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SUMMARY

Free-living amoebae are known to facilitate the growth of water associated pathogens. This study, for the first time, explored the diversity of free-living amoebae in a dual distribution (potable and recycled) water system in Rouse Hill NSW, Australia. Water and biofilm samples were taken from the tertiary recycled water treatment plant and from within the two distribution systems (using a Modified Robbins Device). Amoebae were not detected entering the distribution systems from the tertiary recycled water treatment plant over a 6 week sampling period. However, amoebae were isolated within the recycled water distribution system (0.1 amoebae/ml). Furthermore, amoebae were isolated from within the potable water distribution system (0.2 amoebae/ml). Chlorine concentrations appear to explain the different amoebae numbers. More research is required to determine the relationship between these free-living amoebae and water associated pathogens within this water distribution system.

INTRODUCTION

A range of **water associated pathogens** have been reported to grow intra-cellularly in free-living amoebae:

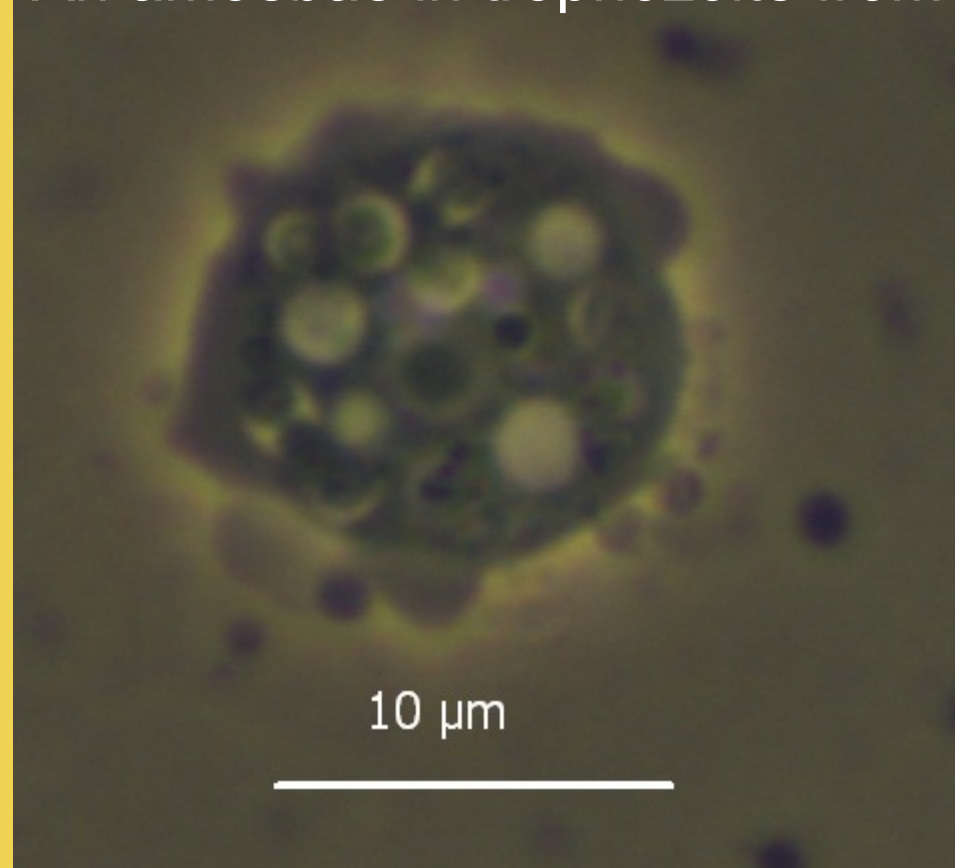
- *Legionella pneumophila*
- *Mycobacterium avium*
- *Campylobacter jejuni*
- *Listeria monocytogenes*
- *Escherichia coli* O157:H7
- *Salmonella enterica*
- *Pseudomonas aeruginosa*
- *Helicobacter pylori*

(Snelling et al., 2006, *Microbes and Infection* **8**: 578)

