Enhanced chemiluminescence as an indicator of air pollution

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Abstract

Investigations were conducted to determine the pollution levels of air condensate and rain samples using the enhanced chemiluminescence (ECL) reaction. Air condensate samples were initially collected from roadside sites, non-roadside sites, smoking-exposed sites and non-smoking-exposed sites using an automatic dew collector. Rainwater was also collected throughout the sampling period. Samples were subsequently analysed using the ECL reaction, and pollution levels (Eclox Units) were calculated. Air condensate samples collected from roadside- and smoking-exposed sites generally had higher pollution levels than those from non-roadside- and non-smoking-exposed sites. Samples from both smoking-exposed and traffic-exposed sites generally had higher pollution levels than rainwater. Finally, in samples collected from roadside-exposed sites and rainwater, the main polluting constituents were determined to be cationic/anionic components. In samples collected from smoking-exposed sites, the main polluting constituents were determined to be organic components.

Introduction

Enhanced chemiluminescence (ECL) reactions have well-established clinical and analytical applications. The reaction is based upon the oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in the presence of the enzyme horse-radish peroxidase (HRP) and involves free radicals, derived from luminol and oxygen (Misra & Sauamito 1982; Kricka & Thorpe 1990) (Fig. 1). A high-intensity, prolonged and stable light emission profile can be obtained from this reaction at pH 8.6, by the inclusion of an enhancer such as 4-iodophenol (Thorpe et al. 1985; Thorpe & Kricka 1987).

Contaminants interfere with light emission from the reaction by several mechanisms. Radical scavengers (chain-breaking anti-oxidants), competitive and non-competitive enzyme inhibitors and the many compounds that act as substrates for HRP, can alter the enzyme activity or influence the reactions leading to reduced light emission. A variety of substances will influence the light emission from an ECL reaction, and the adaption of the ECL test to water quality monitoring has shown that pollutants in domestic sewage or industrial effluence may suppress or completely inhibit light emission (Whitehead & Thorpe 1990; Billings et al. 1994). The ECL test has been shown as a potential routine screening method for detecting such pollutants in watercourses (Sawcer 1999). However, it is hypothesised that the ECL test may be a suitable screening method for the detection of air pollutants within air condensate samples. Air condensate samples may contain fine particles that are harmful for human health and environment. This represents a new approach to air pollution monitoring; the collection of air condensate samples and their subsequent analysis using the ECL test could provide rapid identification of air pollution hot spot in comparison with traditional air sampling techniques.

This aim of this study was to determine air quality at different sample sites by collecting rain and air condensate samples and performing the ECL test on them. Furthermore, samples were characterised with respect to their anionic/cationic and organic content. Samples were exposed to different anionic/cationic and organic extractants and the effect on the light emission profile of the ECL reaction was subsequently investigated (Whitehead et al. 1992; Gebicka & Gebicki 1998).

Materials and methods

Instrumentation and materials

A Bio-orbit 1250 luminometer (Thermo Electron Corperation, Waltham, MA, USA) was used to measure the ECL reaction light emission which is maximal at 425 nm. The kinetics of light emission were recorded on a chart recorder and a computer. The ECL water quality kit ECLOX (Aztec reagents) was obtained from Capital Controls Ltd (Sutton Coldfield, UK).
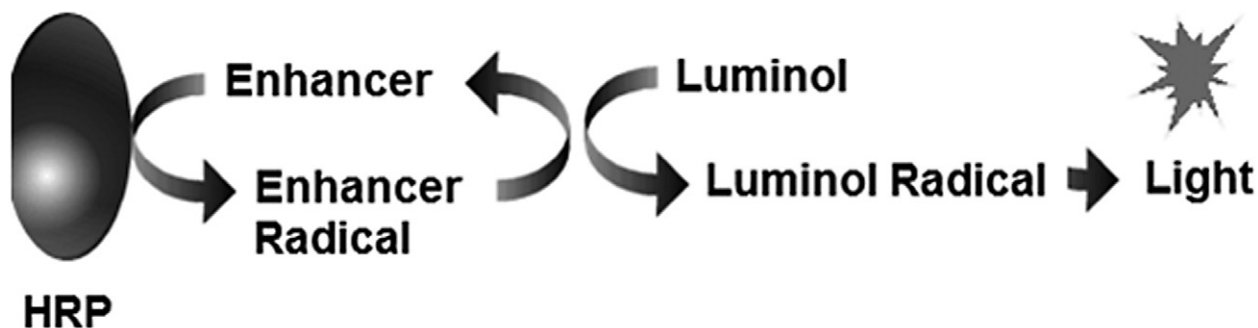
Fig. 1. The enhanced chemiluminescence reaction.

