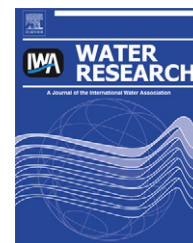




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A probabilistic model of norovirus disease burden associated with greywater irrigation of home-produced lettuce in Melbourne, Australia

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ABSTRACT

The reuse of domestic greywater has become common in Australia, especially during periods of extreme drought. Greywater is typically used in a raw, untreated form, primarily for landscape irrigation, but more than a quarter of greywater users irrigate vegetable gardens with the water, despite government advice against this practice. Greywater can be contaminated with enteric pathogens and may therefore pose a health risk if irrigated produce is consumed raw. A quantitative microbial risk assessment (QMRA) model was constructed to estimate the norovirus disease burden associated with consumption of greywater-irrigated lettuce. The annual disease burdens (95th percentile; DALYs per person) attributed to greywater irrigation ranged from 2×10^{-8} to 5×10^{-4} , depending on the source of greywater and the existence of produce washing within households. Accounting for the prevalence of produce-washing behaviours across Melbourne, the model predicted annual disease burdens ranging from 4×10^{-9} for bathroom water use only to 3×10^{-6} for laundry water use only, and accounting for the proportionate use of each greywater type, the annual disease burden was 2×10^{-6} . We recommend the preferential use of bathroom water over laundry water where possible as this would reduce the annual burden of disease to align with the current Australian recycled water guidelines, which recommend a threshold of 10^{-6} DALYs per person. It is also important to consider other exposure pathways, particularly considering the high secondary attack rate of norovirus, as it is highly likely that the estimated norovirus disease burden associated with greywater irrigation of vegetables is negligible relative to household contact with an infected individual.

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1. Introduction

Views about greywater have shifted in recent years, with developed countries such as Australia, the USA and Japan leading the way with reuse (Domènech and Saurí, 2010). Predominantly used for landscape irrigation (Casanova et al., 2001), the practice is becoming more common, particularly in arid and semi-arid regions (Roesner et al., 2006; Wiel-Shafran et al., 2006) and has undergone a resurgence of use in Australian households. With recent extended drought conditions and water restrictions severely limiting outdoor tap water use, many Melbourne households turned to greywater (up to 71% in 2007; ABS, 2007), including water bucketed from showers and washing machines (ATA, 2005; Pinto and Maheshwari, 2010).

Greywater is often perceived as relatively harmless and is typically used untreated, either immediately after generation or after some period of storage, yet it has been well established that it can be contaminated with a wide range of chemicals and microorganisms (Eriksson et al., 2002; Maimon et al., 2010). Viruses are assumed to be present in greywater, simply as a function of human excreta including faeces and vomit (Lopman et al., 2012), although only a few studies have tested for viruses (Birks and Hills, 2007; O'Toole et al., 2012) (Birks and Hills, 2007; O'Toole et al., 2012). Viruses can be shed during bathing and can also be transferred on fomites, such as clothing and towels (Boone and Gerba, 2007) resulting in contamination of laundry water. Rose et al. (1991) demonstrated that viruses could survive in greywater with no change in seeded virus numbers over 2 days at 17 °C.

Enteric viruses are a major concern because they typically have a low ID₅₀, high shedding rate and high persistence in the environment. Norovirus is a major cause of gastroenteritis worldwide (Boone and Gerba, 2007; Matthews et al., 2012). It is transmitted faecal-orally, can survive in water, and is highly resistant to treatment (Lodder and De Roda Husman, 2005; Ueki et al., 2005). Previous studies have demonstrated potential waterborne transmission of norovirus via drinking water (Åstrom et al., 2007; Masago et al., 2006) and recreational waters (Viau et al., 2011), but there are no published studies measuring norovirus concentrations in greywater.

Several quantitative microbial risk assessment (QMRA) studies have investigated risks associated with greywater irrigation (Jackson et al., 2006; Surinkul and Koottatep, 2009) although only two have attempted to estimate viral risks. Ottoson and Stenström (2003) used rotavirus as a model viral pathogen (determined from estimates of faecal contamination, based on coprostanol values and epidemiological data) and predicted risks associated with direct exposure due to irrigation, while Barker-Reid et al. (2010) used published thermotolerant coliform concentrations for source-separated greywater and estimated annual probability of enteric virus infection. The scale of reuse is an important consideration in terms of the overall context of risk. At small scales of reuse (such as a household), person-to-person contact may be the predominant exposure pathway given the high secondary attack rates for norovirus (Alfano-Sobsey et al., 2012). As well, only a few assessments of norovirus risks (Ashbolt et al., 2010; Mara and Sleight, 2010; Schoen et al., 2011; Soller et al., 2010; Viau et al.,

2011; Yang et al., 2011) have been published since the development of the norovirus dose-response model (Teunis et al., 2008). In an effort to contribute to this gap in knowledge, we used QMRA to estimate the disease burden from norovirus associated with greywater reuse behaviours in Melbourne households.

2. Model construction

2.1. Hazard assessment and exposure model

Lettuce was chosen as the representative food crop because it is a common plant for home production. About 15% (20,982 tons) of all lettuce consumed by Australians is grown in backyards (ABS, 2000) and, of those who eat home grown produce, nearly 20% rank lettuce among the top five vegetables grown (Langley et al., 1998). Lettuce is predominantly eaten raw (i.e. no pathogen reduction from cooking) and it retains a relatively large volume of water on the surface of the plant, thus conferring greater potential for transfer of pathogens from irrigation water. Norovirus was chosen as the microbial hazard to model because it is the most common cause of community gastroenteritis in Melbourne (Sinclair et al., 2005). The model was constructed as a sub-component of a larger project on greywater reuse in Melbourne, and data on *Escherichia coli* counts (O'Toole et al., 2012) and reuse behaviours and practices (Sinclair et al., in press) have been drawn from the broader project. Given that two studies have shown that less than 5% of greywater users in Melbourne use any form of greywater treatment (ATA, 2005; Sinclair et al., in press), the model assumed that greywater was not treated prior to use. Kitchen greywater may be heavily contaminated with food particles, detergents and oils and grease (EPAV, 2006; Travis et al., 2008) and may have high faecal indicator counts (Friedler, 2004). Kitchen greywater was excluded from the model because (i) it accounts for a very small proportion of total use (~8% by volume (Sinclair et al., in press)), (ii) its reuse, especially for purposes where human exposure is likely, is strongly advised against by various authorities (EPAV, 2006), and (iii) human excreta inputs to kitchen greywater are less likely than for bathroom and laundry greywater. Bathroom water and laundry water, the predominant greywater sources, were considered in the model as well as average greywater – a representation of the proportionate use of individual greywater sources across the broader Melbourne population.

