. 29737)) was evaluated. The main objective of this experiment was to determine the effective dose of Nanosil which could be used for disinfecting the environment of swimming pools and other surface area. The following steps present the procedure of the experiment.

The above mentioned bacteria were cultivated on Tryptic Soy Agar (TSA, Merck) for 24 h at 35-57°C. The *C. aibicans* and *A. niger* were cultivated on Sabouraud Dextrose Agar (SDA, Merck) at 20-25°C for 48 h and 72 h respectively. After incubation, the bacterial and fungal inocula were prepared by suspending single colonies in 0.9% saline. The inocula were adjusted by plate count method and photometrically at 600 nm to a

cell density equivalent to 10^8 CFU/mL for bacteria and 10^7 CFU/mL for fungi (Block, 2000).

In order to evaluate the antimicrobial activity, Nanosil stock solution was diluted with distilled water to obtain test solutions containing 1%, 2% and 3% of Nanosil.

Then aliquots of 100 µL from each microbial suspension were added to 1 mL of Nanosil solutions separately. The number of viable cells contacted with test disinfectant solution was about 10^7 CFU/mL for bacteria and 10^6 CFU/mL for fungi. Nanosil solutions were in contact with the microbial suspensions for different time periods of 5, 10, 15, 20, 30 and 60 min at room temperature. At the end of each contact time, 100 µL of microorganism-disinfectant mixture was transferred to 900 µL of neutralizer (sodium thiosulfate solution) and mixed thoroughly.

The number of viable bacteria and fungi was determined by spreading 100 µL of the neutralized mixture on the surface of above mentioned agar plates. After incubation of the plates for related time period and temperature, the plates were examined for microbial growth. No visible colonies, corresponded to the number of viable microorganisms in 1 mL of microorganism-disinfectant mixture was reduced to lower than 10^2 CFU/mL (about 5 log decrease in bacteria and 4 log in fungi viable counts).

Evaluation of Nanosil for disinfecting Swimming pool water

Swimming pools and disinfection strategy

The disinfection effect of Nanosil was evaluated in two private and one public swimming pool. One of the private swimming pools which were used by a family was located in open space area and it was only in use during summer. The other private swimming pool was built in the closed area which could be used in all seasons. The private pools were used by five bathers before sampling and during the day. The public swimming pool was located in an open space area and used by about 50-70 bathers in the working hours. The volume of indoor private pool, outdoor private pool, and the public swimming pool were 31.2, 27, 350 m^3, respectively. It should be noted that the pools were cleaned and filled with fresh water prior to the study and no disinfection were experienced in the pools before the application of Nanosil. Microbial counts were detected after several days of swimming in the pools and then application of the disinfectant started and monitored periodically. The recommended dose of Nanosil for swimming pools varies from 20 to 70 mg/L according to the documents of supplier. The purpose of this study was to determine the effective dose of Nanosil to keep the microbial indicators as low as possible in order to provide the best microbial water quality in swimming pools. Therefore, Nanosil dose in the private pool (which was located in close space) increased until the results of microbial indicators were acceptable. Then the dose was applied to the other pools to provide more reproducible evidence. The water samples were taken before adding the Nanosil to take the record of microbial indicators prior to the disinfection. The required amounts of Nanosil were added to the pool to make the given concentration. Then, after 24 h the residual amount of Nanosil and microbial indicators were determined to evaluate the efficiency of disinfection practice. Residuals of Nanosil in swimming pool water were tested using the Model HYP-1 hydrogen peroxide test kit of Hach Company Model HYP-1. After the quantification of hydrogen peroxide, the reduced concentration of hydrogen peroxide were calculated and added to the swimming pool as a make up dose. Parameters such as turbidity, temperature, ORP (Oxidation Reduction Potential) were determined at the time of sampling.

Microbial indicators and performed tests

In this study, it was intended to monitor 'indicator' microorganisms which were mentioned in the recent publication of World Health Organization rather than specific microorganisms which may cause hazards. The indicator microorganisms and the methods used for detection are presented below.

- Heterotrophic plate count (HPC): This microbial index can be considered as an indication of the overall bacterial population within the pool. It is also useful for evaluating any kind of practice

which are supposed to be done to optimize the sanitary conditions of swimming pool. This test has been performed according to the method 9215 B which was presented in the "Standard Methods for the Examination of Water and Wastewater" (APHA, 2003). It should be noted that HPC test was performed using plate count agar and pour plate method.