Fig. 2. The automatic dew collector.

Reagents were prepared according to the manufacturer's instructions, and fresh deionised water was used for reagent preparation and control samples.

Air sampling equipment

The automatic dew collector (Fig. 2) (designed at the Wolfson Applied Research Laboratory, University of Birmingham) was a portable, battery-powered machine used to collect air condensate samples. The automatic dew collector consisted of a battery, connected to a peltier (heat exchanger). A condensation plate was present on the front side of the peltier; a heat plate and fan were present on the reverse. The application of a current from the battery, across the peltier, caused a reduction in temperature of the condensation plate and an increase in temperature of the heat plate. Excessive heat was removed from the heat plate by the fan. If a sufficient voltage was applied across the peltier, a temperature would be obtained on the condensation plate that would be below the dew point of the ambient air. This would result in the formation of air condensate on the condensation plate that subsequently dripped into a sample collector.

Two automatic dew-collecting machines were employed for air condensate collection at two different locations throughout the same time period. Before sampling, the condensation plate on each machine was cleaned with deionised water and dried by removing water droplets by paper tissue absorption. A clean glass bottle, which had first been washed with deionised water and subsequently dried, was placed under the condensation plate. A temperature sensor was connected to the condensation plate and a second measuring device was used to determine the dew point of the air to be sampled. When sampling, the equipment was switched on and the voltage control of the peltier was set up to give a condensing plate temperature of approximately 5°C below the dew point and the apparatus was left running for approximately 3 h.

ECL procedure

HRP type XII (500 units, Sigma-Aldrich, Dorset, UK) was diluted into 10 mL of phosphate buffered saline/bovine serum albumin (5 mg/mL) buffered at pH 7.6, aliquoted and stored at −20°C. Before use, an aliquot of this stock solution was left to defrost for 10 min. From this stock solution, a working dilution of HRP was prepared fresh each day by diluting 20 μL of the HRP stock solution with 2.5 mL of deionised water. ECL signal reagent was first prepared by adding 60 mg of reagent A and 60 mg of reagent B to 30 mL of signal reagent buffer.

Sequentially, 600 μL of deionised water and 400 μL of sample were pipetted into a cylindrical reaction cuvette. One-hundred microlitres of signal reagent was subsequently added, and the ECL reaction initiated with 20 μL of HRP. The reaction cuvette was shaken by hand for approximately 2 s and subsequently placed in the luminometer. The intensity and kinetics of light emission were recorded for 4 min from the moment the glowing sample was rotated into the enclosed measuring position of the luminometer. The percentage of light inhibition induced by the contaminated sample was measured by subtraction of the computer-generated integration values under the emission curves over

the 4-min period from that measured for control samples. The level of pollution of the samples was subsequently calculated by multiplying the percentage inhibition by the total working volume and then dividing by the sample volume to provide a measure of pollution (Eclox Units). Eclox Unit values between 1 and 20 typically represented a low level of contamination and are indicative of good-quality water; however values between 21 and 50 typically represent a medium level of contamination and are indicative of poor quality water. Eclox Unit values of 51 to 100 represented a high level of pollution; such levels are found within industrial effluent. Finally, the ECL reaction has different sensitivities to different pollution constituents. For example, sensitivity is in the range of 1 to 5 p.p.m. for metal ions (copper, mercury, lead) and 0.001 to 0.1 p.p.m. for organic components (uric acid, phenol) (Sawcer 1999).

