Toxicity Assessment of a Former Manufactured Gas Plant

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Contaminated groundwaters and soils contain a complex mixture of chemicals. Their characterization provides a qualitative and quantitative approach to understanding groundwater and soil geochemistry. However, this does not account for the complex chemical interactions (synergistic, antagonistic and additive) that occur in such heterogeneous systems (Boyd et al. 1997; Dewhurst et al. 2002). Chemical characterization is also restricted by detection limits, and, cannot indicate the toxicity of complex pollutant mixtures. In contrast to this, toxicity testing does not reveal information on the specific contaminants present in the groundwater, however, its relatively low cost and rapidity in comparison to full chemical characterization means that it can be used as a rapid "environmental screening system" (Boyd et al. 1997). This provides a convenient means to prioritize samples for mandatory chemical analysis.

Traditional approaches to toxicity testing employ a range of organisms (invertebrate, alga, and a fish species) and are time consuming and costly. Consequently, the employment of rapid diagnostic methods is becoming more widespread (Wells et al. 1998, Mowat and Bundy, 2001; Cotou et al. 2002; Dewhurst et al. 2002). The Microtox™ bioassay (Azur Environmental Ltd., Wokingham, U.K.) employed here, evaluates the response of the luminescent marine bacteria *Vibrio fischeri* following the addition of an unknown environmental sample (Bulich, 1979). The degree of light loss shown by the organism is indicative of toxicity, which in turn can be expressed as an EC_{50} value, i.e., the concentration of the tested material that reduces *Vibrio fischeri* light emission by 50%. This toxicity bioassay was used to determine the acute toxicity of groundwaters and soils obtained from a former Manufactured Gas Plant situated in Portadown, Northern Ireland. Potential correlations between toxicity, and chemical characterization data, or total microbial counts were investigated. The validity and value for employing the toxicity bioassay in the assessment of polluted industrial and brownfield sites, prior to more complex chemical analysis, is described here.

MATERIALS AND METHODS

Groundwater and soil samples were obtained from a former Manufactured Gas Plant in Portadown, Northern Ireland, (Ordnance Survey Sheet 200NE, 3016-3543) (Figure

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1). Geological and hydrogeological data has been described previously (Doherty et al. 2001). Multi level boreholes (BH) and trial pits (TP) were positioned at various locations as shown in Figure 1. Two minor aquifers within a complex alluvial - periglacial sequence were identified. Prior to groundwater collection from the boreholes, three borehole volumes were purged using a peristaltic pump (Watson Marlow 505S) and allowed for representative groundwater sample collection. Groundwater samples from BH and TP were collected in acid washed 500ml glass bottles (Merck Ltd., U.K.), while surface soil samples, 20cm below surface, were collected in 500ml polypropylene containers. Samples were stored at 4°C during transportation to the laboratory and storage. Analysis of samples was carried out within 24hrs of collection using the Microtox[TM] Model 500 Analyzer (Azur Environmental Ltd., Wokingham, U.K.). The toxicity of the groundwater and soil samples was determined in accordance with the protocols outlined in the Microtox[TM] users manual (Azur Environmental Ltd., 1998). Three different protocols were used (i) Microtox[TM] protocol for Basic Test (ii) Microtox[TM] protocol for extended dilution range Basic Test, used for groundwater sample analysis; and (iii) Microtox[TM] solid-phase test, for soil samples. The Microtox[TM] protocol for extended dilution range Basic Test was only employed for analysis of BH 8-10 groundwater due to the high colouration of the sample. *Vibrio fischeri* luminescence was measured after 5 mins exposure with the unknown environmental sample. Additional experimentation where luminescence was measured after 15 mins revealed no significant difference in sample toxicity (data not presented). Full chemical analysis (UKAS accredited laboratory) of environmental groundwater and soil samples, taken from all BH and TP positioned at various depths across the site, was carried out (Doherty, 2002).

Enumeration of microorganisms was carried out on R2A plates (OXOID Ltd.). Soil slurries (20g in 100ml sodium pyrophosphate, pH7.0) were shaken at 200 rpm for a period of 20 mins. Serial dilutions (10^0-10^{-6}), prepared in $^1/_4$ strength Ringers solution (pH7.0) and carried out in triplicate, were incubated (25 °C) for a period of 10 days.

