Mansoura Journal of Biology Vol. 35 (2) December, 2008.

MICROBIAL CONTAMINATION OF HOUSEHOLD DRINKING WATER FILTERS AND EFFECT OF SOME PHYSICAL CONDITIONS ON ISOLATED FUNGI

Hoda H. AL-Homeidan
Botany Department, College of Education - Riyadh University for Girls
P.O. Box 27104, Riyadh 11417 Saudi Arabia

ABSTRACT

Fifty samples of drinking water were collected from filters of different houses during June and July 2006, in Riyadh City, Saudi Arabia. No coliform were isolated on endo agar medium from any samples while other bacterial counts ranged between 12-72 cfu per 100 ml, after 24 hours and 300 cfu/100ml after 48 hours of incubation at 37°C on nutrient agar. Nine samples yielded fungi on Czabeks Dox medium at 25°C. The effect of some physical factors *viz*: incubation temperatures, boiling for different times, pH and microwave on growth of fungal isolates was studied. The results revealed that 30°C was found to be optimum temperature for *Paecilomyces carneus* and *Penicillium* spp. while 25°C was optimum temperature for *Penicillium jensenii*. *P. carneus* and *P. jensenii* were unable to grow at 0°C, -10°C and -17°C. While *Penicillium* spp. Shows a very weak growth at these temperatures. A ten minutes boiling checks the growth of *P. carneus* and *Penicillium* spp. While 5 minutes for *P. jensenii*. The optimum pH 7.5 for *P. carneus* and *Penicillium* spp. while 6.5 for *P. jensenii*. The microwave treatments of 1sec reduced the growth: *P. carneus* 57.69%, *Penicillium* spp. 47.62% and. *P. jensenii* 55.56%, the growth were completely stopped at 4, 5 and 3 sec. respectively

INTRODUCTION

Fungi, including yeasts and filamentous species or moulds have been found in potable water and on the inner surfaces of distribution system pipes. Either they survive water treatment or they enter the

system after treatment and remain viable. The most frequently isolated fungal genera from household drinking water were reported to be *Penicillium*, *Sporocybe*, *Acremonium*, and *Paecilomyces* **[Nagy & Olson (1982 &1985)]**. Filamentous fungi were detected in 104 out of 126 (82·5%) water samples, 95·2% of community water samples and 76·2% of hospital water samples. Prevailing genera were *Penicillium* spp., and *Aspergillus* spp. **[Arvanitidou *et al.*, (1999)]**. In order to assess the dissemination of hygienically relevant fungi via the public drinking water distribution system, a 12-month survey was performed on groundwater-derived drinking water from 29 water supplies in Germany. No correlation with standard hygiene indicators, such as *E. coli* or other coliform bacteria was observed. Common opportunistic and allergenic *Aspergillus* species were encountered only rarely. A limited number of species of *Acremonium*, *Exophiala*, *Penicillium* and particularly *Phialophora* dominated the fungal flora **[Gottlich *et al.*, (2002)]**. To evaluate the efficacy of preservation of fungi in distilled water for 12 months. 43 species of fungi were maintained in glass flasks with sterile distilled water. These fungi showed growth after 12 month of preservation **[Diogo *et al.*, (2005)]**. Water samples collected from one hundred different points around the water distribution system in different sections of the hospital, yielded, sixteen species of fungi, the most frequent being *Penicillium* spp. (24 %), *Aspergillus* spp. (8 %) and *Acremonium* spp. (5 %) **[Hapcioglu *et al.*, (2005)]**. A. number of 94 mold species belonging to 30 genera were isolated from Norwegian water supply and drinking water **[Hageskal *et al.*, (2006)]**. The mycobiota was dominated by species of *Penicillium*, *Trichoderma*, and *Aspergillus*, with some of them occurring throughout the drinking water system. Several of the same species isolated from water may have the potential to cause allergic reactions or disease in humans. Other species are common contaminants of food and beverages, and some may cause unwanted changes in the taste, odour or smell of water **[Hageskal *et al.*, (2006)]**. Health problems are possible, originating from mycotoxins, animal pathogens and allergies. These fungi are associated with the production of the mycotoxin and the odour secondary metabolite geosmin **[Ana *et al.*, (2006)]**. Microwave sterilization and pasteurization had been used for stabilization of different fruit juices. Abu Tava Khan and Ostapenkov (1995) have demonstrated the influence of the microwave treatment on moulds (*Aspergillus niger*, *Penicillium glaucum*) and yeasts (*Saccharomyces cerevisiae*) at different

