International Journal of Geology, Earth & Environmental Sciences ISSN: 2277-2081 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jgee.htm
2017 Vol. 7 (2) May-August, pp. 63-69/Kumar et al.
Research Article

DISTRIBUTION AND ECOTOXICOLOGICAL RISK ASSESSMENT OF PRIORITY PHENOLS IN RIVER BANK SEDIMENTS

***B. Kumar, V. K. Verma, Premanjali Rai, Sanjay Kumar and C. S. Sharma**
Central Pollution Control Board, East Arjun Nagar, Delhi, India
**Author for Correspondence*

ABSTRACT
Phenolic compounds are released into the environment through various anthropogenic activities and found in all environmental compartments such as water, sediment and soil. This study was carried out to determine eleven priority phenolic compounds in Yamuna River Bank sediments. Determination of priority phenolics (phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol) was carried out using ultrasonication and manual liquid-liquid shaking extraction technique and high performance liquid chromatography (HPLC) equiped with diode array detector (DAD) for qantification. The concentration of total eleven phenolics ranged between 2.09-5.25 mg kg^{-1} with the mean and median value of 3.46 mg kg^{-1} and 3.50 mg kg^{-1} (SD, ±1.26 mg kg^{-1}), respectively. The observed average levels of individual phenolic compounds concentrations from this study were lower than the intervention values. Further, concentration levels of total phenolic compounds were lower than consensus based sediment quality guidelines (CBSQGs) values in terms of threshold effect concentration (TEC), midpoint effect concentration (MEC) and probable effect concentration (PEC) for the protection of environmental and human health.

Keywords: *Priority Phenols, River Sediment, India, Consensus Based Sediment Quality Guidelines (CBSQGs), Threshold Effect Concentration (TEC), Midpoint Effect Concentration (MEC), Probable Effect Concentration (PEC)*

INTRODUCTION
Priority Phenols are ubiquitous environmental contaminants found in all water, sediment and soil. Phenols are organic compounds similar to alcohols, but characterized by the hydroxyl (-OH) group attached to a carbon atom in an aromatic ring. The structural formula of phenol (C_6H_5OH) is replaced for subsequent phenolic compounds with structure R- C_6H_4OH, where R represents some groups including halogenated (chlorophenols), nitrated (nitrophenols), alkylated (methylphenols) and ether (methoxyphenols) derivatives.

Phenols mainly occur in nature as a product of coal tar or crude petroleum. Phenolic compounds are used or produced in many industrial processes. These are commonly released into the environment through various anthropogenic activities, such as manufacturing of dyes, pulp, paints, polymer intermediates, flame retardants, herbicides and wood preservatives, chemical, pharmaceutical, plasticizers, pesticide, metallurgical and domestic sewage (WHO, 1989; Santana *et al.*, 2009). Some phenolics are also originated from the transformation of pesticides and phenolic biocides (Daviá and Gnudi, 1999; Marianna, 2004). The sources of nitrophenols and methylphenol have been related to vehicular emissions (Michalowicz and Duda, 2007). However, some phenols may occur naturally via biodegradation of humic products, tanins and lignins (Sim *et al.*, 2009).

The most studied phenolic compounds are chlorophenols, nitrophenols, alkylphenols and bisphenols (Padilla-Sánchez *et al.*, 2010). Some phenolic compound, especially the chlorophenols and bisphenols, are known for their toxicity, mutagenicity, carcinogenicity, endocrine disrupters and vasodilatory activities, and persistence in the environment (Michalowicz and Duda, 2007; Olujimi *et al.*, 2010). Due to their toxicity, persistence and potentially health effects, many phenolic compounds namely; phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol have been

included in the lists of priority pollutants in many countries by World Health Organization (1989), European Community (2001) and United States Environmental Protection Agency (2014).

