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ORIGINAL ARTICLE

Occurrence of moulds in drinking water

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Abstract

Aims: In order to determine the occurrence of filamentous fungi in public drinking water systems in Norway, water from 14 water supply networks from all over the country was sampled and analysed. Networks with both ground and surface water sources were included in this study.
Methods and Results: During a one-year period, 273 water samples were collected. Frequencies of fungi in samples from raw water, treated water and from home and hospital installations were determined on the basis of incubation of 100 ml membrane-filtered samples on dichloran 18% glycerol agar media. Filamentous fungi were recovered from 62% of all samples. In ground water 42·3% of the samples were positive for mould growth, while surface waters yielded 69·7% positive samples.
Conclusions: The risk to recover moulds from surface water is three times higher compared with ground water. It is more likely to detect moulds in cold waters and showers than in hot waters.
Significance and Impact of the Study: By analysing the water reaching the consumers, the results reported in present study indicate that filamentous fungi in drinking water is not negligible, and that moulds should be considered as part of the microbiological analysis parameters in drinking water.

Introduction

A high proportion of the drinking water supply in Norway is derived from surface water. While 90% of the population get their water from surface lakes and rivers, only 10% get their water from ground water sources. Raw water of varying qualities is used. Surface water in Norway is generally considered to be sufficiently clean for the purpose of drinking. Nevertheless, 12% of the water works reporting bacteriological quality in 2002 failed to satisfy the criteria of bacterial content in the water (Einan *et al.* 2004). The treatment and disinfection procedures differ from one plant to the next; approximately 25% have no water treatment at all. UV-radiation is the most widely used treatment method. Some of the larger plants apply chemical treatment, some use sand filters, but many water works only treat the water with straining and disinfection, mostly applying chlorine. Ground water is usually distributed without disinfection (Einan *et al.* 2004).

The quality of drinking water is regularly expressed in terms of microbiological parameters, usually in terms of bacteria present in a given volume of water (Anon 2001). In recent years viruses and parasites have also been more or less accepted as quality parameters. Occurrence of filamentous fungi in drinking water has been reported in several studies world wide, although the frequencies of mould recovery are most variable, ranging from 82·5% to 17·6% (Rosenzweig *et al.* 1986; West 1986; Franková and Horecka 1995; Arvanitidou *et al.* 1999). Hinzelin and Block (1985) reported mould contents of 81% in chlorinated water from France, while Göttlich *et al.* (2002) only detected 7·5% fungal positive samples from ground water in Germany. In a study comparing occurrence of filamentous fungi in distribution systems in ground water and surface water in California, no significant differences between the two categories could be established, even though the mean number of colony forming units (CFU) in surface water

samples were nearly twice the numbers in ground samples (Nagy and Olson 1982). Franková (1993) identified mould colonies in all of the surface water samples analysed in Slovakia, while the ground water revealed 40% positive samples. From an investigation in Finland (Zacheus and Martikainen 1995), mesophilic fungi were detected in an average of 32 CFU l^{-1} in water. The numbers of fungi were higher in cold waters than in hot waters. It was also indicated, although not established, that the amount of moulds was higher in surface waters than in ground derived waters.

In Norway, little attention has been paid to the occurrence of filamentous fungi in water supply networks. In a study on heterotrophic micro-organisms in distribution systems for drinking water in Oslo, Ormerod (1987) found that all the waters investigated contained microfungi in much higher quantities than those reported from Swedish waterworks at that time (Åkerstrand 1984). Warris *et al.* (2001a) investigated occurrence of filamentous fungi in water sampled from taps, showers and main pipe in a paediatric bone marrow transplantation unit at Rikshospitalet University Hospital, Norway. The results revealed 94% mould positive samples from inside of the hospital, at the intake reservoir fungi were recovered from all samples. In another study Warris *et al.* (2002) concludes that ground water in Nijmegen, the Netherlands, does not contain moulds, and suggest that type of natural reservoir that serves as water source has a significant impact on recovery of filamentous fungi from the water system installations.

There are few guidelines as to what is considered as normal or acceptable levels of moulds recovered from water. This will probably be the most important question for the consumers. Limited information is given in literature regarding this issue. From a mould situation case in Råbacka, Sweden (Åslund 1984), one of the concluding comments was that mould quantities of 1000 CFU l^{-1} in water can be compared with those found in air in certain working environments (10^6 CFU m^{-3}), which is known to give immune response by exposure. Pursuant to the Swedish drinking water regulations (Anon 2003), the criteria of microfungi in water before esthetical and technical impact are 100 CFU per 100 ml sample. The Norwegian drinking water regulations (Anon 2001) does not include microfungi.

