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Nova Biotechnol Chim (2019) 18(1): 59-65
DOI: 10.2478/nbec-2019-0008

Nova Biotechnologica *et* Chimica

Effect of fly ash-lime treatment on the acute toxicity of greywater towards *Daphnia magna*

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Article info

Article history:
Received: 31st December 2018
Accepted: 12th May 2019

Keywords:
Daphnia magna
Mixed effluent
Acute toxicity
Small-scale irrigation

Abstract

Acute toxicity of raw and treated greywater towards *Daphnia magna* was assessed in this study. Treatment was performed with exposure of greywater to the fly-lime mixture After 48 h of exposure, 100 % mortality of *D. magna* was recorded when testing the following volumetric fractions of the raw greywater streams in the tested liquid medium (%; v/v): 10 % for kitchen greywater, 5 – 10 % for bathroom greywater and 1.25 – 10 % for laundry greywater. After greywater treatment with the fly-ash-lime mixture with pH adjustment to 7.0, 80 % of neonates of *D. magna* survived after exposure to treated laundry greywater in all dilutions at 48 h. At the same time, 100 % of neonates survived exposure to treated bathroom and kitchen greywater at all volumetric fractions. Therefore greywater had acute toxicity to *D. magna*, i.e. greywater treatment was required before its discharge or reuse. Values of the Pearson's correlation coefficient between the chemical components of the raw greywater and treated greywater and the survival of *D. magna* indicated a lack of statistically significant correlation at 5 % level of significance (p-value > 0.05 in all cases), i.e. the survival of *D. magna* was independent of the concentration of chemical constituents in greywater samples tested. Further studies will have to be conducted on the chronic toxicity of the greywater effluent after treatment with the fly-lime mixture. Experiments from this study will have to be re-run for the fully scaled-up version of the fly-lime mixture-based greywater treatment systems.

Introduction

Greywater accounts for a large volume of the drinking water that is consumed daily in a household (Nondlazi *et al.* 2017). Composition of greywater undergoes fluctuations and it can be considered a complex waste (chemical and biological components; Abed and Scholz 2015). If greywater is to be reused, it must be classified as an effluent and its potential toxicity to the environment must be carefully evaluated. Toxicity can be evaluated by measuring concentrations of the following parameters: Chemical Oxygen Demand (COD), heavy metals, organic pollutants and faecal coliforms (Hernandez Leal *et al.* 2012).

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However, measurement of at least some of these parameters is time-consuming and expensive; and it is fair to say that it is almost impossible to achieve complete (chemical) characterisation of effluents with complex chemical composition (complex effluents; Villegas-Navarro *et al.* 1999; Li *et al.* 2009). Greywater would be an example of such a complex effluent and its toxicity must be characterised using holistic approaches.

Biomonitoring is an important tool used to evaluate environmental changes based on biological responses (Oertel and Salánki 2003). *Daphnia spp*has long been considered and used as a standard organisms in water ecotoxicology (Adema 1978), in part due to its sensitivity and toxicity profile which is compatible with the toxicity profiles of higher organisms (Martins *et al.* 2007). In addition, this organism is highly responsive to exposure towards toxic components of effluents, its parthenogenesis and other factors result in quick reproduction of the crustacean; and *D. magna* is also easy to cultivate (in a laboratory; Sakai 2001; Gopi *et al.* 2012). Nondlazi *et al.* (2017) reported on the efficiency of a pilot-scale version of the fly-ash-lime-filter tower (FLFT) in greywater treatment. This system can be classified as a sanitation technology and thus toxicity testing with *D. magna* can be used to characterise the FLFT effluent before discharge (Tyagi *et al.* 2007). Here the authors seek to perform the acute toxicity testing of the simulated FLFT effluent.

