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RESEARCH ARTICLE

Assessing toxicity of two industrial zone effluents reaching Kelani River, Sri Lanka

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Abstract: Evaluation of the efficacy of waste treatment technologies of industrial waste is a major challenge for sustainable industrial development world-wide. Hence, new strategies are needed to assess interactive toxic effects of all substances present in the treated waste. This study assessed potential toxic hazards of treated effluents discharged from common wastewater treatment plants of two industrial zones located in the Kelani River basin using *Allium cepa* (common onion) test system. The results showed that the final effluents of both industrial zones under undiluted and diluted (1:8 v/v) conditions induced cytotoxicity on all occasions, with evidence of significant ($p < 0.05$) mito-depression in the root meristem and retardation of root growth in *A. cepa*. Genotoxic hazard of the effluents was evident by frequent increase of nuclear and chromosomal abnormalities, and occasional development of micronuclei in the root meristem. Dilution of the effluents to 1:8 reduced the genotoxic effects generated in *A. cepa* roots by the final effluents. The results revealed that waste treatment technologies in these two industrial zones need to be upgraded in order to eliminate cytotoxic and genotoxic hazards associated with the treated effluents. The results highlight the importance of incorporating practically feasible bioanalytical tools such as *A. cepa* root based test system on a regular basis for evaluating the efficacy of waste treatment technologies.

Keywords: *Allium*, industrial zone effluent, Kelani River, toxic hazard identification.

INTRODUCTION

Evaluating the effectiveness of waste treatment technologies is a major challenge for sustainable industrial development world-wide. Different types of anthropogenic chemicals including the 'contaminants of emerging concern' are being detected in treated wastewaters globally and there is increasing evidence of adverse environmental effects related to wastewater treatment plant discharges (Prasse *et al.*, 2015). Conventional effluent monitoring approach using a selected set of physico-chemical parameters is unable to detect all potentially hazardous substances present in complex industrial wastewater samples including 'transformation products' that can be formed during wastewater treatments (Vasquez & Fatta-Kassinos, 2013; Prasse *et al.*, 2015; Papa *et al*., 2016). In view of sustainable industrial development, new strategies are needed to assess interactive toxic effects of all substances present in the industrial waste even after the final treatments.

For regulatory purposes, tolerance limits have been established for a set of physico-chemical and microbiological parameters (Anonymous, 2008) of industrial effluents discharged into inland surface waters of Sri Lanka (BOI, 2011). However, no attention has yet been given in the national regulations for assessing toxic hazards of these industrial effluents using bioanalytical tools. Among the major rivers in Sri Lanka, Kelani River is considered as the largest recipient of industrial waste (Ileperuma, 2000; Mahagamage *et al*., 2016). The river serves as the habitat for diverse flora and fauna (Silva, 1996) and also as a main drinking water source to general public in the area (Mahagamage & Manage, 2014). Effluents generated from common wastewater treatment

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plants (CWWTPs) of export processing zones/industrial parks may contain complex chemical mixtures which may pose cytotoxic and genotoxic hazards to biota under chronic exposure. However, conventional monitoring methods are not effective in identifying toxic effects associated with the effluents originated from diverse industrial processes in the industrial zones which are being discharged into riverine ecosystems (Hemachandra & Pathiratne, 2017a). Hence, incorporation of sensitive and practically feasible bioanalytical tools are needed for the identification of cytotoxic and genotoxic hazards associated with these effluents.

Allium cepa (common onion) test system is a simple, sensitive and economical tool for rapid screening of general toxicity, cytotoxicity and genotoxicity of environmental chemicals (Leme & Marin-Morales, 2009; Hemachandra & Pathiratne, 2015). Recently *A. cepa* root based test system was used to assess the toxicity of textile and tanning industry wastewaters reaching the Dandugan Oya (Kannangara & Pathiratne, 2015) and cytogenotoxicity of textile industry effluents and drinking water treatment plant effluents discharged into the Kelani River (Hemachandra & Pathiratne 2016; 2017b). The objective of the present study was to use the *A. cepa* root based test system for assessing the potential cytotoxic and genotoxic hazards posed by treated CWWTP effluents generated from two export promotion industrial zones/parks located in the Kelani River basin, Sri Lanka.

