MICROBIAL RISK ASSESSMENT AND HAZARDS ASSOCIATED WITH GREYWATER AND WASTEWATER RECYCLING IN SOUTH AFRICA: A REVIEW

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ABSTRACT. **Microbial risk assessment and hazards associated with greywater and wastewater recycling in South Africa: a review**. Sustainable water security and management in South Africa have challenges that include climate change impacts, increasing water demand, and dysfunctional infrastructure. These challenges result in the necessity for additional water management and reclamation strategies. Greywater (domestic wastewater) has been recognized for its potential for treatment and reuse for non-potable purposes such as toilet-flushing, irrigation, and firefighting. This wastewater management strategy has been highlighted as important because greywater is produced in large quantities and has low to medium pollution levels. The benefits of this strategy are not only related to the decreasing of freshwater use but also to preparedness for emergency disasters such as disease outbreaks (provision of consistent sanitation and hygiene) and urban/rural fires. Various greywater treatment systems (GWTs) have been operated, evaluated, and implemented. The threat from the implementation of GWTs is the recalcitrant microbial content in treated greywater which pose health risks to the environment and when reused for mentioned non-potable purposes. This is assessed by the low microbial removal efficiencies of different GWTs. Risk assessment methods from previous studies are considered, for example, the Quantitative Microbial Risk Assessment (QMRA), and Cumulative Risk Rating (CRR). This review presents a framework for a microbial risk assessment approach in the context of greywater treatment and reuse in South Africa.

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INTRODUCTION

Population growth has increased water demand due to domestic, agricultural and industrial usage (Wolski *et al.,* 2020). The water supply systems are under pressure from the increased demand. The old and dysfunctional infrastructure and energy crises also negatively affect the efficient functioning of conventional wastewater treatment plants, causing waste, contamination, and uneven water distribution for the communities (Dube*,* 2024). Apart from rainfall, water availability and access in South Africa is also dependent on the Strategic Water Source Areas (SWSAs) which are defined as regions (i.e. rivers, catchments and aquifers) that supply large amounts of water to the water supply systems (i.e. small rivers, boreholes, dams and reservoirs) (Nel *et al*., 2017; Maitre *et al*., 2018; DWS, 2020; Stats SA, 2022). In addition to the water demanding sectors, climate change also has a significant impact on SWSAs (Molobela and Sinha, 2011; Stats SA, 2022). The inconsistent access to water, sanitation and hygiene (WASH) directly affects emergency and public health services; for example, communities tend to be prone to disasters such as fires and spread of diseases due to compromised sanitation and hygiene for example COVID-19. The major national and local socio-economic sectors, such as agriculture, industry, and tourism, are also severely affected by the improper management and scarcity of water. Improvement and teaching of the existing water policy to the communities is crucial for efficient management of water availability and quality (DWS, 2020). Investment in water research institutions and innovation are also important approaches for efficient and sustainable water security and quality management in South Africa.

Due to the highlighted water challenges and impacts, greywater treatment and reuse have emerged as a promising sustainable approach for mitigation. This approach has been conceptualized in the field of wastewater treatment worldwide as it leads to a decrease in the demand, pressure and dependency on freshwater sources (i.e. SWSAs, underground water and rainwater harvesting), and desalination technology (i.e. reverse osmosis) (Friedler, 2004; Carden *et al*., 2007a; Langergraber and FucWolski, 2009; Zuma *et al*., 2009; Rodda *et al*., 2011; Blersch *et al*., 2014; Al-Ismail *et al*., 2017). Greywater can be described as an opaque or grey-coloured domestic wastewater from either the bathroom, kitchen, laundry or mixed origin, and it excludes toilet wastewater (Griffoen and Natha, 2013; Pachkor and Parbat, 2017; Irshad and Mansoor, 2021). The potential for remediation and reuse of greywater is influenced by its low to medium level of pollution and because it is generated at significant volumes in households (Oh *et al*., 2018; Kapata *et al*., 2021; Madikizela *et al*., 2022a; Van de Walle *et al*., 2023).

According to the South African guidelines for greywater treatment and reuse, greywater is categorized as light and dark (Bakare *et al*., 2017; Carden *et* *al*., 2018; Kurniawan *et al*., 2023). Light greywater is divided into classes 1 and 2, which are sourced from the bathroom and laundry, respectively, while dark greywater is sourced from the kitchen (Ghaitidak and Yadav, 2015; Carden *et al*., 2018; Kurniawan *et al*., 2023). Dark greywater is the most polluted due to high concentrations of organic materials, while light greywater has low organic content (Carden *et al*., 2007a; Li *et al*., 2009; Carden *et al*., 2018). The classification of greywater according to its source is important because it helps predict contamination, remediation, management and reuse (Carden *et al*., 2018).

The aim and objective of this literature review is to evaluate microbial health risk assessment approaches for water and wastewater treatment systems (i.e. Greywater treatment systems for this study) and establish metabarcoding as a robust preliminary, and applicable method for assessing the sustainability in terms of biological treatment and pathogen related risks in decentralized GWTs. This review is addressing the limited application of QMRA in South African context due to data requirements such as pathogen specific data and dose response models. The key research gaps include seldom integrated microbial risk assessment frameworks (i.e. microbial profiling in GWTs) and reliance on culture-based techniques, which do not fully profile the diversity of bioremediating and pathogenic microbial communities. For instance, Ilemobade *et al.* (2013) is the solely South African study that conducted health risk assessment (i.e. diarrheal disease through ingestion scenario during toilet flushing) in GWTs. The components of this review include description of greywater quality and quantity, performance and sustainability of GWTs, microbial risk assessment methods, and applied microbial risk assessment methods in South African water systems.

METHODOLOGY

This literature review was conducted using a PRIMSA informed/guided strategy comprising identification, screening and eligibility assessment, and inclusion of relevant studies. The conventional full PRISMA 2020 framework was implemented, however substantial literature search was conducted to identify studies relevant to greywater quality and quantity, greywater treatment technologies, microbial risk assessment methods in greywater treatment technologies, and metabarcoding and metagenomics applications in water and wastewater systems. The employed literature databases include Academia.edu, the Directory of Open Access Journals (DOAJ), Google Scholar, ScienceDirect, Scopus, and Water Research Commission (WRC) intranet. Studies from year 2003 to 2025 were considered according to the thematic area under review. Upon screening and eligibility assessment, 25 studies were cited in the introduction section, while 20, 24, and 45 studies relevant to quality and quantity of greywater, performance of greywater treatment technologies, and microbial risk assessment approaches were cited respectively. The methodology flow diagram is shown in Figure 1.