The dose of norovirus (λ ; no. ingested person⁻¹ d⁻¹) resulting from the consumption of greywater-irrigated home grown lettuce that an individual is exposed to was modelled as

$$\lambda = V I c e^{-kt}, \quad (1)$$

where V is the volume of greywater caught on the surface of a lettuce plant following irrigation (mL g⁻¹), I is the mean per capita intake of lettuce (g person⁻¹ d⁻¹), c is the concentration of norovirus in the greywater (no. mL⁻¹), k is the in-field virus kinetic decay constant (d⁻¹), and t is the withholding period (d), i.e. time between last greywater irrigation event and harvest. This exposure model considered overhead irrigation only because Sinclair et al. (in press) found that the majority

of greywater users used either a bucket (76%) or a garden hose (21%) to distribute greywater. Post-harvest decay of enteric viruses was not considered as it is likely to be insignificant (Badawy et al., 1985) and particularly unlikely to be consequential in backyard food production because food is likely to be harvested close to the time of consumption. The zero-truncated Normal distributions of Hamilton et al. (2006) and Petterson et al. (2001a, b, 2002) were used to represent V and k , respectively (Table 1). In the absence of data on backyard withholding periods, we chose to represent t with a Uniform distribution covering zero days (i.e., consumption on the day of last irrigation) through to a maximum of 2 days, which is reasonable because plants are unlikely to thrive for more than 2 days without irrigation during warm weather – the time of the year when greywater use is most likely to occur.

There is very limited Australian information on vegetable consumption; therefore, to derive a distribution for I we relied on a mixture of Australian and American data. Our approach assumes that home lettuce growers in the USA and Australia have similar lettuce production and consumption habits, which is supported by the fact that total lettuce consumption statistics are similar (30.2 (USEPA, 2009) and 26.03 (Ausveg, 2009) g person⁻¹ day⁻¹, respectively). I was estimated as

$$I = \left[C_{\text{EPA}} - C_{\text{EPA}} \left(\frac{L}{100} \right) \right] m, \quad (2)$$

where C_{EPA} is the USEPA's home-produced lettuce intake rate (g (kg-person)⁻¹ day⁻¹), represented by a Lognormal distribution and based on food as brought into the household, L is the loss of lettuce due to food preparation (%), and m is Australian body mass (kg person⁻¹), weighted by age, gender and population. The Mixture distribution for L was developed by combining two PERT distributions, which were constructed from data on two different lettuce cultivars (Matthews and Garrison, 1975, Table 1). The Mixture distribution for m was constructed using a number of different data sources on body mass for all age groups. Children under the age of 1 were excluded from this analysis because it was assumed that they would not be consuming solid food. Body mass values (mean and standard deviation) were obtained for children (DoHA, 2010) and adults (ABS, 1998), determined by sex and age ranges. Body mass was assumed to be Lognormal (Penman and Johnson, 2006; Walls et al., 2010) and distributions were constructed for each age range, weighted by proportion of the population (ABS, 2010b). Random samples of weighted body mass from each age range and sex were summed to get a distribution of estimates of the whole of population weighted mean body mass. A set of 10,000 estimates of m was determined and random samples (with replacement) were drawn for use in the calculation of I .

Post-harvest practices such as washing and sanitising have been shown to reduce bacterial loads on produce with varying degrees of efficiency (Fatica and Schneider, 2009; Gil et al., 2009). Relatively little work has been published on the efficacy of washing treatments against viruses, although they have been observed to be more resistant than bacteria (Allwood et al., 2004) and norovirus may be particularly resistant to disinfection (Mattison, 2011). To account for reduced viral load, the dose of norovirus consumed by

greywater users that wash lettuce prior to consumption (λ_{wash}) was modelled as

$$\lambda_{\text{wash}} = Vlc10^{-w}e^{-kt}, \quad (3)$$

where w is the log₁₀-reduction in virus concentration from washing of lettuce prior to consumption. The WHO Guidelines for Wastewater Use in Agriculture (WHO, 2006) assign a 1-log₁₀ bacterial reduction to the washing of salad crops, and previous studies (Mara and Sleight, 2010; Seidu et al., 2008) have assumed that this value can be applied to viruses as well. A few studies (Baert et al., 2008, 2009; Butot et al., 2008; Croci et al., 2002; Gulati et al., 2001; Predmore and Li, 2011) have investigated the efficacy of washing produce with tap water and have reported virus reductions from 0.1 to 2 log₁₀ units, of which nine were reported as 1 ± 0.2 (mean \pm sd); this was used as the most likely value in the PERT distribution used to represent w (Table 1).

2.1.1. Estimation of norovirus concentrations (c)

Owing to the absence of information on norovirus concentrations in greywater, construction of a distribution for c was complex. The enumeration and identification of viruses from wastewater is hampered by a number of methodological challenges: virus detection efficiency is often very poor, test methods can be expensive and in some cases, as for norovirus, there is the added complication of no available cell culture method to determine infectivity (Atmar, 2010). Attempts to circumvent this in previous wastewater studies have employed methods of estimating virus concentrations from measurements of indicator organisms such as *E. coli* or faecal coliforms (Seidu et al., 2008; Shuval et al., 1997), typically using a linear relationship of 1 virus to 10⁵ indicator organisms (Howard et al., 2007; Shuval et al., 1997; Zhao et al., 2006), likely originating from a study of waste stabilization ponds in Brazil that measured faecal coliforms and enterovirus (Oragui et al., 1987), and making the assumption that enterovirus concentrations are equal to norovirus concentrations (Mara and Sleight, 2010). In this analysis, for concentrations of norovirus in bathroom and laundry water, we used different estimation methods, assuming a household size of four people.