METHODOLOGY

Sources of CWWTP effluents

The effluents originating from CWWTPs in two selected export promotion industrial zones (INZ1 and INZ2) which discharge their treated effluents continuously in high volumes into the Kelani River, Sri Lanka were selected. The CWWTP of the INZ1 discharges the treated effluents into a tributary of the Kelani River whereas the CWWTP of the INZ2 discharges the effluents into a canal which eventually confluence with the Kelani River. In the INZ1, textile/apparel industries, pharmaceutical industries, and rubber product industries are the main effluent generating industries. In the INZ2, effluent generating industries include textile/apparel industries, rubber glove manufacturing industries and food processing industries. The effluents generated by these specific industries are expected to be treated in their in-house treatment systems prior to discharge into the common sewer line leading to the respective CWWTP (BOI, 2011). In the respective CWWTP, wastewater is subjected to biological treatment. The final effluents from CWWTPs are expected to conform to the established specific national tolerance limits for discharge of industrial wastewaters to inland water resources (Anonymous, 2008; BOI, 2011). The final treated effluents from the two CWWTPs are discharged into canals/tributaries of the Kelani River where several water extraction points are located along the water course for provision of public drinking water supplies to several townships and suburbs in the area.

Effluent collection

Effluent samples from CWWTPs of both industrial zones were collected in the year 2013 from the respective outfalls (industrial effluent INZ1: 6º 58′ 2.476″ N; 80º 12′ 43.274″ E, industrial effluent INZ2: 6º 57' 18.59" N; 79º 58' 8.18" E) to polyethylene bags separately in two sampling events covering the dry season (in February and July) and one sampling event in the rainy season (in November). In each sampling period, selected physico-chemical parameters *viz*. temperature, pH, conductivity, salinity, total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), spectral absorption coefficients for colour, cadmium, chromium, copper, lead and zinc in the composite effluent samples were measured using standard analytical methods. Physico-chemical characteristics of the effluents collected in these sampling periods have been reported elsewhere (Hemachandra & Pathiratne, 2017a). For toxicity assessments, effluent samples were transported to the laboratory under chilled condition.

Toxicity assessments

Effluent exposure tests were started on the day of collection using a commercially available local variety of *A. cepa* bulbs adopting the previously described procedures (Pathiratne *et al*., 2015; Hemachandra & Pathiratne, 2016). Healthy onion bulbs (6 – 8 g in weight and 1.7 – 2.3 cm in diameter) were placed in glass test tubes filled with the respective exposure media (n = 10). For each CWWTP effluent collected from one sampling period, undiluted effluent and the diluted effluent (1:8 v/v dilution with aged tap water) were tested along with the control (aged tap water). The test tubes were kept in the dark at 25 – 27 ºC to protect the bulbs from direct sunlight. The exposure media were renewed daily. After 48 h of exposure, 10 root tips from each onion bulb from five randomly selected onion bulbs from each exposure condition including the controls were processed for microscopic studies as described by

Hemachandra and Pathiratne (2015). Briefly, the stained slides prepared from the root tips of the respective onion bulb were coded randomly and examined blindly under the light microscope. Microscopic analysis (× 400 magnification) in each slide included scoring of the number of cells undergoing mitotic stages in at least 1000 root tip cells (mitotic index test), micronuclei and nuclear abnormalities in at least 1000 interphase cells (micronuclei and nuclear abnormalities tests) and chromosomal aberrations in at least 100 mitoses excluding prophasic cells (chromosomal abnormalities test). The mitotic index was calculated as the percentage of cells undergoing mitosis in comparison to the total cells examined per each onion bulb. Frequencies of micronuclei and different types of nuclear abnormalities were estimated with respect to 1000 interphase cells per onion bulb. Different types of chromosomal abnormalities were categorised based on the descriptions given earlier (Leme & Marin-Morales 2009; Pathiratne *et al*., 2015) and presented as numbers of the specific type and total abnormalities in 100 mitoses per onion bulb. A separate set of *A. cepa* bulbs was concurrently exposed to a known mutagen, ethyl methane sulphonate (EMS, purity ≥ 98 %, Sigma-Aldrich) at 10 mgL^{-1} as the positive control in the third sampling event following the same procedures. For evaluation of root growth responses (root growth inhibition test), a set of onion bulbs (n =10 per exposure medium) was submerged in exposure media in separate glass tanks (undiluted effluents, 1:8 diluted effluents and aged tap water control) continuously for 7 ds in the dark at 25 – 27 ºC with daily renewal of exposure media. For each onion bulb, the lengths of the whole root bundle were measured after 7 ds of exposure for calculation of mean root length (Fiskesjo, 1985). Morphological abnormalities (if any) of the roots were also recorded.

