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RESEARCH ARTICLE

Toxicity assessment of industrial wastewaters reaching Dandugan Oya, Sri Lanka using a plant based bioassay

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Abstract: Industrial waste may contain complex chemical mixtures with potential cytotoxic and genotoxic effects. The Dandugan Oya, a water canal located in the Western Province of Sri Lanka receives industrial waste from multiple sources. In the present study potential toxicity of selected industrial wastewaters reaching the Dandugan Oya, and the downstream water was assessed using a plant based bioassay with onion (*Allium cepa* L. var. *ascalonicum*) as the test organism. Of the physico-chemical characteristics tested, temperature, pH, biochemical oxygen demand, chemical oxygen demand, cadmium and chromium levels of the wastewaters collected during three sampling occasions in the year 2012 were within the national tolerance limits specified for the discharge of industrial effluents into inland surface waters. The exposure of *A. cepa* bulbs to wastewater and downstream water from the Dandugan Oya resulted in the reduction of root growth (24 – 62 %) and mitosis (31 – 55 %), induction of micronuclei (up to 0.6 %), nuclear abnormalities (3 - 14 folds) and chromosomal aberrations (3 - 21 folds) in the root tip meristematic cells compared to those exposed to the control and the upstream water, indicating cytotoxic and genotoxic effects. No significant difference between the control and the upstream water was found in relation to the measured biological effects ($p > 0.05$). The present study revealed that the tested wastewaters contained cyto-genotoxic contaminants and, the inherent dilution/detoxification capacity of Dandugan Oya during the study period was not adequate to eliminate the toxic effects in the downstream water. In addition to the conventional physico-chemical analyses, inclusion of suitable bioassays as additional assessments in water quality monitoring programmes could alert cyto-genotoxic impacts in wastewater receiving inland surface waters.

Keywords: Bioassay, cytotoxicity, Dandugan Oya, genotoxicity, wastewater.

INTRODUCTION

Industrial wastewaters may contain complex chemical mixtures including metallic and organic compounds with potential cytotoxic and genotoxic effects. The evaluation of hazardous wastes and effluents by genotoxicity assays may provide data useful for hazard identification and comparative risk assessment (Claxton *et al*., 1998). In a review of mutagens in surface waters, Ohe *et al.* (2004) emphasized the importance of conducting mutagenicity/ genotoxicity assays in addition to the analysis of conventional water quality parameters to efficiently assess the presence of mutagens in water.

Higher plants are recognized as excellent genetic models to detect environmental mutagens and may serve as a warning to other biological systems, since the target of the mutagens is DNA, which is common to all organisms. Among the plant species, *Allium cepa* ($2n = 16$) bioassay is frequently used for *in situ* environmental monitoring studies of waste, surface water and groundwater quality assessments (Grant, 1982; Smaka-Kincl *et al.,* 1996; Leme & Marin-Morales, 2009). The *A. cepa* bioassay is a low cost and easily handled toxicity test, which has advantages over other short-term tests that require previous preparations of tested samples. The *A. cepa* bioassay facilitates testing different toxicity endpoints *viz*. root growth inhibition, mitotic index alterations (Fiskesjo, 1985), chromosome aberrations, nuclear alterations and micronucleus analysis (Grant, 1982; Rank & Nielsen, 1993; Ma *et al.,* 1995). A combination of these toxicity

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endpoints can be used to evaluate general toxicity, cytotoxicity, genotoxicity and to verify mutagenicity (Leme & Marin-Morales, 2009). Moreover, this test system provides information to evaluate the genotoxic mechanisms of an agent (clastogenic and/or aneugenic effects) on the genetic material (Leme & Marin-Morales, 2009). Recently, cytotoxic and genotoxic effects on *A. cepa* have been reported following exposure to a number of pollutants including heavy metals (Barbosa *et al.,* 2010; Hemachandra & Pathiratne, 2015), pesticides (Liman *et al*., 2011; Türkoğlu, 2012), complex mixtures such as industrial and municipal wastes (Chandra *et al.,* 2005; Radic *et al.,* 2010; Siddiqui *et al*., 2011) and emerging contaminants (Herrero *et al.,* 2012).

In Sri Lanka, inland surface waters specially in urban areas are being polluted with domestic sewage and industrial effluents (Bandara, 2003). For regulatory purposes, national tolerance limits have been established for a set of physico-chemical and microbiological parameters for discharge of industrial effluents into inland surface waters of Sri Lanka (Anon, 2008). However, no attention has been given for assessing the biological effects of inland surface waters impacted by industrial waste. Complementary to the conventional physico-chemical analysis, biological tests for toxicity assessments are important for evaluating the response of the living organisms in complex mixtures of pollutants and assessing their potential synergistic effects. The Dandugan Oya, a water canal located in the Western Province, Sri Lanka is a recipient of industrial waste from multiple sources. It also serves as a raw water source for public water supply in some suburban areas in the Gampaha District. In the present study the potential cyto-genotoxic effects of wastewaters of two industries discharged into the Dandugan Oya and the downstream water were assessed using *A. cepa* bioassay along with some physico-chemical characteristics to evaluate the quality of wastewaters and receiving water.

