

RESEARCH ARTICLE

Toxic hazards of industrial waste receiving canal system in the lower catchment of Kelani River basin, Sri Lanka

HD Kuruppuarachchi and A Pathiratne*

Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Kelaniya.

Submitted: 11 June 2018; Revised: 22 October 2019; Accepted: 27 September 2019

Abstract: Assessing toxic hazards associated with polluted riverine ecosystems is essential for the development of effective strategies for their management. The present study explored the combined utility of bioassay responses of the plant, *Allium cepa* and surface water physico-chemical characteristics to assess the toxic hazards of an industrial waste receiving canal system located in the lower catchment of the Kelani River basin, Sri Lanka. Surface water samples from seven sites, viz. Maha Ela upstream (Site A), Manikagara Ela (Site B), Manikagara Ela - Maha Ela confluence (Site C), Maha Ela downstream (Site D), Maha Ela - Kelani River confluence (Site E), River down-reach (Site F) and upper-reach (Site R) were analysed on three occasions in 2015 covering dry and wet periods. Irrespective of the sampling periods, exposure of *A. cepa* bulbs to water from the Sites B, C, D and E resulted in root growth retardation and mitosis depression ($p < 0.05$) in the root meristem signifying toxic/cytotoxic hazards. Occasional micronuclei evolution and nuclear bud induction were also found in the root cells exposed to Site B and C samples indicating genotoxicity. Toxic hazards were somewhat reduced towards down-reach of the river, which may be associated with self-depuration effects. The principal component analysis based on surface water characteristics and bioassay responses revealed clear separations of Sites B and C from the other sites. The results revealed that water quality of Manikagara Ela and Maha Ela needs improvements considering toxic hazards to the riverine ecosystem and human health.

Keywords: Kelani River, principal component analysis, toxic hazard, water pollution.

INTRODUCTION

Pollution trend assessments are important for effective management of riverine ecosystems impacted by the contaminants from anthropogenic activities (Kara *et al.*, 2017; Barrenha *et al.*, 2018; Wu *et al.*, 2018). Bioassays with model organisms are considered as one of the green chemistry tools in assessing the environmental quality (Wieczerszak *et al.*, 2016). The plant, *Allium cepa* has been identified as an effective, economical and sensitive model to detect cytotoxic and genotoxic hazards in environmental samples (Leme & Marine-Morales, 2009; Pathiratne *et al.*, 2015) and can be used as a low cost and simple tool for toxicity assessment of surface waters in developing countries (Hemachandra & Pathiratne, 2017a).

Kelani river basin is one of the largest water sheds in Sri Lanka. The river water is used for drinking, domestic, agricultural and other purposes (Silva, 1996). Kelani River is considered as the largest recipient of industrial waste in the country (Ileperuma, 2000). The River is becoming polluted with multiple sources including industrial, urban and agricultural waste (Mahagamage & Manage, 2014; Mahagamage *et al.*, 2016). Several water intake points are located in the Kelani River for provision of drinking water supply to the general

* Corresponding author (asoka@kln.ac.lk ;  <https://orcid.org/0000-0002-2961-9064>)



public in the cities and suburbs. In the proposed ‘multi-stakeholder strategy and action plan for management and conservation of the Kelani River basin 2016–2020’, the importance of pollution impact assessments has been emphasised (Mallawatantri *et al.*, 2016). A large number of industries including several export oriented industrial parks are located in close vicinity of Kelani River (Gunawardena *et al.*, 2017). The industries located in these industrial zones/parks are required to treat their wastewater chemically in in-house treatment plants until specified national tolerance limits (Anonymous, 2008) are met. Industrial wastewater discharged into the common wastewater treatment plants (CWWTPs) are subjected to biological treatment (BOI 2011) prior to discharge to canal systems. Recent bioassay studies revealed that final effluents of the CWWTPs of some industrial zones located in the Kelani river basin pose cytogenotoxic hazards (Pathiratne *et al.*, 2015; Hemachandra & Pathiratne 2017b, 2018). Discharge of such industrial effluents from the industrial zones may pose potential

negative impacts on the quality of water in effluent receiving canal systems, which eventually link with the Kelani River. However, scientifically based evidences on potential toxic hazards of these industrial waste receiving canal systems in the Kelani River basin are meagre. The objective of this study was to explore the combination of *A. cepa* bioassay responses and physico-chemical analyses of surface waters to evaluate the pollution status and toxic hazard pattern of waterways impacted by the treated effluent discharges from a leading industrial zone located in the Kelani River basin, Sri Lanka.

METHODOLOGY

Water sampling sites

Seven sampling sites were selected in the Kelani River basin mainly based on a specific point source of pollution, *viz.* a continuous discharge of treated effluents from a leading export processing industrial zone operated by

