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Distribution of eleven priority phenolic compounds in soils from mixed landuse and assessment of health hazard for human population

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ABSTRACT

This study was carried out to determine eleven priority phenolic compounds in soils from rural-urban area in northern Uttar Pradesh, India. Further, a probabilistic approach was used to determine the human and ecological health effects based on the concentrations of phenolic compounds in soils. The task of determination involved the use of ultrasonication and manual shaking extraction technique and high performance liquid chromatography (HPLC) equipped with diode array detector (DAD) for qantification. The Concentration of total eleven phenolic compounds ranged between BDL-7.08 mg kg^{-1} with a mean value of 1.92 mg kg^{-1} (±0.35 mg kg^{-1}). The average daily dose for lifetime exposure through soil for adults and children was $3.0x10^{-6}$ mg kg^{-1} d^{-1} and $1.1x10^{-5}$ mg kg^{-1} d^{-1}, respectively. A non-cancer health hazard in terms of the hazard index (HI) due to phenolic compounds through soil ingestion was $2.6x10^{-4}$ and $9.7x10^{-4}$ for human adults and children, respectively. The observed concentration levels of phenolic compounds in soils were lower than stipulated guideline values for the protection of environmental and human health. Health hazard was much lower than acceptable safe risk level (HI≤1). Therefore, it can conclude that concentrations of eleven priority phenolic compounds in soil and their hazardous effects to human population were low.

Keywords: Priority pollutants, Phenolic compounds, Soil, Health hazard

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INTRODUCTION

Phenols and phenolic compounds are of environmental concern due to their toxicity and being as ubiquitous contaminants in the environment. The presence of phenolic compounds in soils is due to different sources including industrial activities of chemical, textile, pharmaceutical, polymers, pulp and paper, woods, plasticizers, pesticide, and metallurgic industries or by the release of industrial effluents and domestic sewage [1-2]. Some phenols in the soils originate from the transformation of pesticides such as 2.4-dichlorophenoxyacetic acid (2.4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4,5-trichloro-phenoxyacetic acid (2,4,5-T), 2-buthyl-4,6-dinitrophenol (dinoseb), and phenolic biocides like pentachlorophenol (PCP) and from atmospheric depositions [3]. Moreover, nitrophenols and methylphenol sources have been related to vehicular emissions [4]. Phenols may occur naturally via biodegradation of humic products, for example tanins and lignins [5].

Some phenolic compounds exhibit high toxicity, mutagenicity and carcinogenicity, endocrine disrupters and vasodilatory activities [4,6]. Their Phenol toxicity may be related to the formation of electrophilic metabolites that may bind and damage DNA or enzymes. The most studied phenolic compounds; depending on their physical-chemical properties are chlorophenols, nitrophenols, methylphenols, alkylphenols and bisphenols [7]. The relatively high stability of some of these compounds is responsible for their long term persistence in the soil. Therefore, some

phenols like chlorophenols and nitrophenols, due to their high toxicity, poor degradability and being potentially carcinogenic, have been classified as priority pollutants (Table 1) by World Health Organization (WHO) [1], European Community [8], Environment Agency, United Kingdom [9] and United States environmental protection agency (USEPA) [10].

Human exposure to organic pollutants occurs mainly through occupational exposure and dietary intake of contaminated water and food [11], but a significant exposure may also take place by intake of contaminated soil via ingestion, inhalation or dermal contact a. Human exposure to organic pollutants in soils takes place through various pathways due to close proximity of soils to humans, which may cause toxicological effects on human health. Soils are good adsorbents of the phenolic contaminants due to their high surface area and surface activity and play a very important role in their fate and distribution in the environment. Soils may act as re-emission sources for the pollutants through volatilization, degradation and leaching [12]. Therefore, soil could be considered in any risk assessments involving potentially harmful toxic organic pollutants [13].