- Thermotolerant coliforms: This index was considered to indicate the faecal contamination and the laboratory test was performed according to the multiple tube fermentation method which was presented in the "Standard Methods for the Examination of Water and Wastewater" (APHA, 2003).
- *Pseudomonas aeruginosa*: Routine monitoring of this indicator is recommended for public and semipublic hot tubs and natural spas by World Health Organization (WHO, 2006). In this research, *Pseudomonas aeruginosa* was detected using Millipore kit with Catalogue Number of MHA000P2P.
- *Staphylococcus aureus*: Although this indicator is not tested routinely, it is useful to investigate of water quality when health-related problems with the pool is suspected. For *Staphylococcus aureus*, Baird-Parker Agar Base was used with EY (Egg Yolk) Tellurite Enrichment as culture medium which conforms to specifications of the United States Pharmacopeia (USP).

RESULTS

The sensitivity of the species to different Nanosil concentrations at various exposure times is summarized in Table 1. The variations of microbial indicators to the residual concentrations of Nanosil are presented in Figs. 1 to 3 for the experiment which was performed in the indoor private pool. As shown in Fig. 1, there is a sharp decrease in HPC when the Nanosil residual concentration reaches to 20 mg/L and above this level it is feasible to keep the HPC at zero level.

The results of experiments which were performed in the outdoor private swimming pool are presented in Figs. 4 to 6. As shown in these figures, initial

Table 1: Sensitivity of target microorganisms to different Nanosil concentration at various exposure times

Candida albicans (ATCC No. 10231)

Exposure time (min)	Nanosil Conc. (%) 1	2	3
5	+	+	+
15	+	+	+
30	-	-	-
60	-	-	-

Aspergillus niger (ATCC No. 16404)

Exposure time (min)	Nanosil Conc. (%) 1	2	3
5	+	+	+
15	+	+	-
30	+	+	-
60	+	-	-

Pseudomonas aeruginosa(ATCC No. 9027)

Exposure time (min)	Nanosil Conc. (%) 1	2	3
5	-	-	-
15	-	-	-
30	-	-	-
60	-	-	-

Serratia marcescence (PTCC No. 1111)*

Exposure time (min)	Nanosil Conc. (%) 1	2	3
5	+	+	-
15	+	-	-
30	+	-	-
60	-	-	-

Klebsiella pneumonia (ATCC No. 10031)

Exposure time (min)	Nanosil Conc. (%) 1	2	3
5	+	-	-
15	+	-	-
30	+	-	-
60	-	-	-

Staphylococcus aureus (ATCC No. 29737)

Exposure time (min)	Nanosil Conc. (%) 1	2	3
5	+	+	-
15	+	+	-
30	+	-	-
60	+	-	-

*PTCC: Persian Type Culture Collection
+shows that microorganism after incubation period was detected;
-Shows that no microorganism could be detected after incubation period

Nanosil residual concentration tried to be kept as high as 30 mg/L to be able to meet the recommended values for all microbial indicators. Then, the residual value was changed (successively decreased and increased successively) to study the capability of Nanosil to control the microbial quality of swimming pool. As shown in Fig. 4, HPC

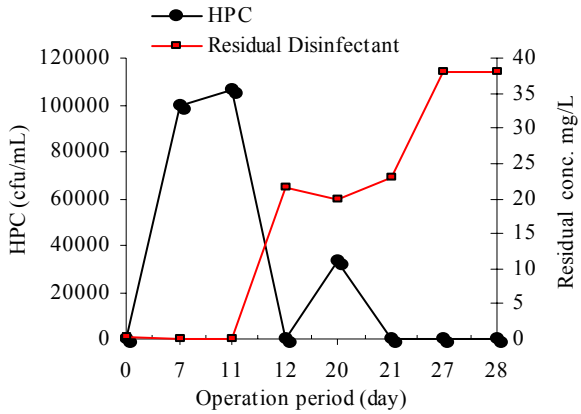

Fig. 1: Variations of HPC in different residual Nanosil concentrations during the experiment (Indoor Swimming pool)

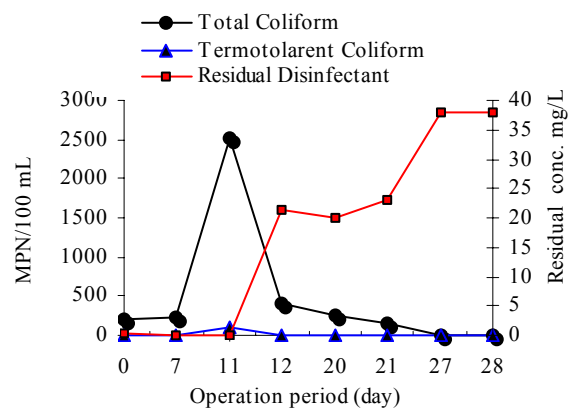

Fig. 2: Variations of total coliform and thermotolerant coliform in different residual Nanosil concentrations during the experiment (Indoor Swimming pool)