Experimental

Consumables

Concentrations of the following chemical parameters were determined in greywater samples: ammonium (NH_4^+), chloride (Cl^-), phosphate (PO_4^{3-}), nitrate (NO_3^-), and chemical oxygen demand (COD) as outlined by Nondlazi *et al.*, 2017). The pH value was measured using the Hanna Comb pH meter (Hannah Instruments, South Africa). The turbidity values were measured using the Lutron TU-2016 portable turbidity meter (The Instrument Group, South Africa). Mechanical shaking was done using the TS-520D orbital shaker (Already Enterprise Inc., Taiwan). All masses were measured using a Pioneer™ PA214 analytical balance purchased from Ohaus Corporation (USA). Hydrochloric acid (HCl) 32 % (w/v) was purchased from Merck (Pty.) Ltd. (South Africa). The DAPHTOXKIT F ™ *Magna* was purchased from Toxsolutions (South Africa – an authorized distributor of Microbiotests in South Africa). The DAPHTOXKIT F ™ *Magna* contents were used as purchased from the supplier. Fly ash and lime were procured as outlined earlier (Nondlazi *et al.* 2017).

Greywater sampling

The greywater samples were collected in 1 dm^3 Schott bottle, which were sterilised as outlined by Nondlazi *et al.* (2017). The individual greywater streams were collected separately, i.e. as laundry greywater, kitchen greywater and bathroom greywater. All Schott bottles were filled to the brim with the respective streams of greywater, the bottles were capped and stored at 4 °C until analysis/use was performed. The following operations were then performed according to the Standard Operating Procedure developed by the manufacturer (Microbiotests 2015): preparation of the Standard Freshwater Solution according to ISO 6341, the hatching of the dormant eggs of *D. magna* (Ephippia) and the range-finding test. The number of dead or immobilised neonates was recorded after 24 h and 48 h. Volumetric fractions and greywater concentrations are considered synonymous in the text of this article.

Toxicity testing

To assess the treated greywater toxicity, treatment of the sampled greywater with the fly-ash/lime layer of the FLFT was simulated (Nondlazi *et al.* 2017). For this, 10 g of fly ash/lime was weighed out using a Pioneer™ PA214 analytical balance and transferred into a 100 cm^3 Erlenmeyer flask. Next, 30 cm^3 of greywater was added into the Erlenmeyer flask and the pH of the liquid phase/suspension was adjusted to 7.0 using 5 M HCl. Treatment was performed by shaking of the Erlenmeyer flasks on the mechanical orbital shaker at 150 rpm at 25 °C for 96 h. The pH values of all suspensions of greywater and the fly-ash-lime mixture were monitored every 24 h and re-adjusted to 7.0 using 5 M HCl as needed. After 96 h, the samples were removed from the shaker and left to stand at room temperature for 30 min to settle the particulate matter. Then the supernatant was transferred to volumetric flask, and relevant dilutions were performed. The experiments were conducted in 2016 and all *D. magna* were procured from a commercial source. Therefore no wild crustaceans were captured, harmed or sacrificed during conducting of this study. Next, the definitive and acute toxicity test was performed with *D. magna* according to the manufacturer's instruction (Microbiotests 2015).

Statistical evaluation of the measured data

Paleontological Statistics software for education (PAST) version 2.17c was used to conduct the statistical analysis of the data. The mortality and immobility data of the greywater samples were compared to the control by means of the Mann-Whitney Test at 5 % level of significance. Further statistical analysis was done to determine the correlation between the concentration of the chemical constituents of the greywater and the survival of *Daphnia magna*. This was done by means of Pearson's Correlation Coefficient Calculator (Stangroom 2016). The correlation coefficients were calculated to indicate, whether the raw and/or treated greywater characteristics are likely important causes for the observed toxicity levels towards *D. magna*.

Results and Discussion

Physicochemical analyses results

Results of physicochemical analyses for the raw greywater and the treated greywater are shown in Table 1 and 2.

The physicochemical constituents of the raw greywater fell inside the following intervals: pH < 6.83±0.06, 7.13±0.06 >; turbidity < 51.3±0.6, 85±2 > NTU; COD < 571±22, 658±20 > mg.dm^{-3}; NO_3^- < 18±0.6, 20±1> mg.dm^{-3}; PO_4^{3-} < 0.54±0.03, 0.84±0.03 > mg.dm^{-3}, Cl^- < 8.94±0.07, 9.9±0.3 > mg.dm^{-3} and NH_4^+ < 44.7±0.5, 62.1±0.6 > mg.dm^{-3}.