temperatures (55-80 DEG C). Microwave-treatment at 90 DEG C for 30-40 s provided excellent chemical, biological and sensory qualities for all experimental juices during 6-month storage at 12-35 DEG C. [**Abu-Tavakhin & Ostapenkov (1995)**]. Bacteria were reported to be fully destroyed within 20 seconds and all the fungi within 1 second of microwave treatment [**Park *et al.*, (2003)**].

MATERIALS AND METHODS

Water sampling

Samples of drinking water were collected during June and July 2006, from household water filters from different houses in Riyadh city. Sterile sampling bottles (500 ml) were used to collect water samples. Taps of the filters were cleaned with 70% ethyl alcohol, then the water was allowed to run for at least 3- 4 minutes, and the tap did not touch the bottle before and during sampling. Saples transport to the laboratory as soon as possible. A total of 50 samples were Colleeted from different houses.

Microbiological analysis

All the filtered water samples were cultured on three media (endo agar , nutrient agar and Czabeks-Dox agar). The presence or absence of coliform bacteria in drinking water sources were detected using the endo agar medium, the plates were incubated at 37°C for 24 h and 48 h. Nutrient agar was used for enumeration of the total viable bacterial count expressed as colony forming units (cfu /l) [**Ana et al, (2006)**]. The Czabeks Dox agar plates were incubated at 25°C for 1 week for isolation of fungi.

Identification of fungi

The fungal isolates were identified by Prof. Dr. Youssuf Gherbawy, Department of Biological Science, Taif University.

Effect of incubation temperatures on dry weight of filtered water fungi:

The effect of incubation temperature on the dry biomass weight of fungi isolated from filtered water was studied using ten different incubation temperatures namely : -17, -10, zero, 5, 10, 15, 25, 30, 40, and 45°C). The fungal isolates were cultured in 250 ml-capacity Erlenmeyer

flasks containing 50 ml of Czapeks-Dox liquid medium for 5 days, five flasks were used for each treatment. The cultures were filtered using weighed filter paper, then dried in oven at 60°C for 24 h, and the dry weights of mycelia were determined.

Effect of boiling on dry weight of water fungi.

The fungal isolates were inoculated, separately, in 50 ml of Czabeks Dox medium (in 250 ml- Erlenmeyer flasks), 21 flasks were used for each fungus, of which 18 flasks were maintained in a boiling water bath (at 100°C); after 1, 5, 10, 15, 30, and 60 minutes3 flaqsks were removed for each time, and leave 3 flasks without heating as control test. All flasks incubated at 25°C for 5 days, then filtered, dried and the mycelial dry weights were determined.

Effect of hydrogen ion concentration on dry weight of water fungi:

The fungal isolates were grown in 250ml-Erlenmeyer flasks containing 50 ml Czabeks-Dox medium with hydrogen ion concentrations at pH 3, 4.7, 5.5, 6.5, 7.5, 8.1, 9.2. The culture flasks were incubated at 25°C for 5 days, and the dry weight of mycelia were determined.

Effect of microwave treatments on growth of filtered water fungi:

The inoculated flasks were exposed for house microwave treatments at 1, 2, 3, 4, 5, 6, 10, 15, 20, 30 sec., then incubated at 25°c for 5 days, filtered, and the dry weight of mycelia were determined as previously described.