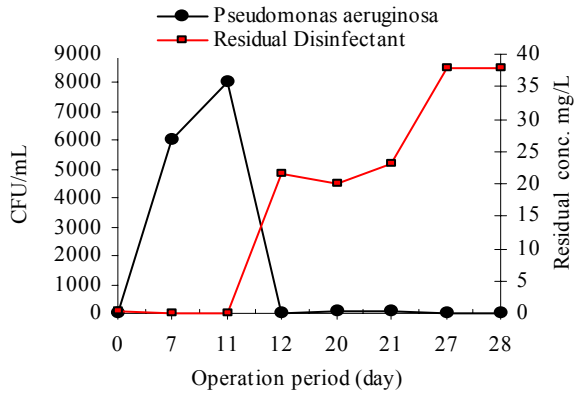

Fig. 3: Variations of *Pseudomonas aeruginosa* in different residual Nanosil concentrations during the experiment (Indoor Swimming pool)

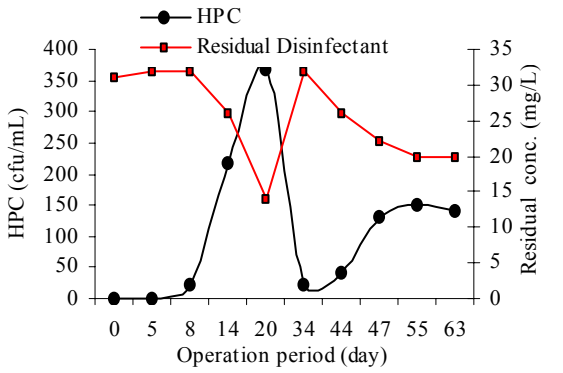

Fig. 4: Variations of HPC in different residual Nanosil concentrations during the experiment (Outdoor Swimming pool)

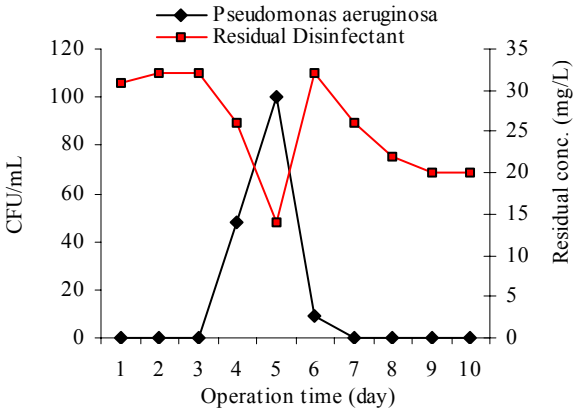

Fig. 5: Variations of *Pseudomonas aeruginosa* in different residual Nanosil concentrations during the experiment (Outdoor Swimming pool)

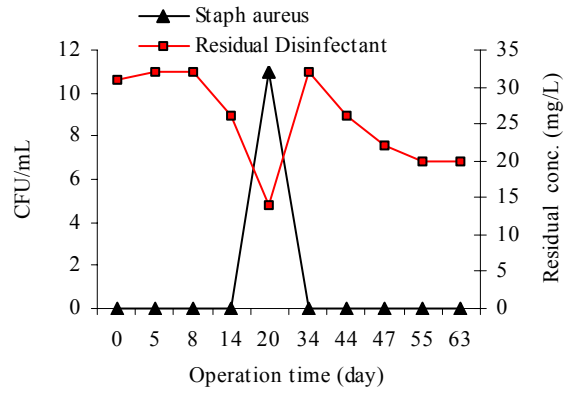

Fig. 6: Variations of *Staphylococcus aureus* in different residual Nanosil concentrations during the experiment (Outdoor Swimming pool)

value increased when the residual value of Nanosil decreased from 30 mg/L. But, HPC decreased to zero when the residual value was increased to its previous concentration (30 mg/L). Furthermore, due to second reduction of Nanosil residual level from 30 mg/L to 20 mg/L, the HPC values increased; but were still less than 200 CFU/mL which is recommended by WHO. As illustrated in Figs. 5 and 6, the levels of *Pseudomonas aeruginosa* and *Staphylococcus aureus* increased as the Nanosil residual was reduced from 30 mg/L to less than 15 mg/L. But, the value of both mentioned indicators reached to the zero level as the Nanosil residual increased to 20 mg/L. It should be noted that there was no significant increase in thermotolerant coliforms following the reduction of Nanosil residual dose and applied dose was enough to meet the WHO recommendation for thermotolerant coliforms. The results of experiments which were performed in the outdoor public swimming pool are presented in Table 2. The results showed that in the Nanosil residual of 20 mg/L and more all the mentioned microbial indicators were in the acceptable range as WHO presented.