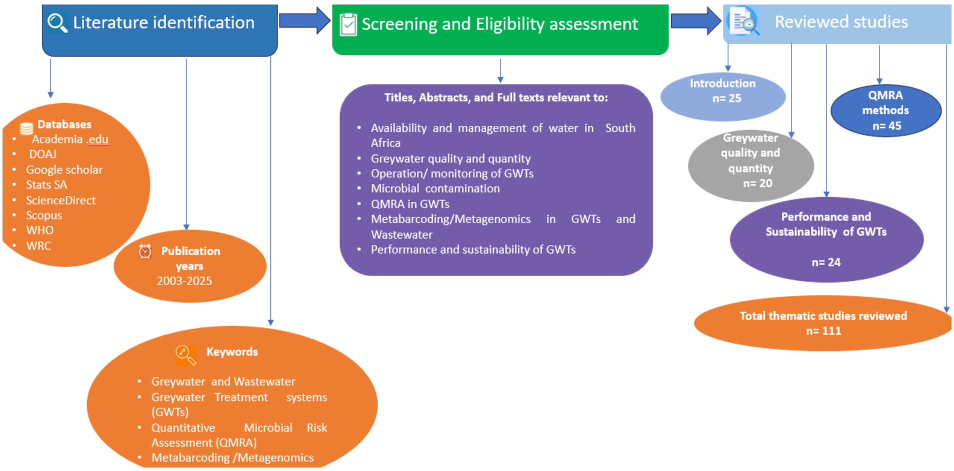

Fig. 1: Methodology flow diagram of the review. *The review was conducted using PRISMA informed strategy, the number of reviewed/cited studies per section of the review are shown (n=). The total thematic studies reviewed refers scientific studies including organisational reports/guidelines.*

RESULTS AND DISCUSSION

3.1. Quality and Quantity of Greywater

Understanding the quality parameters and quantity of greywater is important for optimizing efficient GWTs (Bakare *et al.,* 2017; Oteng-Peprah *et al.,* 2018; Adesoji, 2022; Filali *et al.,* 2022). The quality of greywater is described in previous South African studies (Nel *et al.,* 2022) and elsewhere. Table 1 also presents averages and ranges of greywater quality reported in various studies.

Additionally, Kozyatnyk and Njenga (2023) also reported greywater to have organic constituents such as pharmaceutical products, pesticides, agrochemicals, and surfactants. Judging by the reported quality parameters, greywater treatment technologies are important to effectively remediate greywater before reuse to minimize possible health risks to humans and the environment (i.e. soil and plants).

Table 1: Reported greywater quality

Quality parameter	Average/Range	References
BOD5 (mg/L) COD (mg/L) EC (mS/cm) TSS (mg/L) Turbidity (NTU)	185.25 1426.37 156.00 504.00 120.00	Bakare *et al.,* 2017
Faecal coliforms/*E.coli* (CFU/100ml) *Klebsiella* *Enterobacter cloacae* *Corynebacterium* *Bacillus*	2.80 $x10^7$ Identified, not quantified	Leuta *et al.,* 2020
pH Turbidity (NTU) Total coliforms (CFU/100ml) *E. coli* (CFU/100ml)	5.80 to 9.00 20.10 to 814.00 $6.83x10^5$ to $3.26x10^9$ $0.11x10^4$ to $0.77x10^8$	Kapata *et al.,* 2021
BOD5 (mg/L) COD (mg/L) Chloride (mg/L) TDS (mg/L) TSS (mg/L) Turbidity (NTU) Sulphate (mg/L) Fluoride (mg/L) Nitrate (mg/L) Anionic surfactants (mg/L) Total coliforms (MPN/100ml) *E. coli* (MPN/100ml)	265.73 1754.67 438.00 1837.73 469.13 281.80 87.93 0.87 6.83 16.45 73.40 386.20	Khapra and Singh, 2023

BOD5 (Biological Oxygen Demand), COD (Chemical Oxygen Demand), EC (Electrical Conductivity), TSS (Total Suspended Solids), TDS (Total Dissolved Solids).

Volumes of greywater are different depending on the availability, uses of water and population in a community. For example, Carden *et al.* (2007a) reported an average of 2962.00 litres per hectare per day of greywater volumes from different communities across the South African provinces. While Bakare *et al.* (2016) and Kurniawan *et al.* (2023) reported that the generation of greywater is related to the water service provision, water consumption and environmental health awareness of the communities. Bakare *et al.* (2016) also investigated the community's perceptions of the treatment and reuse of greywater and reported that about 80.00% of the population agreed to install GWTs in their homes for safe and efficient reuse. Additionally, Hassane *et al.* (2021) and Irshad and Mansoor (2021) observed greywater generation of approximately 88 and 62 litres per person per day, respectively, while during the COVID-19 lockdown in South Africa the single household greywater was estimated to account for about 85.00% of the municipal drinking water consumption and bathroom, kitchen and laundry greywaters were reported to be produced, on averages ,71.00 ± 16.00, 27.00 ± 31.00, and 9.72 ± 0.90 L per person per day, respectively (Madikizela *et al* .,2022a). Therefore, in addition to the low contamination, the high volumes and perceptions for the reuse of greywater show the need for efficient greywater treatment technologies (Chaillou *et al.,* 2011; Ilemobade *et al.,* 2013; Elhegazy and Eid, 2020; Akter, 2022; Khan and Kaafil, 2020).

Further insights come from surveys conducted at Rhodes University dining halls and residences, which revealed that greywater production amounted to between 65.00% and 90.00% of blackwater production. These estimations were based on observations of kitchen activities (dishwasher and sink capacity), bathroom usage (showers and basins), and laundry operations (washing machine capacity), all adjusted for the number of students. The interest in greywater treatment and reuse is driven by its large volumes and the unique characteristics of greywater itself. Among the quality parameters of greywater, microbiological content remains a primary concern due to the potential health risks of greywater reuse. The high microbial levels in greywater underscore the necessity for efficient and sustainable treatment, alongside careful risk assessment based on the intended reuse purpose. Expanding knowledge of greywater's microbial quality is crucial, as it can foster positive perceptions of its treatment and reuse (Bakare *et al.,* 2016), ultimately ensuring community safety and sustainability. Additionally, understanding the microbial quality of greywater is essential for designing effective treatment systems, guiding both system composition and operational strategies.

