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Effects from log-yard stormwater runoff on the microalgae *Scenedesmus subspicatus*: Intra-storm magnitude and variability

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ABSTRACT

This paper describes the effects posed by stormwater runoff from an industrial log-yard on the microalgae *Scenedesmus subspicatus*. The effects of stormwater runoff sampled during two rain events were determined by exposing *S. subspicatus* cells to different concentrations (% v:v) of each sample. The effects were measured as the percentage change in growth rates in relation to a control culture after exposure times of 24, 48, 72 and 96 h. The runoff from the first rain event had no negative effects to *S. subspicatus*, posing in most cases growth stimulation, whereas the runoff from the second rain event inhibited algae growth. Differences in runoff physico-chemical characteristics combined with the hydrological factors of each rain event explained these opposite effects. The hypothesis of toxic first flush phenomenon was confirmed in the second rain event on the basis of normalized inhibitory effects and runoff volume. It was found that 42, 51 and 50% of the inhibitory effects during exposures of 24, 48 and 72 h were associated with the initial 4% of the total discharged volume. The fact that negative effects were observed in the two runoff events analyzed, raises concern about the potential environmental threats posed by runoff originated from wood-based industrial areas during the entire hydrological year.

1. Introduction

It is widely known that non-point source pollution from urban stormwater runoff discharges a significant number of xenobiotics to water recipients [1], affecting aquatic organisms and deteriorating the ecosystem [2,3]. Although development and knowledge of stormwater runoff and pollutants transport from urban areas increase, little is known about stormwater runoff generated at industrial sites and their respective environmental impacts.

Among a number of industrial activities that might pose harmful effects to aquatic ecosystems, industrial areas that handle and store wood materials such as log-yards, sawmills and other wood-based by-products, raise concerns due to potential release of organic and inorganic compounds. According to [4], toxic effects on fishes and invertebrates are related to naturally occurring wood-related compounds such as aldehydes, phenols, terpenes and others. Furthermore, tannins, lignin; tropolones, resin acids and toxic metals such as zinc, aluminium and copper are of greatest concern due to their contribution to log-yards runoff toxicity [5–7].

Physico-chemical characteristics of stormwater runoff from log-yards are related to (i) the species of trees stored; (ii) the proportion of runoff that comes in contact with stored wood; (iii) log-yard size; (iv) the period the logs are stored; (v) the ratio between logs surface area to log-yard area, etc. Consequently, stormwater runoff from log-yards might have different characteristics and pose varied environmental effects as well.

The intra-storm variability of toxic effects posed by rainfall-runoff of highway areas has been previously described [8]. Those authors observed that higher toxic effects on the early stages of the runoff were gradually reduced, and that 90% of toxic effects were associated with the first 20% of the total runoff volume. Even though the toxic effects posed by wood-related compounds have been demonstrated [9–11,6,4], little is know concerning the intra-storm magnitude, variability and possible existence of toxic first flushes from stormwater runoff generated in log-yards.

The aim of this paper was to evaluate the magnitude and the intra-storm variability of the toxic effects posed by stormwater runoff originated from a log-yard during single storm events. Most studies previously carried out throughout entire hydrographs have focused on specific contaminant loads and less attention has been given to toxicity evaluation. Despite the useful information on levels of contamination provided, the effects on the biota are not obtained. It is essential to better understand the

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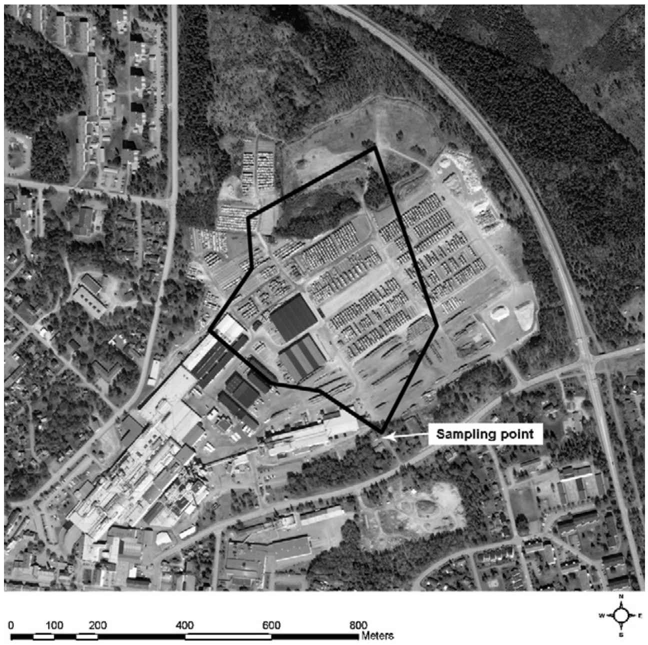

Fig. 1. Aerial view of the log-yard drainage basin with the drainage boundaries. Scale 1:5000.

dynamics of adverse effects of these types of water and their magnitudes, to provide a basis for an appropriate proposal, design and implementation of stormwater runoff management strategies. For the purpose of this study the freshwater, unicellular microalga *Scenedesmus subspicatus* was selected as the test organism. Microalgae are considered ideal organisms for toxicological studies once they are primary producers and any adverse effects towards them, might influence higher levels of the food chain. Besides, microalgae have a short life cycle and are able to quickly respond to the environmental changes over a number of generations [12].

2. Materials and methods

2.1. Industrial site

The physico-chemical characterization of the stormwater runoff was carried out at the industrial log-yard belonging to AB Gustaf Kähr and Kalmar Energi Värme AB located in Nybro, southeast of Sweden. The drainage basin has a total area of approximately 117,000 m^2 (Fig. 1) and stores the following tree species: maple (*Acer* sp.), beech (*Fagus silvatica*), cherry (*Cerasus* sp.), oak (*Quercus* sp.), jatobá (*Hymenaea courbaril*), merbau (*Intsia bijuga*), walnut (*Juglans* sp.), ash (*Fraxinus excelsior*), birch (*Betula* sp.), jarrah (*Eucalyptus marginata*), rosewood (*Dalbergia* sp); oak being the most common (40–50%) [13].