The phenolic compounds in the aquatic environment can arise from natural substance degradation, industrial activities and agricultural practices. These compounds are of particular interest and concern to the environment because they are toxic to aquatic organisms (Vidal *et al.*, 2004). Therefore, this study was carried out for the assessment of the occurrence of eleven priority phenolic compounds in bank sediments from Yamuna River in Delhi.

MATERIALS AND METHODS

Study Area and Sampling

The River Yamuna is a major tributary of River Ganges which originates from the Yamunotri glacier of the lower Himalayas and enters Delhi near Palla village. Delhi, the capital of India having a total area of 1483 km^2, houses a population ~18 million. The city lies between 28^0 36' 36"N to 77^0 13'48"E on the banks of River Yamuna. Delhi experiences a hot and humid climate with ambient temperature rising up to 40-45^oC during summers and dipping up to 4 to 5^oC during winters. The average annual rainfall is ~714 mm during monsoon season. During 2000, the numbers of in-use vehicles were ~3.05 million which increased to ~6.5 million in 2010. There are a number of designated industrial areas with various activities, including power plants (DoEF Delhi, 2010). The River is a major source of water supply from Wazirabad/Sonia Vihar barrage to Delhi, meeting greater than 70% of the total water demand. More than 3,000 MLD (millions liter per day) of domestic and industrial wastewater is generated in Delhi. The available water treatment facilities are not adequate to remove all the pollutants. Consequently, partly treated and untreated wastewater laden with the biological and chemical wastes enters the river every day through several major and minor drains (ENVIS, 2013). After a stretch of 22 km, downstream of Wazirabad barrage, there is Okhla barrage where water is not allowed to flow through the barrage during dry season.

The total catchment area of Yamuna River in Delhi stretch is about 1485 Km^2.

During 2014, sampling was carried out from five sampling locations at Palla (S1), Sonia Vihar (S2), Rajghat (S3), Nizamuddin (S4) and Okhla (S5) on Yamuna River. The bank surface sediment samples were collected in duplicate from each location. Collected sediment samples were mixed thoroughly, and about 500 g of sediment aliquot was taken into cleaned wide mouth amber glass bottle with Teflon lined screw cap. The samples were transported to the laboratory and air-dried in clean dark space at room temperature. The dried samples were sieved through a 1 mm mesh screen and stored in glass bottles in refrigerator at ~4 ^0C until extraction and analysis.

Chemicals, Solvents and Standards

HPLC grade solvents (dichloromethane and methanol), analytical grade chemicals (sodium sulphate, sulfuric acid, and *ortho*-phosphoric acid) and HPLC water were procured from Rankem, India. Individual eleven priority phenols (phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol) and EPA phenol mixture standard solutions were procured from Supelco (Bellefonte, PA, USA).

After dilution of the stock standard solution, intermediate and working standard solutions were prepared daily in methanol and stored at 4°C in the dark.

Instrumentation

Glassware involved in the method was cleaned with detergent followed by deionised water and finally rinsed with solvents and dried in hot air oven. Ultrasonication and LLE (liquid-liquid extraction) technique was followed for phenolic compound extraction. Vacuum rotary evaporator (Eyela, Tokyo, Japan), Turbovap (Caliper, USA) and Minivap (Supelco, USA) were used for extract concentrations. HPLC system (Series 1100, Agilent Technology Inc., Santa Clara, CA, USA) equipped with a quaternary pump with vacuum degasser, auto sampler, column oven and DAD (diode array detector) (λ=280 nm) was used for the chromatographic analysis.