Table 1: Selected properties of priority phenolic compounds [6]

Compounds	CAS No	Molar mass (g/mol)	Melting point (^{0}C)	Boiling point (^{0}C)	pKa	Log Kow	Solubility (g/L at 20 ^{0}C)
Phenol	108-95-2	94.11	40.8	181.8	10.0	1.46	83
4-nitrophenol	93951-79-2	139.11	110-115	279	7.08	2.04	11.6
2,4-dinitrophenol	51-28-5	184.11	114-115	113	3.94	1.67	5.45
2-nitrophenol	88-75-5	139.11	43-45	215.5	7.23	1.89	2.50
2-chlorophenol	95-57-8	128.55	7	174	8.56	2.15	28.5
2,4-dimethylphenol	105-67-9	122.16	25	211	-	-	-
2-methyl-4,6-dinitrophenol	534-52-1	198.14	82-85	312	10.58	2.30	0.05
4-chloro-3-methylphenol	59-50-7	142.58	63-65	235-239	9.6	3.10	4
2,4-dichlorophenol	120-83-2	163	40-43	209-211	7.85	3.06	4.5
2,4,6-trichlorophenol	88-06-2	197.44	65-68	244-246	69	6.15	3.69
pentachlorophenol	87-86-5	266.34	190-191	309-310	4.7	5.12	0.01

The aim of this study was to assess the distribution of eleven priority phenolic compounds in soils. Health hazard due to ingestion of studied soils was also estimated for human adults and children. For this purpose, we estimated lifetime average daily dose (LADD) of 11phenolic compounds through soil ingestion [14]. LADD is the amount of a chemical intake by a person per kg of body weight per day which may indicate the adverse health effects when absorbed into the body over a long period of time. Then, potential health hazard for human population was assessed in terms of hazard quotient (HQ) [14]. Hazard is the properties of pollutant or mixture of pollutants that makes its capable of causing adverse health effects to human or microorganisms or the environment.

MATERIALS AND METHODS

Study Area and Sampling

The sampling locations were in border districts (Bagpat, Gaziabad, Gautam Budh Nagar) of Northern Uttar Pradesh in National Capital Region (NCR), India. Utter Pradesh shares a large area of 10,853 km^2 in NCR (33,578 km^2) with Delhi (1,483 km^2), Haryana (13,413 km^2) and Rajasthan (7,829 km^2). Area is a dynamic mixture of rural-urban settlement and characterized by the presence of ecologically sensitive areas like forests, wild life, bird sanctuaries, Rivers (Yamuna and Hindon) and fertile cultivated land [15]. There are several types of ongoing industrial activities including manufacturing of electroplating, electronic, ceramic, textiles, food products, rubber and plastic, vegetable oil, paints, chemicals, agro-chemicals, automobiles, steel, metal recycling, pharmaceuticals, and distillery.

Area experiences a typical version of the humid sub-tropical climate with hot and long summers (early April to mid-October) and the monsoon season in between. Winter season starts in late November, peaks in January and is very often surrounded by heavy fog. Ambient temperature during summers and winters ranges between 27-45 ^{0}C and 4-25 ^{0}C, respectively. Wind direction changes in early March, from north-western to south-western. The average annual rainfall in the region varies greatly from as low as 300 mm in the western parts to about 850 mm in the central and north-eastern parts, most of which falls during the monsoon season [15].

Twenty five soil samples of different landuse pattern collected during February 2014 from urban-rural locations in Baghpat, Ghaziabad and Gautam Budha Nagar districts of Uttar Pradesh. Approximately, 1/2 kg of soil was

collected from each sampling location. After collection, unwanted materials were removed manually. The soil collected from sampling points at each location was mixed thoroughly to ensure the true representative samples of that location. A sufficient quantity of mixed soil was taken into a clean wide mouth amber glass Teflon lined bottles. The collected samples were transported to the laboratory and stored in refrigerator at ~4 ^{0}C until extraction and analysis.

Chemicals, Solvents and Standards

Solvents (dichloromethane and methanol), chemicals (sodium sulphate, sulfuric acid, and *ortho*-phosphoric acid) and water procured from Rankam, India. All solvents and water was of HPLC grade and chemicals were of analytical grade. 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, an intermediate mixed standard solution was prepared. Intermediate and working standard solutions were prepared 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 the solvents were rinsed and dried in hot air oven. LLE (liquid-liquid extraction) technique using separating funnel (1L) was followed for phenolic compound extraction from water. 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) was used in combination along with a quaternary solvent delivery system and a vacuum degasser unit, auto sampler, column oven, and DAD (diode array detector) (λ=280 nm) for the chromatographic analysis.