2.2. Stormwater sampling and characterization

Stormwater runoff generated by two single rain events during the summer of 2009 (June/July) were studied: (a) rain 1, on June 18 and; (b) rain 2, on July 1. An automatic portable sampler SIGMA 900 MAX was installed at the outfall of the studied drainage area and was triggered by: (i) precipitations higher than 0.6 mm during a period of at least 15 min and; (ii) drainage system levels higher than 3.7 cm. A time-paced sampling program was kept to withdraw samples at 25-min intervals. All bottles used for collecting runoff samples (575 mL polyethylene bottles) were previously washed with 10% HCl solution and thoroughly rinsed with deionized water. The characterization of the stormwater runoff samples was performed for pH and conductivity with a Digital pH meter (WTW Multi 340i); total metals (As, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn) according to the ISO 17297-m method using an inductively coupled plasma-mass spectrometry (ICP-MS), and total chemical oxygen demand (COD) (method LCK 114 according to Dr Lange Dusseldorf, Germany). In order to keep the original composition, the stormwater runoff samples were not filtered. Microscopic examinations of the samples at the beginning and end of the tests (0 and 96 h, respectively) were unable to detect the presence of microalgae others than the test organism *S. subspicatus* and potential algal grazers.

2.3. Rainfall and flow measurement

On-site precipitation was digitally recorded by an external "tipping bucket" rain gauge (SIGMA Model 2149). The standard area-velocity and Doppler principle method of flow measurement was used by a velocity submerged sensor (SIGMA Area velocity submerged sensor).

2.4. Test organisms and experimental procedures

The freshwater unicellular green alga *S. subspicatus* was selected in this investigation. The stock culture was purchased from the algal culture collection at the University of Göttingen, Germany (SAG). The algae were grown in the laboratory in JM (Jaworski's Medium) at $20 \pm 2\,^{\circ}C$, photoperiod of 16:8 h light:darkness and light intensity between 5000 and 8000 lux by using cool-white fluorescent lamps. Stock cultures were done by transferring 50 mL to 450 mL of fresh medium in a sterile 1-L Erlenmeyer flask. The toxicity tests were initiated after 3–4 days of algae cultivation to ensure the organisms were in exponential growth phase.

S. subspicatus cells were continuously exposed over a 96-h period under static conditions to five concentrations of the stormwater samples (%, v:v) using a dilution series of 0.25 (0.4, 1.58, 6.25, 25 and 100%). All dilutions were tested in triplicates and Mili-Q water was used as a negative control. Potassium dichromate ($K_2Cr_2O_7$) was used as a reference toxicant in concentrations of 2, 0.5, 0.125 ($mg\,L^{-1}$), 31.25 and 7.8 ($\mu g\,L^{-1}$). The tests were initiated by transferring 2.5 mL aliquots of stormwater runoff samples previously prepared according to the dilution factors into 6 mL glass test tubes. In sequence, the test tubes were inoculated with 2.5 mL aliquots of stock culture containing an initial density of 20,000 cells mL^{-1}. To avoid external contamination all procedures were carried out in a vertical laminar flow sterilization chamber. All stormwater concentrations (%, v:v) and the initial density of algae culture were two-fold decreased as soon as algae stock culture was poured into the test chambers. Therefore, the initial stormwater runoff concentrations (v:v) and the cell densities were 0.2, 0.79, 3.125, 12.5 and 50%, and 10,000 cells mL^{-1}, respectively.

All the tubes were randomly arranged in tube racks and covered with Parafilm M. The tests were carried out under temperature, light intensity and light cycles as described above. The test flasks were hand-shaken once a day. The parameter used to measure the algal responses was *in vivo* chlorophyll fluorescence measured in a 10-AU Fluorometer (Turner Designs, Sunnyvale-California) at exposures of 0, 24, 48, 72 and 96 h.

2.5. Data analysis

The effects of different concentrations of stormwater runoff were determined by comparing growth rates of exposed *S. subspicatus* to non-exposed (negative controls) in different exposure periods (24, 48, 72 and 96 h). The average specific growth rate for