Except for the two former studies, it is largely unknown what amounts of filamentous fungi are resident and capable to survive water treatment and contaminate the drinking water in Norway. Information on mould behaviour in the network is limited, and guidelines regarding acceptable mould values do not exist. There is no routine in analysing water for moulds, even though reported cases of mould contamination in water distribution systems are emerging. Thus, increased knowledge on fungi throughout the water system, from source to consumer is needed; both in ground and surface water-derived supplies. The aim of this study was to determine the occurrence and distribution of filamentous fungi in drinking water systems in Norway, and also to identify possible factors influencing the level of moulds reaching the consumers.

Materials and methods

Water sampling

The water sampling included 14 water supply networks from all over Norway; three from the Northern part, four from the West-coast, three from the East-inland region and four from the South-east region. Ten of these had surface water source and four had ground water source. A total of 273 water samples were collected from raw water and treated water at the water works, and from hot and cold taps and showers in homes and hospitals. Half of all the water supplies were connected to hospitals, therefore a reduced amount of water samples were collected from this location compared with private homes. The sampling was carried out in December 2002, June and September 2003 and repeated three times for each of the 14 water supplies, so that water from the same location had three repeated measures. All together 91 samples were gathered per repetition. The sampling was performed by employees at the water works. All water samples were collected in sterile 500 ml polyethylene bottles (Sterilin®, Bibby Sterilin, Staffordshire, UK), containing sodium thiosulfate (120 mg l^{-1}) for residual chlorine neutralization, and kept in cool conditions during transportation and storage. The analysis was commenced within 24 h after sampling.

Isolation of moulds

The isolation procedure was based on a Norwegian standard method for analysis of microfungi in water (Anon 1991), with some modifications regarding use of media and incubation temperature and length. Isolation of fungi was preformed by use of a membrane filtration apparatus (Microfil® Filtration System, Millipore, Billerica, MA, USA). A volume of 100 ml of the sample was filtrated through sterile 0·45 μm cellulose filters (Millipore). The filters were placed directly on dichloran 18% glycerol agar (DG18) plates (Hocking and Pitt 1980). The plates were incubated at 20 ± 1°C for up to 2 weeks, and examined weekly. The number of colonies on the agar plates was expressed as number of CFU per 100 ml water sample.

Statistical analysis

The frequencies and distributions of mould in the samples were calculated using Microsoft® Excel spreadsheet software for Windows®XP. The results from the three repeated samplings at the same locations are likely to be correlated, and therefore a repeated measures analysis was performed using PROC GENMOD in the SAS-PC System® Version 9·1 for Windows with Enterprise Guide 3·0 (SAS Institute Inc., 2004). The binary outcome variable was mould recovery or not in each sample, and the independent variables were water supply (14 levels), origin source (surface water/ground water), building (private home/hospital), sampling location (hot tap/shower/cold tap), raw water quality (mould positive/mould negative). All the independent variables were expressed as dummy variables, and taken into the models as fixed effects. The time of sampling was the repeated factor and an exchangeable symmetry structure within time was chosen. The elimination criterion was the likelihood-ratio test (LRT), a *P*-value of 0·1 was used as the level for exclusion from the model. The two-way interaction term was also tested for significance. The odds ratios were estimated for all levels of the significant independent variables in the final model.

Results

Frequencies of moulds

Of the total water samples examined, filamentous fungi were recovered from 169 samples (Table 1). Mean CFU were 9 per 100 ml water for all the positive samples. Moulds were recovered from 69·7% of the surface water samples, while 42% of the ground water samples were positive for fungi, although the total mean CFU per 100 ml for the positive samples were 9·5 and 8·4 in surface and ground water respectively, thus distinguished by only 1·1 colonies. Highest mould recovery was observed in hospital cold taps with over 90% of the samples being positive. Lowest mould recovery was observed in the hot taps.

Table 1 Moulds recovered from water samples

Location	Total number of samples	Positive samples (%)	Mean* CFU per 100 ml	Min–Max† CFU per 100 ml
Raw water	42	81	15	2–36
Treated water	42	69	9	1–31
Private home				
Cold tap	42	71	7	1–28
Hot tap	42	21	2	1–5
Shower	42	69	10	1–100
Hospital				
Cold tap	21	90·5	9	1–28
Hot tap	21	23·8	6	1–16
Shower	21	66·7	6	1–13

*Mean colony forming units (CFU) per 100 ml expressed as total number of colony forming units divided by the number of positive samples.

