COMPARING THE ENVIRONMENTAL IMPACTS AND TOXICITY OF LAUNDRY DETERGENTS WITH SOAPNUTS AND OTHER TREE-BASED NATURAL SURFACTANTS

Archana Waran[*], Syamkumar Reghunandan Pillai[*], Swati Kulkarni[*], Preethy Chandran[*]

[*] Cochin University of Science and Technology, School of Environmental Studies, Kalamassery, Kochi, Kerala, India

corresponding author: Preethy Chandran, e-mail: preethychandran@cusat.ac.in

Original scientific paper
Received: October 17th, 2024
Accepted: January 22nd, 2025
HAE-24112
https://doi.org/10.33765/thate.15.4.1

ABSTRACT

The extensive production of petroleum-based synthetic detergents worldwide presents a huge challenge to sustainability and environmental safety. Efforts are being made to find alternatives from the nature. The study tested plant-based surfactants including soapnut from *Sapindus mukorossi* and *Sapindus trifoliatus* and soapbark extract from *Quillaja saponaria* with anionic surfactants (sodium lauryl sulphate and linear alkyl benzene sulphonate) and commercial detergents (powder and liquid) through wastewater quality analysis and toxicity tests on zebrafish (*Danio rerio*). A wastewater quality index was used to compare the physicochemical properties of laundry wastewater. Wastewater from all the surfactants tested were acceptable, but acute toxicity tests confirmed that soapnuts and anionic surfactants are more toxic than commercial detergents and Quillaja. Aerobic treatment using microbial consortia neutralises saponins three times more efficiently than natural biodegradation, with up to 60 % saponin degradation within 15 - 30 days. Soapnuts are a proven source of natural surfactant, with applications in industry, animal husbandry, and agriculture, and they have the potential to meet the demand for green detergents and net-zero carbon emission goals. For soapnuts to be environmentally acceptable, the wastewater generated from them and their products must be treated with a combination of aerobic degradation, adsorption and filtration until the saponins are adequately removed.

Keywords: *saponin, toxicity, soapnuts, detergent, Danio rerio,* LC_{50}

INTRODUCTION

Clean water and sanitation for all is a priority of the United Nations as their sixth sustainable development goal. Paradoxically, in the modern age, sanitation is impossible without detergents, which pollute water, cause eutrophication, and harm aquatic life [1]. The threat of detergent pollution is ever-present and growing due to population explosion, rising consumer awareness about cleanliness, and increased disposable income. The rising dependence on hard groundwater has increased detergent consumption by 10 % to 30 % per wash [2]. Hard water significantly reduces dirt removal and increases redeposition when cleaning with linear alkyl benzene sulphonates (LAS) solutions. Increasing the quantity of a detergent, adding a softener or builder, and increasing the washing temperature or the number of rinse cycles do not compensate for the loss of detergency. Soft water is the best choice for LAS detergents [3]. Foaming, temperature changes, salinity and pH adversely affect aquatic life. Fishes are more susceptible to exposure to anionic detergent in hard water than in soft water [4]. Since detergents have become more complex, the persistence of their components in the ecosystems further increases the threat. Detergents present in wastewater reduce the efficiency of treatment plants [5]. LAS homologues interfere with the anaerobic digestion of wastewater at half-maximal effective concentration (EC_{50}) of 14 mg L^{-1} [6]. Meanwhile, untreated laundry wastewater pollutes groundwater. Alkyl benzene sulphonate (ABS) is difficult to degrade and can be traced 3.5 km down gradient, while LAS and sodium lauryl sulphate (SLS) are detectable 0.6 km down gradient [7].

Efforts are being made to find alternatives from the nature [8, 9]. Surfactant-producing organisms are used in food processing, bioremediation, oil and gas, pharmaceuticals and the cosmetic industry [10, 11]. Natural surfactants contain saponins, a tensioactive ingredient that reduces the surface tension of water and causes abundant foaming [8], and their primary role in nature is self-defence. Soapnut is a saponin-rich fruit of the *Sapindus* species. Its cleansing action are well documented [11]. Aqueous extract of soapnut (*Sapindus mukorossi*) matches the effectiveness of commercial detergent in terms of foamability, emulsion, stability, viscosity, and dirt dispersion [12]. The foaming characteristics of *Sapindus mukorossi* are better than SLS, an anionic surfactant, and Tween-20, a non-ionic surfactant [13]. In cleaning action, surface activity and wettability, *Sapindus trifoliatus* has been proven superior to *Sapindus mukorossi* [14, 15] and *Acacia concinna* [13]. Soapnut is on a par with the commercially available detergent Henko in foamability, emulsion stability, viscosity and dirt dispersion. Dirt dispersion is highest at the critical micelle concentration (CMC), unlike Henko [13]. Several other applications of soapnut trees, including biodiesel, biochar, pest control, and medicinal attributes, indicate its suitability as a sustainable alternative to chemical surfactants [16].

While SLS is banned due to its low biodegradability, soapnut saponin is reportedly toxic to fish and invertebrates [17]. This research used standard toxicity tests to evaluate wastewater quality and eco-toxicity of detergents with plant-based surfactants. The natural surfactants tested were crude aqueous extracts of soapnuts from *Sapindus mukorossi* (SMU) and *Sapindus trifoliatus* (STR) and purified Quillaja saponin (QUI) obtained from the *Quillaja saponaria* tree bark. They were compared with four petroleum-derived detergents: SLS, LAS, a commercial laundry detergent powder (DET) and a liquid detergent of the same brand (LQD). Liquid detergents are gaining popularity with the increasing dependence on washing machines. However, research on their environmental toxicity was not found.

Zebrafish (*Danio rerio*) toxicity test, accepted worldwide for screening toxicants, bioactive compounds and plant-derived crude extracts, was used in this study [18, 19]. The toxicity data available so far are based on half lethal concentration (LC_{50}) endpoints. However, recent reports recommend effects below LC_{50}, which are more environmentally relevant [20, 21].