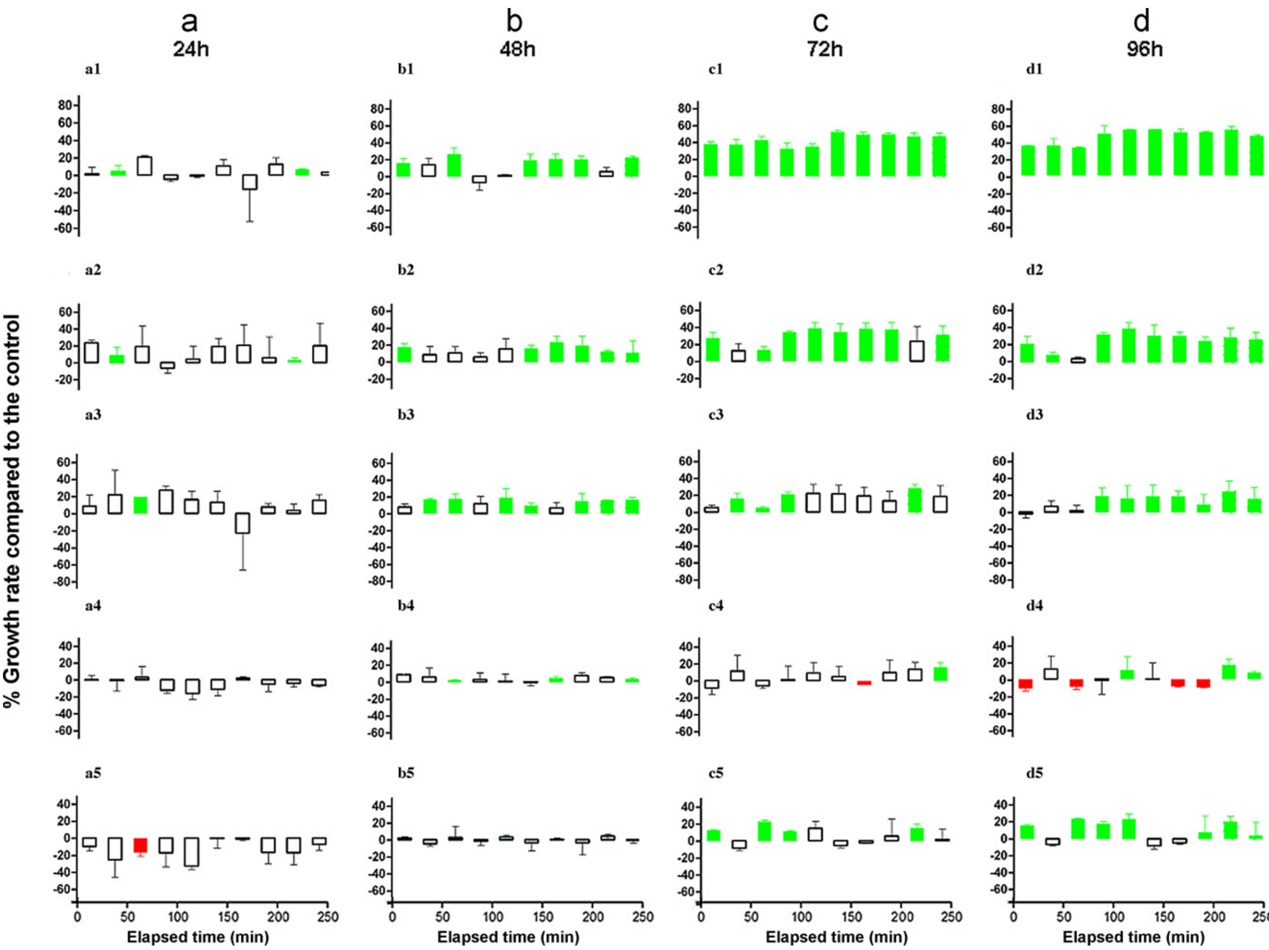

Fig. 2. *S. subspicatus* growth rate % in comparison to the control throughout the rain event of June 18 in exposures of (a) 24 h; (b) 48 h; (c) 72 h; and (d) 96 h. Each row represents stormwater concentrations of (1) 50; (2) 12.5; (3) 3.13; (4) 0.79 and; (5) 0.2% (v:v). Whiskers – standard deviation. White bars – not significant (one-sample *t*-test, $p > 0.05$).

S. subspicatus was given by:

$$\mu = \frac{(\ln F_t) - (\ln F_0)}{\Delta t} \quad (1)$$

where μ is the growth rate (d^{-1}), F_t and F_0 are the biomass measured as *in vivo* fluorescence (RFU) at time t and t_0, respectively, and Δt is the total exposure period ($t - t_0$) in days.

On the basis of calculated growth rates, the % inhibitions were obtained as follows:

$$I\% = \frac{(\mu_c - \mu_e)}{\mu_c} \times 100 \quad (2)$$

where *I%* is the percentage of inhibition, μ_c and μ_e are average growth rates of negative controls and exposed *S. subspicatus*, respectively. $I\% > 0$ and $I\% < 0$ were considered in this current work as inhibitory and stimulatory effects, respectively.

For the samples that caused strong inhibitory effects, the concentration of runoff samples producing a 50% decrease in algal growth in comparison to the controls (EC_{50}) was calculated by selecting non-linear regression models after a visual inspection of concentration–response plots in Excel. The calculation of the no-observed-effect-concentration (NOEC) which is the highest test dilution factor concentration not producing a statistically significant reduction in response compared to the controls was performed by one-way ANOVA followed by Dunnett's multiple comparison tests. In order to verify the statistical significance of the algal growth rate inhibition in comparison to the negative controls, one-sample *t*-test was carried out. The hypothesis of toxic first flush existence was verified by one-way ANOVA and significantly different means were distinguished by post hoc Tukey's multiple comparison test. All the statistical analysis was performed by GraphPad Prism (version 5.02 for Windows, San Diego, USA) assuming Gaussian distribution and variance homogeneity in a significance level of $p \leq 0.05$.

3. Results and discussion

3.1. Effects of stormwater runoff on S. subspicatus growth

Toxicity analyses of stormwater runoff generated in two rain events (June 18 and July 1, 2009) in an industrial log-yard were conducted using the freshwater microalgae *S. subspicatus* as test organism. The intra-storm magnitude and variability of *S. subspicatus* growth rate in comparison to the controls in exposures of 24, 48, 72 and 96-h and stormwater concentrations of 50, 12.5, 3.13, 0.79 and 0.2% (v:v) are summarized in Figs. 2 and 3. Stormwater runoff discharged in June 18 caused stimulatory growth effects to *S. subspicatus* (Fig. 1). On the other hand, inhibitory effects towards *S. subspicatus* due to the exposure to the runoff generated in the rain event of July 1 were observed (Fig. 2).

EC50 and NOEC values of the July 1 rain in different exposures and runoff elapsed times are presented in Tables 1 and 2, respectively. It was not possible to calculate values of $EC50_{96}$ (at the 96^{th} h) due to low inhibitory effects within the entire range of stormwater concentrations. The concentration–response curves with considerable small slopes indicated that regardless of the exposure to

Table 1
EC50 values with 95% confidence intervals calculated for exposures of 24, 48, 72 and 96 h. Stormwater runoff event July 1. Values given in % (v:v).

