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Assessing the impacts of vehicle wash wastewater on surface water quality through physico-chemical and benthic macroinvertebrates analyses

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ABSTRACT

Vehicle wash wastewater (VWW) contains a wide range of contaminants and discharge of such contaminated wastewater into the surface water bodies degrade water quality and affect aquatic ecosystems. This study, presents an impacts of discharging VWW into the stream Olarong Chhu in Thimphu and river Paa Chhu in Paro using water chemistry and benthic macroinvertebrates data sets obtained over a period of 6 months. Water samples and benthic macroinvertebrates were sampled once in a month from upstream, impact, and downstream sites in premonsoon (March to May) and post-monsoon (September to November) seasons in 2016. Significant levels of contaminants associated with vehicle washing were detected in water samples of impact sites whose cumulative effects on benthic macroinvertebrate assemblages were supported by HKHbios index, biological indices, and statistical analyzes. Canonical correspondence analysis indicated that T, dissolved oxygen, pH, biological oxygen demand, chemical oxygen demand, total suspended solids, turbidity, oil and grease, and sulfate significantly alter water quality and affect benthic macroinvertebrate assemblages. With the increasing number of vehicles, management of VWW is becoming a serious issue in Bhutan. Hence, there should be proper enforcement of water and environmental legislations and effective measures like constructions of wastewater treatment facilities should be considered for protecting Bhutan's surface water resources and aquatic ecosystems.

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Bhutan; vehicle washing; vehicle wash wastewater; water chemistry; benthic macroinvertebrate

Introduction

Vehicle washing consumes large quantity of water, involves the use of chemicals, and generates potentially toxic wastewater effluents (Zaneti, Etchepare, & Rubio, 2012). VWW contains a wide range of contaminants such as petroleum hydrocarbon wastes (petrol, diesel, and motor oil), nutrients (phosphorous and nitrogen), surfactants, asphalt, salts, organic matter, and heavy metals (Boluarte et al., 2016; Hamada & Miyazaki, 2004; Smith & Shilley, 2009). Discharge of such contaminants into the surface water bodies degrade water quality which in turn affectaquatic ecosystems and also impair the use of water for household, industrial, agricultural, and recreational purposes. The surface water pollution due to untreated VWW has been mostly reported from developing countries like Ghana (Aikins & Boakye, 2015), India (Mazumber & Mukherjee, 2011), Pakistan (Yasin, Iqbal, Arshad, Rustam, & Zafar, 2012), and Zimbabwe (Danha, Utete, Soropa, & Rufasha, 2014). Corresponding to these studies, Sato, Qadir, Yamamoto, Endo, and Zahoor (2013) have also reported that in low-income countries, only 8% of industrial and municipal wastewaters are treated. Such practices in developing countries could be due to lack of awareness on impacts, unaffordable establishments and operating costs of treatment plants, and less attention from the policy makers.

Usually VWW is greasy, oily, and very turbid due to contaminants washed down from the vehicle's body (Rai, Sharma, Gurung, Sitaula, & Raut, 2018). Oil and grease in water bodies block sunlight, impair photosynthesis, and prevent oxygen replenishment (Diphare, Pilusa, Muzenda, & Mollagee, 2013). Oil and grease also increases biological oxygen demand (BOD) and chemical oxygen demand (COD), temperature, and pH of the water which can consequently trigger aquatic habitat degradation, reduced productivity, and loss of biodiversity (Enujiugha & Nwanna, 2004). Washing of attached muds, sand, and debris from the vehicles contribute to solids (dissolved and suspended) in wastewater. Suspended solids can create stressful environment for aquatic life by increasing BOD, turbidity, reduces available habitat, and clog gills of fish and macroinvertebrates creating respiratory difficulties (Harding, 2005). Heavy metals are also associated with vehicle body parts, fuels, and lubricants (Sansalone & Buchberger, 1997), that get into the wastewater while washing auto brake linings, tires,

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vehicle exhausts, and fluid leakages (Smith & Shilley, 2009). Sensitive macroinvertebrates Ephemeroptera, Plecoptera, and Trichoptera (EPT) are very vulnerable to heavy metal pollution (Qu, Wu, Tang, Cai, & Park, 2010). Synthetic detergents used for washing vehicles are reported to be acutely toxic to fish and macroinvertebrates (Abel, 1974). Use of detergents also contribute to phosphate in aquatic environment that can cause eutrophication (Kundu, Coumar, Rajendiran, Rao, & Rao, 2015).

Bhutan, a small landlocked country in the Eastern Himalayas, situated between longitude 88° 54′ and 92° 10′ East and latitude 26°40′ and 28°15′ North (Figure1(a)) is also challenged by the management of VWW. Rapid economic activities, urbanizations, and continuing population growth are the main reasons for escalation of vehicle numbers and production of VWW (Rai, Sharma, Gurung, & Sitaula, 2016). As per the vehicle statistics recorded on 31 August 2018, Bhutan had 97,900 vehicles (RSTA, 2018). In Bhutan, vehicles are usually washed in private lawns or vehicle wash centers as washing of vehicle in natural water bodies is forbidden. In contrast, vehicle wash centers are usually constructed along the streams and rivers for drawing water as well as discharging VWW into the same water bodies (Rai et al., 2016). As per the study carried out by Asian Development Bank (ADB), Bhutan approximately generates 6.5 million liters of VWW every year (Zangmo, 2016). The river quality assessment carried out by Shrestha et al. (2008) have also reported that vehicle washings and river crossings by vehicles are major stressors of Bhutan's rivers.