Sample Extraction

Air dried soils were smoothly grinded and were made to pass through 1mm sieve and stored in glass bottels in dark. 20-25 g of sample was extracted three times with mixture of 0.1M NaOH in methanol (75 ml) using ultrasonic bath for 30 min and was allowed to settle [16]. Extracted layer was filtered through Whatman 41 filter paper and then transferred to a separatory funnel with adjusted pH (pH <2) with–slow addition of sulfuric acid (1:1v/v). Then sample was three times extracted with 50 ml of dichloromethane for 2 min each. The organic phase was passed through anhydrous sodium sulphate to remove traces of water contents and the extracts were concentrated to near 5 ml by vacuum rotary evaporator (Eyela, Tokyo, Japan). Extract solvent was exchanged to methanol by the addition of 50 ml methanol, and again concentrated to near 5 ml. Meticulous care was taken 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 chromatographic identification and quantification of eleven phenolic compounds was performed using high performance liquid chromatograph (HPLC) (Series 1100, Agilent Technology Inc., Santa Clara, CA, USA), equipped with a vacuum degasser (Agilent, G1379A), quaternary pump (Agilent, G1311A), diode array detector (Agilent, G1315B) and an autosampler (Agilent, G1329B). Sample extract of 10 µL was separated on a C18 reversed-phase analytical column (4.6 mm x 250 mm, 5 µm particles) (Ascentis®, Supelco, USA). Before analytical column, a guard column (4.6 mm x 12.5 mm, 5 µm particles) was used to prevent ~~any~~ contamination into the column. Methanol (0.15% *o*-phosphoric acid) and water (0.15% *o*-phosphoric acid) was used as mobile phase with gradient flow @ 0.7 ml/min. Peaks were determined at 280 nm for all phenolic compounds. The column temperature was controlled at 25±1 ^{0}C. The Chemstation software (Agilent, Rev. B.02.01) was used to control the chromatographic conditions and data acquisition.

Analytical Quality Control

Requisite quality control/assurance (QC/QA) analysis was performed during analysis of soil samples. Individual and mixture solutions of eleven priority phenols certified reference standard solutions were used for quality control analysis. Five levels of calibration working standard solutions for each compound were prepared and chromatographed by injecting 10 µL. A calibration curve for eleven phenolic compounds was prepared separately by plotting peak area (y-axis) versus concentration (x-axis). All curves were constructed using the external standard method by injecting active amount of the five level phenolic compound concentrations as a function of peak area

using linear fit. Method blanks in triplicate were processed as real samples to check any cross contaminations or loss of the analytes. Calibration standard solutions were prepared at the time of instrument calibration with every batch of analysis. The calibration curves followed the Beer's law in the investigation range of phenolic compound. Measurements were repeated three times for each sample and the results were averaged and expressed with respect to the average result for the method blank (concentration, <DL "BDL").

The peak identification of the analytes was done by the accurate retention time of each individual standard. Calibration was verified by analyzing the middle level calibration standard and the relative percent difference between expected concentration and obtained responses from the five-point calibrations (<1%). The Limit of detection (LOD) and limit of quantification (LOQ) were obtained by processing the eight aliquots of a spiked sample with smallest quantity of the standard materials to produce a valid quantifiable peak at signal to noise ratio >3:1 (s/n >3) for a 10 µL of injection. The LOD and LOQ were calculated as per the standard guidelines [17-18]. LOD was calculated as:

Limit of detection (LOD) = SD x $t_{student}$

The limit of quantification (LOQ) was calculated at signal to noise ratio >10:1 as:

Limit of quantification (LOQ) = SD x 10

Where, SD is the standard deviation of response of 8 replicate analysis and $t_{student}$ is n-1 (degree of freedom and n is number of observations) at 99% confidence level. The LOD ranged between 0.11-0.61 µg/ml while LOQ varied between 0.37-2.04 µg/ml. The observed values of LOD were three units lower than LOQ which shows that this method is sensitive for the determination of phenolic compounds in water samples. Accuracy of an analytical method is the closeness of agreement between the conventional true value or an accepted reference value and the value found. Trueness or bias in terms of accuracy/recovery was determined through the percent recovery with addition of the standard solution to the sample in triplicates. The average percent recovery was calculated using the following equation:

Recovery (%) = ($observed_{Concentration}$ / $spiked_{Concentration}$) x 100

Where $observed_{Concentration}$ is the concentration observed in the samples and $spiked_{Concentration}$ is the initial concentration spiked to the sample. The average recoveries ranged between 50%-95% (±1%-6%), except 30%±8% for phenol. Retention times, detection limits and recoveries were presented in Table 2. Moisture content of soils was determined separately to report data on dry weight basis. The results of the analysis are reported in mg kg^{-1} dry-weight (dry wt.) basis.

Table 2: RT, calibration verification, detection limits and recoveries of phenolics

Compounds	RT (Min.)	LOD (µg/ml)	LOQ (µg/ml)	Recovery (%)
Phenol	18.60	0.18	0.61	30 ± 8
4-nitrophenol	21.85	0.61	2.04	60 ± 4
2,4-dinitrophenol	23.45	0.33	1.09	71 ± 5
2-nitrophenol	24.14	0.11	0.37	50 ± 3
2-chlorophenol	24.47	0.11	0.38	51 ± 4
2,4-dimethylphenol	27.52	0.11	0.38	51 ± 5
2-methyl-4,6-dinitrophenol	28.60	0.54	1.81	72 ± 5
4-chloro-3-methylphenol	29.19	0.57	1.89	70 ± 5
2,4-dichlorophenol	30.46	0.32	1.08	70 ± 1
2,4,6-trichlorophenol	33.64	0.38	1.28	73 ± 4
Pentachlorophenol	38.63	0.60	1.98	95 ± 6

Calculation of Results

The results were to be calculated as follows:

Phenolic compound concentration (µg/g or mg/kg) = (AxB/C)

Where:
A^* = Concentration of analyte obtained from instrument (µg)
B = Final extract volume (ml)
C = Initial sample volume taken (g)

*Based upon the average of 3 separate determinations of each solution. Blank value was deducted.

Human Health Hazard Assessment

In this study, ingestion of soils contaminated with phenolic compounds was considered as the main pathway of life-long intake for health risk assessment. Human exposure to phenolic compounds and the consequent health risk was estimated by using recommended guidelines. In this study, we calculated the lifetime average daily dose (LADD) and non cancer risk as hazard quotient (HQ) [14, 19]. Hazard quotient (HQ) for humans was assessed from the estimated lifetime average daily dose (LADD) of phenolic compounds. Health hazard quotient (HQ) was calculated by comparing the estimated average daily dose of the individual compound with the reference dose (RfD) [14]. Reference Dose (RfD) derived by USEPA for oral exposure to a chemical, is an estimate of daily oral exposure to the human population that does not show any appreciable non-cancer effects during lifetime. Where, HQ is known as the magnitude of quantifiable potential for developing non-carcinogenic health effects after averaged exposure period. Total potential for non-cancer risk to humans is the sum of HQ values. This total HQ is referred to as the Hazard Index (HI). It has been suggested that, if HQ is equal to or less than one (≤1) indicates no appreciable health hazard. Hazard index (HI) (∑HQs) value of less than one (≤1) suggests no health hazard either from any chemical alone or in combination with others. The equations used for estimating LADD and HQ were as follows:

$$\text{LADD (mg kg}^{-1}\text{ day}^{-1}\text{)} = (\text{Cs x IR x F x EF x ED})/(\text{BW x AT}) \quad [1]$$

$$\text{Hazard Quotient (HQ)} = \text{LADD/RfD} \quad [2]$$

$$\text{Hazard Index (HI)} = \Sigma\text{HQ} \quad [3]$$

Where, Cs is concentration of pollutant in the soil (mg kg^{-1}), IR is the soil ingestion rate (100 mg day^{-1} for adult and 200 mg day^{-1} for children), F is the unit conversion factor, EF is exposure frequency (365 days/year), ED is the life time exposure duration (70 years and 12 years for adults and children, respectively), BW is the body weight (70 kg and 27 kg for adults and children, respectively), and AT is the averaging time for carcinogens (EF x ED days). RfD is and reference dose for individual phenolic compound (mg/kg/day) [20].