Runoff elapsed time (min)	$EC50_{24}$	95% CI	$EC50_{48}$	95% CI	$EC50_{72}$	95% CI	$EC50_{96}$	95% CI
15	9.5	7.3–12.2	19.3	11.4–32.6	45.9	17.8–118.7	ND	ND
40	10.0	7.5–13.5	31.0	11.3–84.9	60.6	12.6–292.4	ND	ND
65	13.2	9.6–18.5	23.5	6.8–81.0	69.2	7.0–671.0	ND	ND
90	11.9	4.6–31.0	32.5	9.8–108.0	176.1[a]	ND[a]	ND	ND
115	21.2	13.0–34.4	106.8	ND	ND	ND	ND	ND

ND – not determined due to low inhibitory effects.
[a] Imprecise theoretical model.

different stormwater concentrations, the measured effects were nearly constant after 96-h period.

Discussions concerning the different algae responses as a function of exposure time, stormwater concentrations (%, v:v) and elapsed time are presented below in separate sections.

Moreover, the hypothesis of toxic first flush is also discussed on the basis of two different approaches: (a) the intra-storm variability of toxic effects (% inhibition of growth in comparison to the controls) and (b) the measured effects considering the flow rate, as suggested by [8].

Table 2
NOEC values for the July 1 stormwater runoff event. Values are given in % of stormwater runoff (v:v).

Runoff elapsed time (min)	$NOEC_{24}$	$NOEC_{48}$	$NOEC_{72}$	$NOEC_{96}$
15	3.13	3.13	12.5	>50
40	0.79	3.13	12.5	>50
65	3.13	3.13	12.5	>50
90	0.79	12.5	12.5	>50
115	3.13	12.5	>50	>50

3.1.1. Roles of acute exposure and stormwater concentrations

3.1.1.1. Rain event 1 – June 18, 2009. In general, as previously mentioned, the stormwater runoff discharged on June 18, in most cases, stimulated *S. subspicatus* growth (Fig. 2). However, it was observed that significant stimulatory effects (one-sample *t*-test, $p < 0.05$) occurred in most cases, after the first 24 h of exposure. These results suggest the presence of nutrients such as nitrogen and phosphorus and other trace elements in the runoff. Similarly, stimulatory effects and enhanced growth of the green algae *Pseudokirchneriella subcapitatum* due to highway runoff has been reported [8]. A number of trace metals are used by living organisms to stabilize protein structures, catalyze enzymatic reactions and facilitate electron transfer reactions in the photosynthetic and respiratory processes [14,15]. Furthermore, harmless effects towards *Daphnia* sp. reproduction from log-yards runoff in contact with two different wood species (*Picea abies* and *Pinus sylvestris*) have been reported [6].

According to one-way ANOVA and post-hoc Tukey's multiple comparison tests, from 48 h onwards considerable intra-storm variation and significant differences ($p < 0.05$) of the effects on *S. subspicatus* were observed. Runoff samples collected on the last stages of the runoff – the last 110 min – posed significantly higher