The stream Olarong Chhu and the river Paa Chhu are perennial tributaries of the Wang Chhu, the principal river of the Wang Chhu basin, located in western Bhutan that has great environmental and economic importance. Wang Chhu basin occupies around 12 % of Bhutan's area consisting of forest land, agriculture area, wetland, and also supports two hydropower stations (Chukha Hydropower Plant and Tala Hydropower Plant) that are major sources of revenue for the Government of Bhutan (NEC, 2016). Therefore, sustainable management of the Wang Chhu basin is very important for the environmental and economic sustainability of Bhutan. Of late, the Wang Chhu and its tributaries are in focus as several studies have reported degradation in their quality due to increasing economic activities and urbanizations in its watersheds and discharge of VWW (Giri & Singh, 2013; Pradhan & Mandal, 2015; Rai et al., 2018).

Though the Olarong Chhu and the Paa Chhu are being used for discharging untreated VWW, impact of this wastewater on the water chemistry and benthic fauna have not been studied till now. A combination of physico-chemical and biological methods constitutes the best approach for assessing anthropogenic influences on water resources (Xu, Wang, Duan, & Pan, 2014). Physico-chemical indicators reflect the conditions only for that period while the biotic assemblage such as benthic macroinvertebrates provides integrated effects of stressors over time (Korte et al., 2010). Benthic macroinvertebrates are often used in assessing water quality because of their ubiquitous nature (Campbell, 2004), abundance, known pollution tolerances, sedentary habits

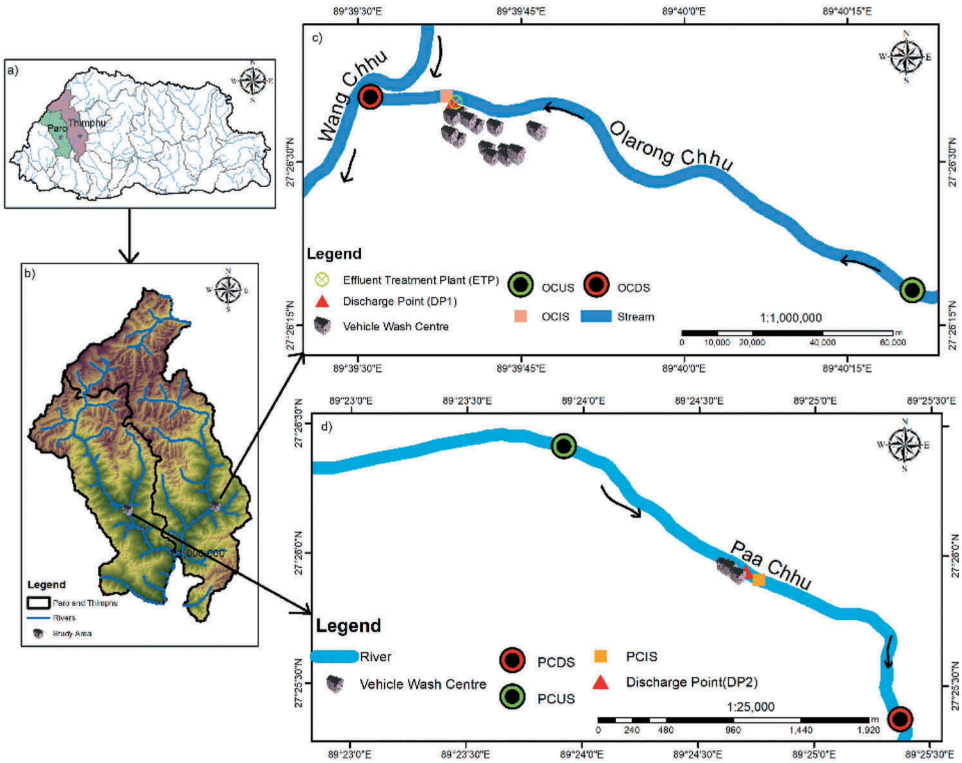

Figure 1. The study area and sampling sites: (a) a map of Bhutan showing the locations of Thimphu and Paro; (b) the location of vehicle wash centers at Olakha (Thimphu) and Paro Town (Paro); (c) sampling sites in the Olarong Chhu; and (d) sampling sites in the Paa Chhu.

(De Pauw, Gabriels, & Goethals, 2006), wide range of feeding habits, varied life spans, and convenient size for field examination with unaided eyes (Chessman, 1995). Though, studies of benthic macroinvertebrates have proved to be very cost-effective, reliable, and robust in monitoring aquatic pollution (Resh, Norris, & Barbour, 1995), its usage in Asian countries is still in its infancy (Korte et al., 2010).

Anthropogenic activities in catchments influence the composition of macroinvertebrate assemblages in adjacent water bodies (Arimoro, Odume, Uhunoma, & Edegbene, 2015). The sensitivities of different macroinvertebrates to pollution indicate the water quality as macroinvertebrates existing in particular water quality don't exist in other water quality conditions (Xu et al., 2014). Hence, this study had hypothesized that because of the discharge of VWW in the Olarong Chhu and the Paa Chhu, the water quality and macroinvertebrate assemblage structures at the impacted sites would differ significantly from the less impacted sites. The objective of this study was to assess the impact of discharging untreated VWW on the water chemistry and macroinvertebrate assemblages of the Olarong Chhu and the Paa Chhu. This study will contribute baseline information on the surface water pollution due to discharge of untreated VWW. It is expected that the findings would guide Bhutan in proper implementation of legislations and appropriate strategies for protecting surface water resources and aquatic ecosystems from the impact of increasing volumes of VW. This study will also facilitate understanding of the efficiency, reliability, and cost effectiveness of using benthic macroinvertebrates in assessing surface water quality especially in developing countries that do not have adequate scientific facilities.

Methods and materials

Study area

The study sites were chosen by considering the locations of vehicle wash centers in Thimphu and Paro (Figure1(b)). Thimphu is the capital city of Bhutan. It is the most urbanized with maximum number of vehicles (RSTA, 2018) and vehicle wash centers (Rai et al., 2016). Paro, a smaller district is also located in the same watershed with Thimphu. During preliminary field survey it was discovered that the Olarong Chhu receive direct discharges of VWW from 21 vehicle wash centers at Olakha, Thimphu (89° 39′ 38.1°44" E, 27° 26′ 36.0° 64" N). Likewise, the Paa Chhu receive VWW from 3 wash centers at Paro Town area (89° 24′ 42.2°77" E, 27° 26′ 36.0°64" N). Olakha vehicle wash center area has one effluent treatment plant (ETP) designed to separate oil, grease, and sludge but it was dysfunctional during the study period. The channels to separating chambers were clogged with debris, plastic bottles and sludges. Hence VWW was flowing through it without any treatment. The study area covered 1.7 km stretch of the Olarong Chhu and 3.3 km of the Paa Chhu.