Sample Extraction

Air dried soil sample of 10-15 g was extracted three times with mixture of 0.1M NaOH in methanol (75 ml) using ultrasonic bath for 30 min and allowed to settle. Methodology in detail has been given elsewhere (Khairy, 2013; Kumar *et al.,* 2014). Aqueous-methanol extract layer was filtered through Whatman 41 filter paper and transferred to separatory funnel. The pH of aqueous-methanol extract was adjusted to <2 with slow addition of sulfuric acid (1:1v/v), then extraction was carried out three times with 50 ml of dichloromethane (DCM) for 2 min each. The DCM (organic phase) extract was passed through anhydrous sodium sulphate to remove traces of water content. The filtered pooled extract was concentrated to near 5 ml by vacuum rotary evaporator (Eyela, Tokyo, Japan). The concentrated extract was solvent exchanged to methanol by the addition of 50 ml methanol, and again concentrated to near 5 ml. Addition of methanol and concentration was repeated two more times to remove traces of dichloromethane. The concentrated extract volume was reduced to 1.0 ml under gentle stream of purified nitrogen gas using Turbo Vap (Caliper, USA) and Minivap (Supelco, USA).

Identification and Quantification of Phenolics

The analysis of eleven phenolic compounds was carried out on high performance liquid chromatograph (HPLC) (Series 1100, Agilent Technology Inc., Santa Clara, CA, USA), equipped with a vacuum degasser, quaternary pump, diode array detector and an autosampler. Sample extract of 10 µL was separated on a C18 reversed-phase analytical column (4.6 mm x 250 mm, 5 µm particle film) (Ascentis®, Supelco, USA). Before analytical column, a guard column (4.6 mm x 12.5 mm, 5 µm particle film) was used to prevent any contamination into the column. A gradient mixture of methanol (0.15% *o*-phosphoric acid) and water (0.15% *o*-phosphoric acid) was used as mobile phase with flow @ 0.7 ml/min. All analyses were undertaken at 280 nm wavelength for all phenolic compounds. The temperature of column thermostat was controlled at 25±1 ^{0}C. The chromatographic conditions and data acquisition were controlled by Agilent Chemstation Software (Rev. B.02.01).

Analytical Quality Control

All analyses were carried out with strict requisite quality control/assurance (QC/QA) performance. Multilevel calibration curves (five levels) were prepared by injecting 10 µL of active amount of the five level phenolic compound concentrations. Calibration standard solutions were prepared at the time of instrument calibration with every batch of analysis. Triplicate method blanks were processed and analyzed as real samples to check any cross contaminations or loss of the analytes. Measurements were repeated three times for each sample. The averaged results of triplicate analysis were expressed relative to the average result for the method blank (concentration, <DL "BDL").

The peak identification of the phenolic compounds was done by comparing the retention time of each individual standard with external standard method. Calibration verification was <±5%. The eight aliquots of a spiked sample with smallest quantity of the standard amount were processed and analyzed for estimation of limit of detection (LOD) and limit of quantification (LOQ) with 10 µL injection. The LOD was calculated using signal to noise ratio >3:1. However, the limit of quantification (LOQ) was calculated at signal to noise ratio >10. The LOD ranged between 0.11-0.61 µg/ml while LOQ varied between 0.37-2.04 µg/ml. The accuracy of the analytical method was determined in the percent recovery with addition of the standard solution to the sample in triplicates. The average recoveries ranged between 50%-95% (±1%-6%), except 30%±8% for phenol. Retention times, detection limits and recoveries were presented in Table 1. Moisture content of sediments was separately determined gravimetrically to report data on dry weight basis. The results of the analysis are reported in mg kg^{-1} dry-weight (dry wt.) basis.

RESULTS AND DISCUSSION

Concentration of Phenolic Compounds in Soils

The statistical summary of concentrations of individual and total eleven priority phenolic compounds in sediments from Yamuna River was summarized in Table 1. However, Whisker's box plot of eleven compounds was presented in Figure 1. The concentration of total eleven phenolics ranged between 2.09-5.25 mg kg^{-1} with the mean and median value of 3.46 mg kg^{-1} and 3.50 mg kg^{-1} (SD, ±1.26 mg kg^{-1}),