3.2 Performance and Sustainability of GWTs

GWTs are essential to sustainable water management, particularly in regions facing water scarcity. Their ability to effectively remove contaminants from greywater is crucial to ensuring the safety and suitability of treated effluent for non-potable uses. However, a critical aspect of greywater treatment is the removal of microbiological contaminants, which is a significant concern, even in well-performing systems. Technologies such as, Adsorption, Advanced Oxidation Ponds, Constructed Wetlands, Filtration systems, Rotating Biological Contactors, Sequenced Batch Reactors, and Upflow anaerobic sludge blanket have been operated (UASB) (Oteng-Peprah *et al.*, 2018; Bani *et al.*, 2025).

Table 2: Advantages and disadvantages of different GWTs

GWTs (description)	Advantages	Disadvantages	References
Mulch Tower filter composed of mulch, coarse sand, fine and coarse gravel.	Easy operation and potential for onsite application. Efficient removal of TSS.	Limited removal of physicochemical constituents. Inefficient removal of microbial contents.	Zuma *et al.*, 2009; Tandlich *et al.*, 2009; Whittington-Jones *et al.*, 2011
Sand filter system.	Low-cost maintenance. Decentralised in student residences and reuse of effluent for toilet flushing. Removal efficiency of Turbidity (53.20 %).	Inefficient removal of microbial contents. Potential to affect occupants/students with ordo and dermal contact, resulting into health risks.	Griffoen and Natha, 2013
Fly Ash/Lime Filter Tower.	Decentralised system. Efficient removal of phosphates, chloride, nitrate, ammonium, and COD at 51%, 40%, 52%, 50%, and 85% respectively. Reported to have 60% average removal efficiency of Faecal coliforms. The effluent is suitable for reuse in irrigation.	Operational challenges such as clogging. Unsustainable removal of microbial contents.	Zuma and Tandlich, 2016; Nondlazi *et al.*, 2017
Hybrid system. Constructed Wetlands/Microbial Fuel Cells/Biosand filter.	Promising performance with COD removal efficiency of 99% and 4-log reduction of *E. coli*. The effluent is reported to be compliant with drinking water standards.	Only evaluated at a laboratory scale for greywater from handwash. High-cost operation and maintenance.	Bolton and Randall, 2019
Horizontal Roughing Filter	Removal efficiency of COD and Turbidity at 90% and 60% respectively. Piloted system for underdeveloped settlements.	Microbial removal is not evaluated and is likely to be inefficient. Operational challenges such as ponding of greywater and evolving of insects.	Mtsweni *et al.*, 2019
Nanofiltration membrane system (UiO-66-NH_2@GO nanocomposites)	The permeate is reported to be compliant with non-potable reuse standards.	The system /membrane is not extensively evaluated for its performance. Fouling and clogging is highly possible. The membranes showed insignificant inhibition of microbial growth.	Matebese and Moutloali, 2021
Biochar-based constructed Wetlands	Efficient removal of physicochemical parameters.	Inefficient removal of faecal coliforms.	Aylan *et al.*, 2023; Kanfade *et al.*, 2023

The design, mechanisms, and performances of various greywater treatment technologies are extensively reported in the review studies conducted by Oteng-Peprah *et al.* (2018), Khalil and Liu. (2021), Kurniawan *et al.* (2023), and Awasthi *et al.* (2024). Table 2 further describes the advantages and disadvantages of different GWTs in terms of operation, maintenance, and removal efficiencies of physicochemical and microbial contaminants.

Among the mentioned and discussed GWTs by Bani *et al.* (2025), Filtration systems are highlighted to have an advantage due to its capability of biological and physicochemical remediation which is assisted by the diverse microbiome attached on the treatment media such as gravel stones. Filtration systems are also versatile as they can be operated using various treatment media such as biochar, gravel stones, and mulch, and also in different configurations such as towers, trickling filters, and hydroponics (Sami *et al.*, 2013; Eregno *et al.*, 2017; Yaseen *et al.*, 2019; Boano *et al.*, 2021; Costamagna *et al.*, 2021; Lakho *et al.*, 2021; Prabha *et al.*, 2021; Çömez *et al.*, 2024; Mendoza *et al.*, 2024). The inefficient removal of microbial concentration is a disadvantage of GWTs, including Filtration systems hence final purification units such as disinfection tanks are essential.

The performance of GWTs reported by Bani *et al.* (2024a), focused on the effectiveness of removing both microbiological and physicochemical constituents, emphasising the continued challenge of microbiological contamination. Removing microbiological contaminants, particularly faecal coliforms, is a concern in greywater treatment. Faecal coliforms, which are commonly found in greywater, are indicators of potential pathogenic contamination that pose health risks when the water is reused without proper treatment. Upon evaluating the microbial removal performance of the GWTs, most systems show limited to moderate success in removing these pathogens. For instance, the Mulch Filter Tower (Zuma *et al.,* 2009) demonstrated insufficient removal of faecal coliforms, indicating that this system was insufficient for microbial safety for direct reuse (Table 2). Similarly, the Lab-scale Gravel Filter Tower and Pilot-scale Gravel/Biochar/Sand Filter (Bani *et al.,* 2024a) showed inefficient removal of faecal coliforms. While the Pilot-Scale Fly Ash/Lime Filter Tower (Nondlazi *et al.,* 2017) reported 60.00 ± 0.010 % removal, suggesting that the combination of fly ash and lime in the filtration process may be more effective in microbial removal (Table 2). However, the general performance in microbial removal across most of the GWTs is not sufficient, with substantial residual microbial content remaining in the treated effluent (Oteng-Peprah *et al.,* 2018; Awusthi *et al.,* 2024; Bani *et al.*, 2025; Table 2).

These findings highlight a critical challenge in the sustainability of GWTs, while progress has been made in reducing the microbiological load, greywater remains a potential vector for opportunistic pathogens. As microbial contamination is often not completely eliminated by the GTWs, microbial content continues to pose a threat to the safety of treated greywater, particularly in cases where it is reused for irrigation, toilet flushing and fire-fighting. Due to the residual microbial content in treated greywater, it is essential to incorporate the QMRA approach into the design and operation of GWTs. The QMRA approach assists in evaluating the potential health risks posed by exposure to pathogens that may be present in GWTs, providing a scientific basis for establishing safety guidelines and operational limits.