Sampling sites

Untreated VWW were collected from the discharge points DP1 (Figure1(c)) and DP2 (Figure1(d)) before their discharge into the surface water. The Olarong Chhu and the Paa Chhu were divided into upstream, impact, and downstream sites: Olarong Chhu upstream (OCUS) or the reference site, impact site (OCIS) or the wastewater discharge zone, downstream (OCDS) or the recovery site (Figure1(c)), Paa Chhu upstream site (PCUS), impact site (PCIS), and downstream site (PCDS) (Figure 1(d)). All the sampling sites were preassessed using Hindu Kush-Himalayan (HKH) screening protocol (Hartmann, Moog, & Stubauer, 2010) to confirm the status of the sites. HKH screening protocol is a macroinvertebrate-based field screening method for assessing the ecological status of rivers in the HKH region.

Sampling and analyzes of wastewater and surface water

Untreated VWW from the discharge points and the surface water samples of the Olarong Chhu and Paa Chhu were collected once in a month in premonsoon (March to May) and postmonsoon (September to November) seasons of 2016. Physico-chemical composition of VWW and its impact on receiving water bodies were investigated by analyzing pH, temperature (T), electrical conductivity (EC), turbidity, total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen (DO), BOD_5, COD, oil and grease, total nitrogen (TN), total phosphorous (TP), total alkalinity (TA), sulfate and heavy metals (Ar, Cd, Cr, Cu, Fe, Mn, Pb, and Zn). Time composite sampling method (APHA, 2005; Maiti, 2004) was followed, considering the variations in number of vehicles washed and resultant volume and composition of wastewater discharged into the water bodies. Composited samples (n = 8) were segregated into different sample bottles and transported to laboratories according to preservation and transportation protocols outlined in APHA (2005).

Physical variables (T, pH, DO, and EC) were measured in situ using HACH portable multi-parameter probe (HQ40d). Turbidity and TDS were measured in laboratory using turbidimeter (HACH 2100Q) and TDS meter (WQTL01510) respectively. TSS, heavy metals, oil and grease, TA, TP, TN, BOD_5, COD, and sulfate were analyzed following the standard procedures outlined in APHA (2005). TSS was analyzed gravimetrically using predried Whatman 4 GF/C filter paper. Heavy metals were determined in atomic absorption spectrophotometer. Oil and grease were determined gravimetrically after extracting with hexane. TA was determined titrimetrically with 0.02 N sulfuric acid using mixed indicator (Bromocresol Green and Methyl Red). TN was measured by Kjeldahl digestion and TP by potassium persulfate digestion followed by spectrophotometer reading. Iodometric Winkler's method was used to determine BOD_5 and COD was determined by open reflux method. Concentration of sulfate was determined in spectrophotometer (DR5000) after reacting the samples with SulfaVer 4 Reagent Powder Pillows.

Wastewater flow and surface water discharge measurements

Wastewater flow rate was measured with volumetric technique using bucket and stop watch (US EPA, 2015). The volumetric flow rate (Q) is equal to the volume of the bucket (V) divided by the average time (t). A 10-liter calibrated bucket was filled with the wastewater for 10 seconds. Five consecutive measurements were averaged to calculate the flow rate.

$$Q = \frac{V}{t} \tag{1}$$

The Olarong Chhu discharge rate was measured using float method (Michaud & Wierenga, 2005). The amount of water passing a point on the stream channel during a given time is a function of velocity and cross-sectional area of the flowing water.

$$Q = AV \tag{2}$$

Where, Q is stream discharge (volume/time), A is cross-sectional area, and V is flow velocity. The Paa Chu discharge measurement was measured through cableway using SEBA current meter and Z6 counter.

Sampling of macroinvertebrates

The benthic macroinvertebrates were sampled once in a month from the sampling sites of the Olarong Chhu and the Paa Chhu in premonsoon and postmonsoon seasons of 2016 using a multihabitat sampling approach (Moog, 2007). Since sampling was done after screening the sites with HKH screening protocol (Hartmann et al., 2010), macroinvertebrates were collected from 10 sampling units covering all representative habitat types with a share of at least 5% coverage within 100 m distance. Benthic macroinvertebrates were collected using D-frame net of 25 cm × 25 cm and a mesh size of 500 μm. Collected organisms were fixed in situ using 70% ethanol in labeled polypropylene plastic containers and transported to the laboratory for sorting and identification. The samples were washed, sieved, sorted, and identified to family level according to Dudgeon (1999), Hartmann (2007), and Thorp and Rogers (2010) in the Biological Laboratory of Samtse College of Education, Royal University of Bhutan.

Data analyzes

The mean and standard deviation for each physico-chemical variables measured in VWW were calculated. Significant spatial variation of physico-chemical variables in the Olarong Chhu and the Paa Chhu were examined with one-way analysis of variance (ANOVA) at 95% confidence level. A paired-sample t test ($P < .05$) was performed between the upstream and impact sites in the Olarong Chhu (OCUS vs. OCIS) and the Paa Chhu (PCUS vs. PCIS) to explain the changes in water chemistry before and after the influx of VWW. Biological metrics including total abundance, taxa richness, EPT abundance %, Shannon diversity, Margalef diversity and Pielou Evenness indices were compared among the sites using nonparametric Kruskal–Wallis test ($P < .05$). Statistical analyzes were done using SPSS software (Version 20).The ecological status of the Olarong Chhu and the Paa Chhu were determined by using HKHbios index (Ofenböck, Moog, Sharma, & Korte, 2010). In HKHbios the average score per taxon of all indicator species present in a sample was calculated according to:

$$ASTP = \frac{\sum_{i=1}^{n} Score_i}{n} \tag{3}$$

Where, $Score_i$ is the score of taxa i and n is the number of taxa. HKHbios was calculated for each site as per the tolerance score of recorded taxa, which is based on 10-point scoring system with high scores indicating high sensitivity (Ofenböck et al., 2010). This value reflects the overall river ecosystem health by accounting the cumulative effects of anthropogenic stresses.