RESULTS AND DISCUSSION

Twenty-eight surface soil samples, obtained from trial pits positioned across the former Manufactured Gas Plant site, were analysed for toxicity. Figure 2 shows the overall distribution of toxicity (EC_{50}) across the site. Historical evidence (Figure 1) revealed prime hotspots for sampling, for example areas around the tar well (TP 30), purifiers (TP 35), furnace chimney (TP 6), and the waste trench positioned in the top right corner of the site (TP 10, TP 36, TP 38, TP 41). Toxicity at these locations was extremely high, ranging from 0.217 % w/v (EC_{50}) for an area previously used for waste dumping (TP 36), to 2.245 % w/v (EC_{50}) for an area where the furnace chimney was formerly positioned (TP 6). The high toxicity observed in the area surrounding the furnace chimney was attributed to the distribution of clinker ash formed during processing. The Microtox[TM] solid-phase test provides a ranking system for contaminated samples, and consequently, trends in toxicity data across a site are evident, only through comparison with concurrent sample points. In relation to our study site, "low" toxicity measurements in surface soils (22.11 % w/v (EC_{50}) TP24) were only apparent through comparison with alternate areas of "high" toxicity (0.217 % w/v (EC_{50}) TP36). Figure 3 showing total organic carbon and Figure 4 showing total cyanide contamination within the TP and BH groundwater give an indication of the widespread spatial distribution of the organic and inorganic contamination across
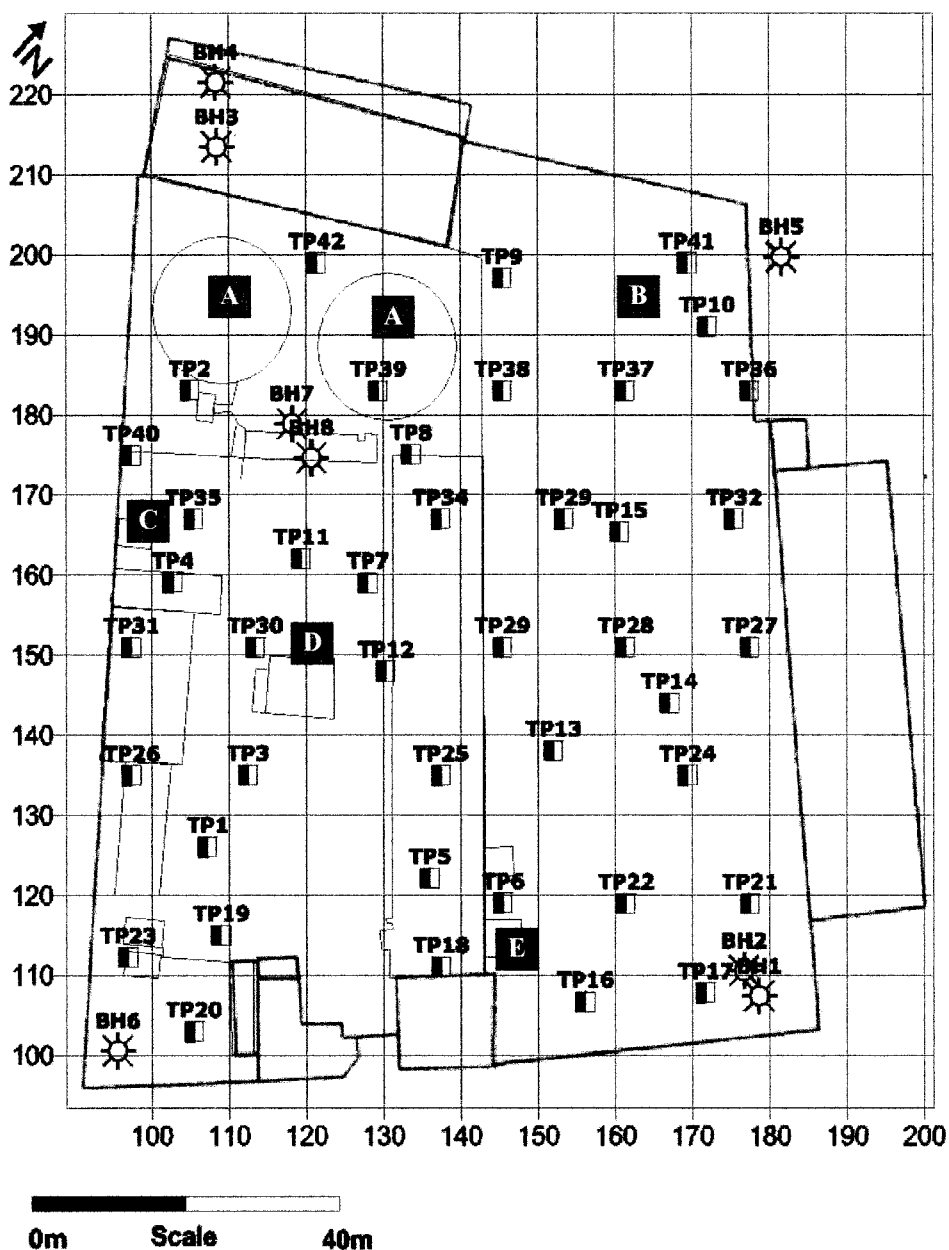