RESULTS AND DISCUSSION

The results in Table (1) shows thatbial population of filtered water samples Coliform bacteria shows no growth in endo agar for all samples incubated at 24 h and 48 h. [**Yamaguchi *et al.*, (2007)**] found coliform in bottled mineral water. The bacterial colonies were grew in nutrient agar with seven samples. Bacterial counts ranged between 12-72 cfu per 100 ml, after 24 hours from incubation at 37°C. While five of these seven samples were contained over 300 colonies in 100 ml after 48 hours and another two samples contained 74 and 82 cfu/ 100 ml. While bacteria were absent in 43 samples. Table (1) further shows that a relatively low number of fungal colonies were developed on only

Czabeks-Dox agar from nine samples of filtered water after incubation for 96h at 25°C. Hapcioglu et al (2005) have shown that more than one type of fungi were present in 13 out of 19 filtered water samples; in the remaining 6 samples, no fungi or bacteria were detected. The fungal species recovered were *Paecilomyces carneus, Penicillium jensenii* and *Penicillium* spp. **Nagy and Olson (1982)** found that *Penicillium, Sporocybe, Acremonium,* and *Paecilomyces* were the most frequently occurring genera in drinking water distribution system. While Arvanitidou, *et al.*, (1999) found that *Penicillium* spp. and *Aspergillus* spp. were the most prevailing genera isolated from hospital water samples . The most frequently isolated fungi from drinking water was *Penicillium* (40.6%) **[Ana et al., (2006)]**. Steven Doggett (2000) found that densities of filamentous fungi in municipal water distribution system ranged from 4.0 to 25.2 cfu cm^{-2}. In the present study, all species isolated were from filamentous fungi.

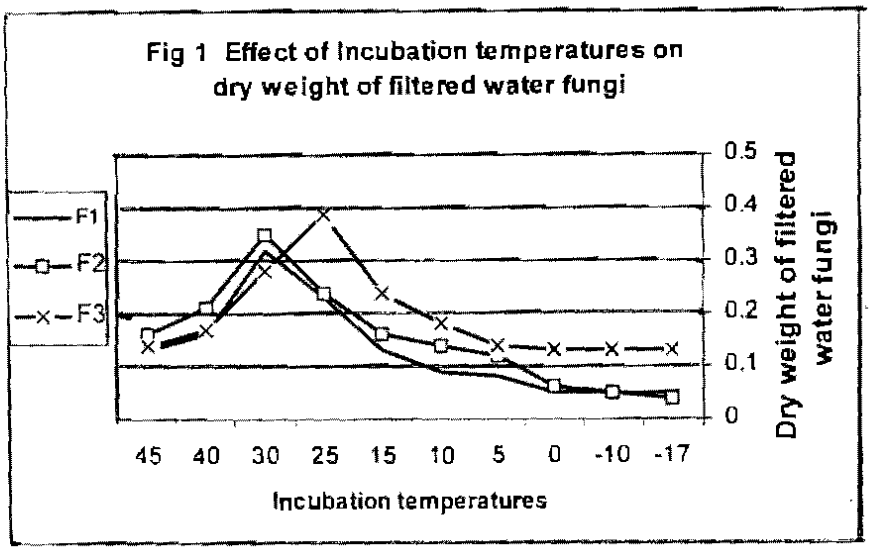
Table (1): Microbial population of filtered water samples in three medium after incubation in 37°c at 24-48 h for bacteria and in 25°c at 96 h for fungi:

Filtered water Sample no.	Bacterial colony on Endo-Agar Medium after 24 h cfu/ml	Bacterial colony on Endo-Agar Medium after 48 h cfu/ml	Bacterial colony on Nutrient agar medium after 24 h cfu/ml	Bacterial colony on Nutrient agar medium after 48 h cfu/ml	Fungal colony on Czapeks-Dox agar after 96 h
1	-	-	72	*	2
2	-	-	72	*	4
3	-	-	63	*	3
4	-	-	51	*	2
5	-	-	32	*	3
6	-	-	30	*	4
7	-	-	23	74	3
8	-	-	12	82	4
9	-	-	-	-	5
10-50	-	-	-	-	-