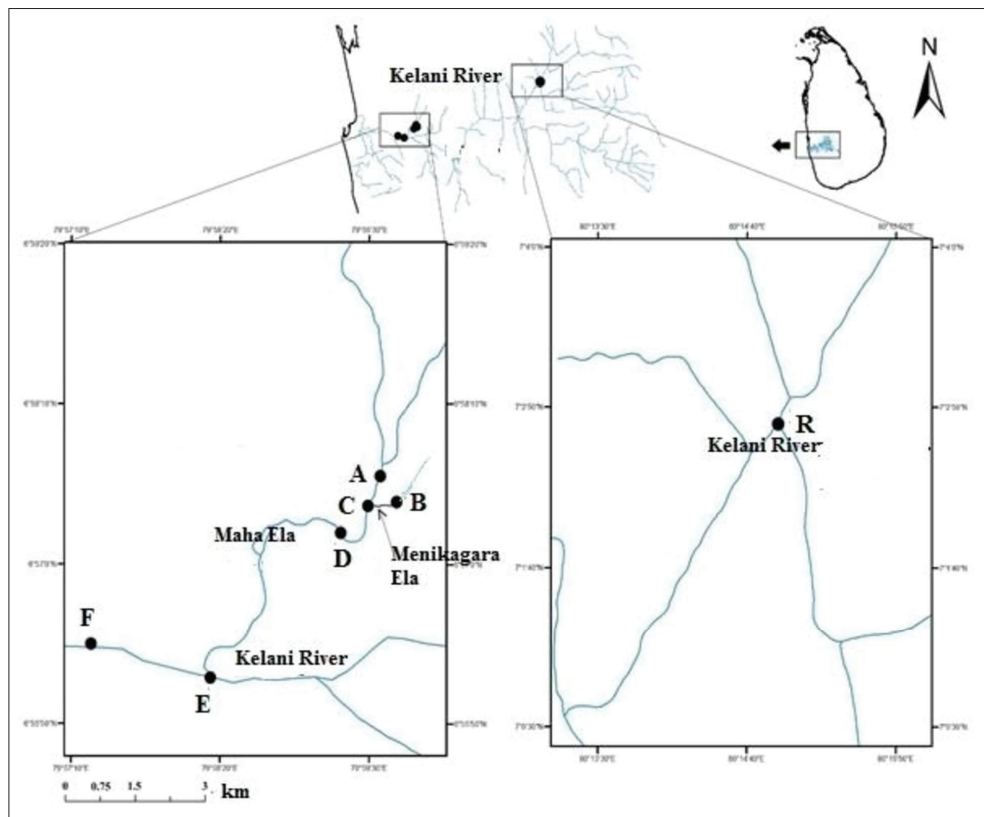


Figure 1: Location of the water sampling sites: Site A - upstream of Maha Ela; Site B - effluent receiving canal (Menikagara Ela); Site C - confluence of Menikagara Ela with Maha Ela; Site D - downstream of Maha Ela; Site E - confluence of Maha Ela with Kelni River; Site F - down reach of Kelani River at Biyagama; Site R - upper reach of Kelani River at Ruwanwella (reference site)

the Board of Investments in Sri Lanka, which consists of many industries including rubber glove, textile, and food processing factories. Following chemical treatments in in-house treatment plants of the respective industries, wastewaters are directed to the CWWTP where biological treatment is required to be carried out prior to the discharge of the final effluent (BOI, 2011) into the Kelani River basin. Of the seven sampling sites selected (Figure 1), six sites (Sites A to F) are located in the lower catchment area of the Kelani River basin. Site A (6° 57' 38.53"N; 79° 59' 35.31" E) is the upstream of a main canal located in a sub catchment called Maha Ela. Site B (6° 57' 27.20"N 79° 59' 42.99" E) is a canal called Menikagara Ela, which continuously receives treated effluents from the specified export processing industrial zone. Menikagara Ela confluences with the Maha Ela at Site C (6° 57' 29.70"N; 79° 59' 35.10" E), which is located nearly 0.5 km from Site A. Site D (6° 57' 27.36"N; 79° 59' 32.12" E) is located in the downstream (0.8 km from Site 2) of Maha Ela. Site E (6° 56' 18.97"N; 79° 58' 8.45" E) is the confluence of Maha Ela with the Kelani River. Site F (6° 56' 35.86"N; 79° 57' 21.84"E) is located in the down reaches of the river near the water intake point of a water treatment plant by the National Water Supply and Drainage Board for treatment prior to distribution for consumption to the general public. Site R (7° 2' 38.56" N; 80° 15' 6.25" E) is located close to the upper catchment area of Kelani river at Ruwanwella, which is a less urbanised area. Site R was considered as the reference site.

Water quality analysis

Water samples were collected from the seven sites on three occasions, which included a dry period (March 2015) and two wet periods (July and September 2015). On each sampling occasion, surface water samples were taken from randomly selected three sub-sampling sites to represent a particular site. *In situ* water quality parameters *viz.* pH, temperature, dissolved oxygen and total dissolved solids, of each sub sample were measured onsite using calibrated multiprobe water quality checker (YSI/Model: 556 MPS, USA). For measurement of heavy metals, water samples (1 L) were collected into acid washed (10 % nitric acid) polypropylene bottles and preserved with concentrated nitric acid. For the determination of other selected pollution indicative chemical parameters, water samples were collected into glass bottles and preserved where necessary as described by APHA (1998). Sample bottles were brought to the laboratory under chilled conditions and stored at 4 °C until analysis. Biochemical oxygen demand (BOD₅),

chemical oxygen demand (COD), nitrate, total dissolved phosphorus, oil and grease levels in the water samples were measured following APHA (1998). Selected heavy metals (cadmium, lead, copper and chromium) in the water samples were analysed using atomic absorption spectrophotometry (Analytikjena, Model: novAA400P) on graphite furnace mode following APHA (1998). Standard solutions were prepared from the reference standards for each metal separately for the generation of standard curves. Deionised double distilled water was used as the blank and 10 blanks were used to estimate the limits of quantification (LOQ = mean +10 times standard deviation) for each metal. LOQ (in µg/L) for Cd, Pb, Cu and Cr were 0.2, 2, 4 and 4, respectively.

Bioassay

On each and every sampling occasion, equal volumes of water taken from three subsampling sites of each site were mixed together to make a composite sample to represent the respective sampling site. Composite water samples from each site were transported to the laboratory in polythene bags within 2 h for the commencement of *Allium cepa* bioassay. The bioassay was conducted as described by Pathiratne *et al.* (2015). Briefly, equal sized healthy onion bulbs were chosen, and the outer scale of the bulbs was removed by gently scraping to expose the apices of root primordia. Onion bulbs were exposed to the exposure media in glass test tubes (60 mL), by submerging one onion bulb up to a depth of one quarter in each test tube (10 onion bulbs per exposure medium). Onion bulbs concurrently exposed to the aged tap water (source: Kelani River) was used as the control. The bulbs with exposure media were kept at 25–26 °C in the dark to avoid direct sunlight. After 48 h of exposure, root tips of 1–2 mm length (5–6 root tips from each onion bulb) of randomly selected five onion bulbs in each exposure medium were processed for microscopic studies. Root tips were fixed in ethanol: glacial acetic acid (3:1, v/v) overnight at 4 °C and transferred into 70 % ethyl alcohol for storage at 4 °C until processing. The remaining five bulbs were exposed to the respective media continuously for 7 d at 25–26 °C in the dark. The exposure media were renewed daily. After 7 d, lengths of all the roots of the bulbs were measured as described by Fiskesjo (1985) for toxicity assessments.

For microscopic analysis, preserved root tips were hydrolysed in 1N hydrochloric acid for 5 min at 60 °C and washed with distilled water. Root tips were then placed in watch glasses containing acetocarmine for 30 min. After staining, root tips were placed on glass slides and a slight

pressure was applied on the cover slip to squash the tip cells over the slide. Prepared slides for each exposure medium (one slide per onion bulb) were observed under the light microscope at 400× magnification to score dividing and non-dividing cells and the cells with micronuclei and nuclear abnormalities (nuclear buds, binuclei and condensed nuclei) in the interphase cells. Mitotic index was calculated as the number of cells in division in 100 total meristematic cells by counting at least 1000 meristematic cells in each slide. At least 1000 interphase cells were observed in each slide for scoring the occurrence of micronuclei and nuclear abnormalities. Root growth data and root meristem analysis data were obtained for five onion bulbs for each exposure.