Statistical analysis

Data were compared using one-way analysis of variance (ANOVA) followed by Tukey's pair wise comparison test ($p \leq 0.05$) after testing the assumptions of the ANOVA. The data with respect to root growth inhibition test were analysed without data transformation. The data with proportions were subjected to arcsine-square root transformations before analysis (Zar, 1998).

RESULTS AND DISCUSSION

In this study, potential toxic hazards associated with the CWWTP effluents originating from two export promoting industrial zones located in the Kelani River basin were assessed for the first time covering different climatic periods using the plant, *A. cepa* root-based test system.

Normal mitotic phases and cytogenetic abnormalities observed in the root meristem of *A. cepa* bulbs after 48 hr exposure to the test media are shown in Figure 1. Observed chromosomal abnormalities in the root meristematic cells were C-metaphases and chromosomal breaks, chromosomal bridges, vagrant chromosomes and polar slips in the anaphase. Observed abnormalities associated with the nucleus were micronuclei, condensed nuclei, nuclear buds and binuclei. The root meristem of *A. cepa* bulbs exposed to the positive control (10 mgL^{-1} EMS) showed suppressed mitotic indices and condensed nuclei induction ($p < 0.05$) in comparison with the control (Table 1). Exposure of *A. cepa* bulbs to CWWTP effluents collected from the two industrial zones for 48 hrs showed significant reduction of mitotic indices in the root meristem under undiluted and 1:8 diluted conditions compared with the respective controls in all sampling events. Among the nuclear abnormalities seen, condensed nuclei occurrence was the most prominent in all cases. The root meristems exposed to undiluted and diluted effluents from both industrial zones showed significantly higher occurrence of condensed nuclei in all sampling events compared to the controls. In the

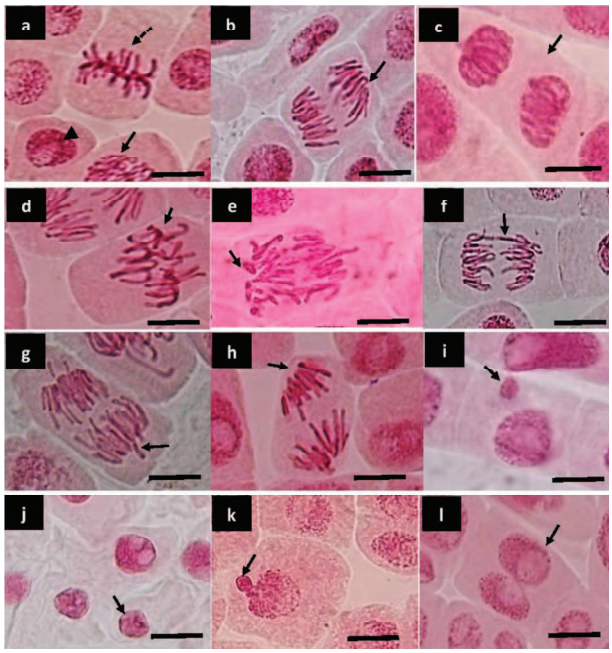

Figure 1: *A. cepa* root meristem showing normal cells (a: interphase -arrow head, prophase-arrow, metaphase-broken arrow; b: anaphase; c: telophase), chromosomal abnormalities (d: C-metaphase; e: chromosomal breaks; f: chromosomal bridge; g: vagrant chromosomes; h: polar slip), micronucleated cell (i: micronucleus-arrow) and nuclear abnormalities (j: condensed nucleus; k: nuclear bud; l: binuclei). Scale bar represents 15 µm