For multivariate analysis the benthic macroinvertebrates data were transformed to log (X + 1) and were not transformed further. The relationship between the physico-chemical variables and the benthic assemblage for study sites was analyzed with canonical correspondence analysis (CCA). CCA works under the principle that species have unimodal responses to physico-chemical gradients. The multivariate analysis was performed in R Software.

Results and discussion

Physico-chemical variables in vehicle wash wastewater

Twenty-two physico-chemical variables associated with vehicle washing were examined in VWW (Table 1). Factors such as chemicals used during servicing and washing, fuels used, road surface travelled, and particulate matters attached to the vehicles may affect the types and concentrations of contaminants in VWW (O'Sullivan, Smalley, & Good, 2011).

Table 1. Physico-chemical variables of vehicle wash wastewater collected from the discharge points DP1of Olakha, Thimphu and DP2 of Paro Town (n = 6).

Variables	Mean±SD VWW (DP1, Olakha)	Mean ± SD VWW (DP2, ParoTown)	Maximum permissible limits by ES 2010 of Bhutan
Temperature (°C)	15.20 ± 0.65	15.56 ± 0.61	No standard
pH	8.83 ± 0.22	8.98 ± 0.14	6.50–8.50
EC (µs/cm)	164.51 ± 3.59	153.60 ± 1.85	No standard
DO (mg/l)	5.53 ± 0.36	6.21 ± 0.24	No standard
TSS (mg/l)	1562.98 ± 3.01	118.53 ± 0.54	80
TDS (mg/l)	201.20 ± 1.79	167.95 ± 1.22	150
Turbidity (NTU)	88.19 ± 4.45	61.19 ± 0.68	No standard
BOD (mg/l)	19.69 ± 0.77	12.39 ± 0.07	30
COD (mg/l)	74.93 ± 2.70	40.30 ± 0.08	150
TN (mg/l)	3.13 ± 0.18	2.58 ± 0.10	20
TP (mg/l)	2.58 ± 0.23	2.18 ± 0.14	No standard
Oil and grease (mg/l)	1394.81 ± 1.86	472.81 ± 1.31	5
TA (ppm)	164.64 ± 4.55	142.83 ± 1.34	No standard
Sulfate (mg/l)	99.36 ± 1.77	42.67 ± 1.49	500
Ar(mg/l)	ND	ND	0.10
Cd (mg/l)	0.04 ± 0.01	0.03 ± 0.01	0.05
Cr (mg/l)	0.02 ± 0.01	0.01 ± 0.00	0.50
Cu (mg/l)	0.22 ± 0.02	0.05 ± 0.01	0.10
Fe (mg/l)	53.79 ± 1.51	7.39 ± 0.06	2.00
Mn (mg/l)	1.54 ± 0.03	0.34 ± 0.01	0.50
Pb (mg/l)	0.04 ± 0.01	0.03 ± 0.01	0.10
Zn (mg/l)	0.42 ± 0.01	0.23 ± 0.05	3.00

ND implies not detected. Detection limit = 0.01 mg/l
ES: Environmental Standards of Bhutan (NEC, 2010)

However, many variables derived from above factors could not be considered and measured within the scope of this study due to lack of laboratory resources.

Environmental Standards of Bhutan (NEC, 2010) does not have specific standards for VWW and the concentrations of physico-chemical variables were compared with generic standards for industrial wastewater. The concentrations of TSS, TDS, oil and grease, Cu, Fe, and Mn in VWW discharged into the Olarong Chhu were greater than the permissible limits of industrial wastewater. Likewise, the mean values of pH, TSS, TDS, oil and grease, and Fe in VWW flowing into the Paa Chhu were not within the permissible limits. Similar result have been also reported by Aikins and Boakye (2015), where variables such as solids, oil and grease, phosphate, and heavy metals exceeded effluent limit for discharge to watercourse. Discharge of VWW into the surface water bodies has the potential to impair water quality and adversely affect aquatic ecosystems. Likewise, the volume of the wastewater discharged into the surface water bodies should also be monitored because, according to Hlthman (2002), if the volume and velocity of the streams or rivers are not sufficient to handle influxes of wastewater, great environmental damage can occur. Though the volumes of the VWW influxed were comparatively lesser than the discharge rates of the water bodies (Table 2), Bhutan should come up with sustainable strategies for protecting surface water pollution from VWW. VWW should be treated with the affordable technology and its contaminants levels should be monitored by setting up VWW standards before discharging into the surface water bodies.

Table 2. Wastewater flow and surface water discharge rates.

Site	Wastewater influx rate (cu.m/sec)	Surface water discharge rate (cu.m/sec)
Olarong Chhu	0.0012	0.539
Paa Chhu	0.0004	4.088

Physico-chemical variables of surface water

All the physico-chemical variables examined in untreated VWW were also analyzed in surface water samples to understand their role in impairment of the water quality and subsequent influence on the benthic macroinvertebrate assemblages. The Olarong Chhu presented significant spatial variation (ANOVA, $p < .05$) in pH, DO, turbidity, BOD_5, COD, oil and grease, and sulfate while the Paa Chhu showed in pH, EC, DO, TSS, TDS, BOD_5, COD, and oil and grease across the sampled sites (Table 3).