METHODS AND MATERIALS

Sampling sites

Sampling sites selected for the present study are given in Figure 1. Wastewater discharged to the Dandugan Oya by industry A (textile dyeing effluent) and B (leachate from a tannery effluent) were collected from site A (E-79°55'24.64" N-7°7'27.45") and site B (E-79°55'17.85" N-7°7'34.98"), respectively along with the downstream water from site C (E-79°55'3.82" N-7°7'39.24"). Sampling was carried out on three occasions in 2012; March, June and September, which was mainly during dry periods. Site C is the raw water intake point by the National Water Supply and Drainage Board (NWSDB) for treatment/distribution. The selection of the two industries was based on the close proximity of their discharges to the raw water intake of the NWSDB. Water samples from upstream of the water course, Attanagalu Oya (Site R, E-80°12'43.19" N-7°7'21.57") were collected in October 2012. The water flows in the direction of site R to site C.

Physico-chemical characterization of water/ wastewater samples

The temperature, pH, electrical conductivity, salinity and total dissolved solids of the water/wastewater samples were measured *in situ* using a multi parameter water quality checker (MPS-556, Yellow Springs Instrument Company, USA). The dissolved oxygen content (DO), biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) of the water/wastewater samples were analysed according to standard procedures (APHA, 1998). Total cadmium and chromium concentrations were analysed following the standard protocols (APHA, 1998) using furnace atomic absorption spectrophotometry with GBC model 932 plus atomic absorption spectrometer equipped with a graphite furnace 3000 system and PAL 3000 auto sampler. The physico-chemical characteristics of the water used for wastewater dilution (aged pipe borne water originated from the Kelani River) were determined concurrently for comparative purposes.

Allium cepa bioassay for toxicity assessments

The *A. cepa* bioassay was performed as described by Grant (1982) with some modifications, which included the use of a commercial variety of red onion (*Allium cepa* L. variety *ascalonicum*) for evaluating different parameters of meristematic cells as indicators of cytotoxicity, genotoxicity and mutagenicity; direct exposure of onion bulbs to the wastewater/water without an intermediary step in the distilled water; and the elevated exposed temperature (25 – 26 ^{o}C) to reflect tropical exposure conditions. Homogeneously sized *A. cepa* bulbs were purchased from the local market. Healthy bulbs (weight 6 - 9 g) were prepared for testing by removing the loose outer scales and gently scraping apices of the bulbs to expose the root primordia. A set of 7 glass aquaria were filled (500 mL) separately with the undiluted and diluted (50 % dilution) wastewater/water samples of each site and the dilution water (control). Tap water originated from the Kelani River was used as the diluents after leaving under continuous aeration for 3 days to release residual chlorine. A series of onion bulbs were installed in a floater and placed in the glass aquaria with the respective exposure medium in order to submerge