Statistical analysis

Bioassay data were analysed using one-way analysis of variance test (ANOVA) after transforming the proportional data to arcsine-square root scale to maintain homogeneity where necessary (Zar, 1998). If there were significant differences, Tukey's pairwise comparison test was used for comparison of means. Accepted level of significance was $p < 0.05$. Site specific variability of chemical characteristics of surface water and bioassay parameters were also analysed by principal component analysis (Minitab version 14). In this analysis, data were transformed into square root scale to reduce the variability of different parameters (Scott & Clarke, 2000). Potential association ($p = 0.001$) of the contribution by chemical characteristics and bioassay parameters to the ordination patterns was examined by Pearson's product moment correlation test between the scores of the first principal components (PC1) of the chemical characteristics and toxicity parameters.

RESULTS AND DISCUSSION

Although export processing industrial zones/ parks considerably contribute to the economic development, diverse industries may generate effluents with complex mixtures of toxicants that may pose health risks to the receiving ecosystems and humans. Recent bioassay studies revealed that final effluents of the common wastewater treatment plants of some industrial zones pose cytogenotoxic hazards (Pathiratne *et al.*, 2015; Hemachandra & Pathiratne, 2017b; 2018). Discharge of such industrial effluents from the industrial zones may pose potential negative impacts on the quality of water in effluent receiving canal systems, which eventually merge with the Kelani River where several water intake points are located for provision of public water supplies. The

present study explored the combined utility of surface water characteristics and bioassay responses of the plant, *A. cepa* to assess for the first time, the pollution status and toxic hazard patterns of industrial waste receiving canal system located in the lower catchment of Kelani River basin, Sri Lanka. The treated effluents discharged from a leading export oriented industrial zone in the country is the main pollution source of this canal system.

Analysis of physico-chemical characteristics of the surface waters in the seven sampling sites in the three sampling events are shown in Table 1. Although the most pollution indicative parameters in the water from the upper reach (Site R) and down reach of Kelani River (Site F) are relatively low, such water cannot be used as portable drinking water without being subjected to treatments as COD values have exceeded the standard limit (10 mg/L) (SLSI, 2013) in all sampling events. Further, oil and grease content and Cd content in the water of Site F exceeded the standard limits in most cases (SLSI, 2013). Water quality analysis consistently showed that the surface water of Site B (Manikagara Ela, the canal which directly receives effluents from the industrial zone) had the lowest dissolved oxygen levels and highest BOD₅, COD, oil and grease, total phosphate, nitrate, Cd, Pb, Cu and Cr levels irrespective of the sampling period (Table 1). Site C (confluence of Manikagara Ela with Maha Ela) also contained considerably high levels of the measured contaminants (Table 1) indicating self-dilution at this site is not sufficient to curtail the incoming pollutant load. In general, some pollution indicative parameters at Site D (Maha Ela downstream) and Site E (confluence of Maha Ela with the Kelani River) are comparatively lower than those in Sites B and C (Table 1) indicating water quality improvements at Sites D and E to some extent with the self-dilution.

In order to protect the riverine water quality, it is important to consider the pollution assimilative capacity of a water body in determining the safe release limits for industrial discharges. Established Sri Lankan tolerance limits for BOD₅ (30 mg/L), COD (250 mg/L), cadmium and lead (0.1 mg/L), chromium (2 mg/L) and copper (3 mg/L) for discharge of industrial effluents to inland surface waters are based on expected dilution of the effluent by at least eight volumes of clean receiving water (Anonymous, 2008). Under this scenario, expected tolerance limits in the receiving water with 1:8 dilution would be one eighths ($\frac{1}{8}$) of the national tolerance limits for discharge of the effluents. Hence, the expected tolerance limits in the receiving water correspond to 3.8 mg/L for BOD₅, 31 mg/L for COD, 12.5 µg/L for cadmium and lead, 250 µg/L for chromium and 375 µg/L

Table 1: Physico-chemical characteristics of water in the sampling sites

Sampling site	pH	Temp (°C)	DO (mg/L)	TDS (mg/L)	BOD ₅ (mg/L)	COD (mg/L)	O&G (mg/L)	NO ₃ ⁻ (mg/L)	PO ₄ ³⁻ (mg/L)	Cd (µg/L)	Pb (µg/L)	Cu (µg/L)	Cr (µg/L)
March 2015 (dry period)													
A - Maha Ela upstream	6.3	30.0	6.6	39	1	94	0	0.4	0.04	28	<2	<4	3
B - Effluent receiving canal	7.6	33.1	2.9	958	51	95	4.4	45.4	0.27	92	67	25	8
C - Canal/Maha Ela confluence	7.2	31.6	4.4	623	15	75	2.4	33.2	0.14	65	28	12	6
D - Maha Ela downstream	7.0	29.9	6.5	94	13	88	1.7	2.8	0.05	53	<2	7	3
E - Maha Ela/river confluence	6.9	30.2	4.5	61	5	54	0.9	0.8	0.04	21	<2	<4	2
F - River down reach	6.8	29.7	5.4	37	1	23	0.1	0.3	0.05	18	<2	<4	<2
R - River upper reach	6.5	28.1	7.6	23	1	25	0.1	0.1	0.04	<0.2	<2	<4	<2
July 2015 (wet period)													
A - Maha Ela upstream	6.6	31.5	6.8	43	3	48	0	0.2	0.02	1	<2	9	3
B - Effluent receiving canal	7.7	32.7	2.9	1316	40	100	1.6	28.4	0.63	118	26	50	6
C - Canal/Maha Ela confluence	7.5	31.5	5.4	337	25	50	1.6	15.8	0.36	73	13	25	4
D - Maha Ela downstream	7.5	31.4	5.8	429	17	33	0.2	1.6	0.23	13	7	12	3
E - Maha Ela/river confluence	6.9	30.7	2.7	253	11	26	0.2	1.2	0.09	13	<2	11	2
F - River down reach	6.9	29.0	6.8	26	2.7	20	0.3	0.2	0.01	18	<2	10	<2
R - River upper reach	6.7	26.1	7.7	18	2.8	21	0	0	0.01	1	<2	11	<2
September 2015 (wet period)													
A - Maha Ela upstream	6.9	29.0	6.9	40	1.7	34	0.2	0.2	0.05	1	<2	8	<2
B - Effluent receiving canal	7.7	31.4	2.4	1192	35	123	1.2	41.5	1.10	51	28	64	11
C - Canal/Maha Ela confluence	6.9	30.2	5.5	402	20	96	0.4	33.5	0.83	39	12	21	5
D - Maha Ela downstream	6.9	29.1	6.6	114	12	37	0.4	2.8	0.15	30	6	19	<2
E - Maha Ela/river confluence	6.9	29.8	2.8	119	13	31	0.4	2.1	0.12	10	6	14	3
F - River down reach	7.1	29.8	6.4	29	2.4	34	0.3	0.1	0.04	4	<2	15	<2
R - River upper reach	7.0	26.1	7.8	18	1.7	31	0	0	0.03	<0.2	<2	9	<2
Wastewater Tolerance limits*	6-8.5	40	-	-	30	250	10	-	5	100	100	3000	500
Drinking water standards**	6.5-8.5	-	-	500	-	10	0.2	50	2	3	10	1000	50