Comparatively higher concentrations of variables related to vehicle washing were detected at the impact sites (Table 3). Other studies have also reported impact of VWW on surface water chemistry with maximum deterioration at the VWW discharge zones (Chukwu, Segi, & Adeoye, 2008; Danha et al., 2014). Paired-samples *t* test (Table 4) also indicated significant impact of influxed VWW in certain water quality variables. Decrease in DO and increase in turbidity, BOD_5, COD, and oil and grease indicated influx of VWW loaded with organic contaminants at the impact sites (OCIS and PCIS). Chukwu et al. (2008) have also reported that VWW disrupts the oxygen balance for the aquatic ecosystems by decreasing DO and increasing BOD and COD.

Both the Olarong Chhu and the Paa Chhu showed minute traces of some heavy metals (Table 3). Discharge of untreated wastewater is one of the probable routes of heavy metal input into the environment (Rule et al., 2006). It is very important to treat VWW before its discharge as the heavy metals in the aquatic environment are toxic, accumulative, becomes bioavailable to aquatic biota, and might enter the human food chain (Akpor et al., 2014). Benthic macroinvertebrates are particularly vulnerable to heavy metal contamination in surface water as they form the base of the food chain (Beasley & Kneale, 2003). The impacts of heavy metals on aquatic fauna have been also reported by Beasley and Kneale (2003) and Tao, Yuan, Xiaona, and Wei (2012).

Benthic macroinvertebrate assemblages

A total of 13640 benthic macroinvertebrate specimens belonging to 8 orders and 46 families were found

Table 3. Mean value (mean) and standard deviation (SD) of physico-chemical variables measured at the sampling sites of the Olarong Chhu and the Paa Chhu (n = 6).

Physico-chemical Variables	Olarong Chhu OCUS (mean ± SD)	OCIS (mean ± SD)	OCDS (mean ± SD)	P value	Paa Chhu PCUS (mean ± SD)	PCIS (mean ±SD)	PCDS (mean ± SD)	P value
T(°C)	11.75 ± 2.32	12.65 ± 2.36	12.27 ± 2.34	0.83	13.92 ± 1.97	15.42 ± 2.24	14.63 ± 2.05	0.53
pH	7.70 ± 0.13	8.42 ± 0.15	7.83 ± 0.07	0.00*	8.46 ± 0.26	9.05 ± 0.48	8.67 ± 0.24	0.04*
EC (µs/cm)	65.53 ± 38.51	103.03 ± 66.69	81.42 ± 69.33	0.61	112.55 ± 5.56	138.82 ± 11.15	116.72 ± 8.47	0.00*
DO mg/l	8.37 ± 0.31	7.70 ± 0.51	8.40 ± 0.50	0.04*	8.53 ± 0.31	7.47 ± 0.43	8.33 ± 0.15	0.00*
TSS mg/l	31.35 ± 24.90	57.62 ± 52.91	37.42 ± 44.28	0.60	19.33 ± 6.37	55.17 ± 13.52	30.27 ± 9.68	0.00*
TDS mg/l	44.22 ± 37.23	54.35 ± 39.36	49.70 ± 44.02	0.92	53.50 ± 6.05	76.08 ± 6.13	58.19 ± 3.59	0.00*
Turbidity (NTU)	5.90 ± 3.43	12.94 ± 3.53	8.28 ± 2.89	0.01*	4.96 ± 2.75	8.76 ± 1.43	6.08 ± 2.74	0.06
BOD_5 mg/l	0.77 ± 0.18	1.45 ± 0.43	0.92 ± 0.09	0.00*	0.52 ± 0.26	1.77 ± 0.25	0.95 ± 0.33	0.00*
COD mg/l	1.29 ± 0.32	3.88 ± 1.38	2.00 ± 0.64	0.00*	0.80 ± 0.59	3.37 ± 1.02	1.68 ± 0.33	0.00*
TN mg/l	1.07 ± 0.51	1.16 ± 0.49	0.78 ± 0.29	0.39	1.11 ± 0.83	1.02 ± 0.40	1.45 ± 0.82	0.61
TP mg/l	0.54 ± 0.71	0.65 ± 0.85	0.26 ± 0.09	0.61	0.11 ± 0.03	0.34 ± 0.13	0.42 ± 0.47	0.23
Oil & grease mg/l	0.00 ± 0.00	74.67 ± 11.29	31.00 ± 7.19	0.00*	0.00 ± 0.00	52.33 ± 7.34	8.07 ± 2.36	0.00*
TA (ppm)	34.47 ± 2.03	50.83 ± 16.60	47.77 ± 30.01	0.40	67.18 ± 15.29	77.53 ± 20.13	69.55 ± 20.09	0.66
Sulfate mg/l	1.18 ± 0.23	3.87 ± 1.37	2.00 ± 0.82	0.00*	8.20 ± 2.08	9.12 ± 2.60	9.18 ± 1.28	0.70
Fe mg/l	0.53 ± 0.20	0.67 ± 0.32	0.49 ± 0.12	0.44	0.43 ± 0.34	0.92 ± 1.16	0.31 ± 0.18	0.38
Mn mg/l	0.04 ± 0.04	0.03 ± 0.03	0.00 ± 0.00	0.12		NOT DETECTED		
Pb mg/l	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.29	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.60
Zn mg/l	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.19	0.02 ± 0.02	0.02 ± 0.03	0.02 ± 0.00	0.91

Table 4. Significant paired-sample t test ($P < .05$) results of water quality variables between the upstream and impact sites of the Olarong Chhu and the Paa Chhu.