The profiling of potential exposures, quantifying risks, and assessing the performance of biological treatment process can guide the development of targeted disinfection strategies to address the residual microbial content, especially for high-risk applications such as the irrigation of crops, toilet flushing and firefighting. Additionally, QMRA can assist in optimizing operational protocols, ensuring that GWTs are adequately monitored and that any residual microbial risks are minimized through additional disinfection methods, such as ultraviolet (UV) disinfection, oxidation ponds or chlorine treatment, which are known to reduce microbial content. This will enhance public health protection and make greywater reuse a more viable, reliable and sustainable water management strategy for water-scarce regions.

While microbiological removal remains a challenge, the GWTs are promising in terms of removing physicochemical constituents. As presented in Table 2, the GWTs have high to moderate removal efficiencies for turbidity and TSS, which contribute to the safe quality of treated water. The previously operated Pilot-scale Gravel/Biochar/Sand Filter had a promising removal of ammonia (85%) and COD (67%), showcasing the effectiveness of biochar in adsorption and enhancing filtration (Bani *et al.*, 2024a). These physicochemical improvements are critical for ensuring that greywater is suitable for non-potable applications. However, the limitations in microbiological removal underscore the need for additional treatments and effective monitoring (i.e. QMRA), particularly for systems targeting broader reuse applications and preservation of the treated effluent.

3.3.Microbial Risk Assessment methods in GWTs

The primary concern of treatment and reuse of greywater include health risks and disasters that may arise and affect humans and the environment (Benami *et al.,* 2016b; Dada and Gyawali, 2021; Marques *et al.,* 2021; Nel *et al.,* 2022). The health and disaster risks must be considered by conducting practical assessment and quantification, which is important for improving operation of sustainable GWTs and safer reuse of the effluents. The WHO and South Africa have provided guidelines for safer reuse of treated greywater (WHO, 2006; Carden *et al.,* 2018). Studies have explored risk assessment and quantification approaches. The most popular approach is the QMRA, which is the prediction of possible health risks related to exposure to pathogenic microorganisms in the water systems (i.e. GWTs) and in the effluents that are to be reused for toilet flushing, fire-fighting, and garden irrigation (Ottoson and Stenstrom, 2003; Maimon *et al.,* 2014; Benami *et al.,* 2016b; WHO, 2016; Shi *et al.,* 2018; Kusumawardhana *et al.,* 2021). As described in the study conducted by Maimon *et al.* (2014) and elsewhere, the QMRA approach covers four steps, namely: hazard identification, exposure assessment, dose-response model(s) and risk characterization. In Figure 2, the QMRA process in GWTs is demonstrated.

Hazard identification refers to the profiling and describing pathogenic microorganisms likely to be present in greywater. These include faecal coliforms/*E coli*, *Staphylococcus aureus*, *Salmonella* and *Campylobacter* (bacteria), Norovirus (viruses) and Cryptosporidium (protozoa) (Ottoson and Stenstroom, 2003; Maimon *et al*., 2014; Dalahmeh *et al*., 2016; Benami *et al*., 2016a, b; Eregno *et al*., 2017; Busgang *et al*., 2018; Shi *et al*., 2018; Nel *et al*., 2022). The exposure assessment focuses on the exposed population in terms of its size, the exposure routes, and the rate of exposure, while the dose-response model provides a description of the mathematical relationships used to predict the exposure to a pathogen and likelihood of infection (Benami *et al*., 2016b; Busgang *et al*., 2018). The risk characterization step is based on the synthesis of the information from the previous steps and the description of the yearly probability of infection, illness and maximum acceptable risks according to the highlighted exposure scenarios (Benami *et al*., 2016a; Benami *et al*., 2016b). The detailed description and application of the QMRA steps in various studies is provided below.

Hazard identification

Hazard identification is important for the observation and management of health disaster risks in biological and chemical-based technologies such as GWT and rainwater harvesting (Eregno *et al*., 2017; Kusumawardhana *et al*., 2021; Madikizela *et al*., 2022b; Mortula *et al*., 2023). There are two approaches for hazard identification, and they include conventional culturing, where spread plating and membrane filtering for colony forming units (CFU) in selective growth media is conducted to observe representative viable cells of microorganisms such as *Salmonella spp*, *Campylobacter*, *E coli*, and Feacal coliforms (Reyes *et al*., 2014; Li *et al*., 2017; Reyneke *et al*., 2022). The second approach is based on molecular microbiology and two techniques such as metagenomics and metabarcoding can be used (Mendoza *et al*., 2015; Chua *et al*., 2021). Metagenomics is briefly defined as the Polymerase Chain Reaction (PCR) independent genomic technique that analyses the whole (meta-analysis) microbial genetic contents from the environmental samples such as water, soil and plants (Osunmakinde *et al*., 2018; Ghosh *et al*., 2019; Li *et al*., 2019; Chua *et al*., 2021; Mzava *et al*., 2022; Archana *et al*., 2024).

In metagenomics short gun sequencing technique is employed and it allows extensive parallel sequencing of all genetic material to produce large taxonomic and functional data output in a short period (Mendoza *et al*., 2015; Dash and Das, 2018; Iwu-Jaja *et al*., 2022; Davis *et al*., 2023; Makuwa *et al*., 2023; Poopedi *et al*., 2023).

Alternatively, metabarcoding is an affordable and robust technique that is valuable in profiling genetic markers in environmental samples (Ruppert *et al*., 2019; Kasozi *et al*., 2020; Rubiola *et al*., 2022). It is performed via PCR amplification of the targeted genes within the extracted DNA sample, meaning PCR is conducted to amplify specific genetic markers such as 16S rRNA for bacteria, Internal Transcribed Spacer (ITS1) for fungi, mitochondrial 16S (mt16S) for mammals, cytochrome c oxidase subunit I (CO1) for insects, and rbcL, trnl and matK for plants (Mendoza *et al*., 2015; Liu *et al*., 2019; Rubiola *et al*., 2022; Serite *et al*., 2023). The amplified markers are then sequenced to identify the phyla and genera present in an environmental or consortium sample. However, this technique has been reported to be PCR bias and that is the disadvantage due to non-detection of unamplified taxa (Bohmann *et al*., 2021; Serite *et al*., 2023).