Table 1. Characteristics of raw greywater.*

Parameter	BGW	KGW	LGW
pH	6.94±0.07	7.13±0.06	6.83±0.06
Turbidity [NTU]	51.3±0.6	68.7±0.6	5.0±2.0
COD [mg.dm^{-3}]	571±22	658±20	616±31
NO_3^- [mg.dm^{-3}]	18.00±0.60	20.0±1.0	19.7±1.0
PO_4^{3-} [mg.dm^{-3}]	0.77±0.04	0.54±0.03	0.84±0.03
Cl^- [mg.dm^{-3}]	8.9±0.1	9.9±0.3	9.7±0.3
NH_4^+ [mg.dm^{-3}]	44.7±0.5	55.2±0.7	62.1±0.6

*BGW: Bathroom greywater, KGW: Kitchen greywater, LGW: Laundry greywater.

Values of the physicochemical parameters of the treated greywater samples (Table 2) fell inside the following intervals: pH < 7.02±0.04, 7.05±0.07 >; turbidity < 21±0.0, 25±0.0 > NTU; COD < 543±13, 552±16 > mg.dm^{-3}; NO_3^- < 17.8±0.2, 19.3±0.7 > mg.dm^{-3}; PO_4^{3-} < 0.08±0.01, 0.60±0.02 > mg.dm^{-3}; Cl^- < 6.35±0.09, 9.9±0.2 > mg.dm^{-3} and NH_4^+ < 22.35±0.38, 22.73±0.38 > mg.dm^{-3}. There was no statistical difference between chemical parameter of the untreated/raw and treated greywater in all the different types of greywater. The *p*-values ranged from 0.076 to 0.081 for the laundry, kitchen and laundry greywater for COD, NH_4^+, Cl^-, PO_4^- and NO_3^-.

Table 2. Characteristics of treated greywater.*

Parameters	BGW	KGW	LGW
pH	7.03±0.05	7.05±0.07	7.02±0.04
Turbidity [NTU]	21±0	21±0	25±0
COD [mg.dm^{-3}]	552±16	552±13	543±13
NO_3^- [mg.dm^{-3}]	17.8±0.2	19.3±0.7	17.9±0.7
PO_4^{3-} [mg.dm^{-3}]	0.08±0.01	0.09±0.01	0.60±0.02
Cl^- [mg.dm^{-3}]	6.35±0.09	9.90±0.20	7.51±0.77
NH_4^+ [mg.dm^{-3}]	22.73±0.38	22.57±0.38	22.35±0.38

*BGW: Bathroom greywater, KGW: Kitchen greywater and LGW: Laundry greywater.

Acute toxicity test with D. magna

It has been shown in literature that the survival rates of *D. magna* reached maximum values, when