Data are presented as the mean value of three sub sampling sites. Temp: temperature, DO: dissolved oxygen, TDS: total dissolved solids, BOD₅: biochemical oxygen demand, COD: chemical oxygen demand, O&G: oil and grease. LOQ (in µg/L) for Cd, Pb, Cu and Cr were 0.2, 2, 4 and 4, respectively. *Tolerance limits for discharge of industrial effluents into inland surface waters assuming at least 8 times dilution with clean receiving water (Anonymous, 2008). **National standards for portable drinking water (SLSI, 2013).

for copper. As shown in Table 1, mean BOD₅ levels in the sampling sites B, C, D and E in all sampling events exceeded 3.8 mg/L. Mean COD values in Sites A, B, C, D in all sampling events and in Site E in the dry period exceeded 31 mg/L. The concentrations of cadmium exceeded 12.5 µg/L in Sites B, C, and D in all sampling events, in Site A only in the dry period and in Sites E and F in the dry period and a wet period (Table 1). The results indicate that the self-dilution capacities of the receiving canal system may not be sufficient to reach the expected tolerance limit levels of some pollution indicative parameters in the treated wastewater discharged from the industrial zone.

Bioassays provide data for hazard identification as they can show integral response to the complex chemical mixtures without prior knowledge of the chemical composition and properties (Claxton *et al.*, 1998; Vasquez & Fatta-Kassinos, 2013). *A. cepa* is a sensitive and economical plant model for assessing general toxicity, cytotoxicity and genotoxicity of wastewater discharged by industries into the surface waters (Leme & Marin-Morales, 2009; Kannangara & Pathiratne, 2015; Hemachandra & Pathiratne, 2016). In the *A. cepa* test system, mitotic index depression indicates cytotoxic effects promoted by environmental pollutants (Leme &

Marin-Morales, 2009). Comparison of mitotic indices of root meristem of *A. cepa* bulbs exposed to the surface waters from different sampling sites (Table 2) indicated no significant differences among the roots exposed to the water collected from the reference site and those exposed to aged tap water in the three sampling events. However, the mitotic indices of the roots exposed to the water collected from Site B (Manikagara Ela) and Sites C, D and E of Maha Ela were significantly lower than those exposed to the water collected from the reference site ($p < 0.05$) in all sampling occasions (Table 2). The results indicate that the contaminants in the surface water of these sites could obstruct the cell division process in the root meristem. Inhibition of DNA/protein synthesis in the cells may cause mitosis depression as indicated by Yildiz *et al.* (2009). Mito-depressive effect of the surface waters in Sites B to E may be associated with the overall interactive effects of the complex assortment of cytotoxic chemicals present in the industry waste. The mitotic index of the root meristem exposed to the water from Site A (upstream of the Maha Ela) was also significantly depressed ($p < 0.05$) compared to the reference site (Table 2) in the two sampling events in the rainy periods. This may be due to cytotoxic pollutant inputs associated with surface run off in the rainy periods.

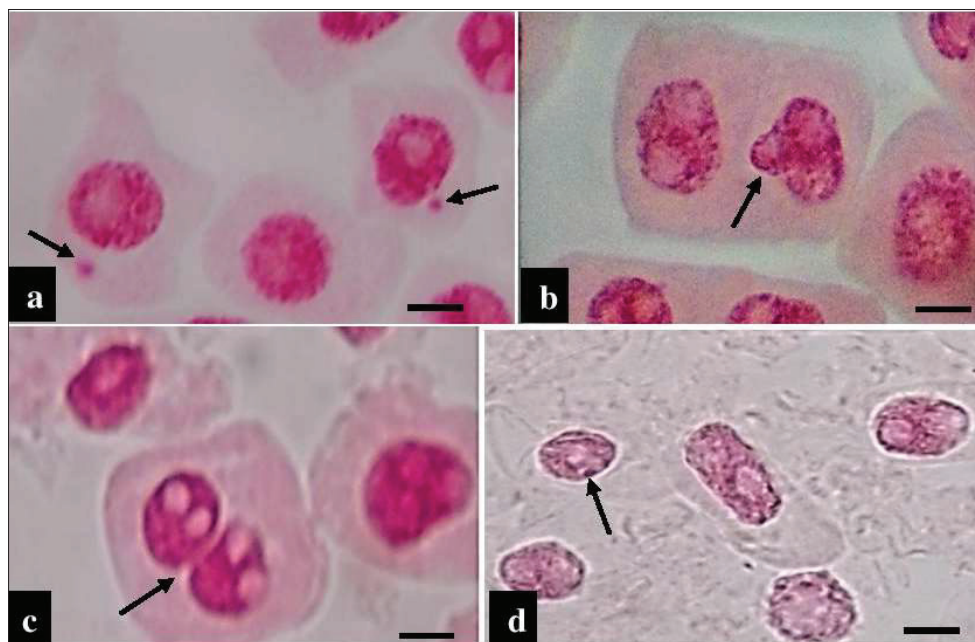


Figure 2: Micronuclei and nuclear abnormalities seen in *Allium cepa* root meristem exposed to water from the effluent receiving canal (Site B): (a) micronuclei; (b) nuclear bud; (c) binuclei; (d) condensed nucleus. Scale bar represents 10 µm

Of the micronuclei and three types of nuclear abnormalities (nuclear buds, binuclei and condensed nuclei) (Figure 2) found in the *A. cepa* root meristems exposed to water samples obtained in the present study, the most prominent type of abnormality was the condensed nuclei (Table 2). Occurrence of condensed nuclei in the root meristems exposed to the water from Sites B and C in all sampling events and those exposed to the water from Sites D and E in the third sampling event was significantly higher ($p < 0.05$) than those exposed to the reference site water and aged tap water. Andrade-Vieira *et al.* (2012) stated that induction of condensed nuclei may indicate nuclear chromatin condensation in meristematic cells following stress conditions, which could lead to cell death. Consistent occurrence of condensed nuclei in the root meristem exposed to the water from Sites B and C at very high levels may indicate

higher frequencies of nuclear condensation leading to cell death, which may be associated with the overall interactive effects of complex assortment of cytotoxic chemicals present in the industry waste.

