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Assessment of Priority Phenols in Various Water and Soil Samples by HPLC

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Abstract: Phenol and substituted phenols are derived from the family of compounds considered as harmful to environment and humans based on their toxicity and carcinogenic effects. In this study, the selected phenols and their derivatives have been identified in water and soil samples. A convenient and simple High Performance Liquid chromatography method was used for detection and quantification of phenols in water, wastewater and soil samples. First, the water (tap water; bottled water samples and drain water samples) and soil samples were collected from different sources of Furniture market. Then the analysis of selected physical parameters was conducted. Based on the field survey information, three phenol were selected (phenol, catechol, *p*-cresol) and the water and soil samples were analyzed against these standards. Two of the Priority phenols namely phenol and *p*-cresol was detected in most of water samples while catechol was not found in any of water sample. In tap water samples T2, T4, T5 and T6, the detected concentration of phenol was 1.91, 1.90, 1.91 and 1.90 ppm respectively. Whereas, phenol was detected in all drain water sample D1, D2, D3, D4 and D5 in concentration of 1.89, 1.86, 1.88, 1.89and 1.89 ppm. In soil phenol was detected in only one sample which was S1 in concentration of 1.89 ppm. Moreover, *p*-Cresol was also found in about all tap water samples, in drain water and one soil sample. *p*-cresol concentration was found in four tap water samples in concentration of 2.17, 2.16, 2.17 and 2.16 ppm while in drain water samples D1, D2, D3, D4 and D5 it was detected in concentration of 2.14, 2.13, 2.13, 2.14 and 2.14 ppm respectively while it was detected in only one soil sample S1 in concentration of 2.14 ppm. Detected concentrations of phenols in all samples were exceeding the standard values set by EU Directive 2455/2001/EC.

Key words: Priority Phenols · High Performance Liquid Chromatography · Catechol · *P*-Cresol

INTRODUCTION

Sufficient amount of safe drinking water is a fundamental right of every person for healthy living. However, unfortunately the availability of safe drinking water is a major problem in the world because now basic water sources hold many contaminants. These contaminants are a major cause of water borne diseases and the related deaths in different parts of the world [1]. Water may get contaminated through the anthropogenic activities. By these activities microorganisms (Bacteria) and chemicals (Benzene, arsenic, phenols etc.) are added in the water sources and cause various problems [2]. By nature soil has some organic compounds in it but the addition of harmful chemicals and other organic contaminants has led to the degradation of the soil hence, creating land pollution. This problem is being reported all around the world though, soil is a renewable resource of energy but excess damage to it can cause alarming conditions. Phenols are also called as phenolics and they belong to a class of an aromatic organic compound. The structure consists of one or more than one hydroxyl group (-OH) and it is attached to an aromatic hydrocarbon group (-CH). The structure of phenols depicts the characteristics and names of different phenols like benzo-phenol, carbolic acid, hydroxybenzene [3]. Phenol has its presence as both naturally and synthetically. Phenol exists as a component of creosote and coal tar naturally [4]. By anthropogenic activities amount of phenol increases in the environment and these activities include usage of phenolic adhesive, resins and in paints etc. As a by-product phenols are

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produced in paper and pulp, nonmetallic mineral, chemical, steel and metal, petroleum and pharmaceutical industries [4]. Phenols are harmful towards human health and environment [5]. Phenols can enter human bodies through pathways like inhalation, skin and ingestion. Its long and short term exposure both have detrimental effect on health but long term has more including cardiac arrhythmias, cancer, gastrointestinal damage, enzyme level increases in liver which causes liver enlargement and many other disorders. Whereas, it's lethal dose can also cause coma or death [6]. Its occupational impacts are also very severe. Phenols are produced during different steps of wood processing. Workers of furniture or wood industry get exposed to wood dust, bacteria and chemicals like phenols which results in severe health impacts. Diseases like impaired pulmonary functions respiratory diseases like chronic bronchitis, cancers including sinonasal cancer, lung cancer, bladder cancer, brain cancer and skin cancer. Below is some health impacts studied in wood workers [7]. Priority phenols are the compounds that consist of substituted phenolic compounds like nitrates, halogenates, ether derivatives etc. There are eleven phenols that are listed by EPA as priority phenols because of their adverse toxic effects even in the trace amount [8,9]. Basically, maximum contaminant levels (MCLs) are the standards that are given by U.S EPA for "drinking water quality". These standards provide the threshold limit by providing the allowable limit of substance in the water systems of public under the Safe Drinking Water Act (1974). The units used for the expression of these limits are milligrams per liter of water (mg/L) or micrograms per liter of water (µg/L). In addition to this, there are also two other parameters threshold limits value (TLV) and recommended exposure limit (REL) used for the employees being exposed to the chemicals on daily basis. For this research, the water quality parameters and priority phenols concentration in water samples were compared with standards provided by world health organization, National Environmental Quality Standards and European standards. Catechol has adverse health impacts on both humans and animals. Specifically in humans, catechol is known as the cause of eye burning, skin irritations and convulsions [10]. In humans, *p*-cresols has health impacts including anemia, paralysis, cramps, vomiting, irritation in mouth and throat, drowsiness, tiredness and hypertension with leads to increased heartbeat rate. It can also damage human internal organs including kidneys, CNS and liver etc [11]. They are also carcinogenic and endocrine disruptors [12].This research work was thus carried out for the qualitative and quantitative analysis of selected priority phenols in water (Tap water, bottled water and drain water) and soil samples collected from different sources of Furniture market.