†Min–Max colony forming units (CFU) per 100 ml expressed as minimum and maximum number of colonies recovered from the positive samples.

Distribution of fungi

The distributions of fungi at the different sampling locations (Fig. 1) illustrated very different results for samples derived from surface water compared with ground water. The raw water from surface origin had 100% of the samples positive and had higher mould numbers than the samples from treated waters and the network installations. Only one-third of the ground water-derived raw water samples were positive. These samples had lower mould numbers than samples from the network installations, although a small reduction of mould numbers was indicated from raw to treated water (Fig. 1). In surface water-derived supplies, all the hospital cold tap samples yielded moulds. Also the ground water samples had high presence of moulds in the hospital cold taps, with 66·7% of the samples being positive. These results were considerably higher than at the same locations in the private homes or any of the other sampling locations.

Factors affecting the risk of mould recovery

The risk to recover moulds from surface water was three times higher than from ground waters (Table 2). Occurrence of moulds indicated significant differences between

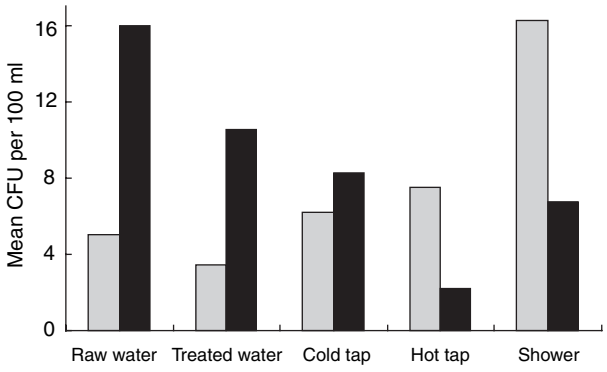

Figure 1 Trends in mould recovery at the different sampling locations in surface water (▒) compared with ground water (■). Mean colony forming units per 100 ml are calculated on basis of the positive samples only. The network installations (hot/cold/shower) represent both private homes and hospitals.

Table 2 The results from the repeated measures analysis of 273 water samples, with mould recovery as outcome variable

Independent variable	Categories	Odds ratio (OR)	LRT 95% CI for OR	*P*-value
Origin source	Surface water	3·1	1·6–6·3	0·03
	Ground water	1	–	
Sampling location	Cold tap	14·1	4·0–50·1	0·08
	Shower	8·5	2·2–32·2	
	Hot tap	1	–	

LTR, Likelihood-ratio test; CI, Confidence interval.

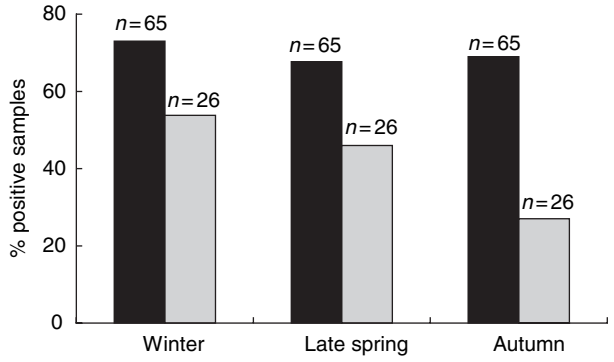

Figure 2 Total % positive samples at the three sampling periods in surface water (■) compared with ground water (▩) (*n*, number of samples).

water sampled from cold taps and samples from hot taps and showers. The odds for recognizing moulds in cold waters were 14 compared with hot waters. It was 8·5 times more likely to recover fungi in showers than in hot taps. There were no significant differences between private homes and hospitals regarding the different installations. No significant differences could be observed between the three sampling repetitions; hence no seasonal variation in mould recovery from water during the year. However, the first water sampling in ground waters resulted in twice as many positive samples as the last sampling (Fig. 2). No significant differences in mould recovery in samples from the different geographical parts of Norway could be observed.

Discussion

The present investigation demonstrates, in conformity with Ormerod (1987) and Warris *et al.* (2001a), that drinking water in Norway regularly contains filamentous fungi. The statistical analysis provides evidence of significant differences in risk of mould recovery from surface water compared with ground water. These results are in general agreement with those reported from other studies (Nagy and Olson 1982; Franková 1993; Zacheus and Martikainen 1995; Warris *et al.* 2002).