For bathroom water, we have assumed that all people in the household have a bath or shower every day and that water from all baths and showers is collected. The average daily concentration of norovirus in bathwater (c_{NV_bath} ; no. mL⁻¹) was estimated as

$$c_{\text{NV}_bath} = \frac{C_{\text{Ecoli}_bath} SR_{\text{NV}} OP}{F_{\text{faeces}} 4} \quad (4)$$

where C_{Ecoli_bath} is the measured *E. coli* concentration in bathroom water (CFU mL⁻¹), F_{faeces} is the estimate of *E. coli* or faecal coliforms per g faeces (CFU g⁻¹), SR_{NV} is the norovirus shedding rate (no. g⁻¹ faeces), O is the daily incidence of norovirus per household (probability of norovirus outbreak), P is the number of people that are ill during the household outbreak, and four is the household size. So $OP/4$ is the probability of a bath/shower having an ill person in it such that if one person in the household is ill, then the contaminated bathroom water from that person will be diluted by the three other uncontaminated baths/showers on that day.

Table 1 – Model input parameters and distributions.

Notation and definition (units)	Distribution type (values) ^a	Source
V = volume of greywater captured by lettuce (mL g ⁻¹)	Normal (0.108, 0.019) – truncated at zero	(Hamilton et al., 2006; Shuval et al., 1997)
I = mean daily consumption of lettuce per person (g person ⁻¹ day ⁻¹)	Mixture ~23.8 (mean) ^d	
C _{EPA} = Consumption of home-produced lettuce (g (kg-person) ⁻¹ day ⁻¹)	Lognormal (0.39, 0.27)	(USEPA, 2009)
L = Loss due to food preparation - lettuce (%) (includes removal of core and wilted leaves; mean used as most likely value)	Mixture (A, B) ~9.1 (mean) ^d A = PERT (5,9,24) ^b butterhead lettuce B = PERT (4,7,12) ^b crisphead lettuce	(Matthews and Garrison, 1975)
m = Australian body mass (kg person ⁻¹), weighted by age, gender and population	Mixture ~67.2 (mean of 10,000 iterations)	(ABS, 1998, 2010b; DoHA, 2010)
C _{NV} = concentration of norovirus in greywater (no. mL ⁻¹)		
C _{Ecoli_Rose} = E. coli concentration in shower water (CFU L ⁻¹)	Point estimate: 6 × 10 ⁴	(Rose et al., 1991)
C _{Ecoli_bath} = E. coli concentration in bathroom water (CFU L ⁻¹)	Lognormal (1.71 × 10 ⁴ , 4.46 × 10 ⁴)	(O'Toole et al., 2012)
F _{faeces} = E. coli in faeces (CFU g ⁻¹)	10 ¹ PERT (7.0, 7.4, 7.9) ^b ~ 2.7 × 10 ⁷ (mean) ^d	(Drasar, 1974; Feachem et al., 1983; International Agency for Research on Cancer Intestinal Microecology Group, 1977)
SR _{NV} = norovirus GII shedding rate in faeces (no. g ⁻¹ , assuming that genomic copies equal number of viruses)	10 ¹ PERT (log ₁₀ (2.5 × 10 ⁴), log ₁₀ (3.0 × 10 ⁸), log ₁₀ (7.7 × 10 ¹⁰)) ^c ~ 2.2 × 10 ⁹ (mean) ^d	(Chan et al., 2006)
O = daily incidence of household norovirus outbreaks (proportion of households)	Mixture ~1.49 × 10 ⁻⁴ (mean) ^d	(Sinclair pers. comm.; Sinclair et al., 2005)
P = number of people involved in household outbreak	Discrete Frequency Distribution ~1.3 (mean) ^d (1, p = 0.75; 2, p = 0.18; 3, p = 0.05; 4, p = 0.02)	(Sinclair pers. comm.)
F _U = faeces on underwear (g pair ⁻¹)	PERT (0.001, 0.1, 10.0) ^b ~ 1.73 (mean) ^d	(Gerba, 2001a)
R _{virus} = viral log ₁₀ reduction from washing	Uniform (1.11, 2.88) ~ 2.00 (mean) ^d	(Gerba and Kennedy, 2007)
V _{laundry} = laundry load volume (L)	Discrete Frequency Distribution ~115 (mean) ^d (75, p = 0.48; 152, p = 0.52)	(Roberts, 2005, 2012)
k = in-field virus kinetic decay constant (days ⁻¹)	Normal (1.07, 0.07) – truncated at zero	(Pettersen et al., 2001a, b, 2002)
t = withholding period (days)	Uniform (0, 2) ~1.00 (mean) ^d	
w = virus reduction due to post-harvest washing of lettuce (log ₁₀ units)	PERT (0.1, 1.0, 2.0) ~1.0 (mean) ^d	(Baert et al., 2008, 2009; Butot et al., 2008; Croci et al., 2002; Gulati et al., 2001; Predmore and Li, 2011)
G _w = proportion of population that wash vegetables prior to consumption	Mixture ~0.8704 (mean) ^d	(Mitakakis et al., 2004)
G = proportion of population engaged in greywater irrigation of lettuce	Mixture ~0.03365 (mean) ^d	
H _{grey} = Percentage of Melbourne households that use greywater (%)	Uniform (28.7, 71.0) ~49.9 (mean) ^d	(ABS, 2007, 2010c; d)
H _{veg} = Percentage of greywater users that use greywater to irrigate vegetable gardens (%)	Point estimate: 22.65 (248/1095 greywater users)	(Sinclair et al., 2012)
H _{lettuce} = Percentage of vegetable gardeners that grow lettuce (%)	Uniform (28.0, 31.6) ~29.8 (mean) ^d	(Cross and Taylor, 1996; National Gardening Association, 2009)
Norovirus dose–response parameters for a + b inoculum	Point estimates: α = 0.04, β = 0.055, a = 0.9997, η = 0.00255, r = 0.086	(Teunis et al., 2008)
d = exposure events per year (days)	Discrete frequency distribution ~251 (mean) ^d	Refer to Table A-1
B = disease burden (DALYs case of illness ⁻¹)	Uniform (3.71 × 10 ⁻⁴ , 6.23 × 10 ⁻³) ~3.30 × 10 ⁻³ (mean) ^d	(Cressey and Lake, 2009; Haagsma et al., 2008; Kemmeren et al., 2006; Lake et al., 2010; Masago et al., 2006)
S _f = susceptibility fraction	Uniform (0.8, 1.0) ~0.9 (mean) ^d	(Atmar, 2010; Denborough and Downing, 1968; Soller et al., 2010; Thorven et al., 2005)

a Distribution types and values: Discrete Frequency distribution – list of values and their associated probability or frequency; Lognormal(mean, sd), where population parameters μ and δ are required and calculated as follows: $\mu = \ln(\bar{x}) - 0.5\ln(1 + s^2/\bar{x}^2)$, $\delta = [\ln(1+(s^2/\bar{x}^2))]^{1/2}$, where \bar{x} is the sample mean and s^2 the sample standard deviation (sd); Mixture – combination of various distributions; Normal(mean, sd); PERT(min, most likely, max); and Uniform(min, max).

b Most likely value represented by the mean.

c Most likely value represented by the median.

d estimated from 3,650,000 iterations of the model.