Free-living amoebae **facilitate the growth** of intracellular pathogens by providing:

- nutritional requirements
 - protection from environmental stressors
 - protection from chlorine disinfection
 - up regulating genes involved in virulence
- (Lau and Ashbolt, 2009, *J. App. Micro.* **106**: DOI: 10.1111/j.1365-2672.2009.04208.x)

An amoebae in trophozoite from



Free-living amoebae are ubiquitous in aqueous environments and have been isolated from:

- treated and untreated water
 - swimming pools
 - air-conditioning units
 - bottle water
 - nasal mucosa
 - healthy human throats
- (Marciano-Cabral and Cabral, 2003, *Clin. Micro. Rev.* **16**: 273)

To date the **diversity and distribution** of amoebae in drinking and recycled water systems has not been well characterised.

AIMS

- To explore the **diversity and distribution** of free-living amoebae in a dual distribution (potable and recycled water) system in Rouse Hill, NSW Australia.
- To determine which **water quality** parameters influence the diversity and distribution of free-living amoebae.

METHODS

Sampling site 1: Tertiary recycled water treatment plant

500 ml samples were taken after four stages of treatment:

- tertiary clarification
- deep bed filtration
- UV disinfection
- super chlorination

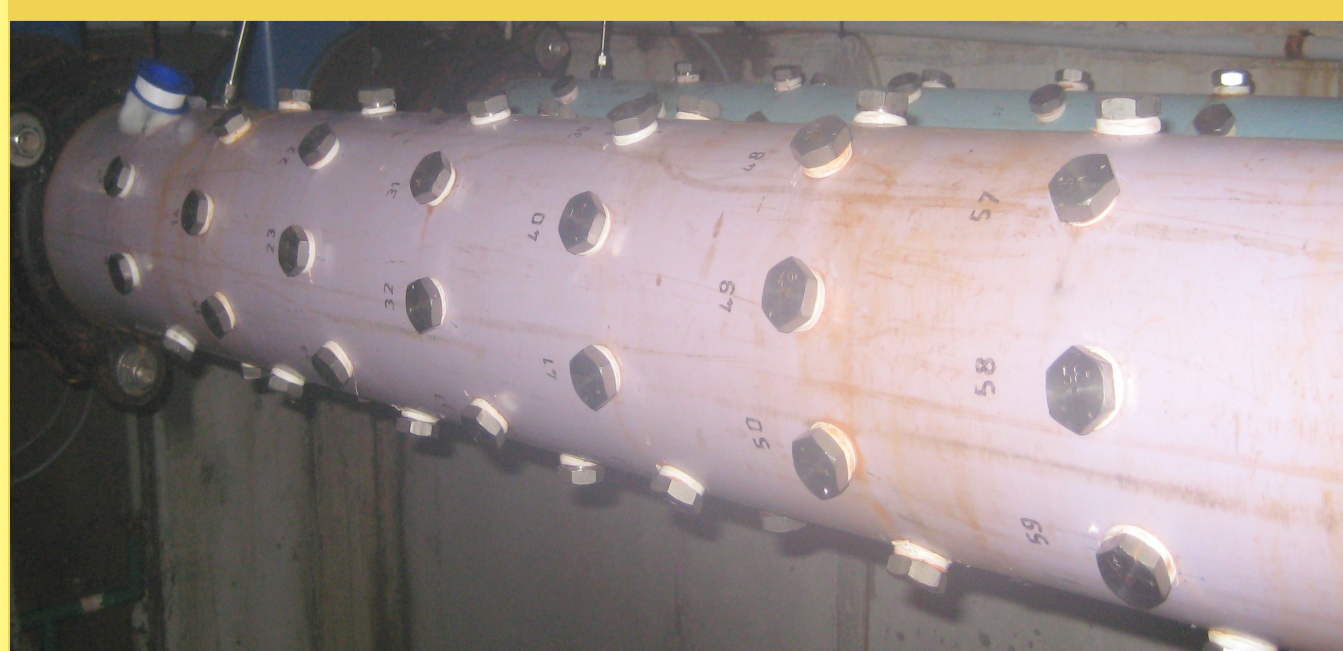
Sampling was undertaken for 6 weeks.