EXPERIMENTAL

Materials

The following washing agents were tested in this experiment: sodium lauryl sulphate (SLS), purchased from Merck, and linear alkylbenzene sulphonate, purchased at Hyderabad (neutralised with 12.5 % NaOH in water to produce linear alkyl benzene sulphonate (LAS)). A popular commercial powder laundry detergent (DET), and liquid laundry detergent (LQD) were purchased in Cochin. *Saponin Quillaja* sp. (QUI) with 20 - 35 % sapogenin content was purchased from Sigma Aldrich. The dried fruit of *Sapindus mukorossi* (SMU), purchased from an herbalist in Coimbatore, was air-dried, deseeded and stored in an airtight jar. Five grams of finely chopped fruit was soaked in 100 mL double distilled water and placed on a shaker for 4 h at 100 rpm. The extract was warmed to 70 °C, centrifuged for 20 min and passed through a pre-weighed Whatman Filter paper #1. 50 mL water was added to the residue, shaken at 100 rpm for 2 h and filtered. The total extract was 150 mL. The filter papers were dried at 50 °C for 24 h and weighed. The fibre weight was subtracted from the initial weight to calculate the concentration of the stock solution. The stock solution was kept in the refrigerator for up to 30 days. Fruit was collected from a *Sapindus trifoliatus* (STR) tree at the Cochin University of Science and Technology (CUSAT) campus. Aqueous extract of *Sapindus trifoliatus* was prepared after shade-drying the fruit.

For the biodegradation study, a composting mix named Bhoomi Dhaari BD-9 was obtained from Pune, which contained a consortium of *Trichoderma viride, Aspergillus niger, Bacillus* sp., *Lactobacillus* sp., yeast, and actinomycetes in a sterilised carrier of wheat bran, carboxymethyl cellulose, jaggery, rock phosphate, calcium carbonate and talc. The consortium's colony forming unit (CFU) count was 1x 10^9 CFU g^{-1}.

Methods

Physicochemical properties

The CMC of detergent samples was tested in distilled water (DW) and at 2000 mg L^{-1}in tap water (TW), which was filtered groundwater with a hardness of 179 mg L^{-1} and a conductivity 0.070 S m^{-1}, and washing machine effluent (WW) washed in tap water. The International Electrotechnical Commission's IEC60456 methodology using a regular cotton program at 30 °C was followed when washing the laundry [22]. To reflect average consumer conditions, 2 kg dry weight of the cotton laundry, including two bed sheets, one pillowcase, one towel, four t-shirts, one terrycot shirt, and a pair of socks used for one day, was soaked in 10 L of 2000 mg L^{-1} concentration of each surfactant for 30 min and run in a top-loading washing machine for one wash cycle followed by two rinse cycles. All items were colour-fast. Water from all three wash cycles was combined in equal measure to obtain the effluent sample.

Temperature, pH, electrical conductivity, total dissolved solids, salinity and dissolved oxygen were measured with a portable ThermoScientific Eutech Cyberscan series 600 waterproof hand-held water analyser. Total hardness, calcium hardness, magnesium hardness, total alkalinity, chemical oxygen demand (COD), and biological oxygen demand (BOD_3) were calculated according to the standard methods prescribed by the American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) [23]. Nitrate, phosphate, chloride and sulphate were tested by ion chromatography. The results of the tests were compared with the wastewater quality discharge norms set by the regulatory authorities of different countries, including the Environment (Protection) Rules, 1986 [Schedule VI], India [24] monitored by the Central Pollution Control Board (CPCB), India; Regulations for wastewater reuse and discharge (145/193, 1993), Oman; Jordanian Standard (JS: 893/2002) for discharge to streams, storage and groundwater recharge, Jordan; and the World Health Organization (WHO) [25], as shown in Table 1.

A wastewater quality index (WWQI), similar to the simple water quality index (ISQA) prepared using the simple water quality index calculator [26] was developed for testing freshwater samples, where *Ii* was the weighted index of each parameter. The value of *Ii* is between 0 and 1. A sample that was within the permissible range received a value of 1, while a sample that was within a relaxed range received a value of 0.5, and samples outside the permissible limit received a value of 0.

The following equation was used to calculate the wastewater quality index (WWQI):

$$WWQI = T \text{ x } \sum_{i=0}^{12} Ii \qquad (1)$$

where *Ii* is the sub-index value of each parameter, and *T* is ambient water temperature in °C.

Twelve parameters were used: pH, redox potential, electrical conductivity, total dissolved solids, salinity, alkalinity, total hardness, calcium hardness, chemical oxygen demand, and 3-day biological oxygen demand. A WWQI of 250 - 300 was considered excellent, 200 - 250 good, 150 - 200 average, and 100 - 150 was fair or below average. A WWQI below 100 indicated poor water quality.

Table 1. Permissible limits for wastewater

Parameter	Unit	Limit	Regulatory authority	Weighted index
Temperature	°C	< 45	CPCB [24]- at the point of discharge	
		< 40	CPCB - at 15 m downstream	
pH		5.5 - 9.0	CPCB	0: > 9.0 1: 5.5 - 9.0
		6.5 - 9.2	WHO [25]	
Redox potential	mV	-150 to -50	CPCB - for wastewater	0: < -150, 0.5: > -150 1: > -50
		> -50	CPCB - for drinking water	
Electrical conductivity	$S\ m^{-1}$	0.2	Oman [25]	0: > 0.2 0.5: > 0.075 1: < 0.075
		0.075	WHO	
Total dissolved solids	$mg\ L^{-1}$	1500	Jordan [25]	0: > 1500 0.5: 1000 - 1500 1: < 1000
		1000	Oman	
Total suspended solids	$mg\ L^{-1}$	100	CPCB - in water bodies	0: > 600 0.5: 200 - 600 1: < 200
		200	CPCB - on land	
		600	CPCB - in public sewers	
NaCl	$mg\ L^{-1}$	250	Varma & Ratan, 2020 [27]	0: > 250, 1: < 250
DO	$mg\ L^{-1}$	> 2	Oman	0: > 4, 0.5: 2 - 4 1: < 2
		> 4	CPCB - for wildlife and fisheries	
Total alkalinity	$mg\ L^{-1}$	200 - 400	CPCB - limit for wastewater	0: > 400, 0.5: > 200, 1: < 200
		100 - 200	CPCB – for aquatic life, drinking	
Total hardness (as $CaCO_3$)	$mg\ L^{-1}$	600	CPCB - for areas with water scarcity	0: > 150, 0.5: 60 - 150 1: < 60
		0 - 60	WHO - Soft water [28]	
Calcium hardness (as $CaCO_3$)	$mg\ L^{-1}$	61 - 120	WHO- Moderate hard [28]	
		150	Oman	
COD	$mg\ L^{-1}$	250	CPCB	0: > 250, 0.5: 150 - 250, 1: <150
		150	WHO	
BOD	$mg\ L^{-1}$	30	CPCB, BOD_3 inland surface water	0: > 100 0.5: 30 - 100 1: < 30
		60	Jordan, for BOD_5	
		80	WHO, for BOD_5	
		100	CPCB, BOD_3 irrigation / coastal	
Total Kjeldahl nitrogen (as N)	$mg\ L^{-1}$	34	WHO	0: > 100 0.5: 34 - 100, 1: < 34
		100	CPCB, inland /coastal water	
Sulphate ion	$mg\ L^{-1}$	400	WHO	0: > 400, 1: < 400
Phosphate ion	$mg\ L^{-1}$	5	CPCB	0: > 5, 1: < 5