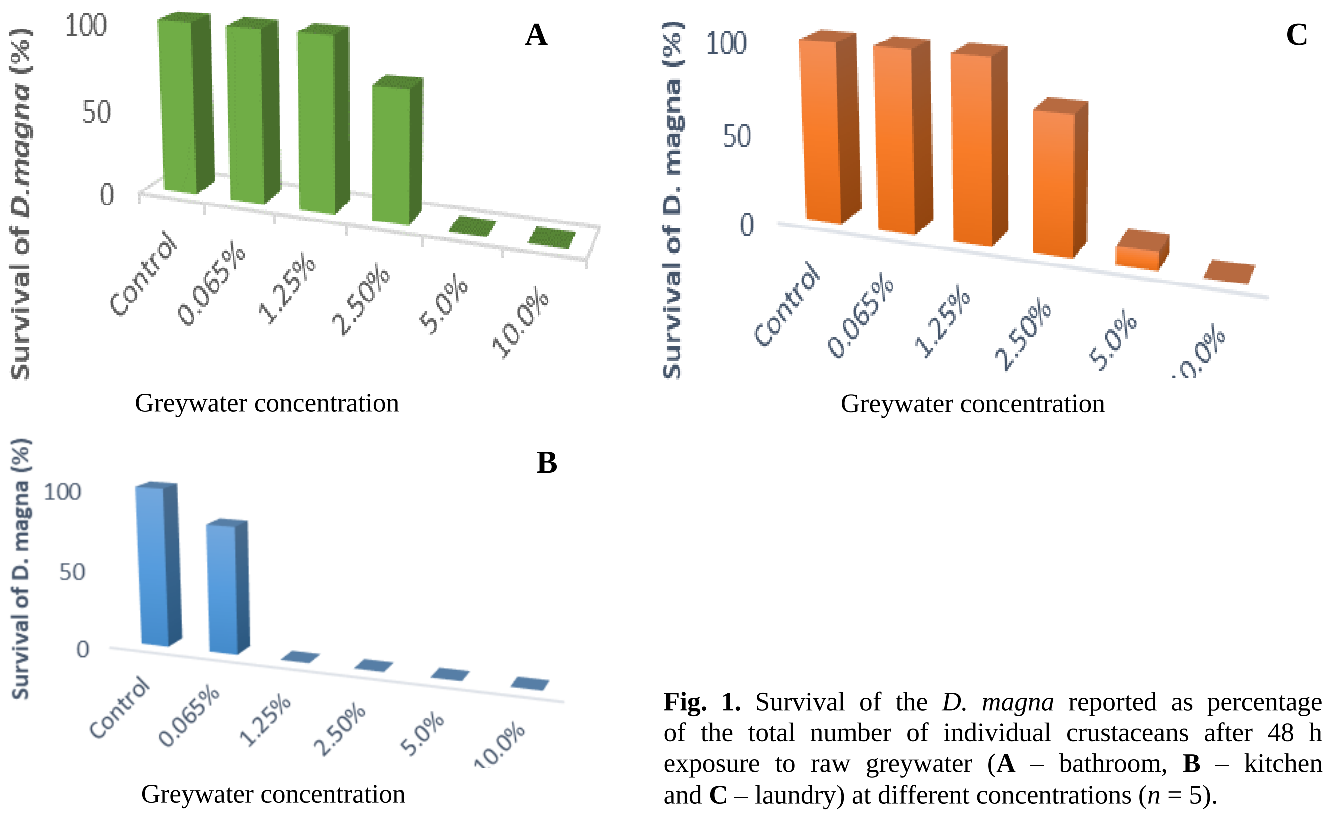

Fig. 1. Survival of the *D. magna* reported as percentage of the total number of individual crustaceans after 48 h exposure to raw greywater (**A** – bathroom, **B** – kitchen and **C** – laundry) at different concentrations ($n = 5$).

the crustacean is exposed to the pH values from 7.00 to 8.33 (Ghazy *et al.* 2011). Because contact with the fly ash-lime particles led to an increase in the pH the greywater to around 13.1 (Zuma and Tandlich 2017), the pH values of the treated greywater was adjusted to around 7.00 to guarantee optimum conditions for the *D. magna* survival. The survival of *D. magna* that was exposed to the control (Standard Freshwater solution) was 100 % (n = 4), *i.e.* the viability of the test organism without any exposure to the (diluted) greywater was likely optimum under the conditions of the acute toxicity test. Results of the toxicity testing are shown in Fig. 1 and 2.

After 48 h of exposure to the individual raw greywater streams, complete mortality of *D. magna* was observed at the following greywater or concentrations of each stream: 10 % for raw kitchen, 5 – 10 % for raw bathroom and 1.25 to 10 % for laundry greywater. Laundry detergents contain surfactants, bleaching agents and additives (Misra *et al.* 2010). Hernandez Leal *et al.* (2012) suggests that the toxic effect of greywater towards *D. magna* was caused by the anionic surfactant concentrations above 80 mg.L^{-1}. This could have been the case in the present study.

The survival of *D. magna* was significantly affected (*p*-values = 0.0124) by exposure to raw laundry greywater as a 100 % mortality is observed from the 1.25 – 10 % concentration, in comparison with the control (Fig. 1C). The treated laundry greywater showed a slight but significant difference against the control with an average of 22 % mortality from the 2.5 – 10 % concentration after 48-hour exposure (*p*-value = 0.0131) (Fig. 2A). There was a significantly lower survival rate of *D. magna* in the raw bathroom greywater (for all measured concentrations) (Fig. 1) when compared to the survival in the treated sample ($p = 0.0147$) (Fig. 2). A 100 % mortality was observed in the 5 and 10 % concentrations, which is significantly lower when compared to the 0 % mortality in the treated samples at the same greywater concentrations.