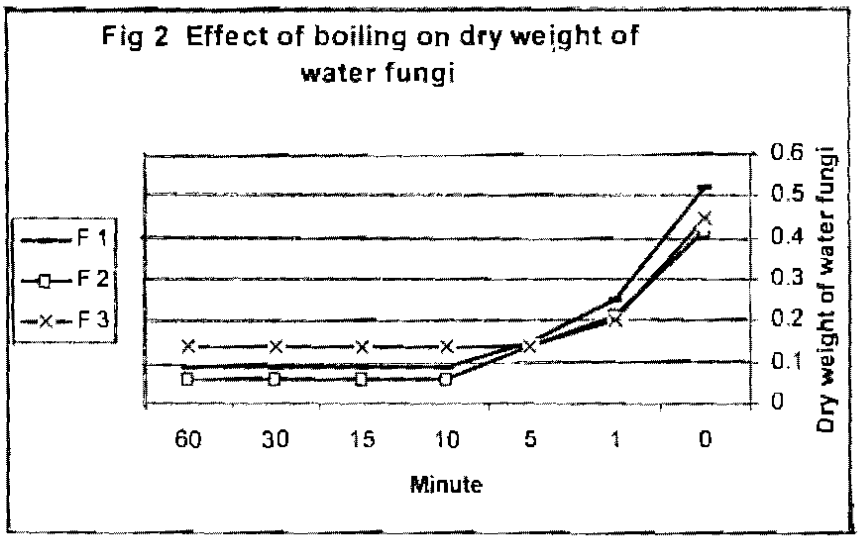
* The colony count of bacteria was over 300 colony/ 1ml.

As regard the effect of incubation temperatures on dry weight of the fungal species isolated from filtered water, the results presented in Fig (1) show that no mycelial growth was produced by *Paecilomyces carneus* at -17, -10 and zero°C; at 5°C very weak growth was observed, and the mycelium growth was increased gradually by rising the temperature up to 30°C ,where highest biomass growth was detected, then decreased at 40 and 45°C, indicating that the optimum temperature is 30°C. The growth of *Penicillium jensenii* was also inhibited at -17, -10 and zero°C, but showed a progressive increase as the temperature was increased up to 25°C where the dry weight of mycelium was culminated, then decreased upon rising the incubation temperature to 40-45 °C, thus, the optimum temperature for *P. jensenii* is 25°C. The growth pattern of *Penicillium* sp. appeared to go parallel with that of *Paecilomyces carneus*, it failed to grow at very low temperature, and its optimum temperature was 30°C. None of the three fungal species isolated throughout this study could grow at very low temperatures (cryophiles), their optimal temperatures being in the range of 25 to 30°C High incubation temperatures do not appear fungus from thermophiles. This is in agreement with the results of Kelley *et al.* (2003) who found that the fungal species isolated from water distribution system seemed to grow well at temperature range of 10-30°C.

The results represented in Fig.(2) shows that the percentage of *P. carneus* growth after exposed to boiling for 1 and 5 minutes was 48.08% and 28.84% (of the control value), respectively, and the fungus was unable to grow when the exposed to boiling for10, 15, 30 minutes. The corresponding growth magnitudes of *Penicillium* spp were 50% and 33.33% *and* it was also unable to survive boiling for 10, 15, 30 minutes. The percentage of growth of *P. jensenii* was 44.44% after exposed to boil 1 minute, and the growth was stoped when exposed to boiling for 5, 10, 15, 30, 60 minutes.