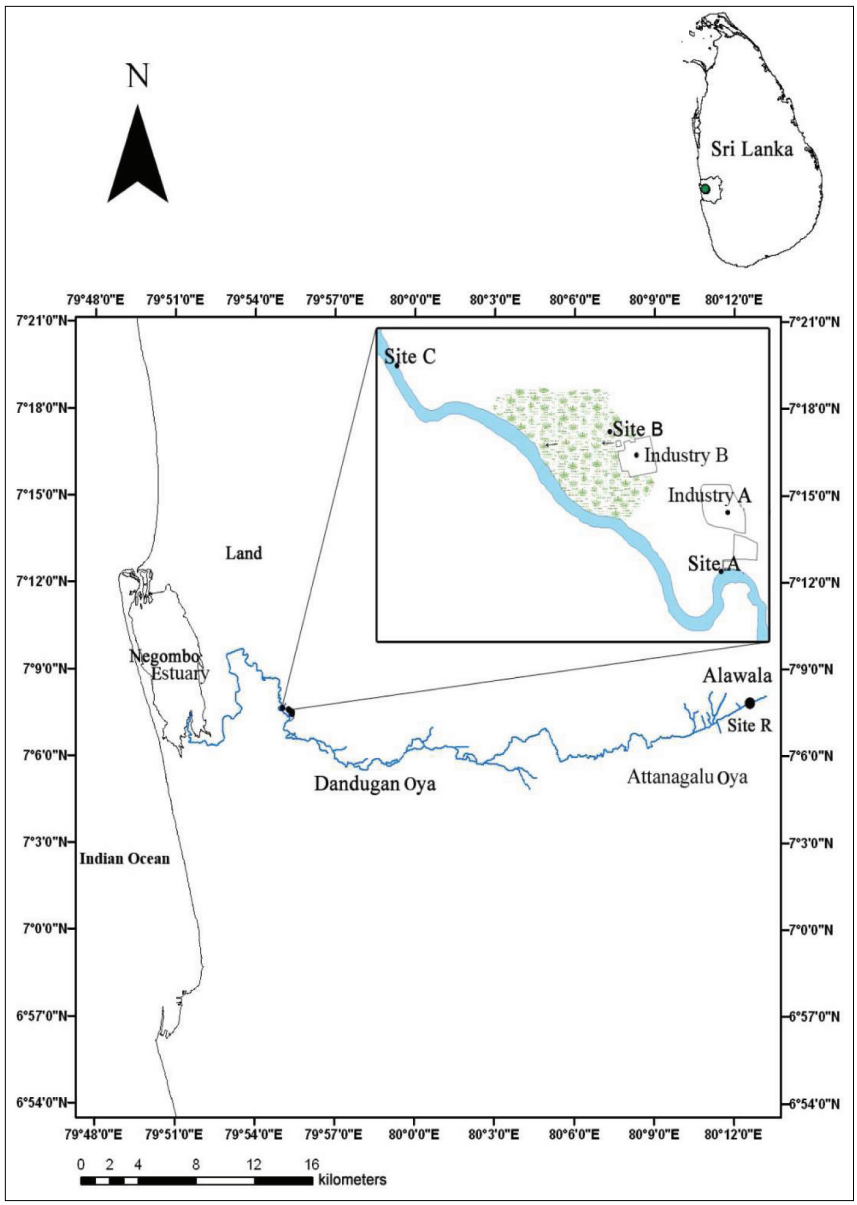

Figure 1: Map of the Dandugan Oya showing sampling sites: A – industry A effluent; B – industry B wastewater leachate; C - downstream water; R – upstream of the water course (Attanagalu Oya)

the onion bulbs to about one quarter of its depth. The exposure media were renewed daily for two days. The bioassay was conducted under laboratory conditions in a shaded area at room temperature (25 - 26 ^{o}C).

After 48 hrs of exposure, ten bulbs with growing roots were randomly selected from each test medium and the control, and several root tips (1 - 2 mm of length) from each bulb were fixed in 3:1 ethanol:glacial acetic acid (v/v) and stored overnight at 4 ^{0}C. The root tips were hydrolysed in 1 N HCl at 60 ^{0}C for 5 min. The acid penetrated root tips were washed with distilled water and stained with 5 % acetocarmine solution for 5 min. The roots were placed on glass slides with a drop of 5 % acetocarmine solution, and a cover slip was placed on the glass slide providing slight pressure to squash the cells on the surface of the slide. One slide was prepared from each bulb. The slides were coded and the root tip meristematic cells were examined under the light microscope (× 400) for scoring of mitotic stages, occurrence of

micronuclei, nuclear abnormalities in the interphase cells and chromosomal aberrations in the dividing cells. The mitotic index was calculated as the percentage of dividing cells of observed meristematic cells by counting 1000 cells per slide (Fiskesjö, 1985; 1988). Nuclear abnormalities were estimated using the abnormalities detected in 1000 interphase cells in each slide. The frequencies of chromosomal aberrations were based on the number of specific chromosomal abnormalities (Leme & Marin-Morales, 2009; Barbosa *et al.,* 2010) corresponding to 100 examined dividing cells in each slide. The frequency of micronuclei was scored in 1000 interphase cells per each bulb (Ma *et al*., 1995). The same procedure was followed with *A. cepa* onion bulbs exposed to the upstream water and the dilution water (control) for comparison.

Another set of *A. cepa* bulbs were continuously exposed to the wastewater/water samples and the control media as described above for 5 d with daily renewal of exposure media for root growth inhibition test. After 5 d of exposure, the lengths of the whole root bundle from each bulb were measured as described by Fiskesjo (1988), for assessing phytotoxicity using growth inhibition as the toxicity endpoint.

Data analysis

Physico-chemical parameters of water/wastewater samples were compared using one-way analysis of variance (ANOVA). If differences were significant, they were compared using Tukey's pairwise comparison test (Zar, 1999). Same statistical tests were used to examine the differences in tested parameters relevant to the onion bulbs exposed to the wastewater/water samples. The data were transferred into $\log_{10}(X+1)$ before analyses. The accepted level of significance was $p < 0.05$.

RESULTS

Physico-chemical characteristics of wastewater/ water

Salinity, conductivity, total dissolved solids and total chromium content of wastewater from site B were significantly higher than those of site A and the water samples from site C downstream of the Dandugan Oya (Table 1). In the downstream water, elevated total dissolved solid levels, BOD_5 and COD concentrations, detectable chromium and cadmium contents were found in comparison to the dilution water and the upstream water (site R).

Of the tested physico-chemical characteristics, temperature, pH, BOD_5, COD, total chromium and cadmium levels of the wastewater samples from sites A and B were within the national tolerance limits specified for discharge of effluents to inland surface waters (Anon, 2008). No national tolerance limits are available

Table 1: Physico-chemical characteristics* of wastewater/water samples collected from the study sites of the Dandugan Oya during the study period