Measured *E. coli* values ($n = 36$) (Appendix 2; O’Toole et al., 2012) were used to define a Lognormal distribution of $C_{E_{coli_bath}}$. Zero values were replaced with $0.5 \text{ CFU } 100 \text{ mL}^{-1}$ (half the detection limit) and then the mean and standard deviation were calculated.

Feachem et al. (1983) compiled a number of studies that reported counts of Enterobacteria per gram of stool sample. Using the data from “western” countries only (Denmark, England, Finland, Scotland and the United States; Drasar, 1974; International Agency for Research on Cancer Intestinal Microecology Group, 1977), we used a PERT distribution of the \log_{10} -transformed data to represent F_{faeces} and assumed Enterobacteria are representative of *E. coli* (Table 1). The norovirus shedding rate in faeces (SR_{NV}) was reported for both norovirus GI and GII (Chan et al., 2006) and, while both genogroups were detected in the study (O’Toole et al., 2012), we used the shedding rate for GII as it predominates regardless of outbreak setting (health care vs nonhealth care; Bruggink and Marshall, 2011) or mode of transmission (Matthews et al., 2012). We also assumed that genomic copies of norovirus equated to numbers of viruses. Similar to Mokharti and Jaykus (2009) we used a PERT distribution of \log_{10} -transformed values and the resulting distribution had a median value (1.8×10^8) similar to the original values (3.0×10^8) reported by Chan et al. (2006).

Sinclair et al. (2005) determined the incidence of norovirus in Melbourne during a 15 month study of 600 households. As well as the proportion of gastroenteritis cases found to be positive for norovirus, the monthly incidence of norovirus outbreaks per household was recorded, together with the size of the norovirus outbreak or number of people ill per household (P ; unpublished data). As the study spanned more than one year, the incidence values for repeated months were used to define a Uniform distribution for that month (Table 2) and daily household incidence of norovirus (O) was estimated as

$$O = \frac{\text{monthly household incidence of norovirus}}{\text{days per month}} \quad (5)$$

Most household outbreaks affected only one person (75% of outbreaks), while multi-person outbreaks were also reported (18% with two people, 5% with three people and 2% with four people). The size of the outbreak (P) was represented as

a Discrete Frequency distribution and divided by four to get the proportion of baths/showers per household per day that were contaminated by an infected person.

The greywater survey did not ascertain the types of personal care products used in each bath or shower. Therefore, we cannot be certain that biocides (antibacterial soaps, shampoos, etc.) were absent from the samples collected. If present, biocides may reduce the measured *E. coli* concentrations resulting in an underestimate of norovirus concentration. In a previous study, Gerba (2001b) used the (presumed) mean *E. coli* concentration of $6 \times 10^4 \text{ CFU } L^{-1}$ in shower water (Rose et al., 1991) to estimate the faecal load in bathwater. Assuming Rose et al. took samples of bathwater in the absence of biocides (not stated in the paper), we have used this value as a comparison with the Lognormal distribution of *E. coli* ($C_{E_{coli_bath}}$) (noting that this is a deterministic input as no value for variance was reported).

Numerous assumptions were made in the estimate of laundry water concentration. We assumed that laundry loads contained detergent only (no biocides) and therefore no decay or inactivation of microorganisms was modelled. Microbial growth was assumed to be negligible as the majority of households use cold water to wash clothes (50–80% of households; O’Toole et al., 2008; Roberts, 2012; Sinclair et al., 2011). Roberts (2012) reported a mean of 4.7 loads of laundry per week, and using the average number of people per household of 2.6 (ABS, 2010a), this is approximately 1.8 loads per person per week (~7 loads for a four-person household). Therefore, we assumed one load of laundry per day per household with one pair of underwear from each member of the household (total of four) in each load. The estimated norovirus concentration is for average laundry water, assuming all wash and rinse water is collected.

We determined the number of norovirus on underwear in a load of laundry ($NV_{\text{underwear}}$; no. load⁻¹) as

$$NV_{\text{underwear}} = F_u SR_{NV} OP \quad (6)$$

where F_u is the amount of faeces per pair of underwear (g), SR_{NV} is the norovirus shedding rate (no. g⁻¹ faeces), O is the incidence of norovirus per household and P is the number of people that are ill during the household outbreak. The concentration of norovirus in laundry water ($C_{NV_laundry}$; no. L⁻¹) was then determined as

$$C_{NV_laundry} = [NV_{\text{underwear}} - (NV_{\text{underwear}} 10^{-R_{\text{virus}}})] / V_{\text{laundry}}, \quad (7)$$

where R_{virus} is the viral \log_{10} reduction due to washing and V_{laundry} is the volume of water in a laundry load (L).

Gerba (2001a) reported results of a study of students that evaluated the faecal load on underwear (F_u) and while the data was not fully presented, in the absence of any other sources of information, we have used the reported values to define a PERT distribution; the minimum value was described as “quite a clean pair of underwear”. Another study conducted by Gerba and Kennedy (2007) evaluated the reduction of enteric viruses (rotavirus, hepatitis A virus and adenovirus) on fabric swatches after laundering with detergent only (R_{virus}). While they demonstrated that viruses were redistributed throughout the laundry load, we have assumed that all viruses removed from the contaminated item(s) of clothing

Table 2 – Monthly household incidence of norovirus (Sinclair et al., 2005).