RESULTS AND DISCUSSION

Concentration of Phenolic Compounds in Soils

Concentrations of individual and total eleven priority phenolic compounds in soils from Northern Uttar Pradesh, India are summarized in Table 3. Their total concentrations at eleven sampling locations were depicted in Figure 1. Concentration of total phenolics ranged between BDL-7.08 mg kg^{-1} with the mean and median value of 1.92 mg kg^{-1} and 1.56 mg kg^{-1} (±0.35 mg kg^{-1}), respectively. Generally, the contamination was found to be heterogeneous, with observed concentrations ranging from below detection limit (BDL) to 2.72 mg kg^{-1}. Concentrations of individual eleven phenolic compounds ranged BDL-0.60 mg kg^{-1}, BDL-2.72 mg kg^{-1}, BDL-0.26 mg kg^{-1}, BDL-0.89 mg kg^{-1}, BDL-1.74 mg kg^{-1}, BDL-0.22 mg kg^{-1}, BDL-0.24 mg kg^{-1} and BDL-2.67 mg kg^{-1}, respectively for phenol, 4-nitrophenol, 2,4-dinitrophenol, 2-chlorophenol, 2-nitrophenol, 2,4-dimethylphenol, 2-methyl-4,6-dinitrophenol and pentachlorophenol. Concentrations of 4-chloro-3-methylphenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol were BDL at all the sampling locations. The observed concentrations of 4-nitrophenol (1.42 mg kg^{-1}) was comparatively higher than 2-nitrophenol (1.04 mg kg^{-1}), pentachlorophenol (1.00 mg kg^{-1}), and other (<0.5 mg kg^{-1}). The major contributors were 4-nitrophenol, pentachlorophenol, 2-nitrophenol, 2-chlorophenol and phenol which accounted for 32.69%, 26.98%, 21.53%, 10.34% and 6.58%, respectively to total phenolics concentration in soils (Table 3). Other compounds (2,4-dinitrophenol, 2,4-dimethylphenol, 2-methyl-4,6-dinitrophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol) contributed for <1% each to total phenolics.

Table 3: Statistical summary of phenolics concentrations in soils

Name of compounds	Range	Mean	Med	SD	SE	% of total
Phenol	BDL - 0.60	0.40	0.38	0.14	0.03	6.58
4-nitrophenol	BDL - 2.72	1.42	1.12	0.55	0.11	32.69
2,4-dinitrophenol	BDL - 0.26	0.16	0.19	0.12	0.02	0.98
2-chlorophenol	BDL - 0.89	0.26	0.18	0.24	0.05	10.34
2-nitrophenol	BDL - 1.74	1.04	1.14	0.56	0.11	21.53
2,4-dimethylphenol	BDL - 0.22	0.16	0.17	0.07	0.01	0.97
2-methyl-4,6-dinitrophenol	BDL - 0.24	0.20	0.20	0.05	0.01	0.84
4-chloro-3-methylphenol	BDL					
2,4-dichlorophenol	BDL					
2,4,6-trichlorophenol	BDL					
Pentachlorophenol	BDL - 2.67	1.00	0.64	0.77	0.15	26.98
Total phenolic compounds	BDL - 7.08	1.92	1.56	1.76	0.35	100

Human Health Hazard of Phenolic Compounds

Health risk assessment was based on assumption that human adults and children may be exposed to phenolic compounds through ingestion of soil. For this study, human health risk assessment was on the assumption that human adults of 70 years and children of 12 years exposed for all the days in a year during their life span. Health risk was assessed by calculating the life time average daily dose (LADD) and hazardous quoteint (HQ). Estimated LADD of total eleven priority phenolic compounds for human adults and children is presented in Table 4 and Figure 2.