Samples were analysed in triplicate and qualitative comparison of light output curve shapes also indicated differences in sample composition. Subsequently, rain samples and air condensate samples were exposed to organic (Dichloromethane), cationic (Diaion CR11 exchange resin) and anionic (Amberlite IRA 400) extractants. After samples were exposed to each extractant, the percentage inhibition of the ECL reaction was calculated to determine the amount of light recovery (and removal of pollutants) of the samples. Statistical analysis of the Eclox Units of roadside-/non-roadside-exposed samples and smoking-/non-smoking-exposed samples, were compared using one-way analysis of variance statistics.

Air sampling sites and sample transportation

Rain samples were collected at the Wolfsen Applied Technology Laboratory (University of Birmingham) between the 13/7/2004 and 28/7/2004. Samples were collected in a clean 50 mL glass beaker every 24 h; the beaker was subsequently cleaned and returned to its outdoor position.

Air condensate samples were collected in the same time period (3 h) between two different sample sites using two different dew-collecting machines. Air condensate samples were initially collected from a roadside site directly exposed to traffic-related pollution (A38 Bristol Road) and a non-roadside site, not directly exposed to traffic-related pollution (The Winterbourne Gardens) between 3/6/2004 and 2/7/2004. Each sampling site is shown in Fig. 3. Furthermore, air condensate samples were collected from a site potentially exposed to smoking-related pollution (Staff House – University of Birmingham Public House) and a site where smoking was prohibited (corridors within the University of Birmingham) between the dates of 3/7/2004 and 28/7/2004.

Samples for ECL analysis were collected in 15 mL screw-cap glass bottles which were filled by insertion under the condensation plate of the automatic dew collector. After collection, samples were kept in an insulated cool box with an ice pack for 1 to 3 h then returned to the laboratory where they were refrigerated upon arrival and analysed within 4 days of collection. Sampling stability tests were carried out as described by Sawcer (1999).
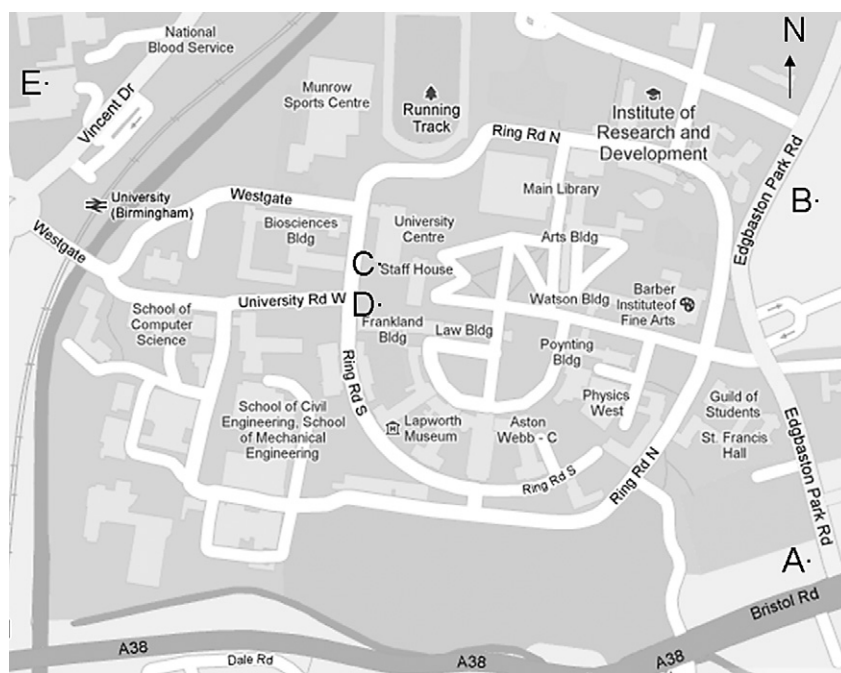

Fig. 3. (a) Roadside environment, (b) non-roadside environment, (c) smoking-exposed environment, (d) non-smoking environment and (e) rain sampling site.