Month	Days per month	Monthly proportion of households with norovirus outbreak
Dec/Jan	62	Uniform (0.007, 0.018)
Feb	28	Uniform (0.002, 0.007)
Mar	31	0.008
April	30	0.003
May	31	0
Jun	30	0.003
Jul	31	0
Aug	31	0.002
Sep	30	0
Oct	31	Uniform (0.003, 0.01)
Nov	30	Uniform (0.005, 0.025)

during washing were suspended in the laundry water, providing a maximum estimate of laundry water norovirus concentration. The popularity of front-loading washing machines has grown in recent years and is nearly equal to that of top loaders (Table 1). Using the typical volume of water for both washing machine types (Roberts, 2005), we constructed a discrete probability distribution accounting for the prevalence of washing machine type and the respective water volume (V_{laundry}).

Householders ($n = 174$) provided information on the sources of collected greywater used to irrigate home produce eaten without cooking. This information, combined with reported months of greywater use per year (Table A-1) and published values of daily per capita greywater generation for each greywater type (Loh and Coghlan, 2003; Roberts, 2004), was used to estimate the proportionate use of each greywater source across the population. Kitchen water and shower wait water (cold tap water discharged while waiting for hot water to reach faucet) were assumed to have zero norovirus. Therefore average greywater quality was determined as: 26.95% bathroom water, 58.96% laundry water and 14.09% clean tap water.

2.2. Dose-response model

The norovirus dose-response models published by Teunis et al. (2008) were used with the fit parameters for the combined inocula dataset (8fIIa + 8fIIb), making no assumption about aggregation state of norovirus particles. One model was developed to accommodate any possibility with respect to the degree of virus aggregation and is therefore the preferred dose-response model to be used where aggregation state is unknown. One of the fit parameters provided by Teunis et al. (2008) exceeds the limits of this model and therefore the Pfaff transformation was used as a very close approximation of the probability of norovirus infection per dose (p_{inf} , person⁻¹ day⁻¹) as follows:

$$p_{\text{inf}} = 1 - \left[\left({}_2F_1 \left(\beta, \frac{\lambda(1-a)}{a}, \alpha + \beta; a \right) \right) \left(\frac{1}{1-a} \right)^{-\left(\frac{\lambda(1-a)}{a} \right)} \right] \quad (8)$$

where ${}_2F_1$ is a hypergeometric function, λ is the dose of norovirus (no. mL⁻¹), α and β are fit parameters and a represents the fit parameter of the (logarithmic series) aggregate size distribution. At doses greater than 33,323 mL⁻¹ the Pfaff transformation fails and so the full Beta-Poisson model (Equation (9)) provides an adequate approximation (Teunis, pers. comm., 19 January 2012)

$$p_{\text{inf}} = 1 - {}_1F_1(\alpha, \alpha + \beta; -\lambda), \quad (9)$$

where ${}_1F_1$ is a confluent hypergeometric function (Teunis et al., 2008). The conditional probability of illness in infected subjects, (p_{ill}), was modelled following Teunis et al. (2008) as:

$$p_{\text{ill}} = 1 - (1 + \eta\lambda)^{-r}, \quad (10)$$

where η and r are described by Teunis et al. (1999). The probability of illness per dose (p_{ill2} , person⁻¹ day⁻¹) was then calculated as

$$p_{\text{ill2}} = p_{\text{inf}} p_{\text{ill}}. \quad (11)$$

2.2.1. Population-level risk

The dose calculations (Equations (1) and (3)) were developed to estimate the risk (p) for an individual consuming greywater-irrigated home-produced lettuce (consumer-only risk). The risk for the average Melburnian (population-level risk; p_{popn}) was estimated as

$$p_{\text{popn}} = pG(1 - G_w) + p_{\text{wash}}GG_w \quad (12)$$

where p is consumer-only daily probability of infection (p_{inf}) or illness (p_{ill2}) for greywater users that irrigate vegetables, p_{wash} is the daily probability for those users that wash vegetables prior to consumption, G is the proportion of the population engaged in greywater irrigation of lettuce and G_w is the proportion of the population that washes vegetables.

The proportion of the Melbourne population that was engaged in greywater irrigation of lettuce (G) was determined as

$$G = \left(\frac{H_{\text{grey}}}{100} \right) \left(\frac{H_{\text{veg}}}{100} \right) \left(\frac{H_{\text{lettuce}}}{100} \right), \quad (13)$$

where H_{grey} is the percentage of Melbourne households that uses greywater, H_{veg} is the percentage of greywater users that uses greywater to irrigate vegetable gardens and H_{lettuce} is the percentage of vegetable gardeners that grows lettuce. H_{grey} was represented by a Uniform distribution that accounted for the variation in Melbourne greywater use behaviours between 2007 and 2010 (ABS, 2007, 2010c, 2010d), H_{veg} was represented by a point estimate obtained from the recent survey of Sinclair et al. (2012) where greywater users indicated their use of greywater on vegetables/herbs/fruit consumed without cooking, and a Uniform distribution was defined for H_{lettuce} , to account for the high proportion of vegetable gardeners that grow lettuce in both the USA (National Gardening Association, 2009) and Australia (Cross and Taylor, 1996). The proportion of the population that washes vegetables prior to consumption (G_w) was also taken into account, using results of a survey of over 500 Melbourne households that reported vegetable washing behaviours (Mitakakis et al., 2004). This survey asked individuals to indicate how often they washed salads and/or vegetables prior to serving. A washing probability was allocated to the qualitative survey responses (Table 3) and the proportion of the population washing vegetables prior to consumption was estimated as

$$G_w = \sum_{\text{always}}^{\text{never}} (\text{washing probability})(\text{response rate}), \quad (14)$$

where *always* and *never* represent the extremes of washing prevalence, *washing probability* is the estimated value attributed to the qualitative prevalence of vegetable washing, and *response rate* refers to the proportion of survey responses selecting the particular washing prevalence. The mean proportion of the Melbourne population that washes vegetables was 0.87, consistent with results of a survey of 2000 households in the United States that found that 81% washed fresh produce just before preparation and cooking (Li-Cohen and Bruhn, 2002).

2.2.2. Annual risk

Annual probability of infection or illness, P , was determined as

Table 3 – Prevalence of vegetable washing in Melbourne households in response to the survey question “Do you wash salads and/or vegetables before serving them?”