A. cepa root-based test system, mitotic index suppression in the root meristem indicates cytotoxicity of chemicals (Leme & Marin-Morales 2009; Hemachandra & Pathiratne, 2016). Significant suppression of mitotic indices in the root meristem of *A. cepa* bulbs exposed to the CWWTP effluents in all cases indicates blockade of mitotic cell division process due to interactive effects of complex mixture of chemicals present in the effluents. The mito-depressive effect could be associated with the cell death process as indicated by induction of condensed nuclei in the root cells exposed to the CWWTP effluents. Andrade-Vieira *et al*. (2012) attributed increased frequency of condensed nuclei in meristematic cells to nuclear chromatin condensation, which is considered as one of the apoptotic markers following stress conditions. In *A. cepa* test system, induction of chromosomal abnormalities and nuclear abnormalities in root meristem indicates genotoxic effects promoted by environmental pollutants whereas the micronucleus test verifies mutagenic effects of the exposed chemicals (Leme & Marin-Morales, 2009). Micronuclei evolution was observed in the root meristem of *A. cepa* following exposure to CWWTP effluents from both industrial zones in both sampling events (Table 1). Micronuclei arise from acentric fragments or whole chromosomes that were not incorporated into the main nucleus during the cell division cycle (Heddle *et al.*, 1991). The frequency of nuclear buds and binuclei in the meristem also increased due to the CWWTP effluent exposure especially, under undiluted condition (Table 1). Nuclear buds may arise due to the removal of exceeding genetic material derived from the polyploidisation process (Fernandes *et al*., 2007). Binuclei may result due to the obstruction of normal cytokinesis process. Statistically significant occurrence of total nuclear abnormalities (with respect to nuclear buds and binuclei) was found

Table 1: Mitotic indices (MI) and occurrence of different nuclear abnormalities in root meristem of *A. cepa* following 48 hour continuous exposure to final CWWTP effluents from selected industrial zones (INZ1 and INZ2)*

Exposure		MI (%)	Condensed nuclei (‰)	Micronuclei (‰)	Nuclear buds and binuclei (‰)
First sampling (Feb 13 2013)					
Control (dilution water)		49.5 ± 2.6^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
INZ1 effluent	1:8 dilution	29.7 ± 1.2^{b} (40)	19.5 ± 6.4^{b}	1.7 ± 0.7^{a}	4.3 ± 1.7^{ab}
	undiluted	25.3 ± 1.3^{b} (49)	30.1 ± 11.2^{b}	4.1 ± 1.9^{a}	8.5 ± 1.1^{b}
INZ2 effluent	1:8 dilution	27.6 ± 1.5^{b} (44)	17.6 ± 6.9^{b}	1.4 ± 0.6^{a}	3.4 ± 1.2^{ab}
	undiluted	26.2 ± 2.1^{b} (47)	60.5 ± 12.4^{c}	3.8 ± 1.2^{a}	11.6 ± 2.3^{b}
Second sampling (July 24 2013)					
Control (dilution water)		53.0 ± 2.5^{a}	0.3 ± 0.1^{a}	0 ± 0^{a}	0 ± 0^{a}
INZ1 effluent	1:8 dilution	36.6 ± 2.9^{b} (31)	8.3 ± 3.5^{b}	0 ± 0^{a}	0.9 ± 0.6^{a}
	undiluted	23.9 ± 3.2^{c} (55)	11.2 ± 3.3^{b}	0 ± 0^{a}	1.9 ± 0.3^{a}
INZ2 effluent	1:8 dilution	25.1 ± 0.8^{c} (53)	7.3 ± 2.5^{b}	0 ± 0^{a}	0.8 ± 0.3^{a}
	undiluted	18.1 ± 0.5^{c} (66)	24.3 ± 6.6^{c}	0 ± 0^{a}	1.8 ± 0.4^{a}
Third sampling (Nov 20 2013)					
Control (dilution water)		46.8 ± 1.2^{a}	0 ± 0^{a}	0 ± 0^{a}	0.4 ± 0.4^{a}
INZ1 effluent	1:8 dilution	31 ± 2.9^{bc} (34)	7.7 ± 1.0^{b}	0.9 ± 0.6^{a}	2.6 ± 0.3^{ab}
	undiluted	30.9 ± 1.7^{bc} (34)	10.3 ± 0.3^{b}	0.9 ± 0.3^{a}	5.5 ± 1.6^{b}
INZ2 effluent	1:8 dilution	35.7 ± 1.2^{b} (24)	9.4 ± 2.0^{b}	0.7 ± 0.4^{a}	2.3 ± 1.1^{ab}
	undiluted	27.2 ± 1.4^{c} (42)	22.6 ± 7.2^{c}	1.9 ± 1.3^{a}	6.1 ± 0.7^{b}
Control (negative control)		50.6 ± 3.1^{a}	0 ± 0^{a}	0.1 ± 0.1^{a}	0.1 ± 0.1^{a}
EMS 10 mgL^{-1} (positive control)		22.2 ± 4.4^{b} (56)	32.2 ± 5.7^{c}	6.8 ± 2.7^{b}	6.8 ± 2.7^{b}