Parameters	Site A	Site B	Site C	Dilution water	Site R	Tolerance limits for industrial effluents#	SLS limits for raw water##
Colour	Red	Light green	Light brown	Clear	Clear		-
Temperature (^{0}C)	31.5 ± 1.7 [a]	28.2 ± 0.6 [a]	29.4 ± 0.8 [a]	30.3 ± 0.5 [a]	25.4	40	-
pH	8.0 ± 0.4 [a]	7.7 ± 0.1 [a]	8.4 ± 0.5 [a]	7.2 ± 0.8 [a]	7.0	6.5 - 8.0 textile 5.5 - 9.0 tannery	6.0 - 9.0
Salinity (g/L)	0.2 ± 0.1 [a]	3.2 ± 0.7 [b]	0	0	0	-	-
Conductivity (µS/cm)	444 ± 312 [a]	6193 ± 1479 [b]	96 ± 4 [a]	95 ± 9 [a]	32	-	-
TDS (mg/L)	247 ± 170 [b]	3893 ± 791 [c]	56 ± 2 [b]	18 ± 3 [a]	21	-	-
DO (mg/L)	2.3 ± 0.3 [a]	3.0 ± 0.1 [ab]	3.5 ± 0.1 [b]	5.1 ± 0.2 [b]	5.6	-	4
BOD_5 (mg/L)	29 ± 3 [a]	19 ± 1 [a]	23 ± 1 [a]	< 3	< 3	60	5
COD (mg/L)	92 ± 2 [a]	96 ± 8 [a]	80 ± 5 [a]	< 9	< 9	250	-
Cadmium (µg/L)	1.5 ± 0.3 [a]	2.0 ± 0.6 [a]	1.2 ± 0.3 [a]	ND	ND	100	-
Chromium (µg/L)	3.0 ± 0.5 [a]	19.4 ± 2.0 [b]	5.5 ± 1.1 [a]	ND	ND	2000	50

* The data are presented as mean ± SEM for 3 sampling events (except site R). For each parameter, means indicated by different superscript letters (a, b, c) are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$).
ND - below the limit of quantification (LOQ: 0.4 µg/L for cadmium; 1.3 µg/L for chromium)
TDS - Total dissolved solids; DO - dissolved oxygen; BOD_5 - biochemical oxygen demand for 5 days; COD - chemical oxygen demand

\# National tolerance limits specified for industrial effluents (Anonymous, 2008)

\## Sri Lanka Standards limits for raw water for public water supply (SLS, 1985)

for salinity, electrical conductivity and total dissolved solid levels for discharge of effluents to inland surface waters in Sri Lanka. Sri Lanka Standards (SLS)(1985) for water quality parameters specified for inland surface waters for use as raw water for public water supplies include pH, dissolved oxygen, BOD_5 and chromium levels. The pH, dissolved oxygen and total chromium levels of the downstream water of the Dandugan Oya (raw water intake area for public water supply) were within the SLS specified limits. However, BOD_5 of the downstream water (22 - 24 mg/L) was higher than the limit specified by the SLS indicating high organic pollution in the downstream water during the study periods. There are no established Sri Lanka standard limits for temperature, salinity, electrical conductivity, total dissolved solids, COD and cadmium for inland surface waters for use as raw water for public water supplies. The cadmium levels of upstream water (site R) and the dilution water were below the detection limits whereas the downstream water (site C) had traces of cadmium (1.2 ± 0.3 µg/L).

Toxicity assessments by *A. cepa* bioassay

The microscopic appearance of normal interphase cells and dividing cells in the *A. cepa* root tip meristematic region are presented in Figure 2. Mitotic indices based on the percentage of mitotically dividing cells in the root tip cells indicate significant inhibition of cell divisions in the root tip cells of the onion bulbs following 2 days of exposure to wastewater (Figure 3). Mitotic indices of the root tip cells exposed to undiluted wastewater from site A and site B and the downstream water from site C were depressed by 34 – 51 , 31 – 42 and 40 – 55 %, respectively in comparison to the onion bulbs exposed to dilution water (control) and the upstream water (site R). Dilution of wastewater to 50 % showed some improvements in the mitotic indices of the root tip cells exposed to the wastewater from site A and site B, but a significant dilution effect on the mitotic indices was not observed for downstream water with 50 % dilution (Figure 3).

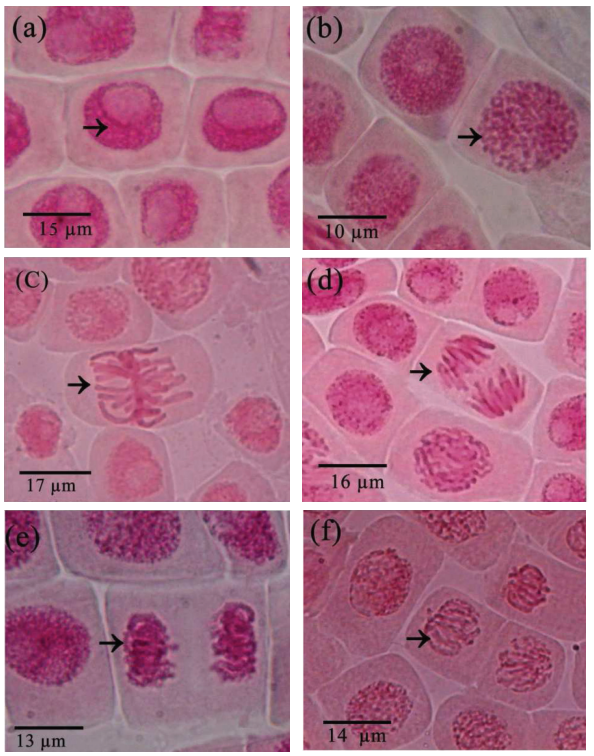

Figure 2: *Allium cepa* var. *ascalonicum* root tip meristematic cells showing normal cell stages (a) interphase; (b) prophase; (c) metaphase; (d) anaphase and (e, f) early and late telophases