Qualitative prevalence of washing – survey responses	Estimated washing probability ^a	Percentage of responses (%) (n = 524) ^b
Always	1.00	66.9
Usually	0.70–0.80	24.3
Sometimes	0.20–0.30	7.6
Rarely	0.01–0.05	0.4
Never	0.00	0.6

a Where vegetable washing probabilities were represented by a range, a Uniform distribution was used.

b (Mitakakis et al., 2004).

$$P = 1 - \prod_{k=1}^d (1 - p_k), \quad (15)$$

where p_k is the k th probability of infection or illness per exposure event and d is the number of exposure events per year (Karavarsamis and Hamilton, 2010). d was represented by a Discrete Frequency distribution (days of greywater use per year), determined from survey results of 575 households (Table A-1), and was randomly sampled (with replacement) for each annual calculation. As a comparison, we evaluated daily greywater irrigation as well ($d = 365$).

Annual disease burden was represented using the Disability Adjusted Life-year, DALY, metric. The DALY is a measure of overall disease burden and is expressed as the number of years lost due to illness, disability or premature death. The annual disease burden (DB; DALYs person⁻¹ year⁻¹) of norovirus illness was estimated as:

$$DB = P_{ill2} B S_f, \quad (16)$$

where P_{ill2} is the annual probability of illness per dose (from Equation (15)), B is the disease burden (DALYs per case of norovirus illness) and S_f is the proportion of the population susceptible to the disease. In the absence of Australian estimates of B , international estimates were used to define a Uniform distribution (Cressey and Lake, 2009; Haagsma et al., 2008; Kemmeren et al., 2006; Lake et al., 2010; Masago et al., 2006). There is evidence of resistance to norovirus infection (Johnson et al., 1990; Lindesmith et al., 2003; Teunis et al., 2008) related to both histo-blood group antigens and secretor status (Le Pendu et al., 2006), although it has been suggested that, due to the variation between norovirus genotypes, it is likely that every person is genetically susceptible to at least one norovirus genotype (Atmar, 2010). The dose-response models of Teunis et al. (2008) were constructed for secretor-positive individuals only, i.e. those individuals thought to be susceptible to norovirus infection, and accounting for approximately 80% of populations of European descent (Denborough and Downing, 1968; Thorven et al., 2005); theoretically the dose-response models should only be applied to that proportion of the population. Since susceptibility to norovirus is uncertain, S_f was represented by a Uniform distribution from secretor-positive individuals (0.8) through to all individuals (1.0).

2.3. Model implementation and evaluation

To account for variability and/or uncertainty in the model, probability distributions have been used for most input parameters and Monte Carlo simulation of 3,650,000 iterations (providing sufficient values for at least 10,000 annual calculations) was used to calculate daily probabilities. For each iteration a set of input variables was drawn from probability distributions (Table 1), accounting for seasonality of norovirus outbreaks. For each estimate of annual probability, d daily probabilities were randomly selected from the appropriate months of the year to obtain a simulated distribution of 10,000 values of annual risk. Sensitivity analyses, using Spearman's rank correlation coefficient, were conducted with values from 1000 iterations of the exposure model, comparing input parameters with daily probability of infection. For all model outputs, confidence intervals were estimated using the percentile method (Buckland, 1984) and 95th percentile values reported unless otherwise stated. In comparing scenarios, the difference was deemed statistically significant if there was no overlap in the 90% confidence intervals. Pseudo-random sampling was used because a set seed was used for each random sampling function to enable repeatability of the model if required. All modelling and analysis was performed in 'R' version 2.12.2 (The R Foundation for Statistical Computing, 2011).

Model outputs were considered against three different health targets. The widely accepted threshold of 10^{-6} DALYs per person per year was used to evaluate tolerable annual burden of disease and the USEPA's original benchmark of 10^{-4} (USEPA, 2002) was used to evaluate annual probability of infection. More recently, Signor and Ashbolt (2009) have suggested that a daily risk target be used to protect against shorter-duration, higher risk events. They proposed a daily health target of 1×10^{-6} probability of infection, derived from the USEPA's 10^{-4} annual infection probability target and assuming exposure 365 days per year. Following this logic, starting from the 10^{-6} tolerable annual burden of disease, and using Equation (15), a daily burden of disease target would be 2.7×10^{-9} . Using Equation (16) and the mean values from Table 1, the tolerable daily probability of norovirus illness would be 9×10^{-7} .

3. Results

Estimated concentrations of norovirus in greywater spanned a few orders of magnitude (Fig. 1), with median concentrations of 4.69×10^{-4} and 8.31×10^{-2} mL⁻¹ for bathroom and laundry greywater, respectively. The bathroom greywater estimate determined from the published *E. coli* concentration in Rose et al. (hereinafter referred to as bathroom_{Rose}) sat between these estimates with a median of 5.28×10^{-3} mL⁻¹.

Median annual disease burden ranged from 2×10^{-10} – 1×10^{-4} , while 95th percentile values ranged from 4×10^{-9} – 7×10^{-4} DALYs (Fig. 2). For the Melbourne population, use of bathroom water (4×10^{-9}) was the safest option. Use of laundry water posed a significantly higher level of risk, in excess of the tolerable burden of disease (3×10^{-6}), and average greywater was similar (2×10^{-6}). Consumer-only

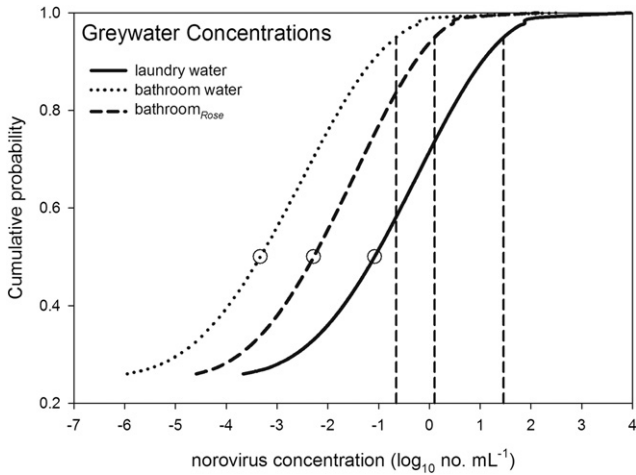


Fig. 1 – Cumulative probability distributions of estimated norovirus concentrations (\log_{10} no. mL^{-1}) in different greywater sources. Open circles are median values and dashed vertical lines are 95th percentile values. For three months of the year norovirus was not detected in households (Sinclair et al., 2005), so ~25% of greywater concentration estimates are zero.

results were similar with the use of bathroom water providing the safest option for vegetable washers (2×10^{-8}) and non-vegetable washers (7×10^{-7} ; Table A-5). Reuse of laundry water exceeded the tolerable level of risk for vegetable washers (4×10^{-5}) and non-vegetable washers (5×10^{-4}). The estimates of annual disease burden for bathroom_{Rose} were approximately 1 order of magnitude higher than our estimates for bathroom water, while the use of survey exposure data (days of greywater use per year) had negligible impact on annual disease burden relative to the use of greywater every day of the year (data not shown).