Sampling site 2. Distribution system pipe sampling device (Modified Robbins Device).

Biofilm and water samples were taken from the:

- potable water pipe
- recycled water pipe

Sampling was undertaken once.

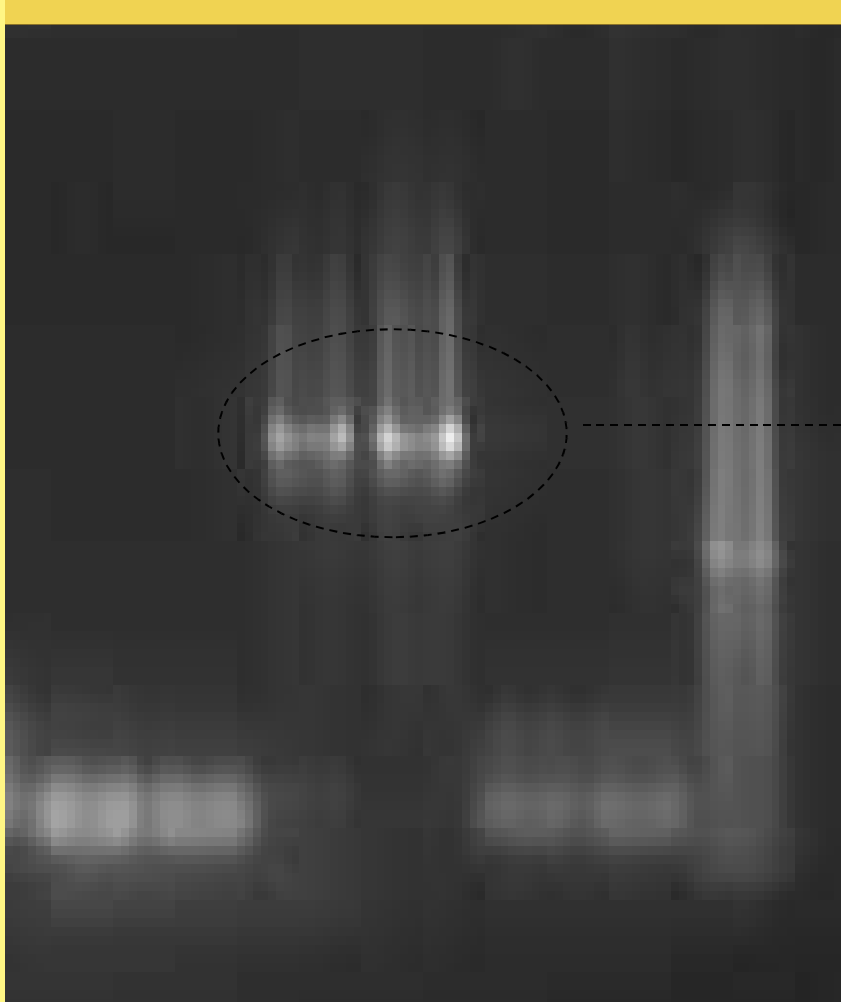


Water quality parameters measured:

- pH
- temperature
- turbidity (NTU)
- total organic carbon
- chlorine (free and total)
- total nitrogen and phosphorus

Free-living amoebae isolation

- *E. coli* overlay on non-nutrient agar plates
- Incubated at 22 °C and 36 °C or 42 °C for 30 days
- Amoebae plaques enumerated
- Amoebae confirmed by microscopy
- Amoebae were subcultured into pure cultures
- Cryopreservation at -80 °C