Toxicity test on zebrafish

The toxicity test was performed on zebrafish (*Danio rerio*) purchased from a pet store (PET). PET zebrafish belong to wild-type, genetically undefined, and genetically heterogeneous stock [29, 30]. Fish acute toxicity test was performed according to the guidelines of the Organisation for Economic Co-operation and Development (OECD) (1992) [31]. The one-up-one-down method helped to identify the range of toxicity in advance. The fish was acclimatised for 7 days, with daily feeding. The batch with less than 5 % fish death during acclimatisation was used for the experiment. The test was conducted in triplicate, with five fish per jar, for 7 days. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, a statutory body under the Prevention of Cruelty to Animals Act, 1960, Government of India (CPCSEA) were followed for the ethical treatment of fish [32]. The criteria for mortality were the absence of gill movement and the absence of spontaneous movement upon gentle prodding. Acute toxicities (96 h LC_{50}) were compared [33]. LC_{90} and LC_{10} were also determined to meet the recent recommendations of different regulatory agencies [20, 21]. Lethal concentration was calculated using R [34, 35].

Aerobic biodegradation of soapnut saponin and toxicity test on zebrafish

Aerobic biodegradation for the two soapnut extracts SMU and STR was carried out in three ways. 600 mg L^{-1} of soapnut extract was incubated in the first set. 100 mL of the same was mixed with 0.5 g of composting mixture (BD-9) in the second set (treatment B). The third set contained 100 mL soapnut extract, 0.5 g of BD-9 and 2 % glucose as an additional carbon source (treatment B+G). These combinations were aerobically degraded for 30 days at room temperature, filtered and sterilised by exposure to UV light for 10 min. The toxicity test was performed according to previous section with samples manipulated as follows: the aerobically degraded samples were diluted to 4 mg L^{-1}(C4), 8 mg L^{-1} (C8), and 12 mg L^{-1} (C12) according to their original concentrations. Pure water was used as a control sample. The experiment compared the difference in toxicity according to these manipulations. Mortality was recorded after 96-h of exposure. Fish mortality in the biodegradability test was analysed using ANOVA [35] and Dunnett's multiple comparison test [36].

RESULTS AND DISCUSSION

Data on physicochemical parameters are presented in Tables 2, 3 and 4, and visualised as heat map to present acceptable (green), slightly high (yellow) and high (orange) values. The plant-based surfactants, QUI, SMU, and STR were acidic, while synthetic detergents were neutral to alkaline. SLS, LAS and DET were extremely hard at CMC. All samples except STR and LQD had very high COD; SLS and LAS had exceptionally high COD: above 5000 mg L^{-1}. The parameters of DET in distilled water were mostly beyond permissible limit for effluent discharge (Table 1), possibly due to various salts that are added to improve the efficiency of the detergent powder. Concentrations of ions (Table 5) were excluded from the WWQI since they were within limit for all the samples.

The effluent from all washing machine samples had an average WWQI and met the standards for effluent discharge at their CMC (Figure 1). Quillaja was not tested as a laundry detergent, but its quality was excellent in distilled water and on par with SMU, slightly less than STR and tap water. As expected, the WWQI of soapnut extracts SMU and STR showed deterioration in the following sequence: distilled water - tap water - wastewater, but DET and LQD showed the opposite result. In the case of DET, this could be due to additives such as water softeners. Overall, LAS, LQD and STR gave the best wastewater quality, followed by DET.

Table 2. Physicochemical properties of detergent samples at CMC in distilled water (DW)

Sample		SLS	LAS	DET	LQD	QUI	SMU	STR	DW
Temperature	°C	28	28	28	28	27	29	28	28
pH		6.2	8.2	11	6.35	4.4	4.2	3.67	5.6
Redox potential	mV	- 18.9	- 132	- 224	- 39.9	84.6	118.6	120	39.4
EC	$S\ m^{-1}$	0.057	0.019	0.206	0.011	0.004	0.027	0.018	0.005
TDS	$mg\ L^{-1}$	936	180	1992	101.8	42.8	254.7	178	43.8
NaCl	$mg\ L^{-1}$	543	175	2113	99.29	46	248	175	47.3
DO	$mg\ L^{-1}$	7.83	7.89	7.92	7.66	7.76	7.9	7.65	7.92
Alkalinity	$mg\ L^{-1}$	31.45	31.5	629	129	62.9	188.7	94.3	BDL
Total hardness	$mg\ L^{-1}$	320	640	320	20.6	12	20	16	20
Ca hardness	$mg\ L^{-1}$	160	320	160	20.6	8	16	12	12
COD	$mg\ L^{-1}$	5236	5527	582	51.84	436	290.9	131	BDL
BOD_3	$mg\ L^{-1}$	40	16.4	32.8	32.82	20.5	49.23	65.6	BDL