Using the USEPA’s threshold of 10^{-4} annual probability of infection, none of the greywater types would be acceptable for use, with 95th percentile values ranging from 2×10^{-2} (average greywater) to 1 (laundry water, no washing of lettuce). Signor and Ashbolt (2009) recommended using a daily risk target of 10^{-6} probability of infection, using both the mean and 95th percentile values to test against the target. All 95th percentile values exceeded this threshold, ranging from 2×10^{-6} (bathroom water) to 3×10^{-2} (laundry water, no washing of lettuce), while all mean values, apart from bathroom water (8×10^{-7}), also exceeded the threshold (Table A-2). Daily probabilities of illness ranged from 6×10^{-11} in bathroom water (population risk) up to 2×10^{-4} in laundry water (consumer-only no washing of lettuce; Table A-3), while average greywater (6×10^{-7}) was below our health target of 9×10^{-7} .

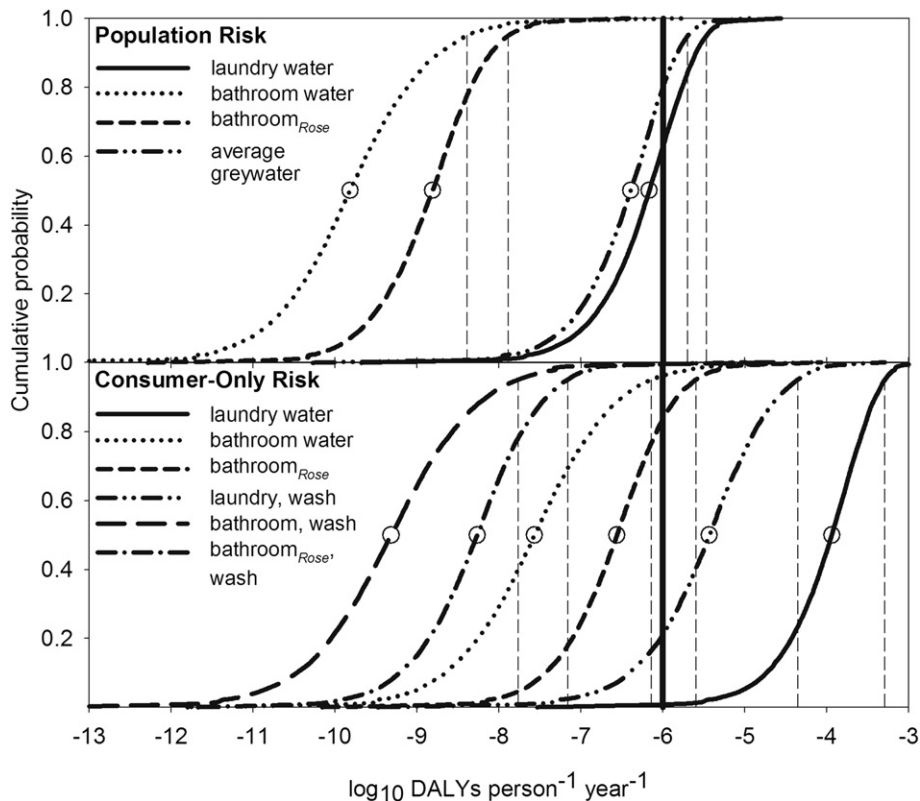


Fig. 2 – Cumulative probability distributions of disease burden (\log_{10} DALYs person^{-1} year^{-1}) for population risk (top) and consumer-only risk (bottom). Consumer-only risk is presented for vegetable washers (“wash”) and non-vegetable washers. Open circles are median values and the dashed vertical lines are the 95th percentiles. The heavy vertical line is the 10^{-6} threshold.

All model input parameters were independent (Spearman's rank correlation coefficient, $\rho < 0.1$) and sensitivity analyses (Table 4) revealed that uncertainty in the norovirus shedding rate (SR_{NV}) overwhelmingly accounted for the variability in daily probability of infection. Faecal contamination of underwear (F_u) and *E. coli* concentration in bathroom water (C_{Ecoli_bath}) also contributed significantly to model output variability, while variability in consumption rate (C_{EPA}), withholding period (t) and \log_{10} reduction from washing of lettuce (w) contributed a smaller amount.

4. Discussion

Greywater reuse is becoming more common, especially in arid cities such as Melbourne, yet there has been limited assessment of human health impacts; our study provides one of the first estimates of viral health risks associated with greywater irrigation. While the use of laundry water posed the highest risk, the median annual disease burden for all population-level risks was estimated to be below the guideline value for acceptable risk (10^{-6} DALYs person $^{-1}$ year $^{-1}$; NRMCC et al., 2006). Disease burden can be reduced further with a shift in greywater preference to use of bathroom water rather than laundry water.

Consideration of alternative health targets results in variable conclusions. The USEPA drinking water health target of 10^{-4} annual probability of infection (USEPA, 2002) is more restrictive and would deem all greywater irrigation scenarios unacceptable at both median and 95th percentile values (Table A-4). Signor and Ashbolt (2009) suggested a risk target of

$<1 \times 10^{-6}$ daily probability of infection while we determined a tolerable daily probability of norovirus illness of 9×10^{-7} . Based on 95th percentile values the former would preclude the use of all greywater sources while the latter would exclude laundry water at the population level (Table A-2). The daily risk target is particularly suited to situations where there is opportunity for risk management. The domestic greywater reuse scenario provides limited control points – apart from choice of greywater source or “opting out” of greywater use on days when a household member is ill. A third option would be to discourage use of greywater during months of higher norovirus incidence, but this is unrealistic as higher norovirus prevalence in Melbourne coincides with the warmer months of the year when greywater irrigation is most popular. Alternatively, while not included in this model, the use of biocides (particularly in laundry water) could reduce norovirus contamination of greywater.