Exposure Assessment

As briefly described by Busgang *et al.* (2018), the exposure assessment is based on evaluating different events where humans and the environment are exposed to the hazards. Exposure assessment requires identification and quantification of the pathogens followed by description and estimation of exposure scenarios such as inhalation, ingestion and dermal contact (Benami *et al*., 2016a; Dalahmeh *et al.,* 2016; Eregno *et al*., 2017; Shi *et al*., 2018; Kusumawardhana *et al*., 2021; Mortula *et al*., 2023). Various QMRA studies have conducted hazard identification and exposure assessment using Equations 11-15, as Appendix A describes. In Equation 16 (Appendix A), the Hazard and Exposure levels are included along with Vulnerability (Tandlich *et al*., 2013a; Tandlich *et al*., 2013b; Madikizela *et al*., 2022b).

Dose-response models

Dose-response refers to quantifying the outcomes and health impacts from exposure to different levels of pathogens (WHO, 2016). The exposure to different doses of pathogens includes infection and illness from the described exposure scenarios (Shi *et al*., 2018). Varying QMRA-based studies have reported exposure scenarios and predicted pathogen doses using different models; this leads to quantification of dose-response using beta-Poisson and Exponential models along with the individual probabilities for counting multiple exposures per year (Benami *et al*., 2016a; Dalahmeh *et al*., 2016; Eregno *et al*., 2017; Busgang *et al*., 2018; Shi *et al*., 2018; Kusamawardhana *et al*., 2021; Mortula *et* *al*., 2023). The cited studies have different expressions for dose-response models using Equations 17-23 (Appendix A), and the model is selected based on the strain of the identified pathogen and exposure scenarios (Kusumawardhana *et al*., 2021).

Risk Characterization

As described by the WHO (2016) and elsewhere, this step synthesizes the outcomes from the previous QMRA steps to estimate the yearly possibilities of infection and illness. The risk characterization models are employed concurrently with the measurement of the burden of disease, which is quantified as disability-adjusted life years (DALY), and refers to the number of years lost because of illness, disability or early death (WHO, 2006; Dawber *et al*., 2009; Benami *et al* .,2016a; Dalahmeh *et al*., 2016; WHO, 2016; Busgang *et al* ., 2018; Shi *et al* ., 2018; Oscar, 2021; Kusumawardhana *et al.*, 2021; Abuzerr *et al*.,2022; Mortula *et al*., 2023). Risk characterization is quantified using Equations 24-34 as Appendix A describes.

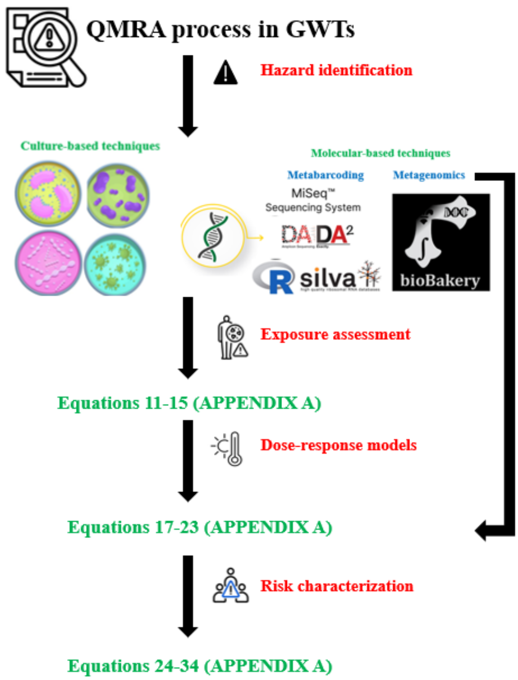

Fig. 2: Quantitative Microbial Risk Assessment process in greywater treatment systems. *The reviewed QMRA process is applicable according to the approaches of hazard identification, exposure scenarios, analysis models and subsequently characterization of the risks. The QMRA process is composed using the described and referenced Equations in APPENDIX A.*

3.2. Microbial Risk Assessment in South African Water Systems

In addition to the reviewed QMRA studies, the local health risk assessment studies include Luyt *et al.* (2012), who suggested the evaluation of health risks in surface water in vulnerable areas using concentration levels of *E coli* as an indicator microorganism, while Nhokodi *et al.* (2023) investigated the effect of storage conditions on microbial quality of drinking water using Hydrogen Sulphide test kits and quantification of faecal coliforms. The suggested health risk estimation was calculated using Equation 35 (Appendix A) (Luyt *et al.,* 2012). Tandlich *et al.* (2013a), Tandlich *et al.* (2013b) and Madikizela *et al.* (2022b) suggested the estimation and characterization of health risks in sanitation systems using Equation 16 (Appendix A) that includes the two steps (Hazard and Exposure) of the QMRA approach. The governmental approaches such as Blue Drop which ensures compliance with the SANS 241 for drinking water, while Green Drop evaluates the performance of the wastewater treatment systems by conducting CRR that is calculated using Equation 36 (Appendix A), and high CRR values indicate high health risks in the wastewater treatment system (Eastern Cape Green Drop Report, 2022; Green Drop Watch Report, 2023). The Green Drop CRR is based on four respective risk indicators: design capacity of the wastewater treatment system, operational inflow, technical skills and quality of the final effluent (Eastern Cape Green Drop Report, 2022; Green Drop Watch, 2023).

The performance of the GWTs can also be evaluated according to the Green Drop Audit score; for instance, an audit score of ≥ 90% shows an excellent performance of the system compared to less than 30% which shows improper operation of the system (Eastern Cape Green Drop Report, 2022). The Green Drop Audit score has been used to track the performance of the municipal and institutional wastewater treatment systems based on graded management components that include capacity (15%), environment (15%), finances (20%), technical (20%), and effluent/sludge quality compliance (30%). For example in 2021, the local Makhanda Municipality was reported to have a 9% Green Drop Audit score, which shows an alarming dysfunctional status of all the three wastewater treatment systems which is a significant health risk to the local communities in terms of consistent access to clean water and proper sanitation (Eastern Cape Green Drop, 2022). The South African guideline for greywater management and reuse (Carden *et al.*, 2018), Green Drop CRR, and QMRA (Equation 16) are suggested to be applied for health risk assessment in preliminary operating GWTs. Additionally, Figure 3 demonstrates a brief conceptual framework/flow diagram of this literature review and its subsequent studies.