Results and discussion

The results in Fig. 4 show the light intensity (%) of the ECL reaction light emission when exposed to samples from different environments (collected on 1/5/2004) as well as deionised water. The results show that there was reduced light intensity (and greater inhibition of the ECL reaction) from samples, initially collected from smoking-exposed and roadside environments (with a mean light intensity of 21.2 and 34.5% respectively), in comparison with the light intensity from samples exposed to non-smoking and non-roadside environments (with a mean light intensity of 51.9 and 70.6% respectively). Furthermore, reduced light intensity (46.4%) of the ECL reaction was measured in samples of rainwater when compared with deionised water. However, the kinetics of the ECL reaction light emission profile for rainwater samples were different to those of samples collected from either smoking-exposed or roadside environments. These results indicated that the nature of the pollution constituents present with in rainwater may be very different in comparison with the other samples. Finally, results also indicated that there was a larger amount of pollution in samples obtained from smoking-exposed/roadside environments and rainwater in comparison with non-smoking-exposed/non-roadside environments and deionised water.

The results in Fig. 5 show that when an air condensate sample obtained from a roadside environment had organic pollutants extracted the mean light intensity of the ECL reaction increased (by 5.3%). Furthermore, removal of cationic and anionic pollutants also caused a further increase in the mean light intensity of the ECL reaction (21.5 and 9.2% respectively). These results indicate that cationic and anionic pollutants contributed to the largest amount of pollution within this sample, as their removal resulted in the greatest increase in the ECL reaction light intensity. The results also correlate with previous roadside air pollution studies carried out in Birmingham (UK), where a variety of inorganic elements (Cu, Zn, Mo, Ba and Pb) together with soluble ions such as SO_4^{2-}, NO_3^- Br^-, Cl^- and NH_4^+ had been identified within samples and could have an impact on public health with particular respect to childhood asthma (Colvile & Hutchinson 2001; Harrison & Deacon 2003; Godri *et al*. 2011).

Similar results were obtained for rainwater samples, as shown in Fig. 6; removal of organic pollutants increased the mean light intensity of the ECL reaction by 6.2%. Removal of cationic and anionic pollutants caused a further increase in mean light intensity by 19.6 and 10.7% respectively.

When cationic and anionic pollutants were removed from smoking-exposed air condensate samples, mean light intensity of the ECL reaction increased by 12.2 and 10.5%
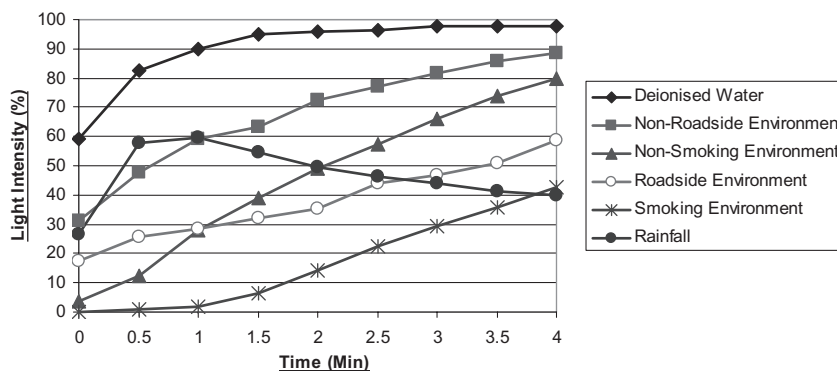