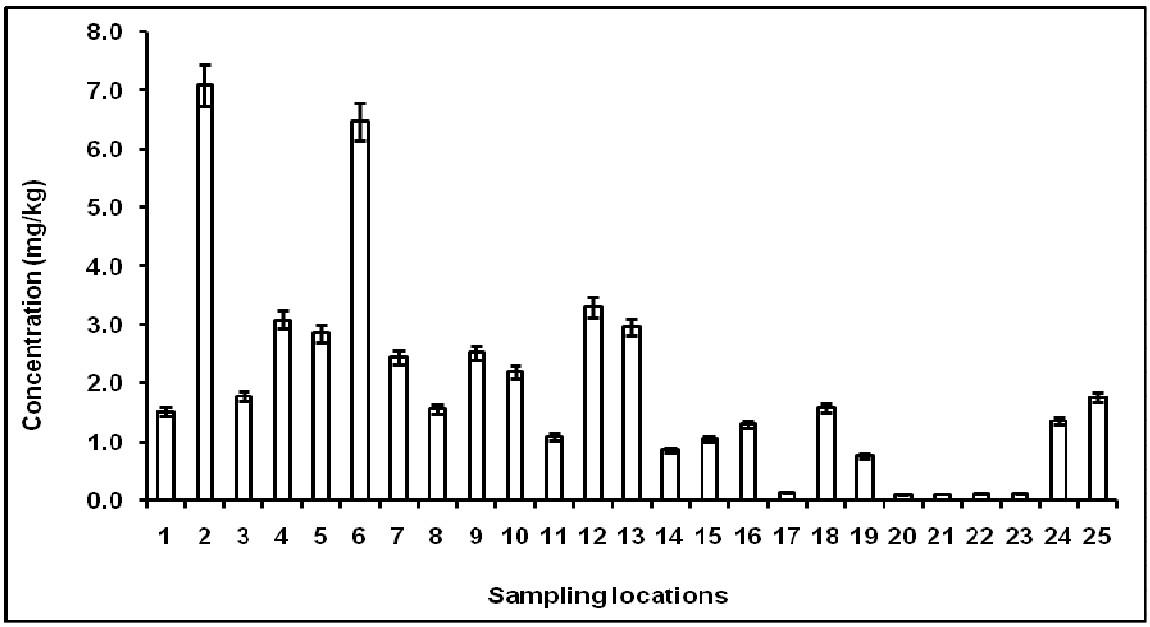

Fig. 1: Distribution of total phenolics in soils at different locations

Table 4: LADD & health hazard index (HI) for human adults and children due to total eleven phenolics through soils

For human adults		For children	
LADD (mg/kg/d)	**HI**	**LADD (mg/kg/d)**	**HI**
$1.4x10^{-7}$ - $1.1x10^{-5}$ ($3.0x10^{-6}$)	$2.6x10^{-5}$ - $1.0x10^{-3}$ ($2.6x10^{-4}$)	$5.1x10^{-7}$ - $4.0x10^{-5}$ ($1.1x10^{-5}$)	$9.6x10^{-5}$ - $3.8x10^{-3}$ ($9.7x10^{-4}$)

The LADD of eleven priority phenolic compounds through soil for adults and children ranged between $1.4x10^{-7}$ - $1.1x10^{-5}$ mg kg^{-1} d^{-1} and $5.1x10^{-7}$ - $4.0x10^{-5}$ mg kg^{-1} d^{-1}, respectively with the mean value of $3.0x10^{-6}$ mg kg^{-1} d^{-1} and $1.1x10^{-5}$ mg kg^{-1} d^{-1}, respectively. There were significant variations in LADD concentrations for adults and children at different sampling locations, which may be influenced by the phenolic sources (Figure 2). Subsequently, on the basis of LADD, the non-cancer health hazard, the hazard quotient (HQ) due to phenolic compounds through soil

ingestion ranged from $2.6x10^{-5}$ to $1.0x10^{-3}$ and from $9.6x10^{-5}$ to $3.8x10^{-3}$ with their mean values of $2.6x10^{-4}$ and $9.7x10^{-4}$ for human adults and children, respectively (Table 4, Figure 2). Therefore, this study bring us to the conclusion that health hazard due to eleven priority phenolic compounds for human population in studied region of India is much lower than acceptable safe risk level (HI≤1). For lack of data about phenolic compounds exposure, we cannot give precise total daily intake. However, according to our results from ingestion of soil, we can conclude that the daily dose of phenolic compounds and non-cancer risk to human adults and children residing in Northern Uttar Pradesh, India is low.