F1= *Paecilomyces carneus*, F2= *Penicillium* sp., F3= *Penicillium jensenii*



F1= *Paecilomyces carneus* F2= *Penicillium* spp. F3= *Penicillium jensenii*

The effect of hydrogen ion concentration on dry weight of water fungi are shows in Fig (3). *P. carneus* growth was weak in pH 3, mycelium growth was increased gradually in pH 4.7, 5.5, 6.5 while the optimum pH value was 7.5, then the mycelium growth was decreased in pH 8.1 and 9.2. Mycelium growth of *Penicillium* spp was very weak in pH 3, 4.7, 5.5, 6.5 and the optimum pH value was 7.5. Whereas *P. jensenii* growth was weak at pH 3, 4.7, reached maximum at pH 5.5 (optimum pH value), then decreased gradually at pH 6.5, 7.5, 8.1 and 9.2.

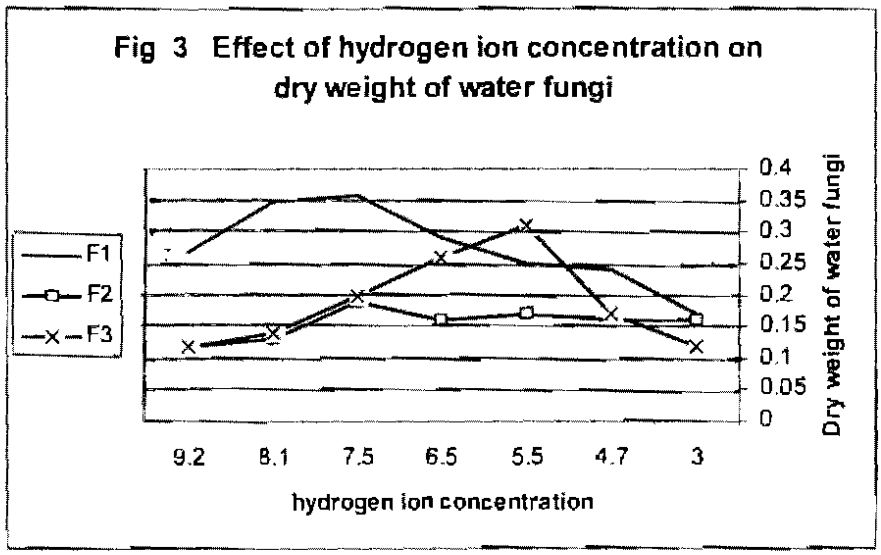
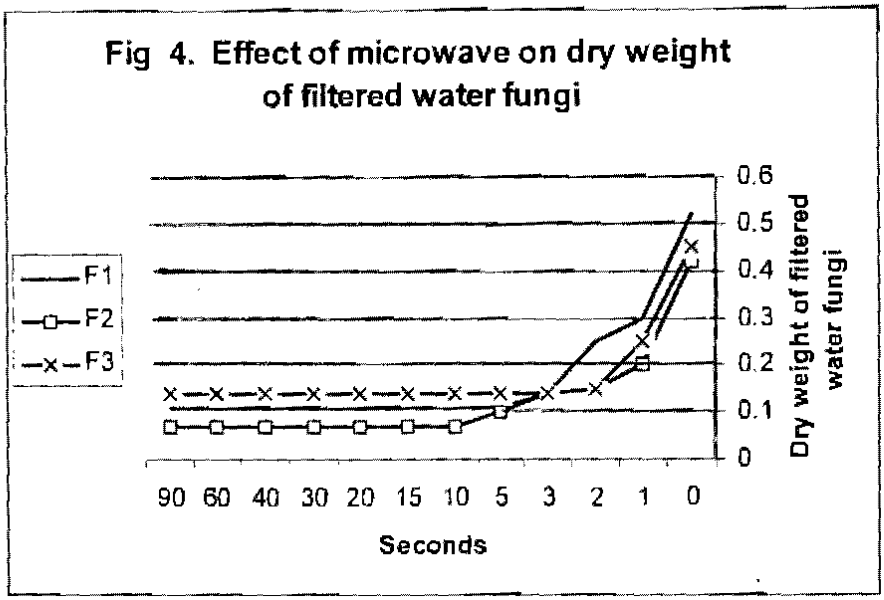