Figure 1. Trial pit (TP) and borehole locations (BH) across the former manufactured gas plant site. Location of original site structures: A- gas holding tanks, B- waste trench, C- purifiers, D- tar well, E- furnace chimney.
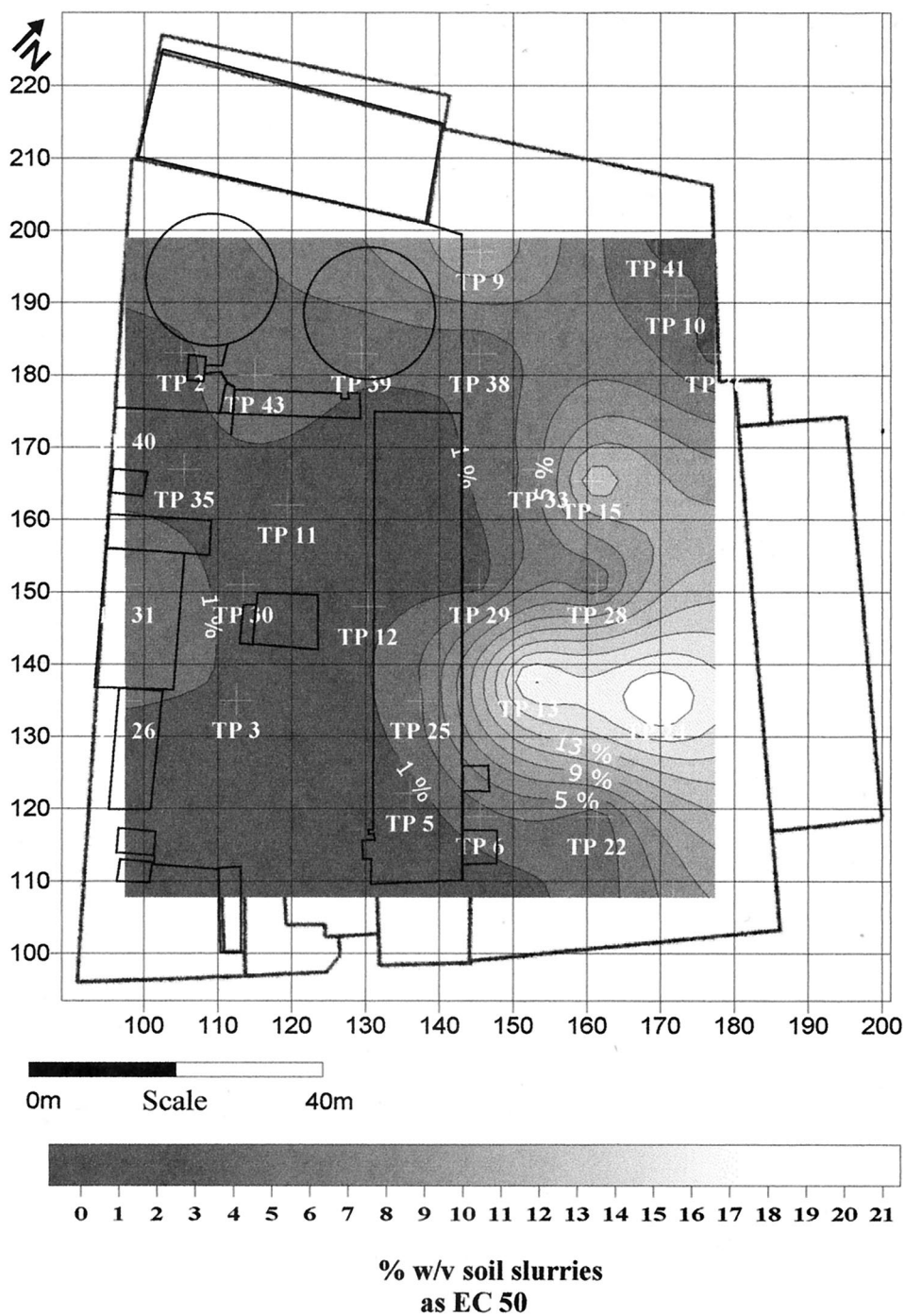
Figure 2. Level of toxicity for surface soil samples across the former manufactured gas plant site, as measured by the Microtox[TM] solid-phase test.
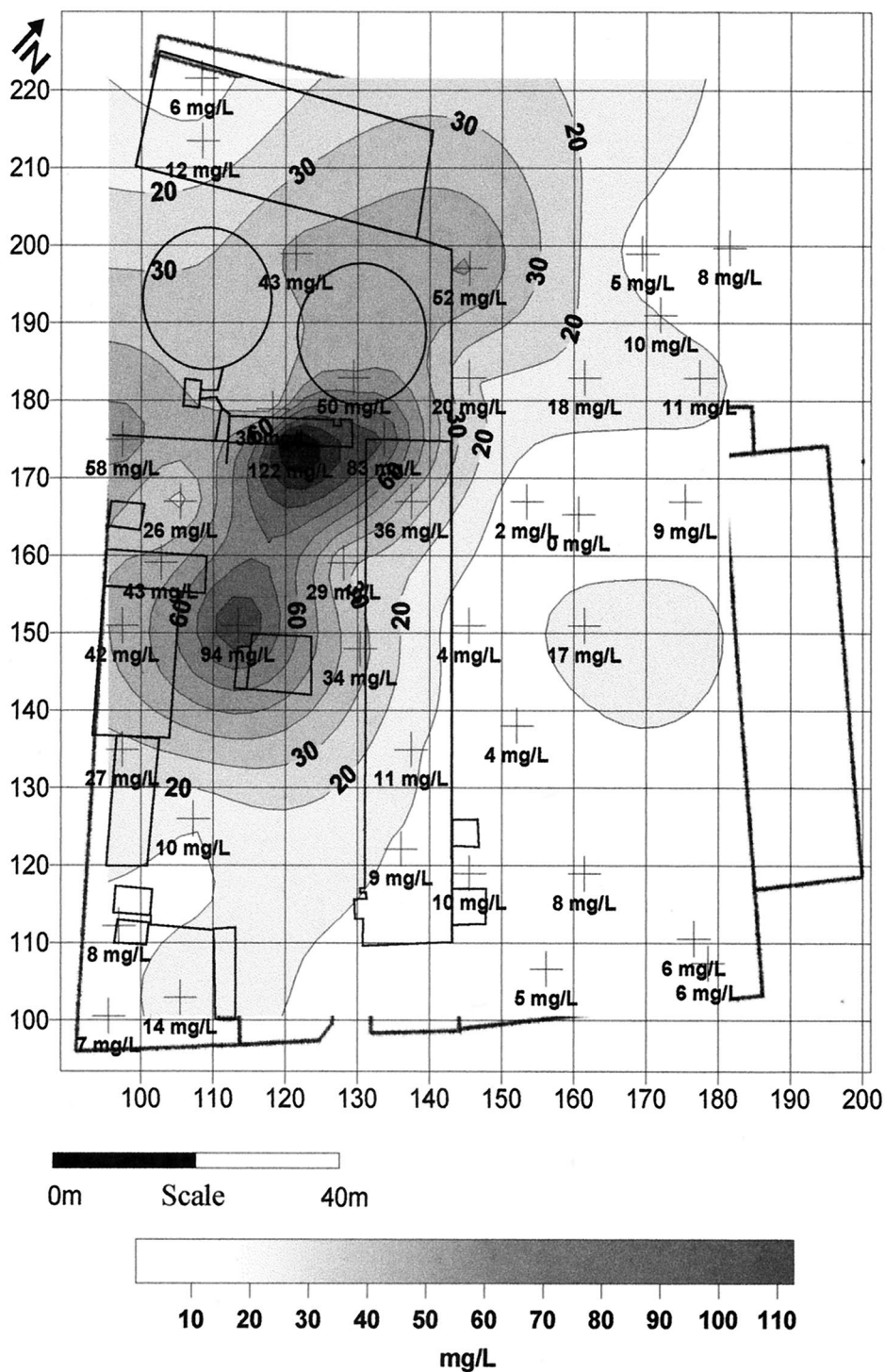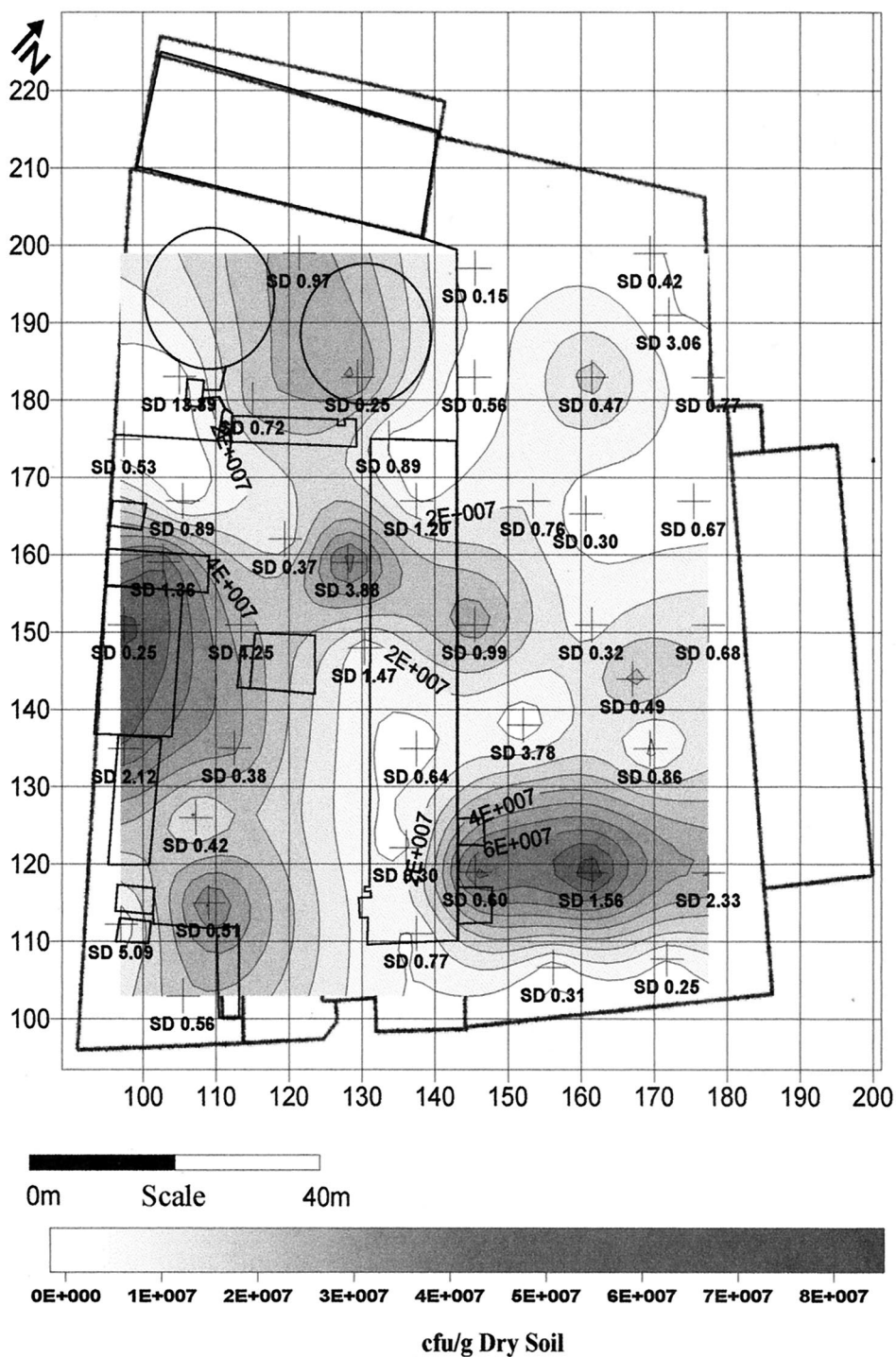
Figure 3. Level of total organic carbon for groundwater across the former manufactured gas plant site.