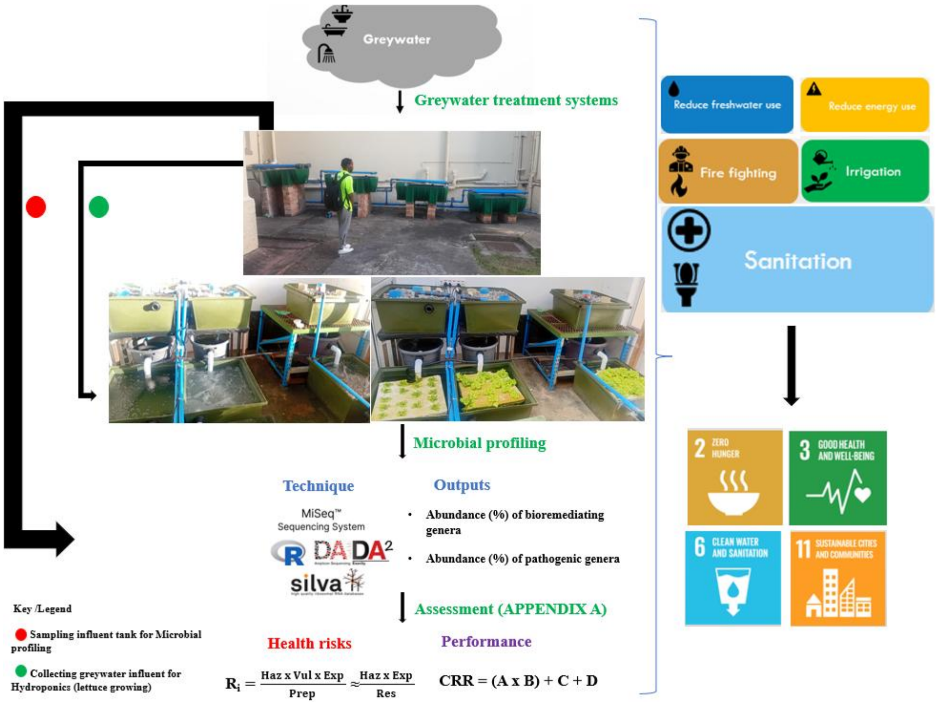

Fig. 3. A brief conceptual framework of the literature review*. The framework outlines greywater sources (e.g. bathroom) and decentralised greywater treatment system (i.e. installed in student residence). Preliminary evaluation of health risks and biological remediation can be conducted via metabarcoding. Upon the formalised and full operation (decentralised Greywater hydroponics) of the system, continuous assessment can be conducted using various applicable (i.e. data availability) equations shown in the Supplementary material. Eventually, sustainable operation and continuous health risks assessment of GWTs can result into mitigation and progress towards sustainable development goals (SDGs).*

CONCLUSIONS

Due to the water challenges and management, greywater treatment and reuse has been noted as an alternative sustainable solution to mitigate effects of water scarcity. A thorough characterisation of the quality and quantity of greywater highlights its significant potential for treatment and reuse, particularly in regions that have uneven distribution and inefficient management of water, such as South Africa. Additionally, the performance and sustainability of GWTs from various authors are reviewed, underscoring the insufficient removal of microbial content as a significant challenge and public health concern. The study further explored QMRA methods that aligns with various greywater reuse purposes. The application of the reviewed QMRA is limited in South African GWTs due to extensive data requirements such as microbial species identification and validated dose-response models. The applicable Microbial Risk and performance Assessment methods currently used in South African water and wastewater systems were mentioned and discussed (Figure 3).

Metabarcoding is recognized as a critical and robust technique for initial microbial risk assessment, to evaluate the sustainability of GWTs and reuse of treated effluent in terms of biological remediation and abundance of opportunistic pathogens. This assessment is conducted by estimating and quantification relative abundance (%) of genetic markers that are associated with bioremediating and pathogenic phyla and genera of greywater reuse systems. This assessment is not only important for observing microbial impact of greywater reuse in sanitation and emergency fire disasters but also in agricultural purposes (i.e. garden soil irrigation and hydroponics). The current assessment is a crucial step towards the implementation process of decentralised GWTs. Application of the QMRA equations (Appendix A) is recommended upon the full operation, implementation, and monitoring of the decentralised GWTs.

Regardless of the exponential attention towards greywater treatment and reuse, there are still knowledge gaps that include metabarcoding, with conventional treatment performance assessments and microbial risk evaluation frameworks. Additionally, microbial species data, dose-response models and long-term monitoring data of GWTs are needed for application of QMRA withing the South African context. This study highlights the importance of decentralised GWTs for sustainable water resource management while emphasizing microbial risk assessment for surveillance of the public and environmental health risks. Policy interventions, stakeholder engagement, updated data on public perceptions are required to support the implementation of decentralised greywater reuse systems through the development and continuous update of greywater reuse guidelines, microbial quality standards, and monitoring requirements of GWTs. The main limitation of this review is the partial application of PRISMA 2020 approach which might have resulted into limited inclusion of other relevant studies to the addressed sections. However, this review provides substantial baseline framework for assessment of GWTs prior the implementation and operation in communities, higher education settings, and industrial institutions.

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Appendix A.

List of Equations for Quantitative Microbial Risk Assessment

Equation	Number	Description of the parameters	References
Microbial Enumeration (Hazard identification)			Mortula *et al* (2023)
Total coliforms, Feacal coliforms $= [\frac{\text{Number of Colonies}}{\text{Volume of the sample}}]\ x\ Dilution\ factor\ x\ 100$	1		
$E\ coli = [\frac{Number\ of\ colonies}{Volume\ of\ the\ sample}]$ x 100	2		
Feacal Load in untreated greywater (g/person/day) = $\frac{E.coli \text{ in greywater (CFU per 100 ml) } x\ Q\ (1\ per\ day)}{E.coli \text{ density in feaces (CFU per g) } x\ NP}$	3	Where: Q is the volume of greywater produced within the household per day and NP is the number of persons served by the treatment	Dalahmeh *et al* (2016)
Concentration of the pathogen in untreated greywater (CFU or PFU 1^{-1}) = $\frac{\text{FL x PD x YI x ET x (1+UR) } x\ NIP}{\text{365 x Q}}$	4	Where: FL is the faecal load in g/person/day, PD is pathogen density in the faeces, also expressed as excretion rate of pathogen in faeces (CFU or PFU/g), YI is number of infection episodes per year per person, ET is the pathogen excretion time (days), UR is the underreporting ration of disease incidence and NP is the number of infected persons.	
	5		
Microbial Dose quantification (Exposure and dose response models)			