Table 2: Variations of microbial indicators in the public swimming pool during the experiment

Days	Turbidity (NTU)	ORP (mV)	Residual Disinfectant (mg/L)	HPC (CFU/mL)	Termotolarent Coliform (MPN/100 mL)	Pseudomonas (CFU/100 mL)	Staph (CFU/mL)
0	0.25	193	24	0	0	0	0
1	0.23	189	20	0	0	0	0
2	0.29	195	16	9	0	0	0
3	0.22	204	14	13	0	0	0
4	0.25	180	15	5	0	4	4
5	0.15	157	14	8	0	297	35
6	0.21	150	8	30000	0	TNTC	51
7	0.23	97	4	61000	0	TNTC	160

pH Variation: 8.1-8.4 and temperature variation ^{o}C: 33-35.5
TNTC: Too numerous to count

DISCUSSION

As it is shown in Table 1, *Pseudomonas aeruginosa* (ATCC No. 9027) showed the maximum sensitivity to Nanosil among the bacterial species. *Klebsiella pneumoniae* (ATCC No. 10031) proved to be the second sensitive microorganism in the experiment. *Staphylococcus aureus* (ATCC No. 29737) was the most resistant bacteria in the study. The results showed that Nanosil concentration of 2% (20000 mg/L) is enough to kill all target bacteria in 15 minutes. According to the more negative results in Table 1, which is more extended for the two tested fungal species (*Candida albicans* (ATCC No. 10231) and *Aspergillus niger* (ATCC No. 16404)), it is concluded that bacterial species are likely to be more sensitive than fungal species to Nanosil exposure. *Aspergillus niger* (ATCC No. 16404) was proved to be the most resistant microorganism among the all microorganisms which were tested in this study. According to the effective concentration and time period required to destroy the *Aspergillus niger* (ATCC No. 16404), it is reasonable to apply the Nanosil concentration of equal or above 3% (30000 mg/L) for contact time

of 30 min or more for practical disinfection in swimming pools environment.

It is recommended by World Health Organization that operational levels of HPC should be less than 200 CFU/mL (WHO, 2006). Therefore, keeping the residual level of Nanosil in swimming pool water at 20 mg/L could effectively meet the recommended level. As shown in Fig. 2, there is a significant decrease in total coliform with the increase of Nanosil residual concentration. Although total coliform is not used these days as the microbial indicator, the trend shows that increasing residual concentration would lead to the effective loss of these bacteria. As illustrated in Fig. 2, there was no significant change in thermotolerant coliforms during the study. Operational levels which is recommended by World Health Organization is less than 1/100 mL which did not appeared to be exceeded by using Nanosil at residual levels of 20 mg/L and more. As illustrated in Fig. 3, by increasing the Nanosil residual concentration to 20 mg/L, the *Pseudomonas* count decreased sharply. So maintenance of 20 mg/L Nanosil as residual is practically enough to keep the Pseudomonas count at zero level. It should be noted that according to World Health Organization Operational, Pseudomonas levels should be less than 1/100 mL for continuously disinfected pools. The results of experiments in public swimming pool showed that in the Nanosil residual of 20 mg/L and more, all the mentioned microbial indicators were in the acceptable range as WHO presented.

According to the findings of this study, it is possible to use Nanosil for disinfection purposes in swimming pools. it is recommended to apply the Nanosil concentration of ⩾3% (30000 mg/L) for contact time of 30 min or more for practical disinfection in swimming pools environment. The acceptable microbial quality is also achievable if the Nanosil residual concentration would be as high as 20 mg/L in swimming pool water. It is recommended by World Health Organization that operational levels of HPC should be less than 200 CFU/mL (WHO, 2006). Therefore, keeping the residual level of Nanosil in swimming pool water at 20 mg/L could effectively meet the recommended level. As hydrogen peroxide is not toxicant for algae, It should be emphasized that its application, especially in outdoor pools, should be combined with an appropriate algal control agent when it is considered as an alternative to chlorine compound. Although Borgmann (2003) stated that "products based on hydrogen peroxide, with or without silver ions, are from a microbiological point of view no real alternative to chlorine disinfectant in swimming pools", the results of this study and the recent publication of World Health Organization in which the application of hydrogen peroxide for small swimming pools is authorized (WHO, 2006) are controversial to the results of mentioned study.

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