In the *A. cepa* test system, micronucleus test is used to verify mutagenic effects of the exposed chemicals whereas occurrence of nuclear buds and binuclei indicate genotoxicity (Leme & Marin-Morales, 2009). As shown in Table 2, no micronuclei, nuclear buds and binuclei were detected in the root meristematic cells exposed to water from the reference site (Site R) or aged tap water in all sampling events. However, micronuclei were detected in the root meristems exposed to the water from Sites A, B, C, E and F in the sampling event carried out in the dry period (Table 2). In the rainy periods, micronuclei were detected only in the roots exposed to the water from Site B

Table 2: Mitotic indices, micronuclei, nuclear abnormalities in root meristem and root lengths of *A. cepa* exposed to water from different sites*

Sampling site	Mitotic index (%)	Micronuclei (‰)	Nuclear buds (‰)	Binuclei (‰)	Condensed nuclei (‰)	Root length (mm)
March 2015 (dry period)						
A - Maha Ela upstream	43.9 ± 5.5 ^{ab}	0.3 ± 0.3 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.0 ± 0.5 ^a	40 ± 7 ^{ab}
B - Effluent receiving canal	24.8 ± 1.8 ^c	1.6 ± 0.5 ^a	3.5 ± 0.5 ^b	1.0 ± 0.8 ^a	12.9 ± 1.1 ^b	18 ± 1 ^c
C - Canal/Maha Ela confluence	19.8 ± 7.2 ^c	1.6 ± 0.5 ^a	3.5 ± 0.7 ^b	1.0 ± 0.8 ^a	13.0 ± 1.8 ^b	29 ± 6 ^b
D - Maha Ela downstream	34.4 ± 5.7 ^{bc}	0.0 ± 0.0 ^a	0.3 ± 0.3 ^a	0.0 ± 0.0 ^a	5.4 ± 3.2 ^a	26 ± 2 ^b
E - Maha Ela/river confluence	38.1 ± 6.2 ^b	0.3 ± 0.3 ^a	0.0 ± 0.0 ^a	0.3 ± 0.3 ^a	5.3 ± 1.6 ^a	30 ± 3 ^b
F - River down reach	43.5 ± 3.5 ^{ab}	0.2 ± 0.2 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.4 ± 0.4 ^a	34 ± 3 ^b
R - River upper reach	53.1 ± 6.9 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.6 ± 0.6 ^a	38 ± 3 ^{ab}
Aged tap water	52.1 ± 4.2 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.4 ± 1.0 ^a	43 ± 6 ^a
July 2015 (wet period)						
A - Maha Ela upstream	41.9 ± 3.1 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.2 ± 0.2 ^a	2.0 ± 1.0 ^a	40 ± 8 ^{ab}
B - Effluent receiving canal	26.3 ± 1.8 ^c	1.2 ± 0.1 ^a	1.8 ± 1.8 ^a	0.4 ± 0.4 ^a	8.9 ± 1.2 ^b	12 ± 1 ^d
C - Canal/Maha Ela confluence	31.1 ± 1.6 ^c	0.0 ± 0.0 ^a	0.6 ± 0.4 ^a	0.2 ± 0.2 ^a	9.0 ± 2.0 ^b	19 ± 1 ^c
D - Maha Ela downstream	37.6 ± 1.3 ^b	0.0 ± 0.0 ^a	0.4 ± 0.4 ^a	0.0 ± 0.0 ^a	3.4 ± 1.3 ^a	18 ± 1 ^c
E - Maha Ela/river confluence	44.3 ± 1.5 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	4.3 ± 2.8 ^a	17 ± 1 ^c
F - River down reach	49.2 ± 6.4 ^{ab}	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.2 ± 0.2 ^a	1.4 ± 0.2 ^a	32 ± 1 ^b
R - River upper reach	53.5 ± 5.3 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.2 ± 0.2 ^a	30 ± 5 ^b
Aged tap water	52.4 ± 4.6 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.0 ± 1.0 ^a	48 ± 8 ^a
September 2015 (wet period)						
A - Maha Ela upstream	31.9 ± 2.6 ^{bc}	0.0 ± 0.0 ^a	0.2 ± 0.2 ^a	0.0 ± 0.0 ^a	1.0 ± 1.0 ^a	42 ± 5 ^{ab}
B - Effluent receiving canal	26.0 ± 2.0 ^c	0.2 ± 0.2 ^a	0.6 ± 0.4 ^a	0.2 ± 0.2 ^a	24.2 ± 5.9 ^c	27 ± 2 ^c
C - Canal/Maha Ela confluence	28.7 ± 1.8 ^c	0.0 ± 0.0 ^a	0.4 ± 4.0 ^a	0.2 ± 0.2 ^a	27.6 ± 8.2 ^c	29 ± 2 ^c
D - Maha Ela downstream	31.0 ± 2.8 ^c	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	12.7 ± 4.7 ^b	36 ± 3 ^b
E - Maha Ela/river confluence	27.5 ± 1.9 ^c	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	10.8 ± 3.6 ^b	32 ± 4 ^b
F - River down reach	41.4 ± 3.7 ^{ab}	0.0 ± 0.0 ^a	0.2 ± 0.2 ^a	0.0 ± 0.0 ^a	2.0 ± 1.5 ^a	31 ± 3 ^b
R - River upper reach	53.1 ± 5.2 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.0 ± 1.0 ^a	42 ± 4 ^{ab}
Aged tap water	54.6 ± 6.1 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.6 ± 0.6 ^a	47 ± 6 ^a

* Data are presented as mean ± SD (n = 5). Root length measurements were taken at 7 days and other parameters were taken at 2 days. In a column for a specific sampling event, data with different superscripts are significantly different from each other ($p < 0.05$).

(effluent receiving canal). Although nuclear buds and binuclei were consistently detected in the root meristems exposed to the water from Sites B and C in all sampling events, a statistically significant ($p < 0.05$) increase was found only for the occurrence of nuclear buds in the root meristem cells exposed to the water collected in the dry period (Table 2). As indicated by Heddle *et al.* (1991), micronuclei formation is induced by clastogenic substances that can make breaks and produce alterations in the chromosome structure as well as aneugenic substances, which can cause alterations in chromosome distribution during the cell division process giving rise to aneuploidies. The nuclear buds may have arisen as a result of the elimination of exceeding genetic material derived from the polyploidization process (Fernandes *et al.*, 2007). Although not statistically significant in most cases, numerical increase in the micronuclei and nuclear buds in the root tip cells compared to those exposed to the reference site water or aged tap water may indicate genotoxic/mutagenic contaminations in the water in the sampling sites, especially Manikagara Ela (Site B) and the canal confluence of Maha Ela (Site C).