The root growth based on increase in root length (Figure 3) of *A. cepa* bulbs showed significant inhibition following 5 days exposure to undiluted wastewater from site A (39 – 40 %), site B (34 – 62 %) and downstream water from site C (24 – 44 %) compared to that of *A. cepa* bulbs exposed to dilution water indicating phytotoxicity. Root growth inhibition was significantly lower in diluted wastewater from site B compared to the undiluted wastewater, whereas no significant dilution effect was observed for the wastewater from site A and downstream water.

Micronuclei were not detected in the root tip cells of *A. cepa* bulbs exposed to dilution water or upstream water, whereas micronucleated interphase cells (Figure 4, Table 2) were observed in the bulbs exposed to wastewater/ downstream water samples from the Dandugan Oya. Occurrence of micronucleated cells was greater in the onion bulbs exposed to undiluted wastewater/downstream water samples compared to those in the diluted exposure media. Nuclear abnormalities *viz*. notched nuclei, binuclei and nuclei with nuclear buds were observed in some interphase cells of the root tips (Figure 4). Occurrence of these nuclear abnormalities were significantly greater in the bulbs exposed to undiluted wastewater from site A (3-8 folds) and site B (4-14 folds) and downstream water (3-7 folds) compared to that of bulbs exposed to dilution water (Figure 5). Except for the wastewater from site B in the third sampling event, dilution of the wastewater/downstream water had no significant effect on the occurrence of nuclear abnormalities.

Chromosomal aberrations observed in the dividing cells of onion root tips were sticky chromosomes in the metaphase, C-metaphase, disturbed anaphase, anaphase bridge, lagging chromosomes in the anaphase and telophase bridges (Figure 6). Occurrence of chromosomal aberrations in the onion root tip cells exposed to dilution

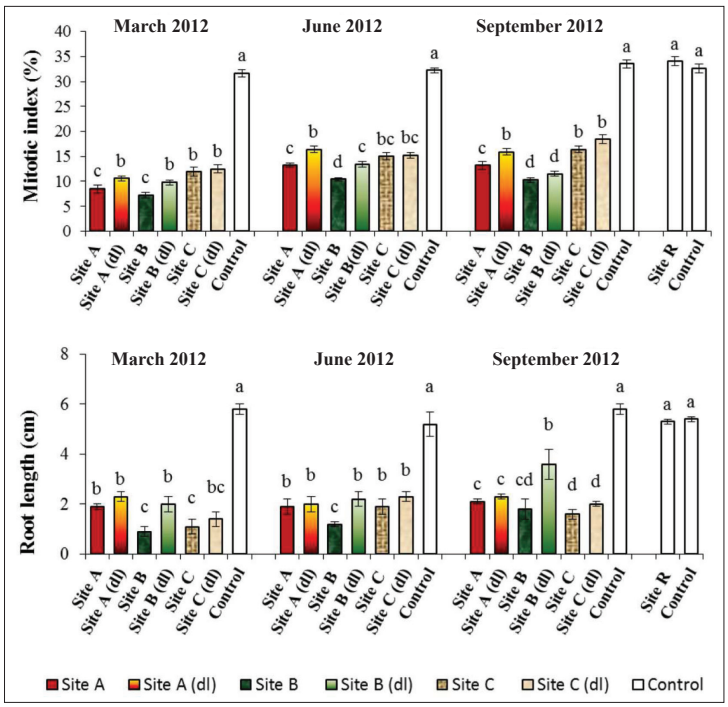

Figure 3: Mitotic index of root tip cells of *Allium cepa* var. *ascalonicum* bulbs and root growth following exposure to undiluted and 50 % diluted (dl) wastewater (site A, B) and downstream water (site C), dilution water (control) and upstream water (site R). For each sampling occasion, results (mean ± SEM, n = 10) indicated by different letters (a, b, c, d) are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$).