BDL = below detection level

Table 3. Physicochemical properties of detergent samples at 2000 $mg\ L^{-1}$ in tap water (TW)

Sample		SLS	LAS	DET	LQD	SMU	STR	TW
Temperature	°C	29	28	29	28	28	28	29
pH		6.69	7.15	9.65	7.95	6.5	6.6	6.73
Redox potential	mV	- 47.8	- 67.7	- 176	-102	- 36	-39.9	- 52
EC	$S\ m^{-1}$	0.083	0.074	0.176	0.075	0.074	0.074	0.070
TDS	$mg\ L^{-1}$	547.5	740	1760	752.1	716	715.7	676
NaCl	$mg\ L^{-1}$	803.8	723	1807	738	729	730.5	676
DO	$mg\ L^{-1}$	6.54	7.25	7.41	6.67	7.11	7.71	7.93
Alkalinity	$mg\ L^{-1}$	240.4	256	609	240.4	156	187.5	156
Total hardness	$mg\ L^{-1}$	212.4	207	181	189.1	195	175.8	179
Ca hardness	$mg\ L^{-1}$	121.7	57	23.3	126.9	52.3	47.5	23.8
COD	$mg\ L^{-1}$	230.4	64	63.4	115.2	338	416.3	0
BOD_3	$mg\ L^{-1}$	63.9	50.8	66.9	67.68	50.5	47.8	38.5

Table 4. Physicochemical properties of wastewater from detergent samples at 2000 $mg\ L^{-1}$ in tap water (WW)

Sample		SLS	LAS	DET	LQD	SMU	STR	TW
Temperature	°C	28	28	28	28	28	28	29
pH		6.85	7.06	7.69	7.08	6.86	6.67	6.86
Redox potential	mV	- 57.1	- 63.5	- 95	- 64.3	- 52	- 48.4	- 53
EC	$S\ m^{-1}$	0.090	0.077	0.101	0.082	0.097	0.073	0.083
TDS	$mg\ L^{-1}$	826.4	773	1008	715.2	972	708.7	829
NaCl	$mg\ L^{-1}$	884.8	753	1004	802.7	959	708.7	810
DO	$mg\ L^{-1}$	0.65	4.23	5.33	6.65	1.34	1.32	2.98
Alkalinity	$mg\ L^{-1}$	156.3	337	337	336.5	641	125	321
Total hardness	$mg\ L^{-1}$	161.5	189	197	204.6	215	185.3	205
Ca hardness	$mg\ L^{-1}$	49.88	18.3	10.4	25.9	75.1	30.88	18.1
COD	$mg\ L^{-1}$	338.2	51.8	40.3	182.1	63.4	169.1	17.3
BOD3	$mg\ L^{-1}$	45.15	54.1	71.4	46.48	67.7	46.28	31.9

Table 5. Concentration of ions in detergent samples in distilled water (DW)

	SLS	LAS	DET	LQD	QUI	SMU	STR	DW
Concentration (mg L^{-1})	2400	650	1910	2020	250	1993	2688	0.0
Fluoride (mg L^{-1})	0.0	0.0	0.0	0.0	0.0	10.3	0.8	0.0
Chloride (mg L^{-1})	0.6	2.1	144.5	5.25	0.1	42.0	0.3	0.0
Nitrate (mg L^{-1})	0.0	2.2	1.3	2.4	0.0	8.0	0.6	0.0
Phosphate (mg L^{-1})	0.1	0.0	0.0	0.0	0.0	4.8	0.6	0.0
Sulphate (mg L^{-1})	2.3	100.5	17.3	4.4	0.0	2.0	0.2	0.0

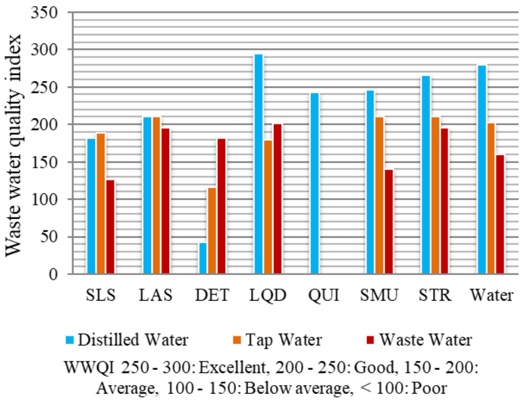

Figure 1. WWQI of detergent samples tested in different waters

One or more water quality parameters could adversely affect aquatic life. The dose-response curves derived from fish toxicity data are shown in Figures 2 and 3. Although the STR and SMU exhibited a slight difference in toxicity after 24 h of exposure, their toxicity levels remained relatively constant as the exposure continued, indicating that the toxins were stable. All exposure durations produced significant slope parameters except, SLS, QUI and SMU. The slope parameter for SLS became significant as the exposure duration extended beyond 24 h.

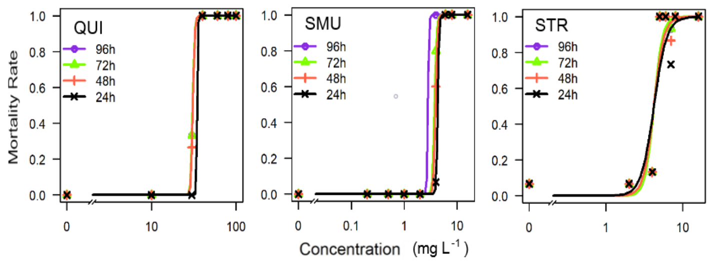

Figure 2. Dose-response curve of plant-based surfactants for *Danio rerio* for 96 h acute toxicity test