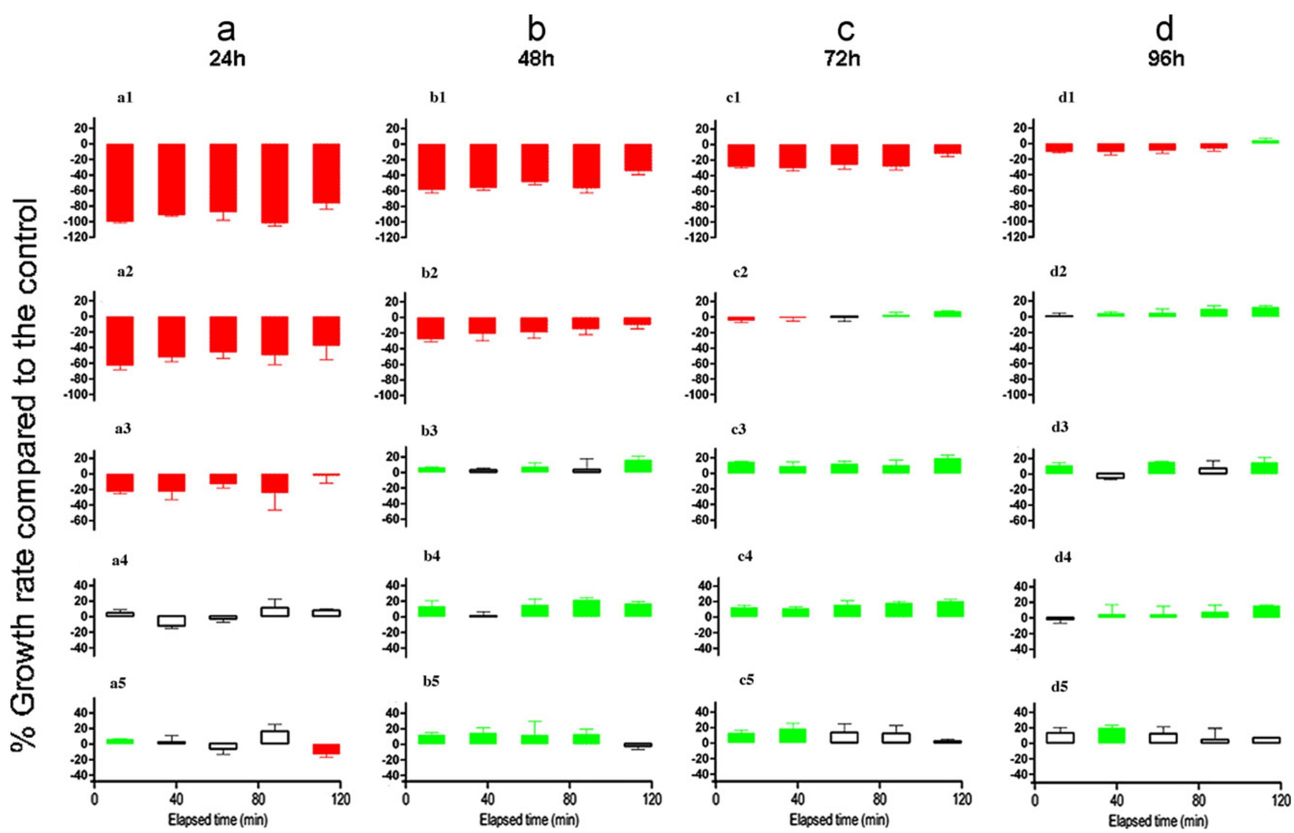

Fig. 3. *S. subspicatus* growth rate % in comparison to the control throughout the rain event of July 1 in exposures of (a) 24 h; (b) 48 h; (c) 72 h; and (d) 96 h. Each row represents different stormwater concentrations of (i) 50; (ii) 12.5; (iii) 3.13; (iv) 0.79; and (v) 0.2% (v:v). Whiskers – standard deviation. White bars – not significant (one-sample *t*-test, $p > 0.05$).

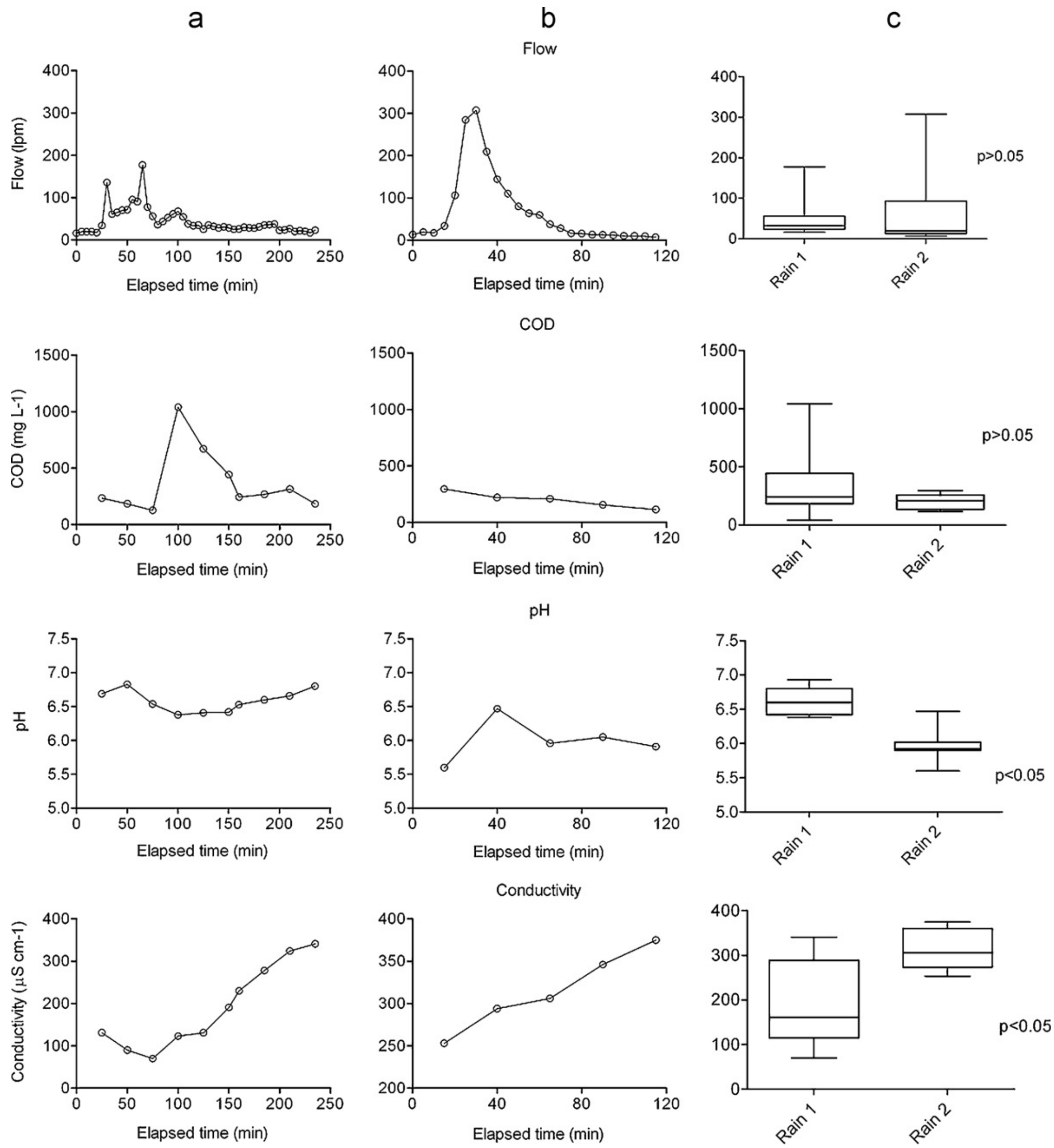

Fig. 4. Hydrograph and pollutographs of (a) rain event 1 (b) rain event 2 and (c) box and whisker plots of water quality parameters observed during rain event 1 ($n = 10$) and rain event 2 ($n = 5$). Average ± SD, 25th and 75th percentiles.

stimulatory effects (Tukey's test; $p < 0.05$) in comparison to the runoff samples collected throughout the first 100 min after 72 and 96 h exposure (Fig. 2c1–c2; d1–d2). Gradual reductions of Cr and Pb concentrations from 10 and 6.6 $\mu g\,L^{-1}$ down to 4.0 and 1.9 $\mu g\,L^{-1}$, respectively during the first 100 min of runoff, then remaining nearly constant throughout the last 110 min, might explain such significant differences. According to [14], in spite of metals being essential to living organisms, Pb may interfere with the proper functioning of enzymes. Even though single metal concentrations in the runoff might explain to a certain extent the intra-storm variability, when dealing with complex mixtures, it is still difficult to draw robust conclusions and state whether the effects are due to either individual compounds or antagonistic and synergistic processes.