Fig. 4. Light intensity (%) of the enhanced chemiluminescence reaction light emission within air condensate samples from different environments.
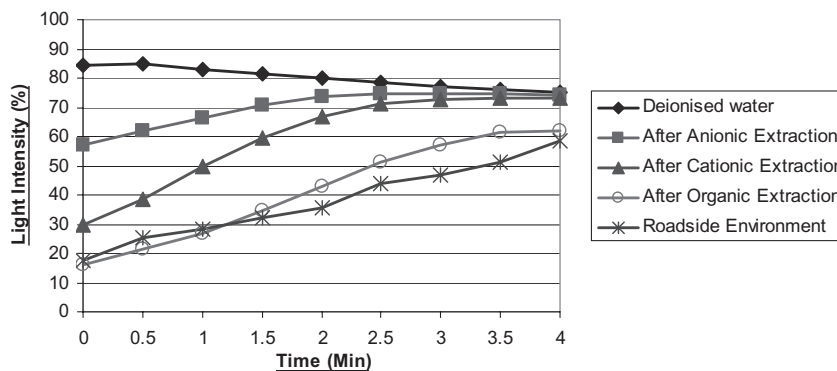

Fig. 5. Light intensity of the enhanced chemiluminescence reaction exposed to a roadside-exposed air condensate sample and subsequently organic extraction and cationic/anionic extraction.
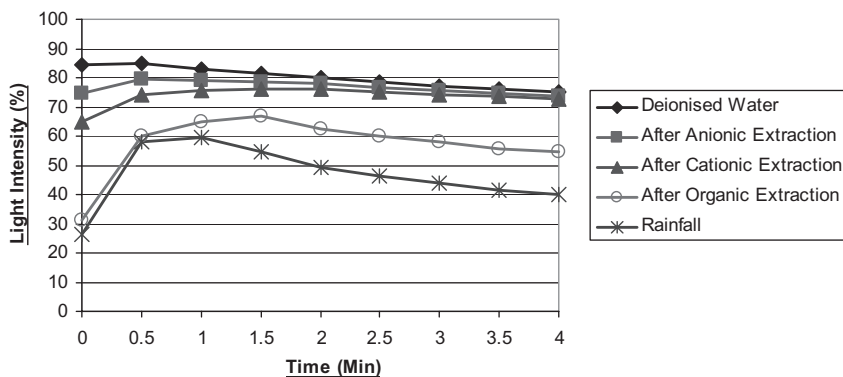
Fig. 6. Light intensity of the enhanced chemiluminescence reaction exposed to a rainfall sample and subsequently organic extraction and cationic/anionic extraction.
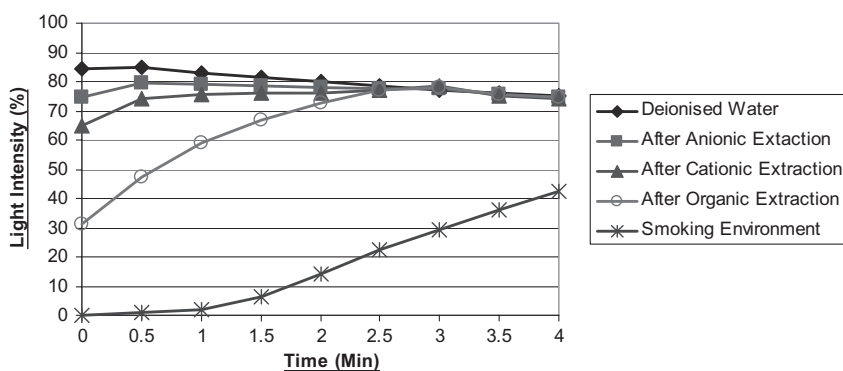

Fig. 7. Light intensity of the enhanced chemiluminescence reaction exposed to a smoking-exposed air condensate sample and subsequently organic extraction and cationic/anionic extraction.

respectively (Fig. 7). Removal of organic pollutants resulted in an increase in mean light intensity by 23.6%. These results indicated that organic pollutants contributed to the largest amount of pollution within these samples. Environmental tobacco smoke has previously been characterised as a complex mixture of over 4000 compounds that include more than 40 known suspected human carcinogens such as n-nitrosamines, benzene, ammonia and a variety of polycyclic aromatic hydrocarbons. These pollutants have raised particular concern with respect to their role in second-hand smoke-related public health issues (Leaderer & Hammond 1991; Luceri 1993; Jinot & Bayard 1994; Gordon *et al*. 2011).