A. cepa root growth inhibition is useful for evaluating general toxicity of chemicals (Leme & Marin-Morales, 2009). In the present study, a statistically significant decrease ($p < 0.05$) in the root lengths of the onion bulbs exposed to water from Sites B, C, D, E and F for seven days was found (Table 2) compared to those exposed to aged tap water in all three sampling events. In most cases the highest percentage root growth retardation after seven days was seen in the onion bulbs exposed to Site B water. The length of the roots exposed to the water in reference site water was significantly lower ($p < 0.01$) than the aged tap water in one sampling event (Table 2), indicating the potential toxicity associated with long-term exposure. Overall root growth retardation effect indicates toxic hazards of the waterways especially in the industrial wastewater receiving canal system after continuous exposure.

In the present study, a multivariate statistical assessment, Principal Component Analysis (PCA) of the measured water quality data and bioassay data in all sampling sites at each sampling event was conducted separately in order to obtain a small number of linear combinations of the variables, which account for most of the variability in the data. For the analysis of chemical parameters, Eigen values for the first and second principal components of the PCA were 9.13 and 0.81 which explained 76 % and 6.7 % of the variation, respectively. According to the score plot and the loading plot of the

chemical parameters (Figure 3), Site B (Menikagara Ela) and Site C (mixing zone of Menikagara Ela with Maha Ela) were clearly separated from the other sites on the principal component 1 (PC1) axis based on high levels of all the measured chemical parameters except dissolved oxygen. As most of these parameters are indicators of organic pollution (i.e. BOD, COD, oil and grease) and heavy metal pollution (Cd, Cr, Pb and Cu), the Sites B and C can be considered as highly polluted sites. Site R (reference site), Site F (Kelani River down reach) and Site A (Maha Ela upstream) were separated from the other sites based on relatively high dissolved oxygen levels and low values of other measured chemical

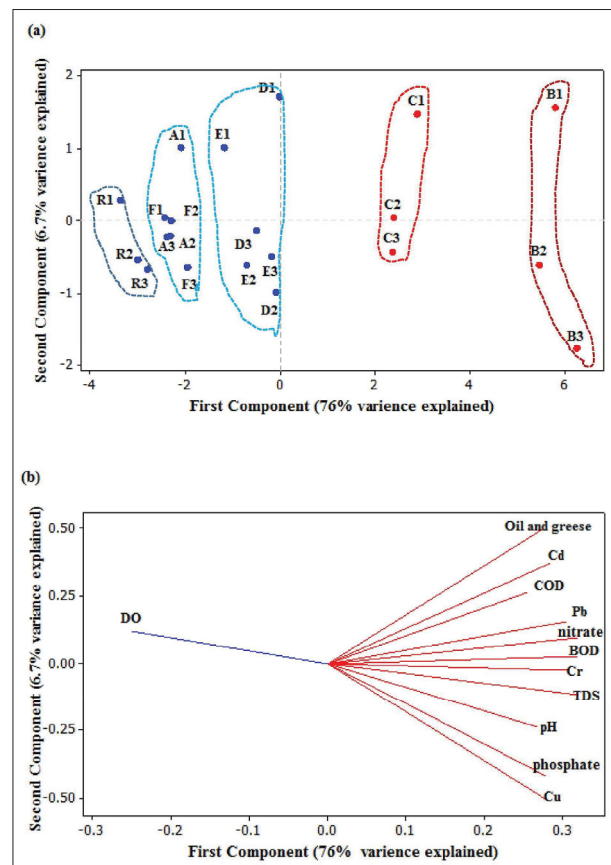


Figure 3: Score plot (a) and loading plot (b) of the principal component analysis of chemical parameters in the sampled water. A1-A3: Maha Ela upstream; B1-B3: Manikagara Ela; C1-C3: confluence of Manikagara Ela with Maha Ela D1-D3: Maha Ela downstream; E1-E3: confluence of Maha Ela with Kelani River; F1-F3: Kelani River down reach; R1-R3: Kelani River upper reach (reference site). A1-R1: samples taken in dry period; A2-R2 and A3-R3: samples taken in wet periods

parameters, which mainly included pollution indicative parameters. Therefore, Sites R, F and A can be considered as relatively less polluted sites compared to the Sites B and C. However, Sites D (Maha Ela downstream) and E (confluence of Maha Ela with Kelani River) showed a separation from these less polluted sites. Considering the pollution indicative parameters, Sites D and E may be considered as moderately polluted sites. In all Sites except Site F, the water quality in the sampling event carried out in the dry period was clearly separated from the water quality in the wet period sampling events on the two principal component axes (Figure 3). This may be partly associated with the water contaminant concentration effect in the dry periods and dilution effect in the rainy periods. For the PCA of bioassay parameters, Eigen values for the first and second principal components of the PCA were 3.82 and 0.91 which explained 63.5 % and 15.1 % of the variation, respectively. The PCA for bioassay parameters (Figure 4) revealed that relatively less polluted sites (Sites R, F and A) were clearly separated from the polluted sites mainly based on high mitotic indices and high root growth effects. In the PCA, a separate group was formed by all sampling events in Site D (downstream of Maha Ela) and the wet period sampling events in Site E (confluence of Maha Ela with Kelani River) but the dry period sampling event of Site E was clearly separated from this group (Figure 4). Highly polluted sites (Sites B and C) were clearly separated from the other sites and were dominated by increase in nuclear abnormalities. However, the dry period sampling event in Sites B and C were clearly separated from the wet period sampling events on the two principal components axes (Figure 4). This may indicate greater toxic hazards associated with the Site B, C and E waters in the dry periods, which may be partly associated with the contaminant concentration effects due to dry climate. A strong correlation (correlation coefficient = - 0.918, degrees of freedom =19, $p = 0.001$) was observed for the first components of the two PCA based on chemical parameters and the bioassay parameters indicating the strong negative association between ordination patterns of the first principal components of the pollution indicative parameters and biological effects. In the PCA, more polluted sites which includes Manikagara Ela (Site B) and mixing zone of Manikagara Ela and Maha Ela (Site C) were clearly separated from the other sites based on chemical characteristics (Figure 3) and bioassay end points in the *A. cepa* test system (Figure 4). Toxic hazards were found to be somewhat reduced towards the downstream in the watercourse, which may be attributed to self-depuration effects.