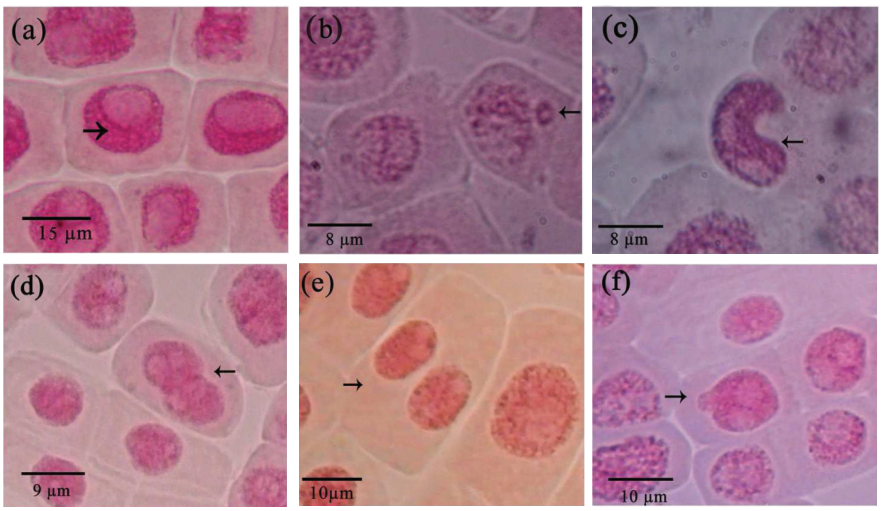

Figure 4: *Allium cepa* L. var. *ascalonicum* root tip interphase cells with (a) normal nucleus; (b) micronucleus; (c) notched nucleus; (d,e) binuclei and (f) nuclear bud following exposure to the selected wastewater samples

water and upstream water was very low (1.6 – 1.8 %) and the differences were not significant (Figure 7). Compared to those exposed to dilution water, the occurrence of chromosomal aberrations were significantly greater

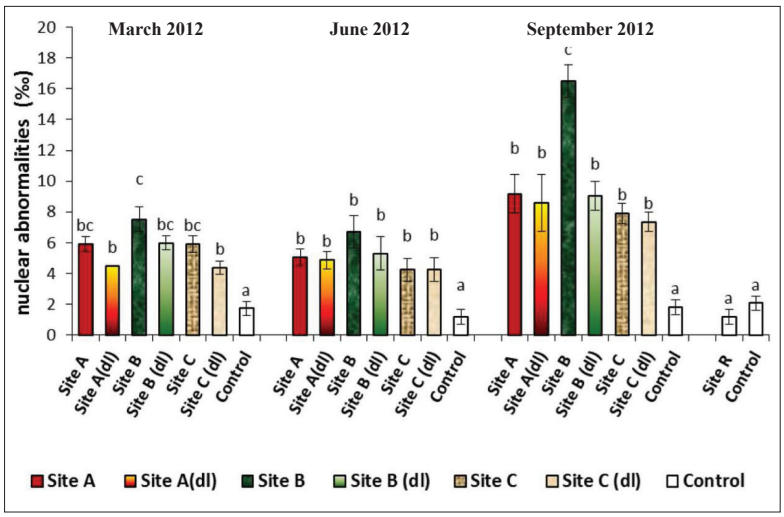

Figure 5: Nuclear abnormalities of *Allium cepa* var. *ascalonicum* bulbs following exposure to undiluted and 50 % diluted (dl) wastewater (sites A, B) and downstream water (site C), dilution water (control) and upstream water (site R). For each sampling occasion, the results (mean ± SEM, n = 10) with different letters (a, b, c) are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$).

Table 2: Occurrence of micronuclei* in *Allium cepa* var. *ascalonicum* root tip cells following exposure to undiluted and diluted (50 % dilution) wastewaters collected from the study sites (site A, B) and downstream water (site C) of Dandugan Oya

Sampling occasion	Tested sample	Occurrence of micronuclei (%)		
		Site A	Site B	Site C
March 2012	Undiluted	0.2 ± 0.2 (0 – 2)	0.6 ± 0.3 (0 – 2)	0
	Diluted	0	0.2 ± 0.2 (0 – 2)	0
June 2012	Undiluted	0.4 ± 0.3 (0 – 2)	0.6 ± 0.3 (0 – 2)	0.2 ± 0.2 (0 – 2)
	Diluted	0.2 ± 0.2 (0 - 2)	0	0
September 2012	Undiluted	0.4 ± 0.3 (0 – 2)	0.6 ± 0.3 (0 – 2)	0.2 ± 0.2 (0 – 2)
	Diluted	0	0	0

* Data are presented as mean ± SEM (n = 10). The numbers in the parentheses indicates the range. No micronuclei were detected in the root tip cells exposed to dilution water or upstream water.