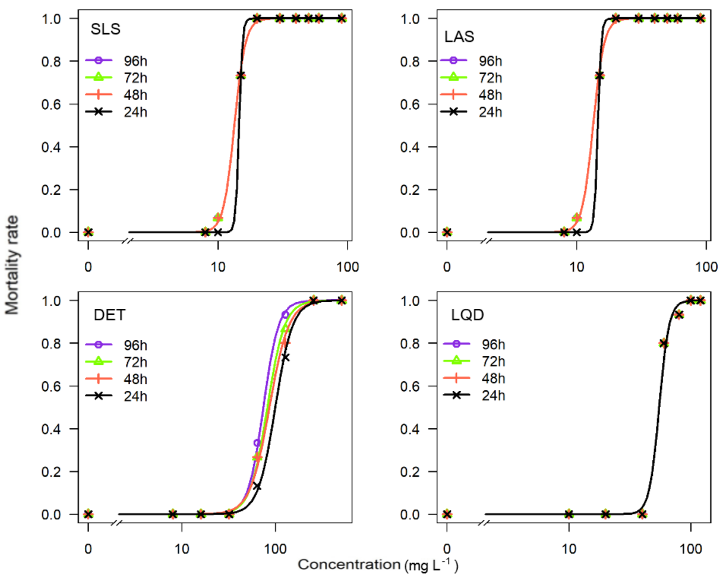

Figure 3. Dose-response curve of synthetic detergents for *Danio rerio* for 96 h acute toxicity test

There was no significant difference between the slope parameters of QUI, STR, and SMU, although their response curves showed different potency levels. QUI was used in a concentration range different from that from STR and SMU.

Despite the good WWQI, plant-based surfactants SMU, STR and QUI were several times more toxic than synthetic detergents (Table 6). Literature on the physiological effects of saponins on fishes or the route of exposure, either oral or dermal, was not available.

Critical micelle concentration indicates the propensity of surfactants to assemble in the water. The surface tension becomes constant when CMC is reached, but the detergent concentration must be above the CMC for adequate cleaning. The maximum detergency for various oils and fats is 6 to 10 times CMC [37]. The CMC of all detergents in this study, according to the literature, is shown in Table 7.

Table 6. Lethal concentration values (LC_{10}, LC_{50}, and LC_{90}) for *Danio rerio* exposed to different detergent samples (in mg L^{-1}) for 24 - 96 h

LC values at 24 h	LC_{10}	Range	LC_{50}	Range	LC_{90}	Range
SLS	13.63	(1.5 - 25.74)	14.55	(10.4 - 18.7)	15.54	(10.4 - 20.7)
LAS	10.77	(5.7 - 15.9)	22.98	(18.04 - 27.9)	49.03	(28.35 - 69.7)
DET	62.49	(43 - 82)	99.75	(79.9 - 119.5)	159.23	(112.6 - 205.9)
LQD	44.74	(36.8 - 52.7)	54.93	(49.4 - 60.44)	67.43	(59.04 - 75.8)
QUI	33.84	(- 68.4 - 136.1)	34.66	(- 68 - 137.3)	35.49	(- 70.4 - 141.4)
SMU	4.05	(3.57 - 4.54)	4.34	(1.67 - 7.00)	4.64	(- 0.57 - 9.85)
STR	2.75	(1.98 - 3.52)	4.2	(3.63 - 4.77)	6.41	(5.33 - 7.49)
LC values at 48 h	LC_{10}	Range	LC_{50}	Range	LC_{90}	Range
SLS	10.81	(9.1 - 12.6)	13.38	(12.0 - 14.8)	16.57	(14.4 - 18.7)
LAS	7.99	(3.22 - 12.75)	19.58	(14.6 - 24.57)	47.99	(24.9 - 71.05)
DET	51.13	(34.9 - 67.33)	86.3	(68.3 - 104.3)	145.67	(98.6 - 192.7)
LQD	44.74	(36.78 - 52.7)	54.93	(49.4 - 60.44)	67.43	(59.04 - 75.8)
QUI	29.13	(21.67 - 36.6)	30.76	(24.04 - 37.5)	32.48	(10.12 - 54.8)
SMU	3.58	(0.48 - 6.69)	3.93	(3.37 - 4.49)	4.31	(1.74 - 6.89)
STR	2.99	(2.27 - 3.71)	4.18	(3.69 - 4.68)	5.84	(5.01 - 6.68)
LC values at 72 h	LC_{10}	Range	LC_{50}	Range	LC_{90}	Range
SLS	10.81	(9.05 - 12.6)	13.38	(12.0 - 14.8)	16.57	(14.4 - 18.7)
LAS	6.57	(1.76 - 11.38)	18.44	(13.1 - 23.78)	51.78	(22.2 - 81.35)
DET	51.45	(36.39 - 66.5)	82.12	(65.84 - 98.4)	131.08	(90.3 - 171.9)
LQD	44.74	(36.78 - 52.7)	54.93	(49.4 - 60.44)	67.43	(59.04 - 75.8)
QUI	28.86	(19.17 - 38. 6)	30.54	(25.77 - 35.3)	32.31	(11.5 - 53.09)
SMU	3.42	(- 2.28 - 9.11)	3.76	(1.33 - 6.2)	4.15	(2.57 - 5.72)
STR	3.17	(2.5 - 3.83)	4.19	(3.75 - 4.63)	5.54	(4.82 - 6.26)
LC values at 96 h	LC_{10}	Range	LC_{50}	Range	LC_{90}	Range
SLS	10.81	(9.1 - 12.58)	13.38	(12.0 - 14.8)	16.57	(14.4 - 18.7)
LAS	6.57	(1.76 - 11.38)	18.44	(13.1 - 23.78)	51.78	(25.8 - 35.3)
DET	49.3	(35.97 - 62.6)	74.4	(60.8 - 87.98)	112.29	(77.3 - 147.3)
LQD	44.74	(36.78 - 52.7)	54.93	(49.4 - 60.44)	67.43	(59.0 - 75.8)
QUI	28.86	(19.17 - 38.6)	30.54	(25.7 - 35.3)	32.31	(22.2 - 81.4)
SMU	2.69	(- 32.1 - 37.48)	2.83	(- 33.35 - 39)	2.98	(- 35.5 - 41.4)
STR	3.17	(2.5 - 3.83)	4.19	(3.75 - 4.63)	5.54	(4.82 - 6.26)

Table 7. Critical micelle concentration vs. lethal concentration of tested surfactants at 96 h