$d_{gw} = V \times C_w$ $d_{tgw} = V \times C_w\ 10^{-R}$ $d_P = V_p \times I \times C_{iw} 10^{-(R_w + R_{decay} \times SH \times T_{withhold})}$	6 7	Where: d_{gw} is the dose of the enteric pathogen in nontreated greywater, V is the volume (ml) of nontreated greywater and C_w is the concentration of pathogen in nontreated greywater. Where: d_{tgw} is the dose of the enteric pathogen in treated greywater, V is the volume (ml) of treated greywater, C_w is the concentration of pathogen in treated greywater and R is the log_{10} removal of the enteric pathogen in the biochar filter. Where: d_p is the dose of the enteric pathogen in the irrigated vegetables, V_p is the volume of irrigation water (ml/g), I is the per capita consumption of vegetables, C_{iw} is the concentration of enteric pathogens in the irrigation water, R_w is the log_{10} reduction in patjhogens due to washing of vegetables, R_{decay} is the decay rate (h^{-1}) of pathogens due to exposure to sunlight and solar radiation, SH is the number of hours per day (h day^{-1}) and $T_{withhold}$ is the withholding period between irrigation and harvest (days).	
Exposure D = C x v x q	8	Where: Exposure D is the mean dose per event, C is the concentration of pathogens in the circulated water applied to the plant via the hydroponic irrigation system, V is the volume of irrigation water that is in contact with lettuce (L/g) and the value is assumed on observation during harvesting and q is the quantity of lettuce consumed per event.	Eregno *et al* (2017)

$Dose_{PEC,toilet} = \sum_{i=0}^{n} C_{PEC,treated}$ x $AerosolDose_{diami}$ x MFR_{air} x T_{toilet} = (C_{PEC} x 10^{-logMF}) x [$\sum_{i=0}^{n}$($C_{aero, diami}$ x $V_{aero, diami}$ x DE_{diami})] x MFR_{air} x T_{toilet}	9	Where: $C_{PEC, treated}$ is the concentration of pathogenic *E coli* in treated greywater (CFU/100 ml), Aerosol $Dose_{diam}$ is the mass of water aerosol (according to median diameter size, i) deposited in extrathoracic region (g/min), MFR_{air} is the mean flow rate of air breathed after toilet flushing (1 of air/min), T_{toilet} is the time spent in the toilet room after toilet flushing (min/flush), log_{MF} is the log_{10} reduction rate of pathogenic *E coli* by microfiltration, $C_{aero, diam}$ is the concentration of aerosols in the air splashed after toilet flushing (number of aerosols -1 of air), $V_{aero., diam}$ is the volume of spherical aerosol (100 ml/aerosol) and DE_{diami} is the deposition efficiency of aerosols in extrathoracic region.	Shi *et al* (2018)
$Dose_{PEC, food\ crop} = C_{PEC, treated}$ x $10^{-log_{decay} \times T_{withhold}}$ x $R_{lettuce}$ x M_{body} x $V_{retention}$	10	Where: log_{decay} is the log_{10} environmental decay rate of *E coli* on lettuce (log_{10}/day), $T_{withhold}$ is the duration of environmental decay (days), $R_{lettuce}$ is the mass of raw lettuce intake per unit body weight per dya (g of lettuce/kg/day), M_{body} is the body weight (kg) and $V_{retention}$ is the volume of water retained on per unit weight of lettuce (100 ml/g of lettuce).	
$d = IVc10^{-w}e^{-kt}$	11	Where: d is the daily dose of the pathogen a person can be possible exposed to, I is the average amount vegetables produced, V is the volume of irrigation water on plant surface (ml/g), c is the concentration of the pathogen in greywater (CFU/ml), w is the log_{10} reduction in pathogenic concentration due to washing of the vegetables, k is the kinetic decay constant (per day) and t is the withholding period (days).	Busgang *et al* (2018)

$D = C \times V_{in} \times N$	12	Where: D is the daily dose of the pathogen (CFU), C is the concentration of the pathogen in water (CFU/L), V is the volume of ingested water per exposure event (L) and N is the number of events per day.	Kusamawardhana *et al* (2021)
$D = C \times PC \times IP \times T \times F_{RA} \times RR \times N$	13	Where: PC is the partitioning coefficient (L/m^3), IR is inhalation rate (m^3/minute), T is the duration of exposure event (minutes), F_{RA} is the fraction of respirable aerosol, RR is the retention rate and N is the number of events per day.	
$D = C \times (10^{-3.38} + h) \times BSA \times N$	14	Where: h is the thickness of water skin after showering (cm), BSA is the body surface area and N is the number of events per day.	
$DLR = D \times 10^{-LR}$	15	Where: DLR is the daily pathogen dose after log removal (CFU), D is the daily pathogen dose, and LR is Log removal.	
Microbial Risk quantification (Risk characterization)		"Where: Ri is the risk on infectious diseases that people are prone to due to sanitation issues, Haz is the potential health threat to human due to improper	Madikizela *et al* (2022b) – text is reused based on the CC BY licence.

$R_i = \frac{\text{Haz x Vul x Exp}}{\text{Prep}} \approx \frac{\text{Haz x Exp}}{\text{Res}}$	16	sanitation/hygiene, Vul is the human vulnerability to infectious pathogens due to improper sanitation, Exp is the level of exposure of the population to infectious pathogens/diseases due to improper sanitation, Prep measures the level of readiness or preparedness of the population to overcome or resolve hazardous issues such as the infectious diseases due to improper sanitation and Res refers to the capability of the vulnerable population/community to resist potential hazardous threats such as exposure to infectious diseases".	
$P_{inf} = 1\text{-exp}^{d/k}$	17	Where P_{inf} is the probability of infection, d is the dose of pathogens that can be exposed to a person (days x number (CFU) of pathogens per m^2) and k= 1.31×10^7 is a shape factor that is characteristic of the process.	Benami *et al* (2016)
$P = 1-(1-P_{inf})^n$	18	Measures the multiple exposures per year and O is the probability of infection from n (number) of exposure events per year.	
$P_{1-k} = 1 - [1 + \frac{dk}{N50} (2^{1/\alpha} - 1)]^{-\alpha}$	19		Dalahmeh *et al* (2016)