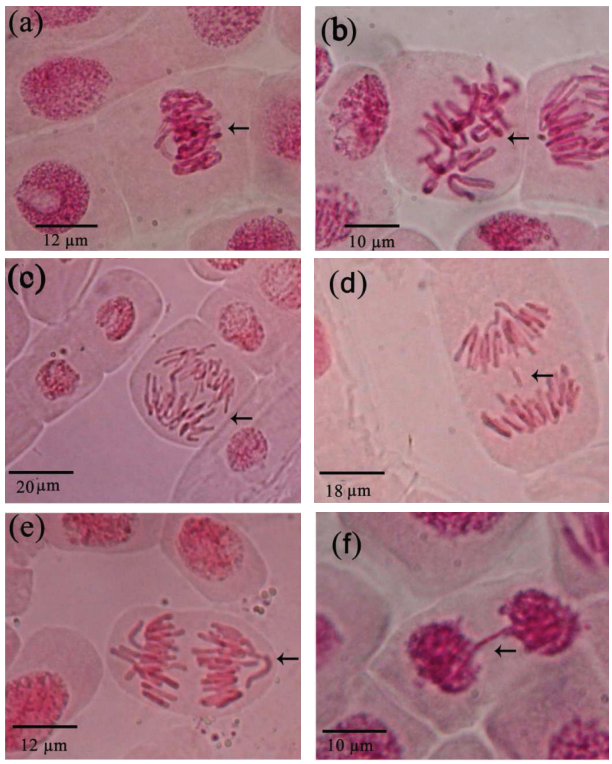

Figure 6: Chromosomal aberrations seen in *Allium cepa* var. *ascalonicum* root tip cells following exposure to selected wastewater samples (a) sticky chromosomes in metaphase; (b) C-metaphase; (c) anaphase bridge; (d) lagging chromosomes in anaphase; (e) disturbed anaphase and (f) telophase bridge

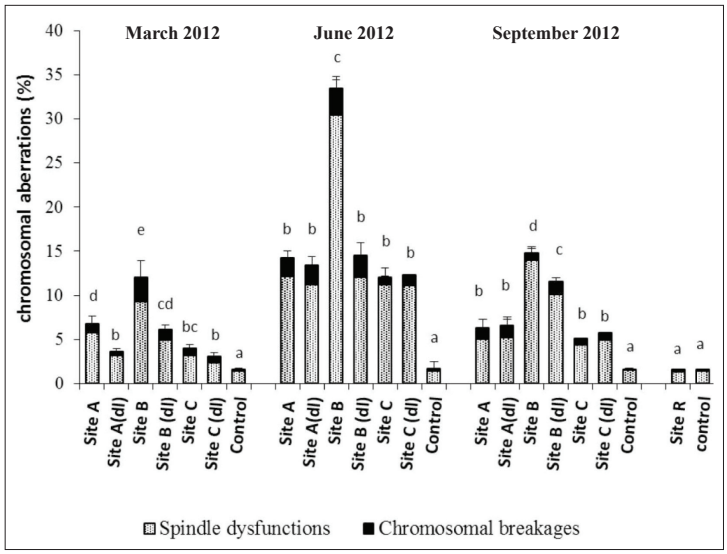

Figure 7: Chromosomal aberrations in root tip cells of *Allium cepa* var. *ascalonicum* bulbs following exposure to undiluted and 50 % diluted (dl) wastewater (site A, B) and downstream water (site C), dilution water (control) and upstream water (site R). For each sampling occasion, the results (mean ± SEM, n = 10) with different letters (a, b, c, d, e) are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$).

in the dividing root tip cells of *A. cepa* bulbs exposed to the wastewater of site A (3 – 9 folds) and site B (8 – 21 folds) and downstream water (site C) from the Dandugan Oya (3 - 8 folds). Dilution of downstream water up to 50 % had no significant reduction on the occurrence of chromosomal abnormalities, whereas wastewater from site B in the three sampling events and wastewater from site A in the first sampling event showed significant reduction in the occurrence of chromosomal aberrations with 50 % dilution. Majority of the chromosomal aberrations observed in the root tip cells exposed to wastewater/downstream water were due to spindle dysfunction effects (sticky chromosomes in the metaphase, C-metaphase, disturbed anaphase and lagging chromosomes), whereas the occurrence of chromosomal damages (anaphase bridges and telophase bridges) were comparatively low (Figure 7). No significant differences between the dilution water and upstream water (site R) were found in relation to the measured parameters.

DISCUSSION

The analyses of samples collected from areas known to receive industrial wastes and effluents have shown that genotoxins can accumulate in the receiving environment and have adverse effects on indigenous biota (Claxton *et al.,* 1998). *A. cepa* bioassay has been used as a tool for environmental monitoring in the last decade, where satisfactory results have been reported (Leme & Marin-Morales, 2009; Radic *et al*., 2010; Masood & Malik, 2013). In the present study *Allium cepa* bioassay with different toxicity endpoints has been applied to assess the toxicity of wastewaters reaching the Dandugan Oya, a raw water source of the pipe borne water supply in some suburban areas in the Gampaha District, Sri Lanka. Although majority of the measured physico-chemical parameters including cadmium and chromium levels were within the national tolerance limits, the bioassay revealed cyto-genotoxic effects of wastewaters reaching the Dandugan Oya at sites A (textile dyeing effluent) and B (leachate from a tannery effluent) and the downstream receiving water (site C).

Depression of mitotic indices and root growth inhibition in *A. cepa* have been reported earlier following the exposure to a number of pollutants including heavy metals (Barbosa *et al.,* 2010; Hemachandra & Pathiratne, 2015), pesticides (Liman *et al*., 2011; Türkoğlu, 2012), complex mixtures such as industrial and municipal waste (Chandra *et al.,* 2005; Radic *et al.,* 2010; Siddiqui *et al*., 2011) and emerging contaminants (Herrero *et al.,* 2012). *Allium cepa* root tip cells exposed to undiluted and diluted wastewater /downstream water samples of the Dandugan Oya exhibited significant depression of mitotic indices in comparison to the dilution water in all sampling occasions. The results indicate mito-depressive effect of the contaminants present in the tested samples. These contaminants may interfere with the normal

process of mitosis, thus preventing a number of cells from entering the prophase and blocking the mitosis cycle during interphase. The inhibition of mitotic index can also be attributed to be an effect of environmental chemicals on DNA/protein synthesis of the biological system (Yildiz *et al.*, 2009). Mitotic index alterations can indicate changes deriving from chemical action in the growth and development of exposed organisms (Leme & Marin-Morales, 2009). Decrease in the root growth of *A. cepa* bulbs exposed to wastewater/downstream water from the Dandugan Oya in comparison to the onion bulbs exposed to dilution water and upstream water can be attributed to the mito-depressive effect caused by cytotoxic contaminations.