Variables	Paired samples of the Olarong Chhu	P value	Paired samples of the Paa Chhu	P value
pH	OCUS and OCIS*	0.000	PCUS and PCIS	0.045
DO mg/l	OCUS and OCIS*	0.038	PCUS and PCIS*	0.002
Turbidity (NTU)	OCUS and OCIS*	0.011	PCUS and PCIS*	0.029
BOD_5 mg/l	OCUS and OCIS*	0.017	PCUS and PCIS*	0.000
COD mg/l	OCUS and OCIS*	0.009	PCUS and PCIS*	0.001
Oil and grease mg/l	OCUS and OCIS*	0.000	PCUS and PCIS*	0.000
Sulfate mg/l	OCUS and OCIS*	0.008	PCUS and PCIS	0.553

during the study period (Table 5). Insects were the dominant group with 44 out of the 46 identified families. The relative abundance of taxonomic groups at the order level revealed Tricoptera were the most diverse order with 13 families, followed by Diptera and Ephemeroptera with 12 families each, Plecoptera with 3 families, Coleoptera, Odonata and Megaloptera with 1 family each and Oligochaeta with two families (Figure 2).

Upstream sites OCUS and PCUS showed good proportion of pollution sensitive macroinvertebrates like EPT (Figure 3). Pollution tolerant taxa were totally absent in these sites. Physico-chemical analysis also did not show any disturbances by variables related to vehicle washing (Table 3). Distribution and abundance of sensitive taxa (Figure 2) confirmed that upstream habitats are intact, heterogeneous, and do not receive any VWW.

Absence of pollution sensitive taxa of Ephemeroptera, Tricoptera, complete absence of Plecoptera, and presence of pollution tolerant Simuliidae and red Chironomidae (Figure 3) were an indication of ecological degradation at the impact sites. Anthropogenic stress in aquatic ecosystems are indicated by changes in species composition and the disappearance of sensitive organisms from aquatic communities (Schindler, 1987). Oily sludge habitat at the impact sites might have favored proliferation of pollution tolerant species such as Simuliidae and red Chironomidae. Shah, Tachamo, Sharma, and Moog (2008) have stressed that, discharges of wastewaters are the major causes of river water quality degradation, as discharge sites become suitable habitat for highly pollution-tolerant species. Arimoro et al. (2015) have also reported similar impact of wastewater on macroinvertebrates assemblages.

In downstream sites (OCDS and PCDS), there were considerable decrease of the concentrations of physico-chemical variables (Table 3), which indicate that restoration of ecological status would be possible. Since the downstream sampling site was just below 0.3 km in the Olarong Chhu, there were still large number of pollution tolerant species. Whereas in the Paa Chhu, since the downstream site was beyond 1.7 km the species richness and composition were very different from the Olarong Chhu downstream (Table 5).

Biological metrics

Summaries of biological metrics including total abundance, taxa richness, EPT abundance (%), Shannon, Margalef diversity indices and Pielou evenness index for the six sampling stations are shown in Table 6.

Except for total abundance (Kruskal-Walis, *P = .06, df = 2, N = 18*), taxa richness (S), EPT abundance (%), Shannon diversity index (*H*), Margalef diversity index (*d*), and Pielou evenness index (*J*) varied significantly among the three sites

Table 5. Distribution and abundance of benthic macroinvertebrates in the Olarong Chhu and the Paa Chhu (premonsoon and postmonsoon of 2016).

		Sampling sites (Olarong Chhu)			Sampling sites (Paa Chhu)		
Order	Family	OCUS	OCIS	OCDS	PCUS	PCIS	PCDS
Ephemeroptera	Ametropodidae				21		16
	Amelitidae				24		
	Baetidae	411	756	977	147	123	76
	Baetiscidae				23		
	Caenidae	32		21	18	23	22
	Ephemerellidae	63	24	32	102	48	86
	Ephemeridae	1					
	Heptagenidae	172			347		190
	Leptophlebidae	107	18	56	195	55	168
	Neoephimeridae	29					
	Oligoneuriidae				34		44
	Potamanthidae				1		
	Siphlonuridae						2
Plecoptera	Chloroperlidae	1			12		9
	Perlodidae	1					
	Perlidae	15			15		2
Trichoptera	Brachycentridae	296	82	142	240	17	63
	Glossosomatidae	77			979		151
	Hydropsychidae	58		27	130		57
	Hydroptilidae				16		3
	Leptoceridae	70		17	25		2
	Lepidostomatidae						1
	Limnocentropodidae	2					
	Limnephilidae						3
	Philopotamidae	28		17	25		31
	Polycentropodidae	17		11	20		15
	Psychomiidae				2		
	Rhyacophilidae	19		13	18		13
	Stenopsychidae	122		32	62		84
Diptera	Anthericidae	11		1	21	9	56
	Ceratopogonidae	1	16	3			
	Chironomidae(w)	199	500	155	92	674	241
	Chironomidae(r)		68	23		95	
	Deuterophlebiidae			16	1		
	Dolichopodidae			1			
	Empididae	25		1			
	Limoniidae	39	419	282	120	26	74
	Psychodidae		27	22			
	Simuliidae	989	109	30	34	24	225
	Tabanidae	1	1	5			
	Tipulidae	42	15	12	15	23	47
Coleoptera	Elmidae	3	2	1	1		1
	Scirtidae				32	16	1
Odonata	Gomphidae	20	4	16			1
Megaloptera	Corydalidae	13		1			
Oligochaetae	Lumbricidae	3	43	6		18	
	Tubificidae		839	239		84	
	Total	2867	2923	2159	2772	1235	1684

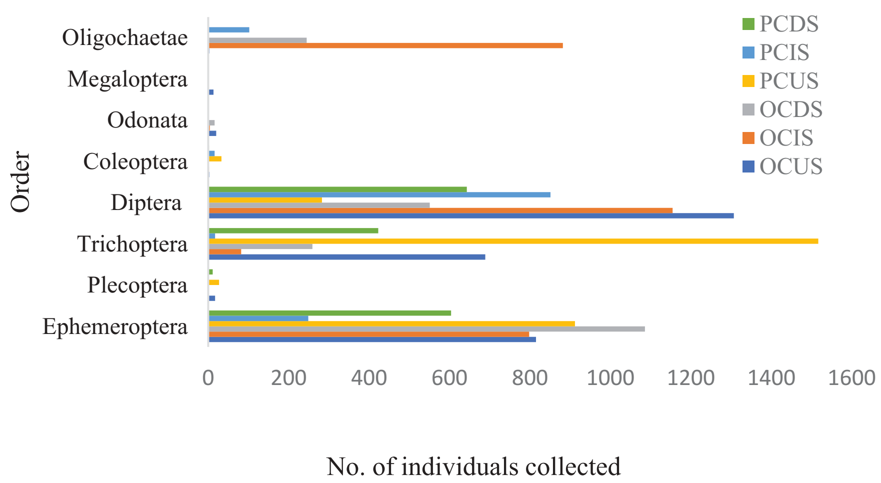

Figure 2. Distribution and abundance of macroinvertebrates in the sampling sites of the Olarong Chhu and the Paa Chhu.