*Data are presented as mean ± SEM (n = 5 onion bulbs for each exposure medium). For a specific sampling event, data in a column with different superscript letters are significantly different from each other ($p < 0.05$). For MI, the number in parentheses indicate percentage mitotic suppression compared to the respective control.

in the root meristems exposed to the undiluted effluents from both industrial zones in both sampling events and the positive control compared to the dilution water controls (Table 1). Although not statistically significant in some cases, effluent induced numerical increase in the micronuclei and total nuclear abnormalities in the root meristem compared to those in dilution water may indicate genotoxic/mutagenic contaminations in the CWWTP effluents. However, with the 1:8 dilution of the effluent, frequency of these nuclear abnormalities is reduced to some extent in comparison to the undiluted effluents.

Table 2: Occurrence of chromosomal abnormalities in root meristem of *A. cepa* following 48 hour continuous exposure to CWWTP effluents from selected industrial zones (INZ1 and INZ2)*

Exposure		Chromosomal abnormalities (%)					
		Breaks	Bridges	C-metaphase	Vagrant chromosomes	Polar slip	Total**
First sampling (Feb 13 2013)							
Control (dilution water)		0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0.7	1.3 ± 0.7^{a}
INZ1 effluent	1:8 dilution	0 ± 0	0.7 ± 0.4	1.0 ± 0.7	1.5 ± 0.8	0.4 ± 0.4	3.6 ± 0.2^{ab}
	undiluted	0.5 ± 0.3	2.7 ± 0.9	0.4 ± 0.4	4.6 ± 1.4	1.9 ± 1.6	10.1 ± 2.9^{b}
INZ2 effluent	1:8 dilution	0 ± 0	0.6 ± 0.6	0.9 ± 0.6	1.2 ± 1.2	0.6 ± 0.6	3.3 ± 2.3^{ab}
	undiluted	0 ± 0	4.4 ± 0.8	0.5 ± 0.5	2.7 ± 1.4	2.4 ± 1.3	10.1 ± 1.6^{b}
Second sampling (July 24 2013)							
Control (dilution water)		0 ± 0	1.2 ± 0.7	0.3 ± 0.3	0 ± 0	0.4 ± 0.3	1.9 ± 1.0^{a}
INZ1 effluent	1:8 dilution	0 ± 0	2.4 ± 0.7	0 ± 0	0 ± 0	0.6 ± 0.2	3.0 ± 0.8^{ab}
	undiluted	1.3 ± 0.5	3.9 ± 0.6	0.3 ± 0.3	1.3 ± 0.5	1.4 ± 0.7	8.3 ± 2.1^{b}
INZ2 effluent	1:8 dilution	0 ± 0	2.6 ± 0.4	0.2 ± 0.2	0 ± 0	0.6 ± 0.4	3.4 ± 0.8^{ab}
	undiluted	2.5 ± 1.1	2.2 ± 0.6	0.8 ± 0.5	2.5 ± 1.1	1.2 ± 0.6	9.2 ± 2.1^{b}
Third sampling (Nov 20 2013)							
Control (dilution water)		0 ± 0	1.4 ± 0.6	0 ± 0	0 ± 0	0.6 ± 0.3	2.1 ± 0.5^{a}
INZ1 effluent	1:8 dilution	0 ± 0	1.9 ± 0.7	1.0 ± 0.5	0.4 ± 0.4	1.2 ± 0.6	4.6 ± 1.5^{ab}
	undiluted	0 ± 0	3.2 ± 0.7	0.5 ± 0.3	4.2 ± 0.2	0.7 ± 0.4	8.6 ± 0.7^{b}
INZ2 effluent	1:8 dilution	0 ± 0	2.5 ± 0.7	1.0 ± 0.5	0.8 ± 0.4	0.9 ± 0.4	5.2 ± 1.2^{ab}
	undiluted	0 ± 0	2.6 ± 0.6	0 ± 0	4.0 ± 0.1	1.1 ± 0.3	7.8 ± 0.5^{b}
Control (negative control)		0 ± 0	0 ± 0	0.0 ± 0.0	0.2 ± 1.9	1.1 ± 0.7	1.3 ± 2.1^{a}
EMS 10 mgL^{-1} (positive control)		0.5 ± 0.5	1.7 ± 1.2	0.5 ± 0.5	16.6 ± 3.0	1.5 ± 1.0	20.9 ± 3.6^{c}