respectively. Pentachlorophenol was the dominant compound with average concentration of 1.37±0.78 mg kg^{-1} (range, 0.54-2.62 mg kg^{-1}) and accounted for 24.28% of total phenolic compounds. The observed concentration of other phenolic compounds was found to be heterogeneous, ranging from 0.44-1.01 mg kg^{-1}, 0.22-1.54 mg kg^{-1}, 0.19-0.22 mg kg^{-1}, 0.14-0.20 mg kg^{-1}, 0.24-1.32 mg kg^{-1}, 0.21-0.21 mg kg^{-1}, 0.01-0.09 mg kg^{-1}, 0.37-0.41 mg kg^{-1}, 0.36-0.92 mg kg^{-1} and 0.31-1.14 mg kg^{-1}, respectively for phenol, 4-nitrophenol, 2,4-dinitrophenol, 2-nitrophenol, 2-chlorophenol, 2,4-dimethylphenol, 2-methyl-4,6-dinitrophenol, 4,chloro-3-methylphenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol. Their contribution accounted for 11.67%, 13.06%, 3.61%, 2.87%, 10.42%, 3.62%, 1.04%, 6.66%, 9.56% and 10.63%, respectively to total phenolics (Table 1).

Table 1: Summary of Priority Phenolics in Sediments from Yamuna River in Delhi

S.No.	Phenolic Compounds	Quality Control			Concentration (mg kg^{-1})					% of ∑
		LOD	LOQ	Recovery (%)	Min	Max	Mean	Median	SD	
1.	Phenol	0.18	0.61	30 ± 8	0.44	1.01	0.68	0.60	0.30	12
2.	4-nitrophenol	0.61	2.04	60 ± 4	0.22	1.54	0.76	0.53	0.69	13
3.	2,4-dinitrophenol	0.33	1.09	71 ± 5	0.19	0.22	0.21	0.22	0.02	5
4.	2-nitrophenol	0.11	0.37	50 ± 3	0.14	0.20	0.17	0.17	0.04	4
5.	2-chlorophenol	0.11	0.38	51 ± 4	0.24	1.32	0.61	0.35	0.48	10
6.	2,4-dimethylphenol	0.11	0.38	51 ± 5	0.21	0.21	0.21	0.21	0.01	4
7.	2-methyl- 4,6-dinitrophenol	0.54	1.81	72 ± 5	0.01	0.09	0.06	0.08	0.04	1
8.	4,chloro-3-methylphenol	0.57	1.89	70 ± 5	0.37	0.41	0.39	0.39	0.03	7
9.	2,4-dichlorophenol	0.32	1.08	70 ± 1	0.36	0.92	0.56	0.40	0.31	10
10.	2,4,6-trichlorophenol	0.38	1.28	73 ± 4	0.31	1.14	0.62	0.51	0.34	11
11.	Pentachlorophenol	0.60	1.98	95 ± 6	0.54	2.62	1.37	1.10	0.78	24
12.	Total compounds	-	-	-	2.09	5.25	3.46	3.50	1.26	100

The location-wise concentration of individual compounds was presented in Table 2. The concentration of total phenolics was low at up-stream location (Palla), which increased at down-stream location (Okhla). The total concentration at different location was 2.48 mg kg^{-1}, 2.09 mg kg^{-1}, 3.47 mg kg^{-1}, 3.50 mg kg^{-1} and 5.25 mg kg^{-1}, respectively at sampling location of Palla, Sonia Vihar, Rajghat, Nizamuddin and Okhla. The average concentration of pentachlorophenol at different location was 0.54 mg kg^{-1}, 1.10 mg kg^{-1}, 2.62 mg kg^{-1}, 1.06 mg kg^{-1} and 1.53 mg kg^{-1}, respectively for Palla, Sonia Vihar, Rajghat, Nizamuddin and Okhla. Their contribution accounted for 21.54%, 52.95%, 75.40%, 30.19% and 29.18%, respectively (Figure 2).