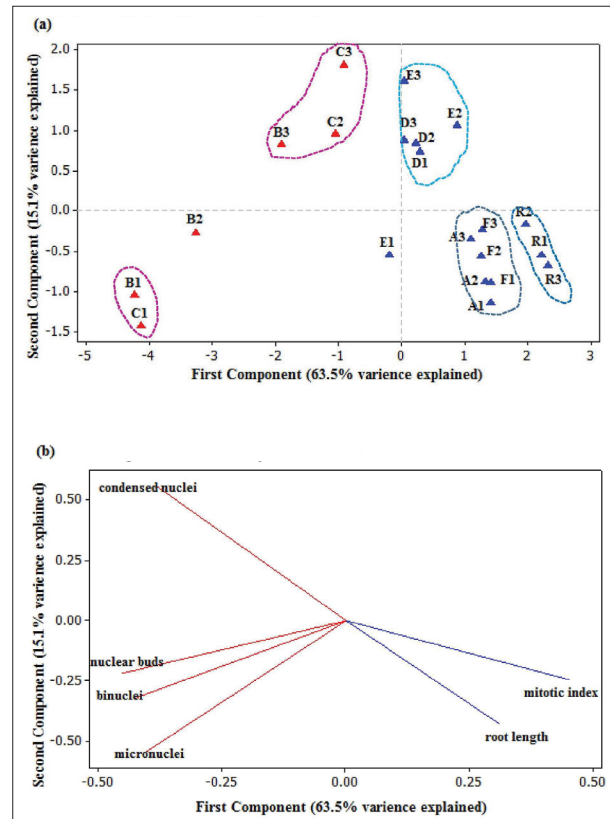


Figure 4: Score plot (a) and loading plot (b) of the principal component analysis of bioassay parameters in the roots of *A. cepa* bulbs exposed to the water of sampling sites. A1-A3: Maha Ela upstream; B1-B3: Manikagara Ela; C1-C3: confluence of Manikagara Ela with Maha Ela; D1-D3: Maha Ela downstream; E1-E3: confluence of Maha Ela with Kelani River; F1-F3: Kelani River down reach; R1-R3: Kelani River upper reach (reference site). A1-R1: samples taken in dry period; A2-R2 and A3-R3: samples taken in wet periods

In an integrated model simulation on Kelani River pollution carried out by Gunawardena *et al.* (2017), the estimated transfer coefficients revealed that industrial parks have a considerably higher impact across all identified zones in the river basin although these parks generally comply with existing effluent concentration standards. In a biomarker assessment study, Ruvinda and Pathiratne (2018) found neurotoxic, genotoxic and liver histopathological effects in the fish Nile tilapia, *Oreochromis niloticus* after short term laboratory exposure to the water samples from the confluence of Manikagara Ela and Maha Ela. In the present study, combined utility of surface water physico-chemical

analysis and *A. cepa* bioassay revealed that the quality of water in the site which receives effluents directly from a leading industrial zone (Menikagara Ela) and the site which receives industrial wastewater from the Menikagara Ela (Maha Ela), need improvements considering riverine ecosystem and human health. In line with the sustainable development goals (UNDP, 2016), it is important to review the existing national environmental regulations on industrial effluent discharge into inland waters in order to safeguard the natural riverine ecosystems and human health. In most developing countries including Sri Lanka, industrial effluent management regulations do not generally require effluent bioassays. Effluent bioassays are being used by jurisdictions in developed countries such as North America, the European Union, and Australia (Power & Boumphrey, 2004). Many jurisdictions include chemical hazard-based systems, effluent bioassays and receiving environment evaluations to predict or measure impacts to biological systems (Power & Boumphrey, 2004). It is prudent to introduce bioassay components to the national effluent discharge regulations in order to assess the human health and ecological health risks associated with the water bodies impacted by the industrial effluents. Further, when imposing tolerance limits for industrial effluent quality management, especially for the high polluting industries in Sri Lanka, more attention needs to be given on pollution assimilation capacities of the waste receiving water course in determining the safe release limits for industrial discharges.

CONCLUSIONS

Surface water quality characterisation with conventional analytical chemistry methods and toxicity assessments with the *A. cepa* bioassay revealed that the quality of water in Menikagara Ela which receives effluents directly from a leading industrial zone and the Maha Ela which receives wastewater from the Menikagara Ela need improvements considering the health of riverine ecosystem and human health. The most cytogenotoxic hazards were associated with the surface waters of Manikagara Ela. Toxic hazards were found to be somewhat reduced towards the downstream in the watercourse, which may be attributed to self-depuration effects. The importance of reviewing industrial effluent management regulations in Sri Lanka considering ecosystem and human safety is emphasised.

Acknowledgement

The authors thank the National Science Foundation of Sri Lanka for funding the atomic absorption spectrophotometer under the equipment grant RG/2011/EQ/16

and Mr. Harsha P. Weeraratna (Technical Officer) for technical assistance in the metal analysis.

REFERENCES

- Anonymous (2008). *The Gazette of the Democratic Socialist Republic of Sri Lanka (Extraordinary) 1534/18*: National Environmental (Protection and Quality) Regulations, No. 1 of 2008.
- American Public Health Association (APHA) (1998). Standard methods for the examination of water and wastewater, 20th edition. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington DC, USA.
- Andrade-Vieira L.F., de Campos J.M.S. & David L.C. (2012). Effects of spent pot liner on mitotic activity and nuclear DNA content in meristematic cells of *Allium cepa*. *Journal of Environmental Management* **107**: 140–146. DOI: <https://doi.org/10.1016/j.jenvman.2012.04.008>
- Barrenha P.I.I., Tanaka M.O., Hanai F.Y., Pantano G., Moraes G.H., Xavier C., Awan A.T., Grosseli G.M., Fadini P.S. & Mozeto A.A. (2018). Multivariate analyses of the effect of an urban wastewater treatment plant on spatial and temporal variation of water quality and nutrient distribution of a tropical mid-order river. *Environmental Monitoring and Assessment* **190**: 43. DOI: <https://doi.org/10.1007/s10661-017-6386-4>
- Board of Investment (BOI) (2011). *Environmental Norms*. Board of Investment, Sri Lanka. Available at: http://www.investsri.lanka.com/images/publications/pdf/environmental_norms.pdf, Accessed 11 May 2017.
- Claxton L.D., Houk V.S. & Hughes T.J. (1998). Mini review-genotoxicity of industrial wastes and effluents. *Mutation Research* **410**: 237–243.
- Fernandes T.C.C., Mazzeo D.E.C. & Marin-Morales M.A. (2007). Mechanism of micronuclei formation in polyploidized cells of *Allium cepa* exposed to trifluralin herbicide. *Pesticide Biochemistry and Physiology* **88**: 252–259. DOI: <https://doi.org/10.1016/j.pestbp.2006.12.003>
- Fiskesjo G. (1985). The allium test as a standard in environmental monitoring. *Heredity* **102**: 99–112. DOI: <https://doi.org/10.1111/j.1601-5223.1985.tb00471.x>
- Gunawardena A.E.M.S., Wijeratne B.W., Atakelty H. & Pandit R. (2017). Industrial pollution and the management of river water quality: a model of Kelani River, Sri Lanka. *Environmental Monitoring and Assessment* **189**: 457. DOI: <https://doi.org/10.1007/s10661-017-6172-3>
- Heddle J.A., Cimino M.C., Hayashi M., Romagna F., Shelby M.D., Tucker J.D., Vanparys P. & MacGregor J.J. (1991). Micronuclei as an index of cytogenic damage: past, present and future. *Environment and Molecular Mutagenesis* **18**: 277–291. DOI: <https://doi.org/10.1002/em.2850180414>
- Hemachandra C.K. & Pathiratne A. (2016). Combination of physico-chemical analysis, *Allium cepa* test system and *Oreochromis niloticus* erythrocyte based comet