*Data are presented as mean ± SEM (n = 5 onion bulbs for each exposure medium). For a specific sampling event, data in a column with different superscript letters are significantly different from each other ($p < 0.05$).

Specific and total chromosomal abnormalities observed in the root meristems are presented in Table 2. Chromosomal breaks were seen only in the root meristems exposed to undiluted effluents in both sampling events and the positive control. Chromosomal bridges in the root meristems appear to increase in effluent-exposed onion bulbs. Chromosome breaks and bridges are indicators of clastogenic action, which reflect the alterations in chromosomal structure due to direct effect of the effluents on the chromosomes. C-metaphases, vagrant chromosomes and polar slips are indicators of an eugenic action which reflects the alteration of the chromosomal distribution due to the effect on microtubule assembly in the dividing cells (Leme & Marin-Morales 2009; Hemachandra & Pathiratne, 2016). Total chromosomal abnormalities in

the meristematic cells exposed to undiluted CWWTP effluents were significantly higher than in the respective controls on all occasions (Table 2) indicating genotoxic hazards. However, with the effluent dilution (1:8), the occurrence of chromosomal abnormalities is reduced but not eliminated completely.

In the *A. cepa* root based test system, root growth inhibition indicates general toxicity of chemicals (Leme & Marin-Morales, 2009). After seven days continuous exposure, *A. cepa* bulbs exposed to the undiluted effluents collected from the two industrial zones showed fragile, thick roots with brown root tips in comparison with the thin, flexible white coloured roots of the onion bulbs exposed to the control media. Significant reduction of root growth in comparison to the controls was found after seven days of continuous exposure of the onion bulbs to the effluents both under undiluted and diluted conditions except for the bulbs exposed to the 1:8 diluted INZ1 effluent in the third sampling event (Table 3).

Table 3: Root growth of *A. cepa* bulbs following seven days continuous exposure to CWWTP effluents from selected industrial zones (INZ1 and INZ2)*