Figure 3. Images of sensitive families of macroinvertebrates: Rhyacophilidae and Perlidae. Images of pollution tolerant families of macroinvertebrates: Simuliidae and Red Chironomidae.

Table 6. Site wise total abundance, taxa richness, EPT abundance %, diversity, and evenness indices of benthic macroinvertebrates.

	Olarong Chhu			Paa Chhu		
Particulars	OCUS	OCIS	OCDS	PCUS	PCIS	PCDS
Total abundance	2867	2923	2159	2772	1235	1684
Taxa richness (*S*)	31	16	28	30	14	29
EPT abundance (%)	53	30	62	89	22	62
Shannon diversity index (*H*)	2.3	1.9	2.1	2.4	1.7	2.7
Margalef diversity index (*d*)	3.8	1.9	3.5	3.7	1.8	3.8
Pielou evenness index (*J*)	0.7	0.7	0.6	0.7	0.6	0.8

of the Olarong Chhu (Table 6, Kruskal-Walis, $P > .00$, $df = 2$, $N = 18$). While in Paa Chhu total abundance, taxa richness (*S*), EPT abundance (%), Shannon diversity index (*H*), Margalef diversity index(*d*), and Pielou evenness index (*J*) varied significantly among the three sites (Table 6, Kruskal-Walis, $P > .00$, $df = 2$, $N = 18$).

Upstream sites (OCUS and PCUS) showed high species richness, EPT abundance %, diversity and evenness indices. Species richness, EPT abundance %, diversity and evenness indices decreased at the impact sites. Sensitive EPT percentages decreased to 30% in OCIS compared to 53% in the upstream of the Olarong Chhu. Likewise, in the Paa Chhu it decreased to 22% at the impact site compared to 89 % in the upstream. Since the EPT are considered good indicator of water quality owing to their sensitivity (Rosenberg & Resh, 1993), this result indicates that impact sites (OCIS and PCIS) have been degraded due to influx of VWW. Compared to the Olarong Chhu downstream, the Paa Chhu downstream reflected high diversity indices ($H = 2.7$, $d = 3.8$, and $J = 0.8$).

Ecological status

According to HKHbios index ecological status of the Olarong Chhu and the Paa Chhu ranged from good (6.4 – 7.5) to moderate (5.3–6.2). This result corresponds to HKH screening findings, where all the river quality class I and II sites achieved good HKHbios class boundaries while river quality class III achieved moderate HKHbios class boundaries (Table 7). Hence it can be concluded that HKH screening protocol, which is based on sensory criteria and biota that can be identified in the field, is equally efficient in evaluating ecological status of streams and rivers. HKHbios indices at impact sites decreased to 6.2 (PCIS) and 5.3 (OCIS) compared to upstream values 7.5 (PCUS and OCUS). This indicates that ecological status of the Paa Chhu and the Olarong Chhu have been deteriorated especially at the impact sites (OCIS and PCIS) which receive direct discharge of VWW. However, there is increase in HKHbios indices in downstream OCDS (6.4) and PCDS (7.5) indicating that there is some extent of ecological restoration.

Identification of stressor

CCA performed on the whole dataset showed that physico-chemical variables namely DO, pH, BOD_5, COD, TSS, Turbidity, T, oil and grease, and sulfate were influencing variables to the biotic assemblages (Figure 4). The model showed significant variation among the sites and tested variables ($F = 3.2484$, $D = 16$, $P = .001$). Canonical axis 1 and axis 2 explained 60.16% and 19.85%, respectively of the variance in the benthic macroinvertebrate assemblages. Three axes of the CCA explained 89.4% of

Table 7. Ecological status of the Olarong Chhu and the Paa Chhu reflected by HKHbios index and HKH screening protocol.

Site	HKHbios index	Class boundaries	HKH Field Screening result
OCUS	7.5	Good	River quality class II
OCIS	5.3	Moderate	River quality class III
OCDS	6.4	Good	River quality class II
PCUS	7.5	Good	River quality class I
PCIS	6.2	Moderate	River quality class III
PCDS	7.5	Good	River quality class II

the variance. Axis 1 is positively correlated to DO, sulfate, and temperature while other remaining significant variables were negatively correlated to Axis 1. Impact sites (OCIS and PCIS) were heavily influenced from the variables such as TSS, turbidity, COD, BOD_5, oil and grease, and pH (Figure 4). Among 16 physico-chemical variables fed in CCA, 9 variables were found significant predictors of benthic macroinvertebrate assemblages in the study sites (Table 8).