In surface water, the amount of fungi detected in samples from treated water, private homes and hospital installations was lower than in the raw water samples, suggesting that despite that the water treatment has some effect on mould contamination; moulds are still present in the water network. The ground water samples indicate opposite results, as the amounts of fungi increased from raw water and throughout the water network, suggesting contamination or regrowth in the network. The statistical results indicates that water sampled from cold waters more often contained fungi than the shower and hot tap sampling locations, which supports Zacheus and Martikainen's (1995) suggestion of the same results analysing cold and hot waters in Finland. Results from the present study could not provide evidence for significant differences, e.g. between the sampling locations or private homes and hospitals. We recognize that the ground water sample size is relatively small when it comes to saying anything about such differences; even though trends suggest that these differences exist. More work has to be conducted to reveal such relations.

In this study, the mean CFU per 100 ml was calculated on basis of the positive samples only. When water samples recover moulds, it is essential to be able to assess the level of fungi in the samples. Because of the relatively large number of negative samples, especially in ground waters, mean CFU of both negative and positive will probably give an incorrect image of the level of fungi. Mean CFU of the positive samples will therefore be desirable and give most information on how the mould situation in the water is.

In contrast to Nagy and Olson (1982) who found the mean number of CFU in surface water nearly twice the number in ground water, the results from the present study demonstrated limited variation in mean CFU between the two water categories. The reason for this result was one ground water sampling point (shower/private home), which yielded 100 colonies of moulds in one of the repetitions. This number gives a bias total mean CFU for the ground water samples, and perhaps an incorrect picture of the difference between ground and surface water. This again indicates growth of moulds in

network installations, which most likely was the situation in this showerhead.

With respect to general microbial load in water, the large amount of moulds isolated from surface water samples could probably be considered as expected. Conversely, the data regarding ground water was surprising, especially the high mould numbers in hot tap samples, indicating some kind of thermophilic species establishing in these facilities. It is interesting to notice that Norwegian ground water regularly contains relatively high amounts of moulds, compared with Germany (Göttlich *et al.* 2002) and the Netherlands (Warris *et al.* 2002). The same ground water trends as in the present study were observed in the survey from Finland (Zacheus and Martikainen 1995) and may be a phenomenon in waters in the north.

Regarding Warris *et al.* (2001b) suggestion of the waters ground or surface origin as explanation for the differences in contamination levels observed in various studies, results from the present investigation indicated that this might not be the entire reason. In the ground water samples it was experienced that the contamination level increased during transportation in the water system. This indicates that the distribution pipeline provided favourable conditions for establishment of moulds. This could also be the case in the surface water samples where contamination had a tendency to maintain in the network. Fungi are able to survive and grow in biofilms inside the water pipe system (Nagy and Olson 1985; Doggett 2000), and may establish in network installations such as cisterns, heating tanks, shower heads or taps and sinks. A survey was recently conducted to investigate moulds in biofilm in two water supply networks in Oslo (Skaar and Østensvik 2005). The results demonstrated considerably variation in amount of moulds recovered from biofilm samples, ranging from 50 to 600 CFU g^{-1} biofilm in the three sampling series. This indicates that moulds have the ability to reside in biofilms; hence be a possible source of contamination in drinking water. This theory could also explain the high recovery of moulds in hospital samples from both ground and surface derived water. Hospitals have large heating tanks where water might be kept for longer periods and offer favourable environments for establishment of thermotolerant species. The water pipe system is often long and complex, with blinded pipe ends where water possibly is stagnated for some time, which provides excellent conditions for biofilm formation. Fragments of biofilm might be released into the water stream from time to time, resulting in increased contamination, or established moulds could serve as conidia reservoirs. In addition to being an integral biofilm component, the possibility that moulds also establish in sludge in the bottom of water pipes is imaginable.