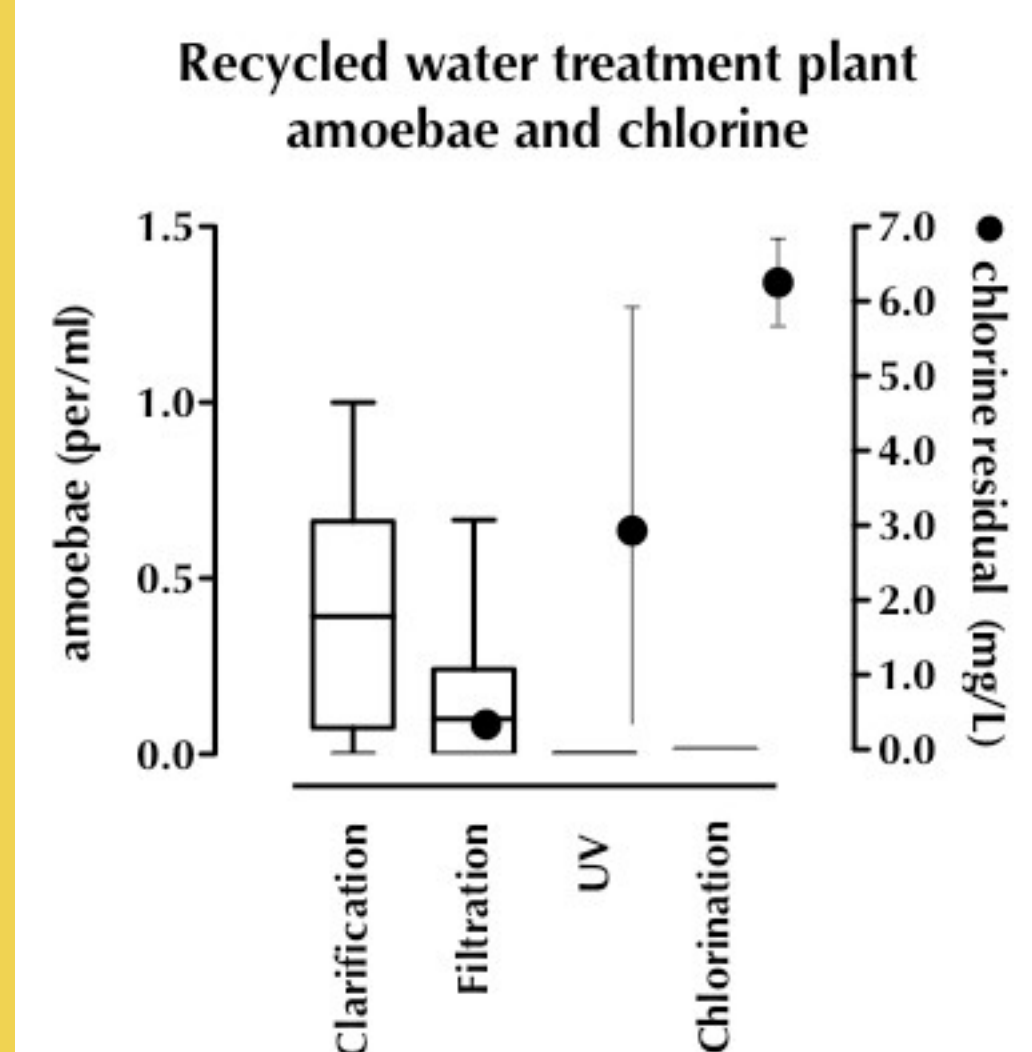
Free-living amoebae identification

- Partial 18S DNA sequencing using published primers for an 1800 bp fragment (Lopez-Garcia et al., 2001, *Nature* **409**: 603)
- Sequenced using an AB3730 sequencer and searched against the NCBI GenBank