Elevation in pH at the impact sites (8.42–9.05) can be attributed to the use of detergents during washing of vehicles. pH levels below 4 and above 9 have been reported to influence the macroinvertebrate assemblages (Berezina, 2001). The mean values of oil and grease in impact sites OCIS (74.67 mg/l) and PCIS (52.33 mg/l) are very alarming as they contain hydrocarbon compounds, some of which can harm aquatic biota even at low concentrations. Negative impacts of petroleum oil on stream macroinvertebrates in terms of abundance and taxonomic richness has been reported by Vinson, Dinger, Kotynek, and Dethier (2008). Impact sites of both the Olarong Chhu and the Paa Chhu showed higher concentrations of TSS (OCIS = 57.62; PCIS = 55.17) and TDS (OCIS = 54.35; PCIS = 76.08). Since the VWW is usually turbid and that might have escalated the TSS and TDS at the impact sites. Installation of treatment plant is necessary for decreasing TSS and TDS in VWW as they are associated with behavioral changes, growth disruptions, survival rates and shifts in community structures of macroinvertebrates (Boehme, Zipper, Schoenholtz, Soucek, & Timpano, 2016; Jones et al., 2011)

Conclusion

Complete analyzes of physico-chemical and benthic macroinvertebrates data reflected that streams and rivers used for discharging VWW are vulnerable to deterioration in their ecological status. Ecological status of the Oarong Chhu and the Paa Chhu have been degraded especially at the impact sites which receive direct discharge of VWW containing various contaminants. Physico-chemical variables related to vehicle washing were present in higher concentrations in impact sites compared to upstream and downstream sites. Deterioration in ecological status at the impact sites were also indicated by less taxa richness, diversity and presence of pollution tolerant benthic macroinvertebrates. CCA revealed that physico-chemical variables associated with vehicle washing such as DO, pH, BOD_5, COD, TSS, Turbidity, T, oil and grease, and sulfate significantly deteriorate aquatic ecological status and affect the benthic macrorinvertebrate assemblages.

Though there is no previous data to compare the results of this study, the present findings clearly showed

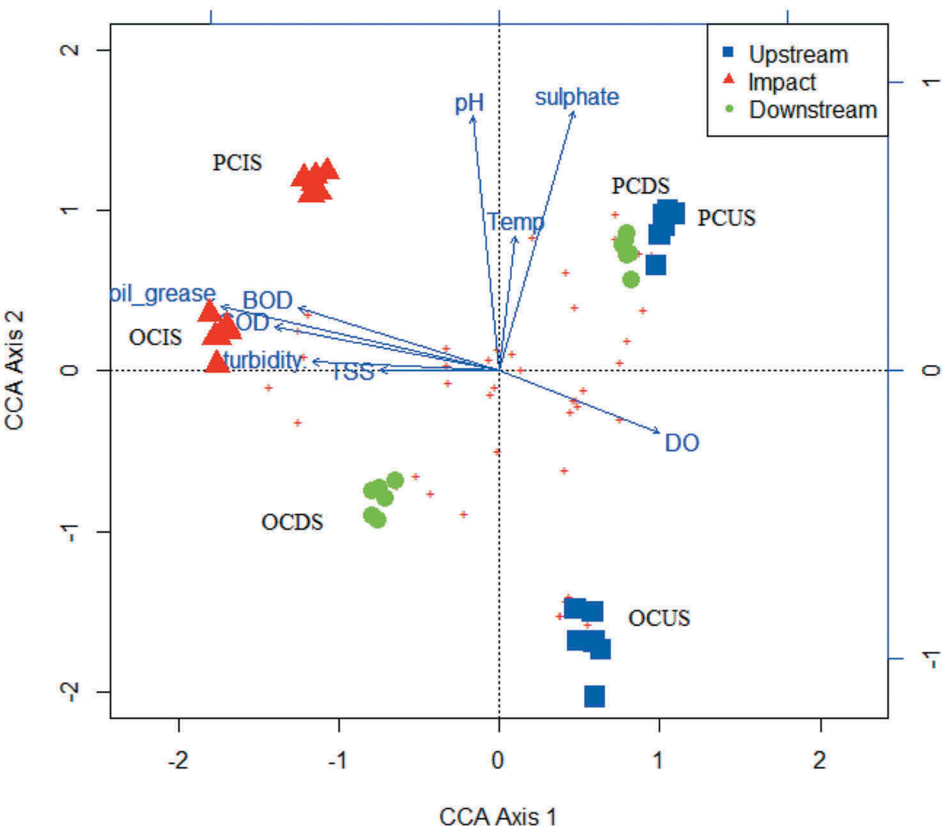

Figure 4. CCA biplot for benthic macroinvertebrates in the Olarong Chhu (OCUS, OCIS, and OCDS) and the Paa Chhu (PCUS, PCIS, and PCDS). Only statistically ($P < .05$) significant variables are shown in CCA ordination plot: DO, pH, BOD_5, COD, TSS, turbidity, T, oil and grease, and sulfate.

Table 8. Number of physico-chemical variables used in the CCA biplot.

Variable	*df*	Chi square	*F*	Pr(>*F*)	Significance codes
Temperature	1	0.03337	2.9184	0.02	*
pH	1	0.07495	6.5542	0.005	**
DO	1	0.10139	8.8663	0.005	**
TSS	1	0.12038	10.5273	0.005	**
TDS	1	0.01279	1.1184	0.33	
Turbidity	1	0.04024	3.5187	0.015	*
BOD_5	1	0.02266	1.9817	0.045	*
COD	1	0.02988	2.6133	0.045	*
TP	1	0.01055	0.9225	0.52	
TN	1	0.01717	1.5014	0.185	
Oil and grease	1	0.07336	6.415	0.005	**
TA	1	0.01048	0.9161	0.5	
Sulfate	1	0.02546	2.2262	0.045	*
Fe	1	0.00383	0.3347	0.965	
Pb	1	0.01112	0.9723	0.435	
Zn	1	0.00671	0.5871	0.745	
Residual	19	0.21727			

*$P < 0.05$, **$P < 0.001$

that discharge of VWW disturb water chemistry, degrade ecological status, and affect macroinvertebrate assemblages. With the increasing number of vehicles in the country, the volume of VWW is going to increase in future. Therefore, information derived from this study can be a baseline data for sensitizing the government in monitoring surface water pollution from the discharge of VWW. VWW should be treated with the affordable technology and its contaminants levels should be monitored by setting up VWW standards before discharging into the surface water bodies. Currently, Bhutan lacks adequate scientific facilities for assessing aquatic health, therefore use of bioindicators could be the best option owing to its efficiency, reliability, and cost effectiveness.

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