Figure 4. Level of total cyanide for groundwater across the former manufactured gas plant site.

Figure 5. Distribution of total viable microbial counts (expressed as colony forming units (cfu/g dry soil) on R2A medium) across the former manufactured gas plant site. Standard Deviation (SD) given in brackets.

the site. When spatial distribution of these indicator plumes are combined the only area generally unaffected correlates with the toxicity data in Figure 2. Indeed, low toxicity in relation to the majority of the Manufactured Gas Plant site was only observed in areas previously left as open space. EC_{50} values for this area were 19.76 % w/v and 22.11 % w/v for TP 13 and TP 24 respectively. Comparison of the toxicity profile with the chemical characterization data obtained for the site (Doherty 2002) revealed that the distribution of polluting compounds across the site was homogeneous with site toxicity.

Microbial viable counts were carried out on surface soil samples, obtained from forty-three trial pit locations (Figure 5). High variation and distribution in microbial numbers across the site was apparent (4.5×10^5 to 8.7×10^7 cfu/g dry soil), with the existence of areas with sparse microbial life. In contrast to an apparent correlation between toxicity and chemical characterization data, no relationship was observed between microbial viable counts and site toxicity (correlation coefficient (r) = 0.052). Indeed, any correlation between microbial numbers and toxicity was not expected due to the methodological basis of the toxicity test. The Microtox[TM] assay employs a sole microorganism (*Vibrio fischeri*) in the determination of toxicity, and consequently, is unable to account for the high variation in toxic chemical tolerance expressed by numerous microorganisms or the bioavailability of nutrients essential for their proliferation.

Table 1. Toxicity of groundwater samples as measured by the Microtox[TM] protocol for basic test

Borehole	Depth of sampling port (mAOD)	EC_{50} (%) v/v	95% Confidence limit (%)
1-2	13.69	100	-
2-1	10.88	100	-
2-2	13.40	100	-
2-3	15.93	100	-
7-1	15.62	3.19	2.04-4.99
7-4	13.42	3.23	2.77-3.77
7-7	12.27	3.78	2.59-5.52
8-1	16.56	100	-
8-2	16.01	30.73	29.06-32.49
8-10*	12.06	0.29	0.28-0.30

*Determined using the Microtox[TM] protocol for extended dilution range basic test

Toxicity assays were carried out on a number of groundwater samples obtained from boreholes positioned across the former Manufactured Gas Plant site (Figure 1, Table 1). Groundwater samples, at any depth, obtained from BH 1 and BH 2 caused no inhibition in the luminescence of *Vibrio fischeri*. The results were further validated through comparison with the chemical characterization data, showing no polluting compounds above Dutch intervention levels (Doherty 2002). This also correlated with groundwater flow modelling, suggesting that groundwater enters the site at this point and flows around the foundations of the gasholding tanks (Doherty et al. 2001). As groundwater flowed across source contaminant areas an increase in toxicity was observed in groundwater samples. Due to the positions of BH 7 and BH 8 in relation to the contaminant plume that flows around the foundations of the gasholding tanks,

differences in toxicity values were expected. BH 7 (5m depth) is relatively shallow in comparison to BH 8 (8m depth) and is located on the fringe of the contaminant plume whereby BH 8 is situated in the centre. The toxicity of groundwater samples obtained from BH 7 at all depths was uniform and suggested that contamination was not restricted to one depth. This result was attributed to contaminant transport from a source area of contamination at depth (the tar well). In relation to BH 8 the toxicity profile is more heterogeneous. A reduction in *Vibrio fischeri* luminescence was not apparent following exposure to BH 8-1 groundwater (the shallowest sampling point). This result was unexpected due to the location of the borehole in relation to the contaminant plume and although correlated well with chemical characterization data, suggested that the majority of plume was located in the identified lower aquifer. The toxicity of groundwater obtained from BH 8-10 was significantly higher then all other samples analysed (0.29 % v/v (EC_{50})) and in conjunction with the chemical characterization data further corroborated the location of the plume and depth of source contamination from the tar well.

Toxicity testing provides a highly sensitive approach to determine the condition of environmental sites. This paper illustrates an apparent correlation between toxicity and chemical charaterization data and consequently provides compelling evidence for the employment of such ecotoxicological techniques in the determination of contaminant hotspots. The rapidity of both sample preparation and analysis, in comparison to complex analytical techniques, allows for the reduction in sample time and also the associated costs. We conclude that toxicity testing provides an ideal assay for initial site investigations.

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