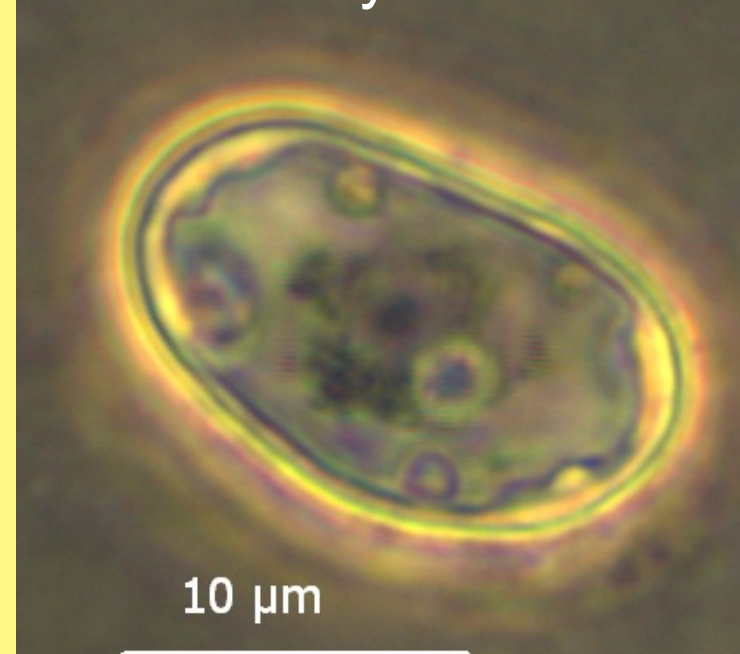
RESULTS

Site 1. Tertiary recycled water treatment plant

- Higher numbers of amoebae were isolated after tertiary clarification (0.39 amoebae/ml)
- No amoebae were isolated after the final disinfection stages (chlorine residuals 2.54 to 5.8 mg/L)