The estimated risk associated with greywater irrigation of home grown lettuce should also be considered within the broader context of community rates of gastroenteritis. Australian studies have consistently reported high rates of gastrointestinal illness, from 0.8 cases per person per year in Melbourne in the late 1990s (Hellard et al., 2001) to 0.92 cases per person per year across Australia between 2001 and 2002 (Hall et al., 2004; Hall et al., 2005). Conservatively using annual probability of infection (rather than illness), the model estimated up to 0.03 additional cases of norovirus infection per person per year (95th percentile values), approximately 1 extra case per person every 33 years.

In the absence of norovirus measurements in greywater, we used two different methods to estimate norovirus concentrations of 5×10^{-4} – 8×10^{-2} mL $^{-1}$ in bathroom and laundry water, respectively (median values). These values seem reasonable, being 4–6 orders of magnitude lower than reported median norovirus values in raw sewage (~ 400 – 600 copies mL $^{-1}$; Haramoto et al., 2006; Katayama et al., 2008). As previously described, our methods used an estimate of faecal load, measured directly on underwear for laundry water or estimated from *E. coli* in bathroom water. Other investigators have also used faecal loading as the starting point of their modelling. Ottosen and Stenström (2003), using two different methods to estimate faecal load in greywater, found that *E. coli* resulted in a daily faecal load of 65 g while coprostanol concentrations resulted in an estimate of 0.04 g per person per day. We substituted these values of faecal load and daily per capita greywater generation (64.9 L; Ottosen and Stenström, 2003) into Equation (4) and, using median values of SR_{NV} , O and P , obtained approximate norovirus concentrations of 2.8×10^{-3} and 4.5 mL $^{-1}$ for coprostanol and *E. coli* methods, respectively. Our sensitivity analysis clearly showed that the output variation was largely attributable to the variation in norovirus concentrations (specifically norovirus shedding rate). Further refinement of the risks associated with greywater reuse will require measurements of norovirus rather than estimates of their concentrations.

E. coli concentrations in laundry water could not be used to estimate faecal load due to the unknown effects of detergents and/or biocides used by householders. In contrast, we assumed that bathroom water samples did not contain biocides as typically only shampoos and soaps are used. As

Table 4 – Spearman rank order correlation coefficients (ρ)^a for daily probability of infection (representative values).

Model input parameters ^b	Laundry water, no washing of lettuce (Dec/Jan)	Bathroom water, washing of lettuce (Oct)	Bathroom _{Rose} , washing of lettuce (Feb)
V	0.055***	0.049***	0.054***
I	0.186***	0.175***	0.191***
C_{EPA}	0.185***	0.174***	0.190***
L	-0.010***	-0.004*	0.002
m	0.003*	0.001	0.003
SR_{NV}	0.870***	0.810***	0.895***
O	0.079***	0.094***	0.104***
P	0.113***	0.104***	0.113***
F_u	0.323***	n/a	n/a
R_{virus}	0.006***	n/a	n/a
$V_{laundry}$	-0.105***	n/a	n/a
F_{faeces}	n/a	-0.108***	-0.119***
C_{Ecoli_bath}	n/a	0.396***	n/a
k	-0.021***	-0.019***	-0.021***
t	-0.187***	-0.174***	-0.190***
w	n/a	-0.229***	-0.250***

a For $\rho \geq 0.5$, the relationship is considered strong. P-values are represented as: *** <0.001, ** <0.01, * <0.05 and indicate if the value of ρ is significantly different from zero.

b Refer to Table 1 for definition of input parameters.

greywater samples were collected fresh and transported (refrigerated) to the laboratory for analysis within 24 h (O'Toole et al., 2012), we have assumed that there was no growth or decay of *E. coli* post-collection. Laundry water values may overestimate norovirus concentration as we assumed that all viruses end up in laundry water, while it has been demonstrated that viruses were redistributed throughout the load and transferred to other items in the load (Gerba and Kennedy, 2007). This could be another exposure pathway but it was not considered in this model.

Greywater can be used for a range of purposes, including landscape irrigation, toilet flushing and laundry washing. Irrigation of vegetables, as considered in this model, represents a high risk reuse activity and may not be indicative of risks associated with other uses.

While previous QMRA models on wastewater irrigation have addressed risks associated with treated municipal sewage (Hamilton et al., 2006; Petterson et al., 2001a, b; van Ginnekan and Oron, 2000) and greywater from a housing development (Ottozon and Stenström, 2003), our model evaluated a very different scale of reuse—that of an individual household. In this context, it is important to consider the findings of this model alongside other possible exposure pathways. In particular, secondary transmission of norovirus within a household can be very high, with reports of 14% secondary attack rates in households with an infected individual (Alfano-Sobsey et al., 2012). As well, the potential human health risks from greywater reuse may be higher in multi-dwelling premises than in a single domestic dwelling (EPAV, 2008); in-house reuse of greywater will result in recirculation of pathogens to which householders are likely exposed through multiple different pathways other than just greywater irrigation (Maimon et al., 2010) while greywater sourced from multiple households may contain pathogens to which some users have not been exposed. It is highly likely that the estimated norovirus disease burden associated with greywater irrigation of vegetables is negligible relative to household contact with an infected individual. Further research is required to allow consideration of the potential differences in risk between single and multi-dwelling reuse schemes.

While this paper has demonstrated the relatively low level of health risks associated with current community greywater reuse practices, future guidelines or policies should consider the implications of possible increases in community greywater reuse as well as other risks not included in this model, such as chemical risks and soil and environmental health (Maimon et al., 2010; Pinto et al., 2010; Travis et al., 2010).

5. Conclusion

To assess human health risks associated with domestic greywater reuse, QMRA was used to estimate the annual disease burden from consumption of greywater-irrigated vegetables. The results of this study showed that:

1. greywater use across the Melbourne population had a median annual disease burden of $<10^{-6}$ DALYs per person, while among those using greywater to irrigate

home grown vegetables median annual disease burdens ranged from 10^{-10} to 10^{-4} depending on the source of greywater and vegetable washing behaviours,

2. a shift in greywater preference to bathroom water could reduce annual disease burden by up to 1000-fold,
3. estimated risks associated with greywater reuse on home grown lettuce were orders of magnitude lower than reported risks of secondary infection within households, and
4. choice of health target has a significant bearing on the conclusions drawn from a study and requires further discussion and thought to provide guidance to water authorities and risk managers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2012.12.012>.

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