Exposure		Root growth (cm)	Root growth inhibition (%)
First sampling (Feb 13 2013)			
Control (dilution water)		10.6 ± 0.1[a]	-
INZ1 effluent	1:8 dilution	7.6 ± 0.1[b]	28
	undiluted	4.6 ± 0.1[d]	57
INZ2 effluent	1:8 dilution	6.3 ± 0.1[c]	41
	undiluted	3.3 ± 0.1[e]	69
Second sampling (July 24 2013)			
Control (dilution water)		8.2 ± 0.2[a]	-
INZ1 effluent	1:8 dilution	6.9 ± 0.2[c]	16
	undiluted	2.2 ± 0.1[e]	73
INZ2 effluent	1:8 dilution	4.2 ± 0.2[d]	49
	undiluted	2.1 ± 0.1[f]	74
Third sampling (Nov 20 2013)			
Control (dilution water)		12.1 ± 0.1[a]	-
INZ1 effluent	1:8 dilution	12.8 ± 0.1[a]	-
	undiluted	4.3 ± 0.1[c]	64
INZ2 effluent	1:8 dilution	8.1 ± 0.1[b]	33
	undiluted	3.5 ± 0.1[d]	71

* Data are presented as mean ± SEM (n = 10 bulbs for each exposure medium). For a specific sampling event, the data in a column with different superscript letters are significantly different from each other ($p < 0.05$).

The percentage root growth retardation (estimated as percentage differences in mean root lengths of the onion bulbs exposed to a specific test medium and the mean root length of the respective controls) in the onion bulbs exposed to the undiluted effluents from INZ1 and INZ2 ranged from 57 – 73 % and 69 – 74 %, respectively. The root growth retardation of the effluent-exposed *A. cepa* bulbs may be associated with the disturbance of mitotic process and root elongation process induced by complex mixtures of cytotoxic chemicals present in these effluents.

The chemical composition of the CWWTP effluents generated by the industrial zones during different sampling seasons can be subjected to continuous variation based on the type and amount of raw materials used, their respective industrial activities, waste treatment methods and the concentration or dilution effect of the wastewaters in the dry and rainy seasons. Hence, the toxicity associated with these complex effluents may fluctuate due to interactive effects of their chemical components. CWWTP effluents assessed

in the present study contained moderate levels of lead (all occasions in both INZs), COD (July 2013 in both INZs) and colour (all occasions in INZ1 February and July in INZ2), which exceeded the respective national tolerance limits (Hemachandra & Pathiratne, 2017a). Lead induced cytotoxicity and genotoxicity in *A. cepa* roots after short exposure to environmentally relevant concentrations have been reported (Kaur *et al.*, 2014). Rather than individual chemical effects, cytotoxic and genotoxic responses observed in the CWWTP effluents could be due to overall interactive effects of all the chemicals including the transformation products in the complex mixture. Consequences of continuous genotoxic stress posed by the CWWTP effluents may eventually affect the health of native flora and fauna in the receiving watercourse. A study carried out concurrently with the model test fish, Nile tilapia also revealed induction of erythrocytic abnormalities and DNA damage responses following CWWTP effluent exposure (Hemachandra & Pathiratne, 2017a). Sri Lankan regulatory limits for industrial effluents discharged into surface waters are based on expected dilution of the effluents to at least 1:8 in the clean receiving waters (Anonymous, 2008). Although genotoxicity was somewhat reduced, cytotoxic responses were frequently detected in *A. cepa* exposed to the expected 1:8 diluted conditions of the CWWTP effluents. Hence, 1:8 dilutions of these CWWTP effluents might not be sufficient to restrict the cytotoxic impacts on the native organisms living in the effluent receiving watercourse.

CONCLUSION

A. cepa root-based test system demonstrated that the CWWTP effluents sampled on all occasions from both industrial zones have a potential to induce cytotoxicity as indicated by the mito-depressive and root growth retardation effects. The genotoxic hazard of the effluents was evident by frequent elevation of nuclear and chromosomal abnormalities and occasional evolution of micronuclei in the root meristem. Dilution of the effluents to 1:8 had reduced but not completely eliminated the genotoxic effects generated in *A. cepa* roots by the final effluents. The results demonstrate that waste treatment technologies in these industrial zones need upgrading in order to eliminate cytotoxic and genotoxic hazards associated with the final effluents to native biota in the Kelani River basin. In view of sustainable development goals, focusing on human and ecosystem safety, the results highlight the importance of incorporating practically feasible bioanalytical tools such as *A. cepa* root-based test system on regular basis for evaluating the efficacy of waste treatment technologies in the industrial zones in reducing the biological hazards of the final effluents discharged into inland water resources.

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