Fig 3 Effect of hydrogen ion concentration on dry weight of water fungi

F1= *Paecilomyces carneus*, F2= *Penicillium* sp., F3= *Penicillium jensenii*

Fig (4) demonstrates the effect of microwave treatments on growth of filtered water fungi, It is apparent that there was drastic decrease in growth of *P. carneus* following exposure to house microwave treatments for 1, 2 and 3 sec as compared to control treatment; the percentage of growth was 57.69%, 48.08% and 26.6% respectively. Prolongation of the exposure time to 4, 5, 6, 10, 15, 20 and 30 sec, the fungus was killed. While in case of *Penicillium* sp. the percentages of growth obtained following exposure to microwave for 1, 2, 3 and 4 sec were 47.62, 35.14, 33.33 and 23.80, respectively, the growth was cited as a consequence of exposure to microwave for 5 sec, whereas the growth of *P. jensenii* was stopped after 3 sec of exposure.



F1= *Paecilomyces carneus* F2= *Penicillium* spp. F3= *Penicillium jensenii*

Waterborne fungi are likely associated with taste and odor problems, contamination in food and beverage preparation, and a variety of health-related effects [**Bays *et al.*, (1970); Geldrich (1995) and Seeliger (1975)**]. Some fungi were dominated in water system, and some were encountered throughout the drinking water system and constituted a residual flora. In addition, soft deposits in pipelines were key sites for fungi [**Zacheus *et al.*, (2001)**]. The fungi *Penicillium* sp. and many others are toxin and antibiotic producers. For example, *P. expansum* is well known to produce patulin. *P. brevicompactum* is suspeeted of producing patulin [**Paterson (2004)**]. Fungi could be used as a biological/chemical weapons against harmful microorganisms contaminating the water supply [**Paterson & Lima (2005)**].

REFERENCES

Abu-Tavakhin and Ostapenkov A.M. (1995): Microwave sterilization of fruit juices. J. Technology, Engineering. 5: 19-20.

Ana, Goncaives, Russell, R.; Paterson, M. and Nelson, L. (2006): Survey and significance of filamentous fungi from tap water. j. hyg. environ. Health. 209: 3 257-264.

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

Bays, L.R.; Burman, N. P., and Lewis, W.M.; (1970): Taste and odour in water supplies in Great Britain: a survey of the present position and problems for the future. Water Treat Exam.19: 136–160.

Diogo, H. C.; Sarpieri, A. and Pires, M.C. (2005): Fungi preservation in distilled water. An. Bras. Dermatol. 80: (6) 591- 594.

Geldrich, E. E. **(1995)**: Microbial quality of water supply in distribution systems. New York, N.Y: Lewis Publishers.

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

Hageskal, G.; Knutsen, A.K. ; Peter Gaustad, P.; Hoog, G.S. and Skaar, I. (2006): Diversity and Significance of Mold Species in Norwegian Drinking Water. Appl Environ Microbiol. 72: (12) 7586–7593.

Hapcioglu, B.; Yegenoglu, Y.; Erturan, Z.; Nakipoglu, Y. and Issever, H. (2005): Heterotrophic Bacteria and Filamentous Fungi Isolated from a Hospital Water Distribution System. Indoor and Built Environment. 14: (6) 487-493.

Kelley, J., Kinsey, G., Paterson, R., Brayford, D., Pitchers, R., Rossmore, H., (2003): Identification and Control of Fungi in Distribution Systems. Awwa Research Foundation and American Water Works Association, Denver.

Nagy, L.A. and Olson, B.H. (1982): The occurrence of filamentous fungi in drinking water distribution systems. Can J Microbiol. 28: (6) 667-71.

Park, B.J.; Lee D.H. and Park, J.C. (2003): Sterilization using a microwave-induced argon plasma system at atmospheric pressure. Physics of Plasmas. 10: (11) 4539-4544.

Paterson, R.R.M. (2004): The isoepoxydon dehydrogenase gene of patulin biosynthesi s in cultures and secondary metabolites as candidate PCR inhibitors. Mycol. Res. 108: 1431–1437.