Detergent	CMC mg L^{-1}	Highest toxic concentration (HT)	Lowest toxic concentration (LT)	CMC : HT	CMC : LT	Source
QUI	250	35.5 (24 h LC_{90})	28.9 (72 h LC_{10})	7.04	8.65	[38]
DET	750	159.2 (24 h LC_{90})	49.3 (96 h LC_{10})	4.71	15.21	[39]
LQD	750	67.4 (24 h LC_{90})	44.7 (24 h LC_{10})	11.12	16.77	[40]
LAS	750	51.8 (72 h LC_{90})	6.57 (96 h LC_{10})	14.47	114.16	[40]
STR	240	6.41 (24 h LC_{90})	2.75 (24 h LC_{10})	37.44	87.27	[15]
SMU	320	4.64 (24 h LC_{90})	2.69 (96 h LC_{10})	68.96	118.96	[15]
SLS	2400	16.6 (96 h LC_{90})	10.81(96 h LC_{10})	144.58	222.02	[41]
SMU*	2400	4.64 (24 h LC_{90})	2.69 (96 h LC_{10})	517.24	892.19	[15]
STR*	2400	6.41 (24 h LC_{90})	2.75 (24 h LC_{10})	374.42	872.73	[15]

* in hard water

Plant-based surfactants are far more efficient in reducing the surface tension of water than synthetic detergents. However, the CMC of soapnuts is directly proportional to pH and hardness [42], and has been found to be 2.4 g L^{-1} in tap water, which is on par with SLS [15]. The minimum dilution required to prevent mass fish kill (CMC: HT) and the maximum dilution needed to make the surfactant safe (CMC: LT) are mentioned. This dilution can occur by decomposition of the surfactant, sufficient addition of water or adsorption of the surfactant on the substratum. Quillaja was the least toxic to zebrafish. SMU and LAS needed similar dilution to be safe. DET and LQD showed low toxicity to *Danio rerio* in this study, but DET was found to be much more toxic to the freshwater fish *Heteropneustes fossilis*, where the LC_{50} for a mixture of two commercial detergents was 9.5, 15.2 and 21.7 mg L^{-1} for 48, 72 and 96 h, respectively [43]. Because toxicity varies among taxa, a more comprehensive approach that includes species from multiple taxa could determine the range of toxicity of detergents across taxa and trophic levels. Aerobic biodegradation of SMU and STR for 30 days followed by fish toxicity test and ANOVA determined the effect of the two extracts was statistically insignificant and small ($F(1, 46) = 2.89$, $p = 0.096$; η^2 (partial) = 0.06, 95 % CI [0.00, 1.00]). The interaction between species and manipulation was also not statistically significant and showed a medium-sized effect ($F(9, 46) = 0.76$, $p = 0.654$; η^2 (partial) = 0.13, 95 % CI [0.00, 1.00]). In contrast, the effect of manipulation was statistically significant and large ($F(9, 46) = 7.98$, $p < 0.001$; η^2 (partial) = 0.61, 95 % CI [0.41, 1.00]).

The Dunnett's multiple comparisons method examined mean differences between the following treatment groups using the emmeans package: C12 + B, C4 + B, C8 + B, C12 + B + G, C4 + B + G, C8 + B + G, C12, C4, and C8 compared to the control group (Figure 4). Here C denotes crude extract of soapnut (SMU or STR) of 4, 8, and 12 mg L^{-1} aerobically degraded for 30 days. B and G denote bacterial mixture and glucose. The results were averaged across factor levels (species). C12 + B + G (estimate = 0.167, $p < 0.0001$) and C12 (estimate = 1.667, $p = 0.0148$) resulted in significantly higher mortality than the control group.

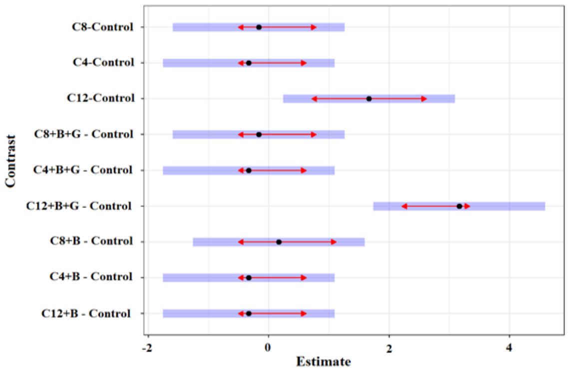

Figure 4. Dunnett's multiple comparisons: treatment effects vs. control (C: crude extract of soapnut of 4, 8, and 12 mg L^{-1}, aerobically degraded for 30 days. B: bacterial mixture, G: glucose. Bands and arrows represent 95 % confidence limit and comparisons)

None of the other treatments showed statistically significant differences ($p > 0.05$), indicating that their effects were similar to those of the control group. These results suggest that aerobic treatment using microbial consortia could neutralise saponins three times more efficiently than natural biodegradation. However, glucose (or an alternative food source) prevents microbes from actively degrading saponins, suggesting that microbes prefer to avoid saponins as a food source.

CONCLUSION

Physicochemical properties alone are not sufficient to decide on the suitability of wastewater for discharge. The tested wastewater from all surfactants was of acceptable quality, but the acute toxicity test confirmed that soapnuts, SLS and LAS were more toxic to *Danio rerio* than other detergents. Modern laundry detergents and Quillaja have proven to be environmentally safe and less toxic. Soapnuts can be considered as suitable precursors for plant-based detergents only if the toxicity of their effluent is neutralised through aerobic treatment. Future studies could evaluate combinations of different sources of natural detergents for efficacy and toxicity to arrive at the most environmentally friendly alternative to laundry detergent.