The differences in results of mould recovery reported in literature may also partly be explained by differences in methodology. There is no international standard method described for analyses of filamentous fungi in water. Most investigations use membrane filter techniques applying volumes of 10–1000 ml water (Nagy and Olson 1982; Niemi *et al.* 1982; Hinzelin and Block 1985; Ormerod 1987; Zacheus and Martikainen 1995; Arvanitidou *et al.* 1999; Warris *et al.* 2001a; Anaissie *et al.* 2002). Others perform direct plate spread with volumes of 0·1–1·0 ml (Franková 1993; Franková and Horecka 1995; Göttlich *et al.* 2002). This results in various detection limits of moulds in the samples. When Warris *et al.* (2002) compared their results with those of Anaissie *et al.* (2001), it is obvious that the natural water reservoir may be responsible for the differences in mould recovery. However, the methodology should also be considered, owing to the fact that the two studies use very different isolation methods. Warris *et al.* (2001a) filtrates 500 ml water sample and incubates at 35°C, while the comparing study apply both membrane filtrating of 1 l water sample and centrifugation of 25 ml with serial dilutions before incubation at 25°C. These differences will probably be of most importance for the recovery of fungi from the samples, and which fungal species that are obtained. Obviously, the incubation temperature will probably select for some species and result in loss of others. The incubation temperature varies from 20 to 37°C in studies worldwide. Preferably, the method for isolation of filamentous fungi from water should be standardized.

The isolation medium is also varying among the different investigations. This might result in selectivity towards some mould genera and loss of others. DG18 is a medium originally developed for enumerating xerophilic fungi in dry foodstuffs (Hocking and Pitt 1980). Independently, DG18 has been tested and compared with other sampling media and found to be most effective in collecting fungal colonies in terms of both quantity and types of genera, hence it is usable as general medium for enumerating moulds in substances from various environments (Smid *et al.* 1989; Verhoeff *et al.* 1990; Wu *et al.* 2000; Viljoen *et al.* 2004). Dichloran rose bengal chloramphenicol agar (DRBC) (King *et al.* 1979) and DG18 are now recommended as general media in purpose of isolation and enumeration of fungi in foods of high water activity ($a_w > 0{\cdot}90$) (Samson *et al.* 2004), and may therefore also be suitable for water analyses. In our study DG18 was selected on basis of several advantages, first of all because of characteristic colony appearance on the agar, and good differentiation of species, making secondary selection and re-isolation of pure cultures easier to accomplish, as compared with DRBC. DG18 also inhibits overgrowth of fast growing genera like *Mucorales* and *Trichoderma*, and does

not easily produce substances toxic to the fungus when exposed to light. Media containing rose bengal are light sensitive and produce inhibitory compounds in significant concentrations after 2 h exposure to light (Samson *et al.* 2004), so use of all media containing rose bengal was avoided in this study. Additionally, we recognize the opinion of some researchers that a combination of media might be needed to obtain the complete cross section of fungi present in water (Kinsey *et al.* 1999; Kelley *et al.* 2003). Employing a low-nutritional medium in addition to DG18 may have given supplementary knowledge about the occurrence of moulds in Norwegian water, but as mould analyses are time and labour consuming, analysing more water samples was preferred instead of extended use of media.

Another factor for explaining differences in mould recovery is the content of organic material and chemical conditions in the water. Norwegian drinking water is often derived from surface water, which generally contains large amounts of organic material, which again may provide nutrients or act as a medium for fungal growth. These surface waters are usually classified as soft as they normally are slightly acidic and do not contain much calcium. Ground water is classified as hard water, although the international classification of water hardness deviates from the Norwegian classification. Water classified as hard internationally is 10 times harder than the Norwegian scale, so that our ground water would be classified as soft outside Norway (Økland and Økland 1998). This fact also makes comparisons of results in ground water between the present study and studies abroad more complicated.

Differences in climate will probably also be of importance for mould frequencies. Scandinavian climate with the change of seasons would be expected to give other results than more temperate climates. Imaginably, snow-melting washing all kind of materials into streams and lakes would make the level of micro-organisms in water increase. In this study, the samples were taken winter, late spring and autumn to balance influences by climate changes during the year. It is surprising that the differences in mould recovery at the different sampling periods are limited. At least in surface waters we expected to discover some variation. The idea was that ground water would be more stable during the year, as the source is less influenced by the climate, but the analysis show quite opposite results, as recovery of fungi in the autumn-sampling was half the amount in winter-sampling. This might be explained by contamination or establishment of certain species somewhere in the supply network.

The present investigation has obtained important information about the occurrence of fungi throughout the municipal drinking water network. The results obtained indicate that filamentous fungi are present in all parts of the water distribution system in Norway. It is important to keep the amount of fungi reaching the consumers under surveillance, because of the ability of different species to cause disease or allergy in humans, act as contaminators in, e.g. food and beverage industry, or reduce esthetical quality of water in regards of smell and taste. More work on species identification is needed to reveal any relations to human health. The study also gives important information about how moulds are distributed in the water network, which will be important to consider in any means of guidance or management in cases of mould contamination problems.

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