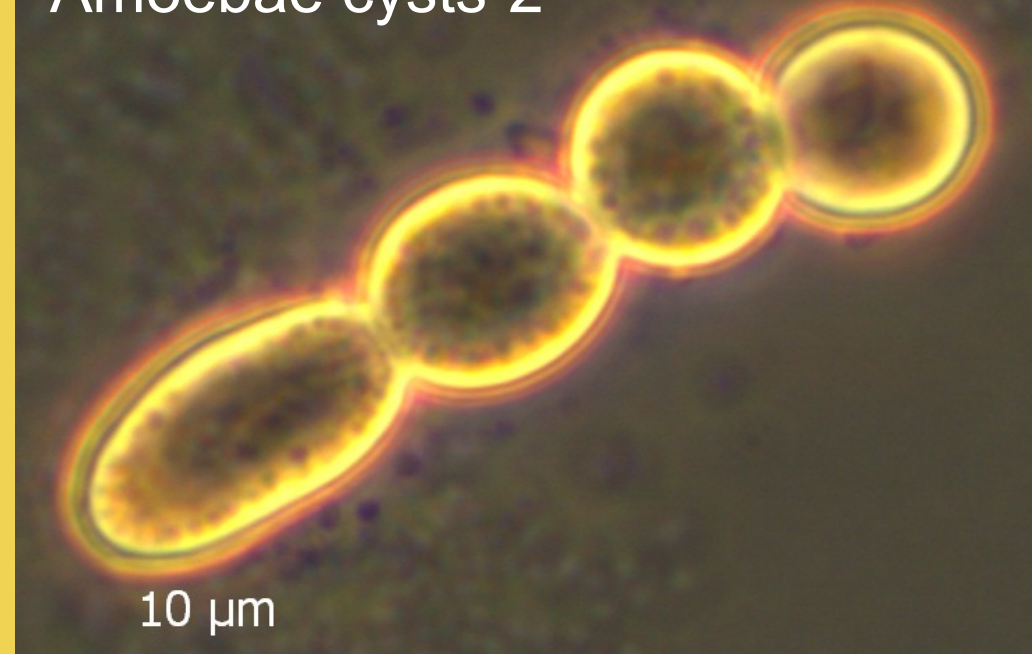


Amoebae cyst-1



Tertiary clarification isolate

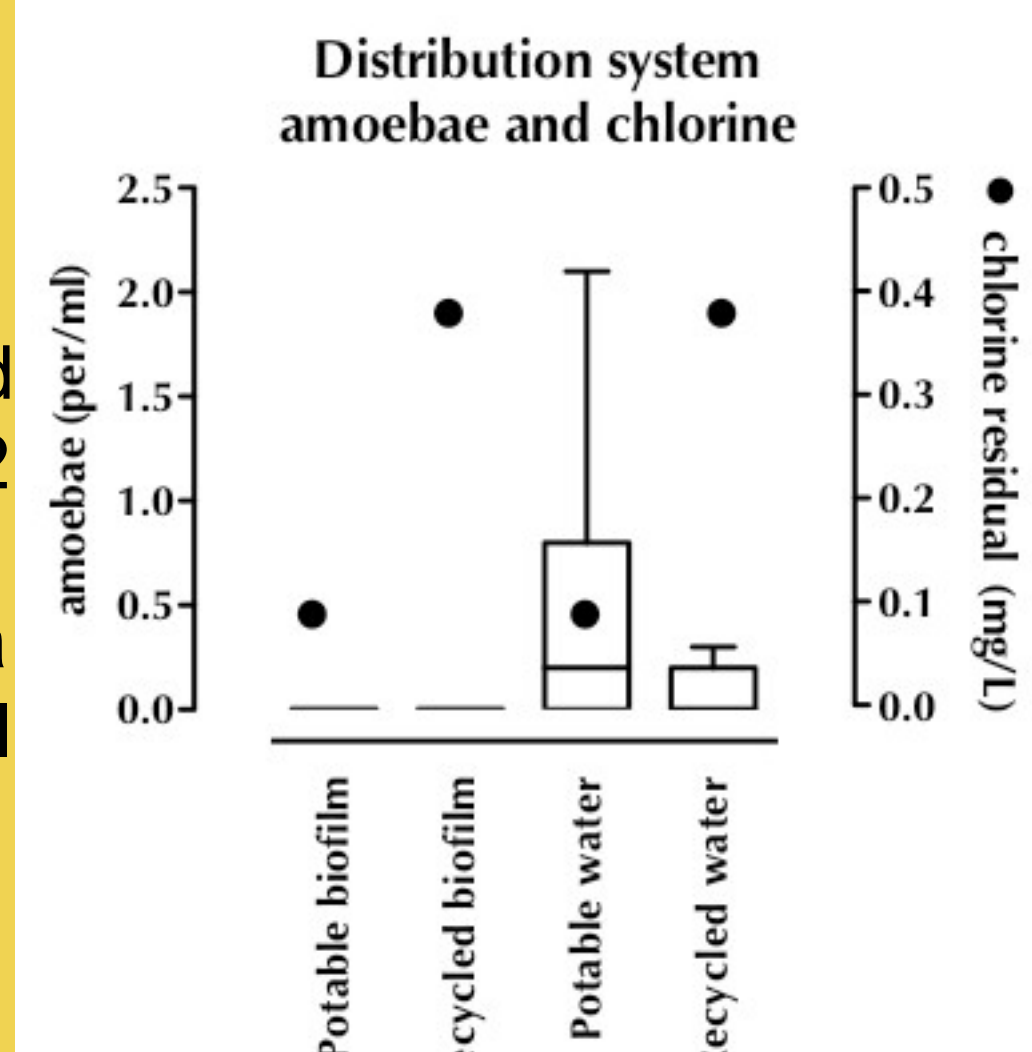
Amoebae cysts-2



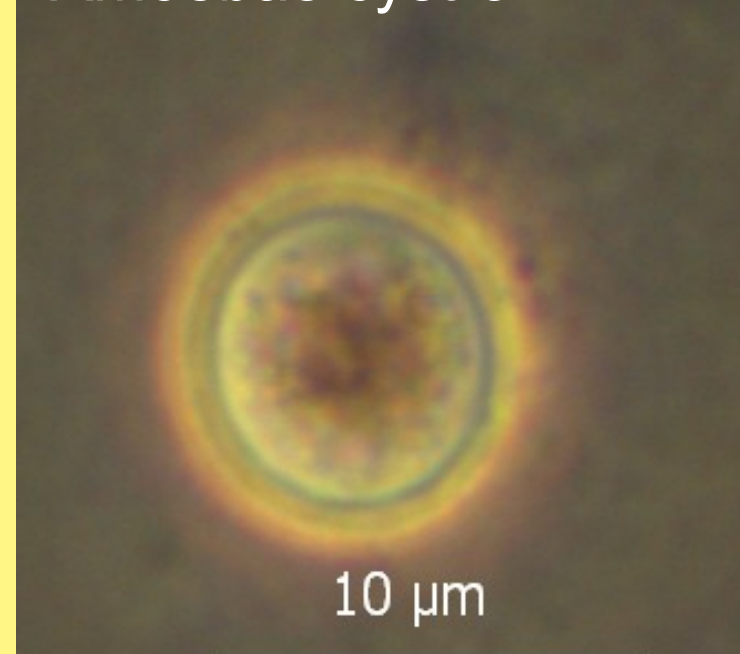
Deep bed filtration isolate

Site 2. Distribution system

- Higher numbers of amoebae were isolated from potable water (0.2 amoebae/ml)
- Recycled water had a higher chlorine residual (0.38 mg/L)
- No amoebae were isolated from the biofilm samples

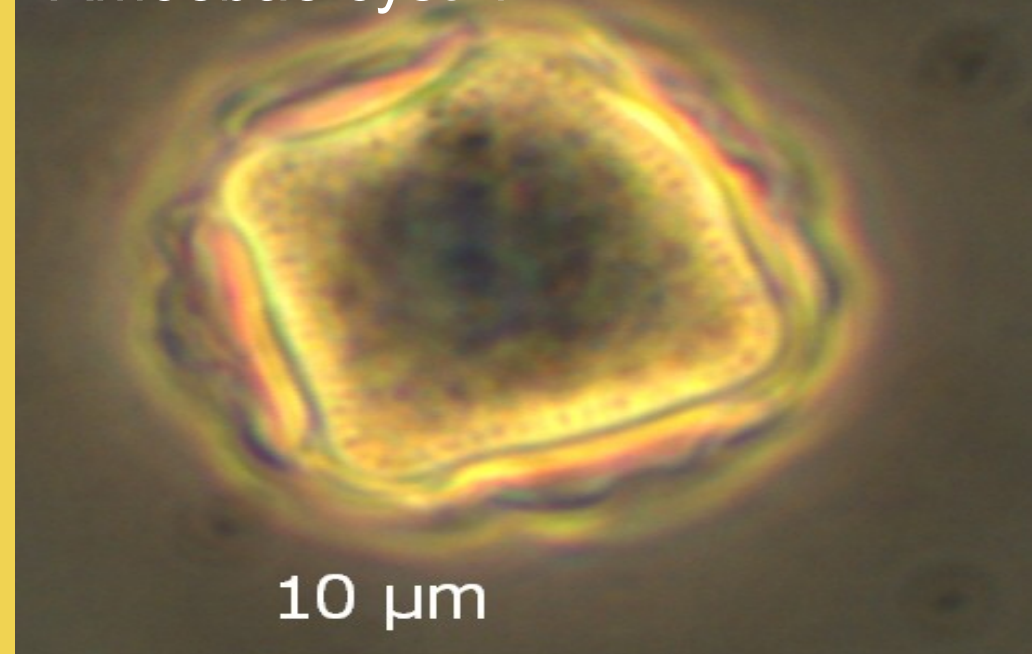


Amoebae cyst-3



Potable water isolate

Amoebae cyst-4



Recycled water isolate

CONCLUSIONS

Free-living amoebae were isolated from both potable and recycled distribution systems. While amoebae were not detected in the outlet of the recycled water treatment plant they were detected within the distribution system, although not from biofilm samples, suggesting that re-growth of these organisms may have taken place. The higher chlorine residual in the recycled water distribution system (0.38 mg/L) compared with the potable water system (0.07 mg/L) may explain the lower numbers of amoebae recovered. More research is required to determine the interaction between the free living amoebae and any water associated pathogens within this system.

ONGOING WORK

At present 18 isolates are being identified by partial 18S sequencing.