REFERENCES

[1] A.C. Kogawa, B.G. Cernic, L.G. Domingos do Couto, H.R.N. Salgado, Synthetic detergents: 100 years of history, Saudi Pharmaceutical Journal 25(2017) 6, 934-938. https://doi.org/10.1016/j.jsps.2017.02.006

[2] B.A. Camerson, Detergent considerations for consumers: Laundering in hard water - how much extra detergent is required?, The Journal of Extension 49(2011) 4, Article 21, 1-11. https://doi.org/10.34068/joe.49.04.21

[3] K. Gotoh, Y. Mei, Effect of washing conditions on cleaning action of linear alkylbenzene sulfonate in hard water, Tenside Surfactants Detergents 54(2017) 4, 291-298. https://doi.org/10.3139/113.110502

[4] P.W.A. Tovell, C. Newsome, D. Howes, Effect of water hardness on the toxicity of an anionic detergent to fish, Water Research 8(1974) 5, 291-296. https://doi.org/10.1016/0043-1354(74)90091-8

[5] S.A. Mousavi, F. Khodadoost, Effects of detergents on natural ecosystems and wastewater treatment processes: a review, Environmental Science and Pollution Research 26(2019), 26439-26448. https://doi.org/10.1007/s11356-019-05802-x

[6] M.T. Garcia, E. Campos, J. Sánchez-Leal, I. Ribosa, Effect of linear alkylbenzene sulphonates (LAS) on the anaerobic digestion of sewage sludge, Water Research 40(2006) 15, 2958-2964. https://doi.org/10.1016/j.watres.2006.05.033

[7] E.M. Thurman, L.B. Barber Jr., D. LeBlanc, Movement and fate of detergents in groundwater: a field study, Journal of Contaminant Hydrology 1(1986) 1-2, 143-161. https://doi.org/10.1016/0169-7722(86)90013-6

[8] S. De, S. Malik, A. Ghosh, R. Saha, B. Saha, A review on natural surfactants, RSC Advances 5(2015) 81, 65757-65767. https://doi.org/10.1039/C5RA11101C

[9] C.B.B. Farias, F.C.G. Almeida, I.A. Silva, T.C. Souza, H.M. Meira, R. de Cássia, F.S. da Silva, J.M. Luna, V.A. Santos, A. Converti, I.M. Banat, L.A. Sarubbo, Production of green surfactants: Market prospects, Electronic Journal of Biotechnology 51(2021), 28-39. https://doi.org/10.1016/j.ejbt.2021.02.002

[10] M. Thakur, M.F. Melzig, H. Fuchs, A. Weng, Chemistry and pharmacology of saponins: special focus on cytotoxic

properties, Botanics: Targets and Therapy 1(2011), 19-29. https://doi.org/10.2147/BTAT.S17261

[11] A. Garcia, S.A. Rhoden, J. Bernardi-Wenzel, R.C. Orlandelli, J.L. Azevedo, J.A. Pamphile, Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant *Sapindus saponaria* L, Journal of Applied Pharmaceutical Science 2(2012) 10, 35-40. https://dx.doi.org/10.7324/JAPS.2012.21007

[12] R. Ghagi, S.K. Satpute, B.A. Chopade, A.G. Banpurkar, Study of functional properties of *Sapindus mukorossi* as a potential bio-surfactant, Indian Journal of Science and Technology 4(2011) 5, 530-533.

[13] A. Pradhan, A. Bhattacharyya, Quest for an eco-friendly alternative surfactant: Surface and foam characteristics of natural surfactants, Journal of Cleaner Production 150(2017), 127-134. https://doi.org/10.1016/j.jclepro.2017.03.013

[14] A. Panda, A. Kumar, S. Mishra, S.S. Mohapatra, Soapnut: A replacement of synthetic surfactant for cosmetic and biomedical applications, Sustainable Chemistry and Pharmacy 17(2020), Article ID: 100297. https://doi.org/10.1016/j.scp.2020.100297

[15] P. Wojtoń, M. Szaniawska, L. Hołysz, R. Miller, A. Szcześ, Surface activity of natural surfactants extracted from *Sapindus mukorossi* and *Sapindus trifoliatus* soapnuts, Colloids and Interfaces 5(2021) 1, Article ID: 7. https://doi.org/10.3390/colloids5010007

[16] A. Waran, P. Chandran, Cradle to grave: The multifaceted soapnut-an update on the applications of *Sapindus* spp., Sustainable Chemistry and Pharmacy 24(2021), Article ID: 100557. https://doi.org/10.1016/j.scp.2021.100557

[17] X. Jiang, H.C.B. Hansen, B.W. Strobel, N. Cedergreen, What is the aquatic toxicity of saponin-rich plant extracts used as biopesticides?, Environmental Pollution 236(2018), 416-424. https://doi.org/10.1016/j.envpol.2018.01.058

[18] K. Howe, M.D. Clark, C.F. Torroja, J. Torrance, C. Berthelot, M. Muffato, J.E. Collins, S. Humphray, K. McLaren, L. Matthews, S. McLaren, I. Sealy, M. Caccamo, C. Churcher, C. Scott, J.C. Barrett, R. Koch, G.-J. Rauch, S. White, W. Chow, B. Kilian, L.T. Quintais, J.A. Guerra-Assunção, Y. Zhou et al., The zebrafish reference genome sequence and its relationship to the human genome, Nature 496(2013) 7446, 498-503. https://doi.org/10.1038/nature12111

[19] A.M. Chahardehi, H. Arsad, V. Lim, Zebrafish as a Successful Animal Model for Screening Toxicity of Medicinal Plants, Plants 9(2020) 10, Article ID: 1345. https://doi.org/10.3390/plants9101345

[20] A.C.S. da Cruz, B.C. Couto, I.A. Nascimento, S.A. Pereira, M.B.N.L. Leite, E. Bertoletti, P. Zagatto, Estimation of the critical effect level for pollution prevention based on oyster embryonic development toxicity test: The search for reliability, Environment International 33(2007) 4, 589-595. https://doi.org/10.1016/j.envint.2006.09.003

[21] G. Azimonti et al., Comparison of NOEC values to EC10/EC20 values, including confidence intervals, in aquatic and terrestrial ecotoxicological risk assessment, EFSA Supporting Publications 12(2015) 12, EN-906, 274 pp. https://doi.org/10.2903/sp.efsa.2015.EN-906

[22] A. Kruschwitz, A. Augsburg, R. Stamminger, How effective are alternative ways of laundry washing?, Tenside Surfactants Detergents 50(2013) 4, 263-269. https://doi.org/10.3139/113.110258

[23] Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association, Water Environment Federation, 23rd edition, 2017.

https://www.standardmethods.org/doi/book/10.2105/SMWW.2882, Accessed: August 13, 2024.