		Where; $P_{1\text{-}k}$ is the risk of infection for k^{th} exposure week, d_k is the dose of pathogens during the k^{th} exposure week, α is the model parameter that represents infectivity constant and N_{50} is the median infection dose.	
$P_{inf} = 1-(1 + D/\beta)^{-\alpha}$ $P_{inf} = 1- \exp^{(-rD)}$	20 21	Beta-Poisson model, where D is the pathogen dose and β and α are infection constants that are different according to the pathogenic strain for example for Norovirus α = 0.04 and β= 0.055 while for *Campylobacter* α = 0.145 and β = 7.58. Exponential model for estimating probability if infection from *Cryptosporidium*, and r = 0.059.	Eregno *et al* (2017)
"$P_{inf} = 1- [\ 1 + \text{Dose}\ \frac{2\ (\text{raise}\frac{1}{\alpha})-1}{N50}\ ]^{-\alpha}$" "$P_{ill} = 1- \exp(-K \times \text{Dose})$"	22 23	"Beta-Poisson model for probability of infection, where α is the infection constant, N_{50} is the dose level at which 50% of the population is expected to be affected and d is the pathogen dose (CFU)". "Exponential model, where P_{ill} is the probability of illness and k is the best-fit parameter that represents the level of pathogens".	Shi *et al* (2018); Kusamawardhana *et al* (2021)
$P_{I\text{-}annual} = 1- \prod_{k=1}^{np}(1-Pk)^{nq}$ $P_{d\text{-}annual} = DI \times P_{I\text{-}annual}$	24	Estimates the annual risk of infection where P_k is the k^{th} periodic infector probability, np is the number of exposure periods per year and nq is the time over which the assumption of daily infection probability is extended.	Dalahmeh *et al* (2016); Busgang *et al* (2018)

DALY = $P_{d\text{-}annual}$ x B x S_f	25	Predicts the yearly risk of disease ($P_{d\text{-}annual}$) where DI is the proportion of infections that are becoming visible.	
	26	Predicts the yearly burden of disease using disability adjusted life years (DALY), where B is the DALY per case of illness for diseases and S_f is the proportion of people who are vulnerable to the disease.	
$P_{inff/year} = 1- (1-P_{inff/event})^{fr}$	27	Predicts annual probability of infection considering frequency per year (fr).	Eregno *et al* (2017)
$P_{ill/exp} = P_{ill/inff} \times P_{inff}$	28	Estimates risk of illness from a single exposure.	
$P_{ill/year} = 1- (1-P_{ill/exp})^{fr}$	29	Predicts risk of illness considering exposure frequency per year.	
$P_{inf,\ annual\ scenario} = \prod_{i=1}^{n=365\ x\ freq\ scenario}(1-Pinf)$	30	Predicts annual probability of infection considering frequency.	Shi *et al* (2023)
$P_{ill,\ annual\ scenario} = \prod_{i=1}^{n=365\ x\ freq\ scenario}(1-Pill)$	31	Predicts annual probability of illness considering frequency.	
$P_{inf,\ ann} = 1- \prod_{1}^{f}(1-Pinf, dialy)$	32	Predicts daily probability of infection considering frequency (f)	Kusamawardhana *et al* (2021).
$P_{inf,\ annual} = 1 – (1- P_{inf})^{365\ x\ F}$	33	Predicts annual probability of infection considering frequency (F)	Federige *et al* (2019); Mortula *et al* (2023).
$P_{ill,\ annual} = 1- (1-P_{ill})^{365\ x\ F}$	34	Predicts annual probability of illness considering frequency (F)	
$PHRI = 100\ x \frac{N(\text{High risk samples})\text{All water uses}}{N\ total}$	35	Quantifies the potential health risk index (PHRI), N is the number of water samples taken at all targeted sampling sites and N_{total} is the number of samples taken annually at all sampling sites.	Luyt *et al* (2012).

CRR = (A x B) + C + D	36	"Calculates the cumulative risk rating (CRR) where A is the hydraulic design capacity of the treatment plant in ML/day, B is the operational flow as % of the installed design capacity, C is the number of non-compliant effluent quality parameters at point of discharge to receiving waterbody and D is the number of technical skills gaps (supervision, operation, maintenance in terms of Reg 2834 AND Draft Reg 813".	Eastern Cape Green Drop Report (2022).
MPI = $[M_1 \times M_2 \times M_3 \ldots\ldots M_j]^{1/n}$	37	Applies Metal Pollution Index (MPI) where M is the average concentration of metal J (mg/kg dry weight) and n represent the number of heavy metals in the analyzed crop.	Eregno *et al* (2017).
$DUR_i = \frac{Cm}{T \times BMp}$	38	Estimates plant uptake rate of heavy metals and DUR_i is the average daily uptake of heavy metals which is standardized by the dry biomass of different types of lettuce i (mg/day), C_m is the concentration of heavy metals in the lettuce tissue (mg/g), T is the total growth time (days) and BM_p is the dry plant biomass (g).	
$DIR = \frac{Cm \times Cf \times IR}{BW}$	39		

		Measures the daily intake rate (DIR) (mg/kg/day), where Cm is the concentration of heavy metals in vegetables (g/kg), C_f is the conversion factor that converts fresh lettuce weight to dry weight (e.g 0.065), IR is the daily intake rate of vegetables(g/day) (e.g 0.05 kg/day) and BW is the average body weight (e.g 70 kg).	
HRI = $\frac{DIR}{RfD}$	40	Measures the health risk index (HRI) where DIR is the daily intake rate and R_fD is the reference dose (mg/kg/day) that is represented as an oral dose per kilogram of body weight.	
THQ= $x = \frac{EF\ x\ ED\ x\ IR\ x\ Cx\ 10E{-}3}{RfD\ x\ BW\ x\ TA}$	41	Measures the Target Hazard Quotient (THQ) which is a ratio between heavy metal concentration and the reference dose, where EF is the exposure frequency (days/year), ED is the exposure duration (years, equivalent to an average lifetime), IR is the vegetable ingestion rate (g/person/day), C is the concentration of metals in lettuce (mg/kg), BW is the average body weight (kg) and TA is the average exposure time for non-carcinogens (days/year x exposure duration).	