Different exposure concentrations (%, v:v) caused variable effects towards *S. subspicatus*. The lower the stormwater concentration (%, v:v): the lower the observed stimulation or inhibitory effects (Fig. 2a–d). The lack of micro nutrients and trace elements might explain reduced stimulation or inhibitory growth conditions in low concentrations. However, by increasing the exposure period, *S. subspicatus* overcame the initial stressful conditions and partially recovered their metabolic processes reestablishing growth.

3.1.1.2. Rain event 2 – July 1, 2009. As shown in Fig. 3, stormwater runoff of July 1 posed in most cases high inhibitory effects (one-sample *t*-test; $p < 0.05$) to *S. subspicatus* growth. Inhibitory effects reaching up to 100% in comparison to the controls with stormwater concentrations of 50% (v:v) were observed (Fig. 3a1). However, the inhibitory effects decreased progressively with exposure time, and a considerable reduction in the effects was observed when comparing exposures of 24 and 96 h. This might be related to an inherent process of the test organisms to detoxify and overcome the initial hazardous environment. According to [16] algae populations adapt their metabolism to new toxic substances due to either physiological acclimatization or genetic adaptation. Furthermore, whereas [15] state that microalgae have molecular mechanisms to discriminate non-essential from essential compounds for growth, [14] report that microalgae employ a variety of biochemical strategies to reduce the toxicity of non-essential trace metals.

In lower stormwater concentrations, the inhibitory effects were in most cases not significant in the first 24 h of exposure (one-sample *t*-test; $p < 0.05$) (Fig. 3a4–a5). However, with prolonged exposure, stimulatory effects took place suggesting that time of exposure plays an important role over the effects posed by the log-yard runoff towards algae growth (Fig. 3b–d) *S. subspicatus* might have adapted their metabolism to overcome initial hazardous conditions, however, the level of acclimatization seems to rely on how long the exposure period is prolonged. The lower the concentrations of stormwater were, the shorter was the period of detoxification and adaptation of the test organisms (Fig. 3a–d). It was found that 50% (v:v) of stormwater runoff still caused 6.5% growth inhibition after 96 h of exposure (Fig. 3d1), compared to an average of 91% of inhibitory effects in the first 24 h (Fig. 3a1). Therefore, *S. subspicatus* reduced the inhibitory effects by 85% during 72 h. On the other hand, Fig. 3a3–b3 illustrates that it took only 24 h for *S. subspicatus* to shift from an average inhibitory effect of 18% towards an average stimulation of 8% when exposed to 3.13% of stormwater concentration (v:v). Despite the indications of positive relations between stormwater concentrations and the period required to overcome toxic effects, the experimental data suggest that toxicity reduction rate on a daily basis was approximately the same.

Another explanation for the shift from inhibition to stimulation of growth during the experiment could be that certain compounds that were causing toxicity in the first days were transformed and/or degraded (by e.g. light, bacteria). Actually the two mechanisms are not mutually exclusive, i.e. is very likely that the algae adapted and hazardous substances became less abundant over time, allowing the cultures to restore growth as the exposure time increased.

3.1.2. Toxic first flush effects

3.1.2.1. First flush effect as a function of stormwater runoff concentrations. The discussions in this section consider only the rain event of July 1, since stimulatory effects were in general, observed in the rain event of June 18.

Rainfall 2: July 1 – The intra-storm variability of the measured effects towards *S. subspicatus* and the possible existence of toxic first flushes were analyzed with one-way ANOVA and post hoc Tukey's multiple comparison tests. There were significant differences ($p < 0.05$) between inhibitory effects posed by samples taken in the early stages of the runoff and those taken in last stages in all exposure times (24, 48, 72, and 96 h) with stormwater concentration of 50% (v:v) (Fig. 3a1, b1, c1 and d1). A shift from inhibitory effects towards stimulation between 90 and 115 min of elapsed was observed after a 96-h acute exposure (Fig. 3d1).

Linear and inverse relationships (one-way ANOVA and post hoc linear trend tests; $p < 0.05$) between toxic effects and runoff elapsed time in the presence of 12.5% of stormwater were observed in all acute exposures (Fig. 3a2, b2, c2, and d2). Inverse relationships are indicated by either shifts from inhibitory effects towards stimulation or increased stimulation in the last stages of the runoff event.

Therefore, the significant intra-storm variability of toxic effects towards *S. subspicatus* growth rate suggests that toxic first flushes occurred during the monitored runoff event. These results are in agreement with [2] that observed runoff samples taken during the first 10 min of a simulated rainfall over parking lots being as twice as toxic from those samples taken in the last stages.