- assay/nuclear abnormalities tests for cyto-genotoxicity assessments of treated effluents discharged from textile industries. *Ecotoxicology and Environmental Safety* **131**: 54–64.
DOI: <https://doi.org/10.1016/j.ecoenv.2016.05.010>
- Hemachandra C.K. & Pathiratne A. (2017a). Bioassessment of the effluents discharged from two export oriented industrial zones located in Kelani River basin, Sri Lanka using erythrocytic responses of the fish, Nile Tilapia (*Oreochromis niloticus*). *Bulletin of Environmental Contamination and Toxicology* **99**: 481–487.
DOI: <https://doi.org/10.1007/s00128-017-2156-9>
- Hemachandra C.K. & Pathiratne A. (2017b). Cytogenotoxicity screening of source water, wastewater and treated water of drinking water treatment plants using two *in vivo* test systems: *Allium cepa* root based and Nile tilapia erythrocyte based tests. *Water Research* **108**: 320–329.
DOI: <https://doi.org/10.1016/j.watres.2016.11.009>
- Hemachandra C.K. & Pathiratne A. (2018). Assessing toxicity of two industrial zone effluents reaching Kelani River, Sri Lanka. *Journal of the National Science Foundation of Sri Lanka* **46**(4): 538–546.
DOI: <https://doi.org/10.4038/jnsfsr.v46i4.8629>
- Ileperuma O.A. (2000). Environmental pollution in Sri Lanka: a review. *Journal of the National Science Foundation of Sri Lanka* **28**: 301–325.
DOI: <https://doi.org/10.4038/jnsfsr.v28i4.2644>
- Kannangara D.N.M. & Pathiratne A. (2015). Toxicity assessment of industrial wastewaters reaching Dandugan Oya, Sri Lanka using a plant based bioassay. *Journal of the National Science Foundation of Sri Lanka* **43**(1): 153–164.
DOI: <https://doi.org/10.4038/jnsfsr.v43i1.7943>
- Kara G.T., Kara M., Bayram A. & Gündüz O. (2017). Assessment of seasonal and spatial variations of physicochemical parameters and trace elements along a heavily polluted effluent-dominated stream. *Environmental Monitoring and Assessment* **189**: 585.
DOI: <https://doi.org/10.1007/s10661-017-6309-4>
- Leme D.M. & Marin-Morales M.A. (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research* **682**: 1–81.
DOI: <https://doi.org/10.1016/j.mrrev.2009.06.002>
- Mahagamage M.G.Y.L. & Manage P.M. (2014). Water quality index (CCME-WQI) based assessment study of water quality in Kelani River basin, Sri Lanka. *International Journal of Environment and Natural Resources* **1**: 199–204.
- Mahagamage M.G.Y.L., Chinthaka S.D.M. & Manage P.M. (2016). Assessment of water quality index for the groundwater in Kelani river basin, Sri Lanka. *International Journal of Agriculture and Environmental Research* **5**: 1158–1171.
- Mallawatantri A., Rodrigo A. & De Silva K. (2016). Medium to long-term multi stakeholder strategy and action plan for management and conservation of Kelani river basin. Central Environment Authority and International Union for the Conservation of Nature Sri Lanka Country Office, Colombo.
- Pathiratne A., Hemachandra C.K. & De Silva N. (2015). Efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. *Environmental Monitoring and Assessment* **187**(12): 730.
DOI: <https://doi.org/10.1007/s10661-015-4954-z>
- Power E.A. & Boumphrey R.S. (2004). International trends in bioassay use for effluent management. *Ecotoxicology* **13**: 377–398.
DOI: <https://doi.org/10.1023/B:ECTX.0000035290.89590.03>
- Ruvinda K.M.S. & Pathiratne A. (2018). Biomarker responses of Nile Tilapia (*Oreochromis niloticus*) exposed to polluted water from Kelani river basin, Sri Lanka: implications for biomonitoring river pollution. *Sri Lanka Journal of Aquatic Sciences* **23**(1): 105–117.
DOI: <https://doi.org/10.4038/sljias.v23i1.7551>
- Scott A. & Clarke R. (2000). Multivariate techniques. In: *Statistics in Ecotoxicology* (ed. T. Sparks), pp.150–178. John Wiley and Sons Ltd., New York, USA.
- Silva E.I.L. (1996). *Water Quality in Sri Lanka-A Review on Twelve Water Bodies, Kandy*. Institute of Fundamental Studies, Kandy.
- Sri Lanka Standards Institution (SLSI) (2013). *Drinking Water Standard - First Revision, Sri Lanka Standards for Potable Water- SLS 614: 2013*.
- United Nations Development Programme (UNDP) (2016). *Sustainable Development Goals*. Available at: <http://www.undp.org/content/undp/en/home/sustainable-development-goals.html>, Accessed 28 May 2018.
- Vasquez M.I. & Fatta-Kassinos D. (2013). Is the evaluation of “traditional” physicochemical parameters sufficient to explain the potential toxicity of the treated wastewater at sewage treatment plants? *Environment Science and Pollution Research* **20**: 3516–3528.
DOI: <https://doi.org/10.1007/s11356-013-1637-6>
- Wieczerek M., Namieśnik J. & Kudlak B. (2016). Bioassays as one of the green chemistry tools for assessing environmental quality: A review. *Environment International* **94**: 341–361.
DOI: <https://doi.org/10.1016/j.envint.2016.05.017>
- Wu Z., Wang X., Chen Y., Yongjiu C. & Deng J. (2018). Assessing river water quality using water quality index in Lake Taihu Basin, China. *Science of the Total Environment* **612**: 914–922.
DOI: <https://doi.org/10.1016/j.scitotenv.2017.08.293>
- Yildiz M., Cigerci I.H., Konuk M., Fidan A.F. & Terzi H. (2009). Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. *Chemosphere* **75**: 934–938.
DOI: <https://doi.org/10.1016/j.chemosphere.2009.01.023>
- Zar J.H. (1998). *Biostatistical Analysis*, 4th edition. Prentice Hall, New Jersey, USA.