[24] General Standards for Discharge of Environmental Pollutants, The Environment (Protection) Rules, 1986, 545-560. https://www.cpcb.nic.in/GeneralStandards.pdf, Accessed: August 13, 2024.

[25] A compendium of drinking-water quality standards in the Eastern Mediterranean Region, World Health Organization, Regional Office for the Eastern Mediterranean, Regional Centre for Environmental Health Activities, 2006. https://applications.emro.who.int/dsaf/dsa1183.pdf?ua=1, Accessed: August 13, 2024.

[26] Simple Water Quality Index Calculator, Online Water Management Tools, Sustainable Water Management in the Arequipa Region, 2020. https://www.agry.purdue.edu/hydrology/projects/nexus-swm/en/Tools/WaterQualityCalculator.php, Accessed: August 13, 2024.

[27] P. Verma, J.K. Ratan, Chapter 5 - Assessment of the negative effects of various inorganic water pollutants on the biosphere - an overview, in: Inorganic Pollutants in Water, eds.: P. Devi, P. Singh, S.K. Kansal, Elsevier, 2020. https://doi.org/10.1016/B978-0-12-818965-8.00005-6

[28] Hardness in drinking-water: background document for development of WHO guidelines for drinking-water quality, World Health Organization, 2010. https://iris.who.int/handle/10665/70168, Accessed: August 13, 2024.

[29] D. Seguin, R. Gerali, Zebrafish prefer larger to smaller shoals: analysis of quantity estimation in a genetically tractable model organism, Animal cognition 20(2017), 813-821. https://doi.org/10.1007/s10071-017-1102-x

[30] G. Audira, P. Siregar, S.-A. Strungaru, J.-C. Huang, C.-D. Hsiao, Which zebrafish strains are more suitable to perform behavioral studies? A comprehensive comparison by phenomic approach, Biology 9(2020) 8, Article ID: 200. https://doi.org/10.3390/biology9080200

[31] Test Guideline No. 203: Fish, Acute Toxicity Test, OECD Guidelines for the Testing of Chemicals, OECD, 2019, 1-23. https://doi.org/10.1787/9789264069961-en

[32] Guidelines of CPCSEA for Experimentation on Fishes, Government of India, Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), 2021, 1-63. https://ccsea.gov.in/WriteReadData/userfiles/file/Guidelines%20of%20CPCSEA%20for%20Experimentation%20on%20Fishes-2021.pdf, Accessed: August 13, 2024.

[33] P.D. Abel, Toxicity of synthetic detergents to fish and aquatic invertebrates, Journal of Fish Biology 6(1974) 3, 279-298. https://doi.org/10.1111/j.1095-8649.1974.tb04545.x

[34] R: A language and environment for statistical computing, The R foundation, 2023. https://www.R-project.org, Accessed: August 13, 2024.

[35] C. Ritz, F. Baty, J.C. Streibig, D. Gerhard, Dose-Response Analysis Using R, PLOS ONE 10(2015) 12, Article ID: 0146021. https://doi.org/10.1371/journal.pone.0146021

[36] R.V. Lenth, B. Banfai, B. Bolker, P. Buerkner, I. Giné-Vázquez, M. Herve, M. Jung, J. Love, F. Miguez, J. Piaskowski, H. Riebl, H. Singmann, Package 'emmeans'. https://doi.org/10.32614/CRAN.package.emmeans

[37] I. Johansson, P. Somasundaran, Handbook for cleaning/decontamination of surfaces, Volume 1, Volume 2, eds.: I. Johansson, P. Somasundaran, Elsevier, 2007.

[38] N. Pagureva, S. Tcholakova, K. Golemanov, N. Denkova, E. Pelan, S.D.

Stoyanov, Surface properties of adsorption layers formed from triterpenoid and steroid saponins, Colloids and Surfaces: A Physicochemical and Engineering Aspects 491(2016), 18-28. https://doi.org/10.1016/j.colsurfa.2015.12.001

[39] M. Nur-E-Alam, M.M. Islam, M.N. Islam, F.R. Rima, M.N. Islam, Quality analysis, micellar behaviour, and environmental impact of some laundry detergents available in Bangladesh, Environmental Science and Pollution Research 23(2016), 5468-5476. https://doi.org/10.1007/s11356-015-5724-8

[40] H.G. Hauthal, Second European Detergents Conference Report: Detergent Polymers, Other Ingredients, Basics, Tests, Assessment of Sustainability and Environmental Evaluation, Tenside Surfactants Detergents 44(2007) 1, 45-61. https://doi.org/10.3139/113.100327

[41] M.W. Sulek, T. Wasilewski, K.J. Kurzydłowski, The effect of concentration on lubricating properties of aqueous solutions of sodium lauryl sulfate and ethoxylated sodium lauryl sulfate, Tribology Letters 40(2010), 337-345. https://doi.org/10.1007/s11249-010-9668-3

[42] S. Balakrishnan, S. Varughese, A.P. Deshpande, Micellar characterisation of saponin from *Sapindus mukorossi*, Tenside Surfactants Detergents 43(2006) 5, 262-268. https://doi.org/10.3139/113.100315

[43] A.Q. Osmani, R. Ahmed, H. Parbin, B. Mahanta, B. Kalita, Toxicity determination of detergent - its effects on the morphology, behaviour and haematology of a freshwater fish, *Heteropneustes fossilis*, International Journal of Life Sciences Research 7(2019) 1, 342-347.

Acknowledgements

We thank the director of the School of Environmental Studies, CUSAT, for providing all research facilities. We also thank the Sophisticated Test and Instrumentation Centre (STIC) and Cochin University of Science and Technology (CUSAT) for analytical support. Rashtriya Uchchatar Shiksha Abhiyan (RUSA) Scheme has supported this research through the Phase 2.0, Major Project T2F, U.O. No CUSAT/PL (UGC) A1/2314/2023 dated 24 May 2023.