The exposure to raw kitchen greywater resulted in a significantly lower survival rate of the neonates (Fig. 1B), when compared to the survival rate of *D. magna* exposed to the treated kitchen greywater ($p = 0.0131$) (Fig. 2B). In the treated greywater a 100 % survival was observed in all the

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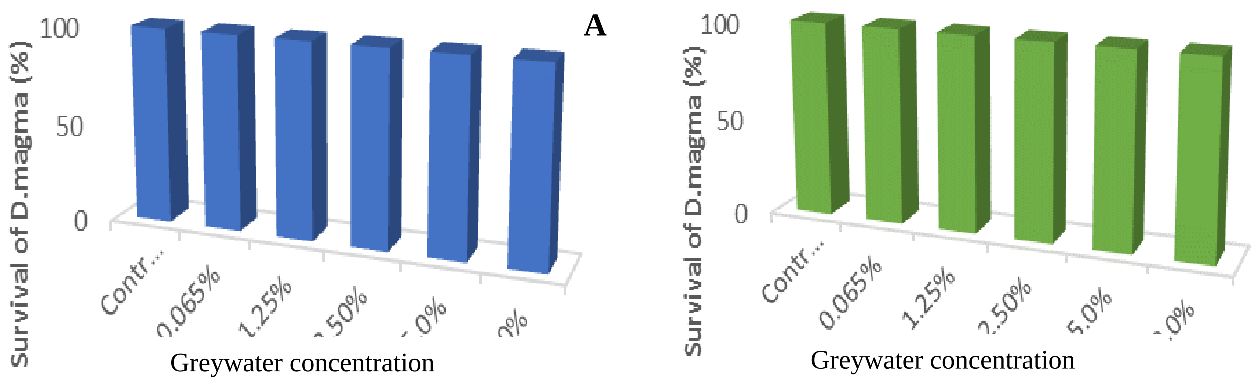

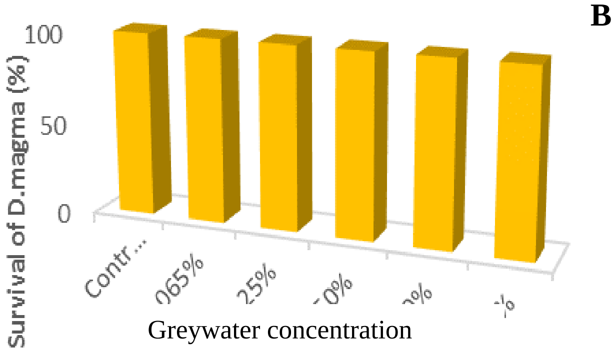

Fig. 2. Survival of *D. magna* after 48 h exposure to greywater leached with fly ash/lime (**A** – Laundry, **B** – Kitchen and **C** – Bathroom greywater) at different concentrations (n = 5).

five concentrations, and for the raw greywater 0 %; 1 % and 4 % survival was observed for the 10 %; 5 % and 2.5 % greywater concentrations respectively. The exposure to mixed greywater was done only for the treated greywater, and a slight but significant difference (p = 0.01312) in the survival of the *D. magna* neonates when compared to that of the control samples. These results suggest that the raw greywater would require dilution before reuse or disposal, as this would ensure that the raw greywater is not toxic to the environment. A 1:1 dilution ration is usually recommended for greywater intended for reuse (Mzini and Winter 2015). An 80 % survival of the neonates was observed at the 10 % greywater concentration. A 0 % mortality was observed for the *D. magna* neonates exposed to the treated bathroom and kitchen greywater at all concentrations.

The increasing concentrations of nitrate found in surface water and groundwater are becoming a concern, yet little information has been published on toxicity of nitrate to common organisms used for toxicity testing (Scott and Crunkilton 2000). Nitrate naturally enters groundwater and surface water via runoff from decomposition of vegetation, natural geological deposits, soil nitrogen, and atmospheric deposition (Scott and Crunkilton 2000). The acute toxicity of nitrate to several species of fish generally falls between 100 and 1,000 mg.L^{-1} NO_3-N (Tomasso and Carmichael 1986). The survival rate of *D. magna* starts to decrease when the concentration of nitrates in the water is 462 mg.L^{-1} NO_3-N (Scott and Crunkilton 2000). In this study the nitrate concentration was calculated to be in the range 17.8±0.2 – 20±1 mg.L^{-1} which would not be toxic to the *D. magna*.