Paterson, R.R.M., Lima, N. (2005): Fungi in drinking water. In: Lehr. J.H., Keeley, J. (Eds.), Water Encyclopedia: The Water Quality and Resource Development. Wiley, New York.

Seeliger, H. P. R. (1975): Increasing spectrum of opportunistic infections. In: Chick E W, Balows A. Farcolow M L. , Springfield, Ill: Charles C Thomas.

Steven Doggett, M. (2000): Characterization of Fungal Biofilms within a Municipal Water Distribution System Appl Environ Microbiol. 66: (3) 1249–1251.

Yamaguchi, M. U.; Yamaguchi, R. C. P.; Yamada-Ogatta, S. F.; Nakamura, C. V.; Ueda-Nakamura, T.; Filho, B. P. D. (2007): Yeasts and filamentous fungi in bottled mineral water and tap water from municipal supplies. Braz. arch. biol. technol. 50: 1 pp. 1-9.

Zacheus, O.M., Lehtola, M.J., Korhonen, L.K., Martikainen, P.J. (2001): Soft deposits, the key site for microbial growth in drinking water distribution networks. Water Res. 35: 1757–1765.

التلوث الميكروبى لمياه الشرب المصفاة فى مرشحات المنازل وتأثير بعض الظروف الفسيولوجية على الفطريات المعزولة منها

هدى الحميدان

قسم النبات – كلية التربية– جامعة الرياض للبنات – المملكة العربية السعودية

جمعت خمسين عينة من ماء الشرب من صفايات المياه من منازل مختلفة فى يونيه ويوليو سنة٢٠٠٦ ميلادية من مدينة الرياض، المملكة العربية السعودية. أتضح من النتائج عدم وجود بكتيريا القولون فى أى من عينات مياه الشرب والتى عزلت على بيئة أندو آجار من جميع العينات. بينما وجد أن معدل الأعداد البكتيرية الأخرى يتراوح ما بين ١٢–٧٢ وحدة تكوين المستعمرات/ ١٠٠مل، بعد تحضينها لمدة ٢٤ ساعة و ٣٠٠ مستعمرة بعد تحضينها لمدة ٤٨ ساعة على بيئة آجار مغذى عند ٣٧°م. كما ظهرت بعض الفطريات في تسع عينات من الماء المصفى عند زراعتها على بيئة تشابك دوكس عند درجة حرارة٢٥°م. اختبرت عشر درجات تحضين على الوزن الجاف للفطريات ووجد أن الدرجة المثلى للتحضين كانت ٣٠°م لفطر بسيلومايسس كارنيس وفطر بنسليوم بينما كانت ٢٥° م لفطر بنسليوم جنسنى، ولم يتمكن فطرى بسيلومايسس كارنيس وبنسليوم جنسنى من النمو عند الصفر المئوى ، –١٠ و–١٧ أما فطر بنسليوم فقد نما نمو ضعيف جدا. عرضت الفطريات للغليان لفترات مختلفة ووجد أن بسيلومايسس كارنيس وفطر بنسليوم لا يمكنهما النمو بعد التعرض للغليان لمدة ١٠ دقائق بينما بنسليوم جنسنى لم يتمكن من النمو بعد ٥ دقائق من التعرض للغليان. كانت الدرجة المثلى لتركيز أيون الهيدروجين لفطر بسيلومايسس كارنيس وفطر بنسليوم هى ٧,٥ بينما كانت الدرجة المثلى ٥,٥ لفطر بنسليوم جنسنى. استخدام الميكروويف المنزلى لمدة ثانية واحدة قللت معدلات النمو على النحو التالى: بسيلومايسس كارنيس بنسبة ٥٧,٦٩ %، بنسليوم بنسبة ٤٧,٦٢ % وبنسليوم جنسنى بنسبة ٥٥,٥٦%، وتوقف نمو كل من الفطريات الثلاثة تماما بعد ٤، ٥ و٣ ثوانى على التوالى.