In an attempt to understand the relationship between water quality parameters and inhibitory effects, Pearson's correlation coefficients were calculated. It was found that inhibitory effects posed by July 1 runoff were significantly correlated ($p < 0.05$) to COD

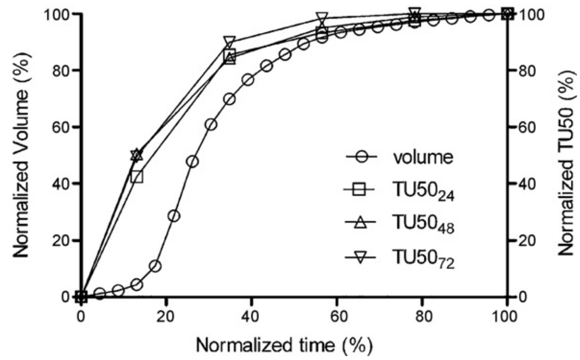

Fig. 5. Discharged volumes and toxic units (TU50) as a function of storm elapsed time on a normalized basis for acute exposures of 24, 48 and 72 h.

($0.981 > r > 0.671$) and conductivity ($-0.977 < r < -0.610$). Plots of COD and conductivity against runoff elapsed time (pollutographs) and the correlation analysis might explain the toxic first flush occurrences during runoff event of July 1 (Fig. 4b). Whereas COD decrease throughout the runoff event, the conductivity increase, indicating then the toxic first flushes.

Toxic first flushes can be also inferred from the fact that EC50 values increased (i.e. toxicity decreased) towards the end of the storm event (Table 1).

3.1.2.2. Toxic first flush effects: normalized toxicity vs normalized runoff volume. To confirm the significant intra-storm variability and the occurrence of toxic first flush, an evaluation of the normalized inhibitory effects during the rainfall-runoff event was carried out. To remove the effects of runoff flow rates on the interpretation of toxicity results, an evaluation on the basis of intra-storm normalized toxicity proportion is recommended [8]. Fig. 5 illustrates the normalized toxicity proportion and normalized discharged volume as a function of normalized elapsed time. The normalized toxicity is expressed in terms of acute toxic units ($TU_{50} = 100/EC50$).

The occurrence of toxic first flush is evident by taking into account 24; 48 and 72-h exposures, once a greater normalized toxicity proportion in the earlier stages of the runoff event in comparison to the normalized discharged volume is observed. It can be observed that 42, 51 and 50% of the toxic effects during exposures of 24, 48 and 72 h, respectively were associated with the first 4% of the total discharged volume and 13% of the total storm duration (Fig. 5). However, as the variation of the cumulative runoff volume as a function of time increases considerably from an approximately 20% of the total duration of the runoff onwards, the toxic first flush phenomenon is partially minimized. The results regarding toxic first flush phenomenon on a normalized basis corroborate the results obtained through the analysis of the intra-storm variability of the toxic effects without considering the flow rates.

3.1.2.3. Rain events characteristics. As previously discussed, whereas the stormwater runoff generated in June 18 stimulated growth, the runoff after rainfall in July 1 caused growth inhibition to *S. subspicatus*. The differences observed in the magnitude and nature of *S. subspicatus* responses – from growth stimulation to inhibition – when comparing both rain events, might have been influenced by the hydrological factors (rain intensity, precipitation heights, antecedent dry period and duration) and the stormwater runoff physico-chemical characteristics. Possible relationships between *S. subspicatus* responses, storm characteristics and chemical pollutant concentrations were evaluated based on the hydrographs and pollutographs (COD, pH and conductivity) presented in Fig. 4.