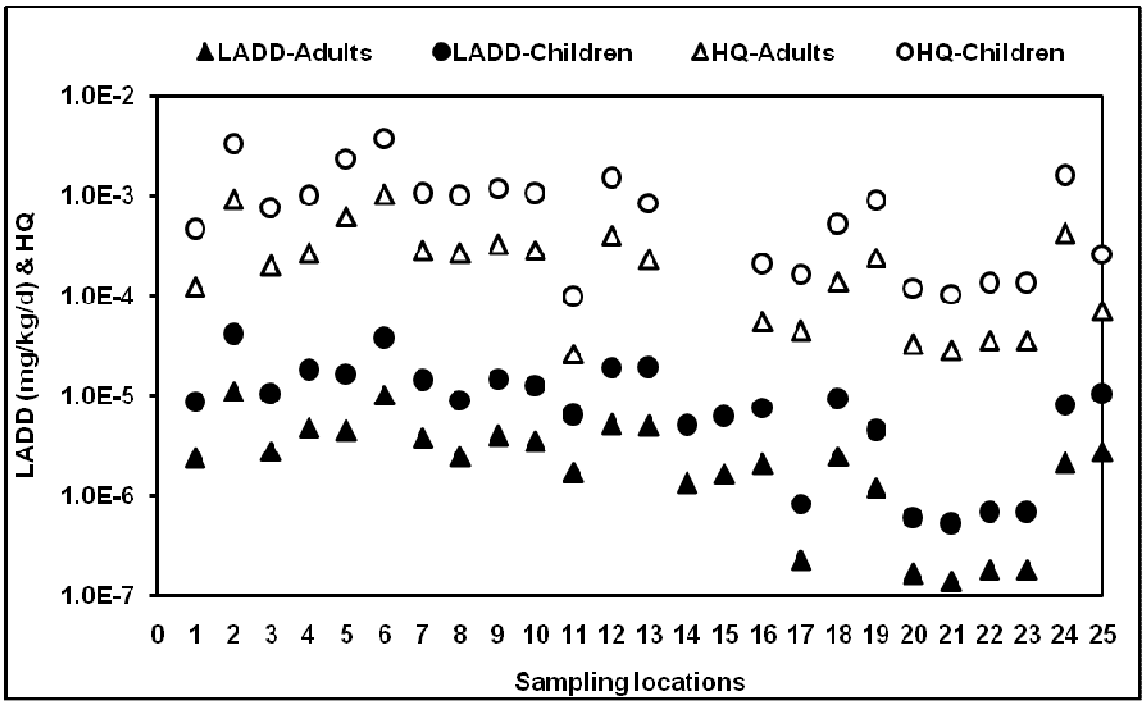

Fig. 2: LADD & HI for children due to total phenolics through soils at different locations

Environmental health Risk of Phenolic Compounds

Environmental health risk assessment was carried out by taking non-carcinogenic effects of phenolic compounds on human and ecological functioning of soil microorganisms into considerations. No environmental guidelines yet have been established in India for phenolic compounds in soil, therefore, established soil quality guidelines from Canadian government [21] were applied for the assessment of ecotoxicological health effect of phenolic compounds. Canadian government recommended environmental soil quality guidelines (SQGs) for phenol and pentachlorophenol as 3.8 mg kg^{-1} and 7.6 mg kg^{-1}, respectively. The levels of phenolic compounds concentrations observed from this study were much lower than the recommended guidelines and indicated no environmental health risk and adverse effects on the soil biota.

CONCLUSION

Phenolic compounds concentrations in soils were lower than the soil guideline limits for the protection of environmental and human health. Non-cancer risk in terms of health hazard index (HI) was lower than acceptable guideline values, which suggested low risk for human adults and children in this area of study.

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