Micronuclei and chromosomal aberration tests in *A. cepa* bioassay provide a rapid screening of genotoxic effects of the chemical substances that are present in the environment (Leme & Marin-Morales, 2009). Micronucleus test has been considered as the most effective and simplest endpoint to analyse the mutagenic effect promoted by chemicals (Ma *et al.,* 1995). The micronucleus is composed either of small chromatin fragments, which arise as a result of chromosomal breakage or of whole chromosomes that do not migrate during anaphase as a result of spindle dysfunction (Leme & Marin-Morales, 2009). Micronuclei (up to 0.6 %) were observed in *A. cepa* root tip cells following exposure to undiluted wastewater/downstream water during all three sampling occasions and diluted wastewater in one sampling event. The micronuclei induction in the root tip cells may indicate the presence of mutagenic contaminations in the tested wastewater samples.

Chromosomal aberrations are characterized by changes in either the chromosomal structure or in the total number of chromosomes, which can occur both spontaneously and as a result from exposure to physical or chemical agents (Russel, 2002). Occurrence of chromosomal aberrations were significantly greater in the dividing root tip cells of *A. cepa* bulbs exposed to the wastewater of site A and B, and downstream water from the Dandugan Oya compared to those exposed to the dilution water. A majority of chromosomal aberrations observed in the root tip cells exposed to wastewater reaching the Dandugan Oya were sticky chromosomes in the metaphase, C-metaphase, disturbed anaphase and lagging chromosome, whereas the occurrence of anaphase bridge and telophase bridges were comparatively low. Sticky chromosome, C-metaphase, disturbed anaphase and lagging chromosome were categorized as indicators of aneugenic action due to disturbances in the mitotic spindle (spindle dysfunction), whereas chromosomal bridges in the anaphase and telophase indicate chromosomal breaks due to the clastogenic effects (Leme & Marin-Morales, 2009; Radic *et al*., 2010; Masood & Malik, 2013). The types of chromosomal abnormalities induced in *A. cepa* root tip cells exposed to wastewater reaching the Dandugan Oya indicate the occurrence of more aneugenic effects due to spindle dysfunctions than clastogenic effects due to chromosomal breaks.

Nuclear abnormalities, which are characterized by morphological alterations in the interphase nuclei have also been included as endpoints in recent studies on testing cyto-genotoxicity of environmental chemicals. Along with chromosomal aberrations analysis, nuclear abnormalities evaluation has shown to be a sensitive analysis for making the investigation of test agent actions even more accurate in relation to their effects on the DNA of exposed organisms (Leme & Marin-Morales, 2009). Nuclear abnormalities of the root tip cells exposed to wastewater/downstream water was induced significantly compared to those exposed to the dilution water. Nuclear buds, which is one of the prominently detected abnormalities associated with pollutant exposure may arise as a result of the elimination of exceeding genetic material derived from the polyploidization process (Fernandes *et al.,* 2007). The induction of nuclear abnormalities in *A. cepa* root tip cells may be associated with the presence of cyto-genotoxic contaminations in the wastewater and the receiving downstream water.

CONCLUSION

The present study applied the *A. cepa* bioassay with different toxicity endpoints (mitotic index, root growth, occurrence of micronuclei, chromosomal aberrations and nuclear abnormalities) for the first time to assess the potential toxicity of wastewaters reaching the Dandugan Oya and downstream receiving water, which is used as a raw water source for public water supply in some suburban areas in Sri Lanka. The bioassay revealed that wastewater samples from the sites A and B (textile dyeing effluent and a leachate from a tannery effluent) consistently contained cyto-genotoxic contaminations over the study period. As the downstream water also exhibited consistent cyto-genotoxic effects, the inherent detoxification and dilution capacity of the Dandugan Oya seems to be inadequate to eliminate the cyto-genotoxicity of the receiving water. In addition to the wastewaters, other pollution sources such as urban waste may have contributed to the cyto-genotoxic contaminations in the receiving downstream water. Dry weather conditions during sampling occasions could have also contributed to the cyto-genotoxicity of the downstream water as toxic substances could be concentrated due to surface

evaporation of the water during dry weather. The results imply that water quality of the Dandugan Oya with respect to cyto-genotoxic contaminations need to be improved considering the potential impacts on human and ecosystem health under chronic exposure. Inclusion of suitable bioassays in water quality monitoring programmes supplementary to the conventional monitoring methods could alert cyto-genotoxic impacts in surface waters.

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