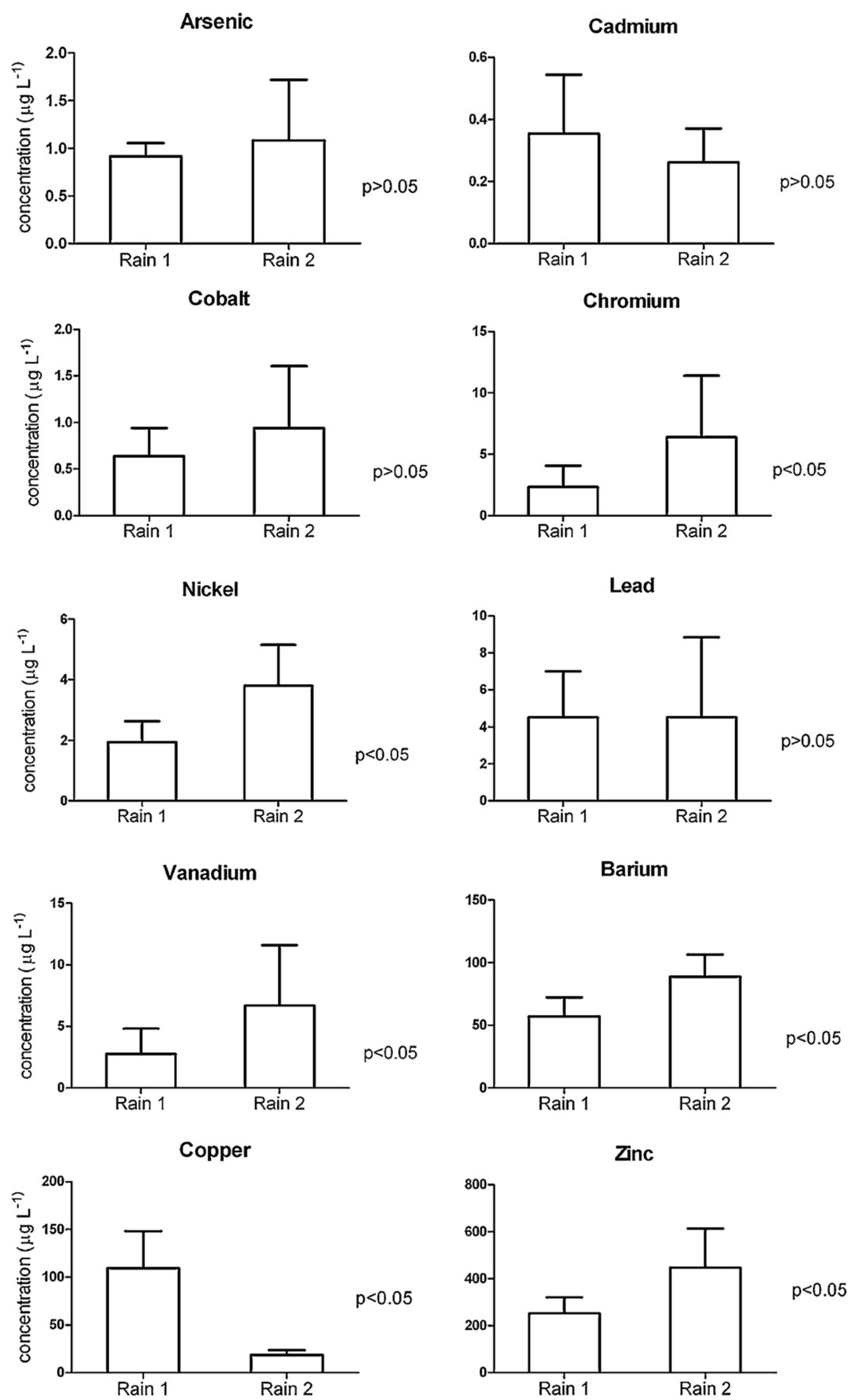

Fig. 6. Average ± SD of various metals concentrations detected in the runoff sample during (a) rain event 1 ($n = 10$) and (b) rain event 2 ($n = 5$).

Significant differences (t-test, $p < 0.05$) observed between metals concentrations, pH and conductivity of the two runoff events, might explain the opposite responses of the test organisms. According to [7] acute toxicity of wood leachates is usually attributed to low pH, that might be a consequence of the presence of wood-related weak organic acids such as tannins and lignins [5]. Fig. 4c shows that the pH of the runoff from July 1 was significantly lower ($p < 0.05$) suggesting that to some extent, low pH might have been responsible to the observed *S. subspicatus* growth inhibition. Moreover, whereas [8] state that toxic effects of some metals increase as pH decreases, [17] observed that growth of a specific strain of the microalgae *Scenedesmus acutus* was considerably affected by the pH, with 50% of growth reduction observed at pH 5.05. Increased toxicity with lower pH′s might be related to an increase of metals solubility and bioavailability of prevailing free metal ions [18].

Besides the differences observed regarding pH values, the runoff waters also differed in metal concentrations. The concentrations of metals such as Cr, Ni, V, Ba, and Zn were significantly higher (*t*-test; $p < 0.05$) in the rain event of July 1, with the exception of Cu ($p < 0.05$) (Fig. 6). The toxic effects due to metal contents in wood-related areas are reported in the literature by [5,6]. The presence of divalent cations, particularly Zn has been reported as the main causes of toxic effects towards juvenile *Oncorhynchys mykiss* (rainbow trout) from runoff waters generated by different sawmills in British Columbia [5,6]. Furthermore, according to [19] metabolic processes such as photosynthesis have been shown to be very sensitive to the presence of toxic metals.

A correlation analysis was carried out to evaluate possible relationships among the different metals discharged within each single rain event. Different metals were positively correlated to each other in the rain event of July 1 (r: 0.694–0.997). However, in the rain event of June 18, some metals were negatively correlated (Cd negatively correlated to Pb and Ni), and as some metals increase, others decrease. Strong association among different metals together with higher concentrations in the runoff of July 1 might explain the observed inhibitory effects on *S. subspicatus* growth.

Other aspects that could have contributed to the responses from *S. subspicatus* are the hydrological factors involved in the rainfall-runoff process such as storm duration, precipitation heights and average rainfall intensity. Whereas 3 mm of precipitation was recorded during 115 min in June 18 (average intensity of 1.56 mm/h), only 1.25 mm was recorded during 45 min in July 1 (average intensity of 1.66 mm/h). Lower amounts of stormwater, but a nearly similar rainfall average intensity during the event of July 1 might be the cause of higher concentrations of metals and H^+ detected within the runoff water. Assuming that similar rainfall intensities as observed, are able to wash-off the log-yard the same mass of contaminants; higher concentrations will be detected in lower amounts of water, posing then higher toxic effects.

Similar results concerning the roles played by hydrological aspects over the toxic effects of runoff have been reported by [2]. The authors also observed that at the same rain intensities, rainfall duration and degrees of toxic effects were inversely related.

4. Conclusions

The runoff originated from two different rain events over the same log-yard area posed different effects on the freshwater, green microalgae *S. subspicatus*. Whereas the runoff of the first rain event had no negative effects to *S. subspicatus* posing in most cases stimulatory effects, the runoff from the second rain event inhibited algae growth. The differences in runoff physico-chemical characteristics combined with the hydrological factors (precipitation and duration) of each rain event explained the opposite effects on *S. subspicatus* observed during the current investigation.

Despite the lack of negative effects to *S. subspicatus* growth, stimulatory effects posed by the first runoff event indicate that the necessity of treating these waters before discharging into natural recipients can not be ruled out since the stormwater runoff might contribute to environmental impacts such as algae blooming and eutrophication.

The intra-storm magnitude and variability of the inhibitory effects posed by the 2nd runoff event confirmed the hypothesis of toxic first flush phenomenon. The evaluation on a normalized basis showed that 42, 51 and 50% of the inhibitory effects during exposures of 24, 48 and 72 h are associated with the initial 4% of the total discharged volume and 13% of the storm duration.

To conclude, the current investigation brings a better understanding of a subject that has not been widely explored: the intra-storm magnitude and variability of the potential environmental effects posed by log-yards runoff. Toxicity studies can provide useful information for an appropriate design and implementation of stormwater runoff management strategies. The fact that negative effects were observed in the two runoff events analyzed in this study, raises concern about the potential environmental threats posed by runoff originated from wood-based industrial areas during the entire hydrological year.

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