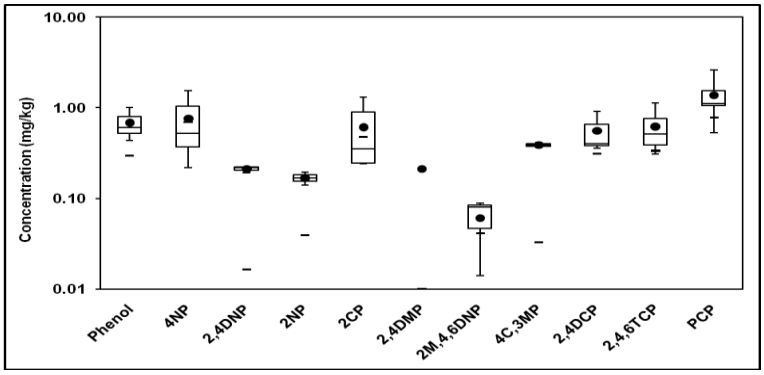

Figure 1: Whisker's Box Plot of Phenolics in Sediments

Table 2: Average Concentration of Priority Phenolics in Sediments at Different Location

S.No.	Phenolic Compounds	S1	S2	S3	S4	S5
1.	Phenol	BDL	BDL	0.44	0.60	1.01
2.	4-nitrophenol	BDL	BDL	0.53	0.22	1.54
3.	2,4-dinitrophenol	BDL	BDL	0.19	0.22	0.22
4.	2-nitrophenol	0.14	BDL	BDL	0.20	BDL
5.	2-chlorophenol	1.32	0.89	0.24	0.24	0.35
6.	2,4-dimethylphenol	BDL	BDL	BDL	BDL	0.21
7.	2-methyl-4,6-dinitrophenol	BDL	0.09	BDL	0.08	0.01
8.	4,chloro-3-methylphenol	BDL	0.37	BDL	0.41	BDL
9.	2,4-dichlorophenol	0.92	BDL	0.40	BDL	0.36
10.	2,4,6-trichlorophenol	0.76	0.31	1.14	0.51	0.39
11.	Pentachlorophenol	0.54	1.10	2.62	1.06	1.53
12.	Total	2.48	2.09	3.47	3.50	5.25

Pentachlorophenol (PCP) is the most studied phenolic compound. PCP and its derivatives sodium pentachlorophenate (NaPCP) and pentachlorophenyl laurate (PCPL) have been used worldwide, mainly in herbicides, biocides, pesticides and wood preservatives since the 1930s. This extensive use has resulted in the contamination of soils, sediments and waters. PCP is the main precursor for the preparation of lower chlorinated chlorophenols. Depending on the environmental conditions, PCP can degrade into as many as 30 different products including dichloro-, trichloro- and tetrachlorophenols; tetrachlorocatechols; dichloro-, trichloro- and tetrachlorohydroquinones; pentachloroanisole and hexachlorobenzene as major products; and polychlorinated diphenylethers and polychlorinated dibenzo-p-dioxins as minor products (McLellan *et al.*, 2007). The lower chlorinated phenols are produced through reductive dechlorination (PCP → TeCP → TCP → DCP). Reductive dechlorination from PCP under anaerobic conditions with transformation rates were highly correlated to carbon, nitrogen and phosphorous content of the sediment. However, persistence of PCP in the environment would suggest its sorption to soils, sediments and dissolved and particulate organic matter in waters, which decreases degradation rates (McLellan *et al.*, 2007).