Air condensate samples were subsequently collected from roadside-exposed/non-roadside-exposed and smoking-exposed/non-smoking-exposed sites over a 12-day period respectively. Rain samples were also collected over a 15-day period. The results in Fig. 8 show that roadside-exposed air condensate samples generally had higher Eclox Unit values (indicating higher pollution levels) than non-roadside-exposed samples throughout the 12-day period; increased pollution levels may therefore be attributed to traffic-related emissions. Furthermore, Eclox Unit values for roadside-exposed samples were significantly ($P < 0.05$) higher than that of non-roadside

samples on days 3, 6, 7, 8, 11 and 12 of the sampling period.

The results in Fig. 9 show that smoking-exposed air condensate samples also generally had higher Eclox Unit values (indicating higher pollution levels) than samples collected from non-smoking environments throughout the 12-day period. Increased pollution levels within smoking environments may therefore be attributed to tobacco-related emissions. Eclox Unit values for smoking-exposed air condensate samples were significantly ($P < 0.05$) higher than that of samples from non-smoking environments between days 3 to 9 and day 11 of the sampling period.

Results in Fig. 10 show that there was variance in the Eclox Unit values (with a mean Eclox Unit value of 22.5) of rain samples, collected over a 15-day period. However, roadside-exposed air condensate samples had a higher mean Eclox Unit value of 47.8, indicating higher levels of pollution in these samples, in comparison with the rain samples. Finally, smoking-exposed air condensate samples had the highest mean Eclox Unit value of 52.8, indicating that pollution levels were generally higher within these samples in comparison with roadside-exposed air condensate samples and rain samples.

Fig. 8. Pollution levels (Eclox Units) of air condensate samples collected at roadside- and non-roadside-exposed sites over a 12-day sampling period. *Significant difference in roadside sample Eclox Unit value compared with non-roadside sample Eclox Unit value ($P < 0.05$). Error bars show +/– standard deviation and sample number = 3.
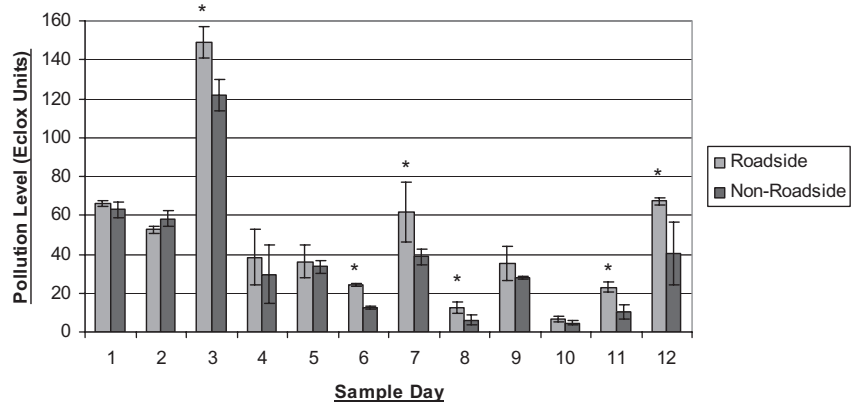