In this study, concentration of phosphate ions were obtained to be in the ranges of 0.08±0.01 – 0.84±0.03 mg.dm^{-3}. Chloride is abundant in natural waters and is an important constituent in biological functions (Elphick *et al.* 2011). It facilitates a variety of ion-exchange mechanisms though trans-membrane chloride channels and it forms salts with each of the major cations (Na, K, Ca, and Mg), but is highly soluble and exists primarily in the environment as a dissociated monovalent anion (Elphick *et al.* 2011). The toxicity of chloride in aquatic environments is a result of its tendency to occur at elevated concentrations in effluents from industrial operations (Van Voast 2003). An acute toxicity of *D. magna* study conducted by Elphick *et al.* (2011) shows that at concentration levels of 0.2 to 1.0 mg.L^{-1} of chloride the survival rate of *D. magna* is within the ranges of 62.5 – 75 % (n = 10 daphnids). The chloride concentration of the greywater samples used in this study was obtained to be in the range of 6.35±0.09 – 9.9±0.3 mg.dm^{-3}, which can still encourage the 62.5 – 75 % survival and growth rate of the *D. magna* in the sample.

Further statistical analysis was done to determine the correlation between the concentration of the chemical constituents of raw greywater and the survival of *D. magna* in raw greywater. This was done by means of Pearson's Correlation (R) and the respective p-values (Stangroom 2016). The correlation between the PO_4^{3-} concentration and the survival of *D. magna* was characterised by the R value of -0.3259, but the negative correlation was not statistically significant at 5 % level of significance as the p-value = 0.3920. The R for NH_4^+ and the survival of *D. magna* was obtained and found to be -0.2537, the R value for COD and the survival of *D. magna* was equal to -0.0695 and finally the R value for the correlation between the concentration of chlorides and the survival of *D. magna* was fund to stand at 0.0363. All these correlations were not statistically significant at 5 % level of significance as the p-value were higher than 0.05 in all cases. There was also no statistically significant correlation between the NO_3^- concentration and the survival of *D. magna* in raw greywater, as indicated by the following values R = 0.2042 with the p-value of 0.598. Therefore, the concentrations of the individual components of raw greywater did not influence the survival/mortality of *D. magna* in the acute-toxicity test performed in this study. Please add the following sentence: It is possible that the cause for the toxicity of raw greywater to *D. magna* might have been a result of the cumulative toxic effect of the greywater components measured.

The correlation between the treated greywater concentrations of PO_4^{3-} and the survival of the *D. magna* had the respective R value of 0.2775, but that correlation was not statistically significant as the respective p-value was equal to 0.3820. Similar trends were observed for the other chemical components of greywater. The R for Cl^- and the survival of *D. magna* in the treated greywater after 48 h was equal to -0.2252 and the p-value > 0.05. For NO_3^- and the survival of *D. magna* in the treated greywater, the R value was 0.2289 and the p-value was again higher than 0.05. Finally, the ammonium ions and the survival of *D. magna* in the treated greywater had the R value of 0.3206 and the p-value > 0.05, while the R value for the survival of *D. magna* and the COD concentration was equal to 0.0253 and the respective p-value of 0.938. These results indicate that there was no statistically significant relationship between the concentration of the chemical constituents and the survival rate of the *D. magna* in treated greywater.

Conclusions

Raw greywater in this study was toxic to *D. magna* in the acute toxicity test. Leaching greywater with fly ash/lime resulted in a significant decrease in the turbidity and other physicochemical constituents of the treated greywater after 96 h. The treatment also significantly reduced the toxicity of greywater, as it was observed in the survival rate of the *D. magna*. Toxicity testing, as performed in this study, can be an effective tool to assess the effluent quality from various greywater streams. Further studies will have to be conducted on the chronic toxicity of the FLFT effluent and on the applicability of this approach in fully scaled up FLFT.

Conflict of Interest

The authors declare that they have no conflict of interest.

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