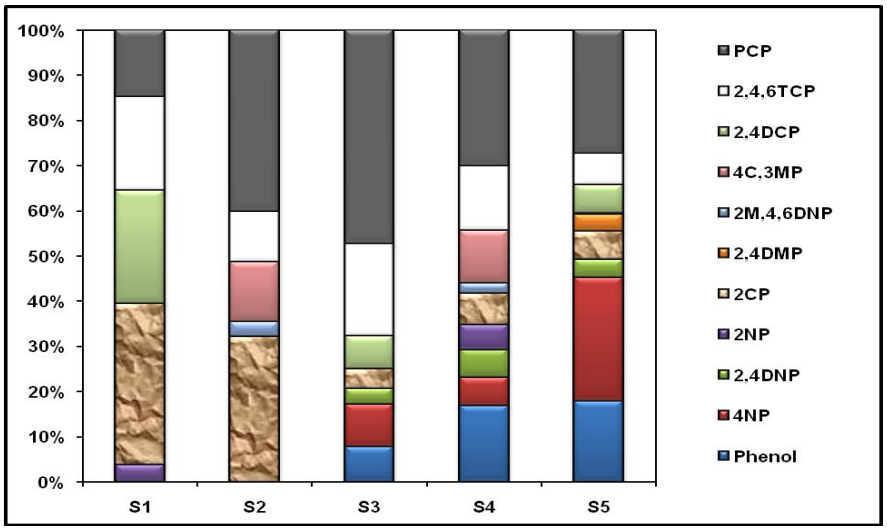

Figure 2: Percent Abundance of Phenolics in Sediments at Different Locations

Eco-Toxicological Intervention

Environmental intervention was assessed for consideration of ecological functioning of sediment microorganisms. No environmental guidelines for phenolic compounds in sediment are available in India.

Therefore, available Dutch intervention values for phenolic compounds were applied for the comparison of observed phenolic compounds in this study. The Intervention values (IV) indicate sediment contaminant concentrations that determine action urgency under the Environment Protection Act. The estimation of these values was based on serious risk concentrations (SRC) derived from ecotoxicological (SRC_{eco}) and human exposure (SRC_{human}) data. SRC_{eco} is the concentration at which 50% of processes in an ecosystem experience unwanted effects. SRC_{human} is based on the human-toxicological maximum permissible risk (MPR) level. The designated IVs for phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol are 14.0 mg kg^{-1}, 7.8 mg kg^{-1}, 8.4 mg kg^{-1}, 110.0 mg kg^{-1} and 8.0 mg kg^{-1}, respectively (Buckman, 2008). Intervention values for nitrophenols and methylphenols in sediments were not available. The observed average levels of individual phenolic compounds concentrations from this study were lower than the intervention values. Therefore, it may be concluded from this study that no remediation action is required for current levels of phenolic compounds in sediment from Yamuna River in Delhi. Further, consensus based sediment quality guidelines (CBSQGs) value and associated level of concern are recommended for phenols in terms of threshold effect concentration (TEC), midpoint effect concentration (MEC) and probable effect concentration (PEC). The TEC and PEC levels are those at which toxicity to benthic-dwelling organisms are predicted to be unlikely and probable, respectively. The MEC is a concentration midway between the TEC and PEC concentrations.

The CBSQGs recommended TEC, MEC and PEC values for phenols were 4.2 mg kg^{-1}, 8.1 mg kg^{-1} and 12.0 mg kg^{-1}, respectively (WDNR, 2003), indicating low toxicity to benthic-dwelling organisms due to phenols in Yamuna River.

Conclusion

Phenolic compounds concentrations in Yamuna River bank sediments were found to be heterogeneous in range. The observed concentrations were lower than the consensus based sediment quality guidelines (CBSQGs) value and intervention values for total phenolics in sediments for the protection of environmental and human health.

ACKNOWLEDGEMENT

The authors are grateful to Competent Authorities of Central Pollution Control Board for providing necessary facilities and infrastructure to carry out present study during training to corresponding author. The views expressed in this paper are those of authors and do not necessarily reflect any organization.

REFERENCES

Buckman MF (2008). NOAA screening quick reference tables (SQuiRTs). NOAA OR&R REPORT 08-1, Seattle Washington, Office of the Response and Restoration Division, National Oceanography and Atmospheric Administration 34.