Fig. 9. Pollution levels (Eclox Units) of air condensate samples collected at smoking-exposed and non-smoking sites over a 12-day sampling period. *Significant difference in smoking sample Eclox Unit value compared with non-smoking sample Eclox Unit value ($P < 0.05$). Error bars show +/– standard deviation and sample number = 3.
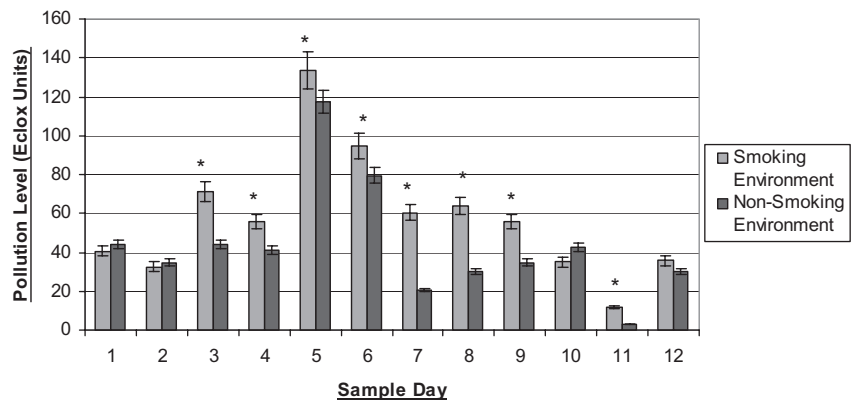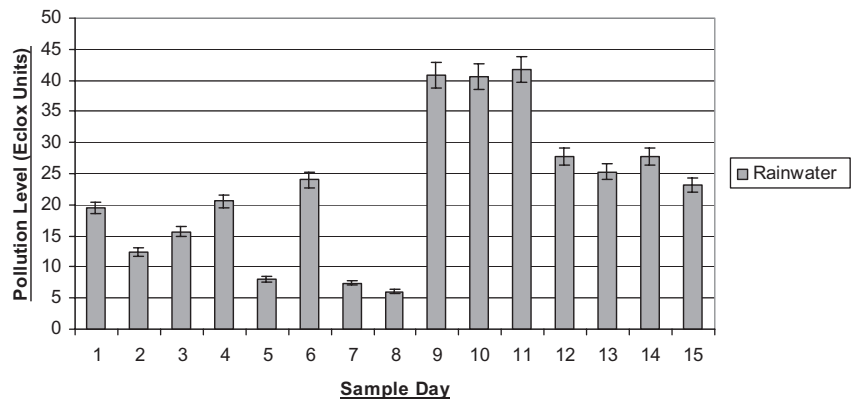

Fig. 10. Pollution levels (Eclox Units) of rainwater collected over a 15-day sampling period. Error bars show +/– standard deviation and sample number = 3.

Conclusions

(1) The automatic dew collector apparatus is suitable for the collection of air condensate samples from a variety of different locations.

(2) Analysis of pollution within air condensate samples using the ECL reaction is able to be carried out; the rapid, reliable and inexpensive test can now potentially be employed in the

identification of atmospheric pollution hot spots as well as those of watercourses.

(3) Air condensate samples collected from smoking-exposed environments, generally had higher Eclox Unit values (and thus higher pollution levels) than sites not exposed to tobacco smoke.

(4) Air condensate samples collected from road traffic-exposed environments generally had higher Eclox Unit

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values (and thus higher pollution levels) than sites not directly exposed to road traffic.

(5) Smoking environments also generally had higher pollution measurements (Eclox Units) than traffic-exposed environments; both sites also had higher pollution measurements than rainwater.

(6) In air condensate samples obtained from road traffic-exposed environments, the majority of light inhibition of the ECL reaction was recovered after samples were exposed to cationic/anionic exchangers. These results indicated that cationic and anionic species contributed to most of the pollution with these samples.

(7) In air condensate samples obtained from smoking-exposed environments, the majority of the light inhibition of the ECL reaction was recovered after samples were exposed to organic extractants; these results indicate that organic species contributed to most of the pollution within these samples.

(8) In rain samples, large fluctuations were measured in pollution levels. The majority of the light inhibition of the ECL reaction was recovered after samples were exposed to cationic/anionic exchangers. These results indicated that cationic and anionic species contributed to most of the pollution with these samples; however, the kinetics of the ECL reaction light emission profile indicated that the nature of these pollutants may be different to those within air condensate samples.

Acknowledgements

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