Daviá ML and Gnudi F (1999). Phenolic compounds in surface water. *Water Research* **33**(14) 3213-3219.

DoEF (Department of Environment & Forest), Delhi (2010). *State of Environment Report of Delhi* [Online]. Available: http://www.indiaenvironmentportal.org.in/content/307917/state-of-environment-report-for-delhi-2010/ [Accessed 1 August, 2017].

EC (European Community) (2001). The list of priority substances in the field of water policy and amending directive, Council directive 2455/2001/ECC. *Official Journal* **vol** L331 1-5.

ENVIS (2013). *Status of Sewage Treatment Plant in Delhi* [Online]. Available: http://www.sulabhenvis.nic.in/Database/STST_statusofsewageindelhi_2091.aspx. [Accessed 1 August, 2017].

Khairy MA (2013). Assessment of priority phenolic compounds in sediments from an extremely polluted coastal wetland (Lake Maryut, Egypt). *Environmental Monitoring Assessment* **185**(1) 441–455.

Kumar B, Verma VK, Sharma CS and Akolkar AB (2014). Quick and easy method for determination of priority phenolic compounds in water and wastewater. *Journal of Xenobiotics* **4**(1) 46-52.

Marianna C (2004). Sources and transformations of chlorophenols in the natural environment. *Science of the Total Environment* **322**(1-3) 21–39.
Mc Lellan I, Carvalho M, Pereira CS, Hursthouse A, Morrison C, Tatner P, Martins I, Romao MVS and Leitao M (2007). The environmental behaviour of polychlorinated phenols and its relevance to cork forest ecosystems: a review. *Journal of Environmental Monitoring* **9**(10) 1055–1063.
Michalowicz J and Duda W (2007). Phenols-Sources and toxicity. *Polish Journal of Environmental Studies* **16**(3) 347-362.
Olujimi OO, Fatoki OS, Odendaal JP and Okonkwo JO (2010). Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring. *Water SA* **36**(5) 671-682.
Padilla-Sánchez JA, Plaza-Bolaños P, Romero-González R, Garrido-Frenich A and Vidal JLM (2010). Application of a quick, easy, cheap, effective, rugged and safe-based method for the simultaneous extraction of chlorophenols, alkylphenols, nitrophenols and cresols in agricultural soils, analyzed by using gas chromatography–triple quadrupole-mass spectrometry/mass spectrometry. *Journal of Chromatography A* **1217**(36) 5724–5731.
Santana CM, Ferrera ZS, Padron MET and Rodrigues JJS (2009). Methodologies for the extraction of phenolic compounds from environmental samples: new approaches. *Molecules* **14**(1) 298–320.
Sim W, Lee S, Lee I, Choi S and Oh J (2009). Distribution and formation of chlorophenols and bromophenols in marine and riverine environments. *Chemosphere* **77**(4) 552–558.
USEPA (United States Environmental Protection Agency) (2014). *CFR Part 423-126 Priority Pollutants* [Online]. Available: http://water.epa.gov/scitech/methods/cwa/pollutants.cfm [Accessed 1 August, 2017].
Vidal JLM, Vega AB, Frenich AG, Gonzalez FJE and Liebanas FJA (2004). Determination of fifteen priority phenolic compounds in environmental samples from Andalusia (Spain) by liquid chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry* **379**(1) 125–130.
WDNR (Wisconsin Department of Natural Resources) (2003). *Consensus-Based Sediment Quality Guidelines (CBSQGs) Recommendations for Use and Application Interim Guidance* [Online] Available: http:// http://dnr.wi.gov/topic/brownfields/documents/cbsqg_interim_final.pdf. [Accessed 1 August, 2017].
WHO (World Health Organization) International Programme on Chemical Safety Geneva (1989). *Environmental Health Criteria 93 Chlorophenols other than Pentachlorophenol* [Online] Available: http://www.inchem.org/documents/ehc/ehc/ehc093.htm [Accessed 9 August 2017].