MATERIALS AND METHODS

Collection of Secondary and Primary Data: Secondary and primary data was collected about priority phenols, their identification and quantification methods etc. Secondary data was collected from different sources like books, published papers; internet etc. and primary data was collected by surveying different areas.

Collection of Samples: Sampling of water (Tap water, bottled water and canal water) and soil was done from a Furniture market of Lahore (Ghora Shah Furniture Market). Water samples were collected in autoclaved bottles by grab sampling method whereas total 10 surface soil samples were randomly collected from market in plastic bags.

Selection of Priority Phenols: Three priority phenols were selected (Phenol, catechol and *p*-cresol) based on the field survey knowledge and nearby activities in the selected areas.

Chemicals, Apparatus and Instrumentation: Selected priority phenols (Phenol, catechol and *p*-cresol) were obtained from Sigma/Aldrich. HPLC grade chemicals i.e. acetonitrile, methanol and distilled water were used for the purpose of research. Main apparatus and instruments used in the experiment were micropipette, vials, High Performance Liquid Chromatography (UV- visible).

Determination of Physical Parameters: The samples were prepared for qualitative and quantitative analysis of priority phenols. The analysis of physical parameters of water samples was done by different tests like turbidity, pH, total suspended solids (TSS) and total dissolved solids (TDS) [13]. Determination of these parameters was very important to check the physical, chemical and biological properties of water and these parameters also helped us to find the water quality. The procedures that were adopted for conducting tests of physical parameters were as follows (Table 1).

pH: pH of water samples was analyzed in the environmental lab after sample collection from different sources. The instrument that was used for pH analysis

Table 1: Test Methods for Physical Parameters

Sr. No.	Physical Parameters	Test Method (Ref.??)
1	pH	US EPA 150.1
2	Turbidity	US EPA 180.1
3	TSS	US EPA 160.1
4	TDS	US EPA 160.2
5	Color	Visual Inspection

was pH meter. Firstly the small amount (10 ml) of water was taken from the collected bottled water samples. It was filtered with the help of filter paper. After filtration, the pH test was conducted by dipping probe of pH meter in water. For proper measurement the probe was rinsed with deionized water (Which removes impurities) before each test.

Turbidity: Turbidity of water samples was determined in the environmental lab. 10 ml of water was taken from different sources of water samples. It was filtered with the help of filter paper. After filtration, turbidity of water was determined with a turbid meter and the readings were noted down.

Total Suspended Solids (TSS): Total suspended solids were analyzed in all water samples that were collected from different areas. 30 ml of water was taken from different sources of water samples. It was filtered through pre-weighed filter paper. The wet filters were then placed in lab aluminum trays inside a drying oven at temperature between 103 to 105°C for one hour. The filters were allowed to cool at room temperature without the risk of being exposed to moisture in the air. When the filters cooled to room temperature, they were weighed again. For quality control purposes, the process was repeated.

Total Dissolved Solids (TDS): Total dissolved solids were analyzed in all water samples that were collected from different areas. For this purpose 30 ml of water was taken out from the collected bottles of water. It was filtered through filter paper. Then a pre-weighed china dish was taken to heat the sample. Water was evaporated due to heat. Again the weight of china dish was taken. The difference between weights of china dish showed the total dissolved solids in water samples.

Analytical Analysis

Preparation of Stock and Pure Water Sample: For HPLC analysis standards/stocks were prepared by dissolving each priority phenol (Phenol, catechol and *p*-cresol) in methanol (10 ml) solution to make 100 µg/mL concentrations. These samples were filtered and ran into the HPLC. Further stocks/standards sample peaks were compared with their respective pure samples for the qualitative and quantitative analysis. For preparation of soil samples ultrasonic Soxhlet extraction was done using Acetone, *n*-hexane as solvent.

High Performance Liquid Chromatography Conditions: For analysis, High Performance Liquid Chromatography (HPLC Aligent 1260, Quaternary gradient system) was used under the following conditions:

The HPLC system having Agilent 1260 Quaternary Pump Gradient System with ODS 18 Column was used for study. Mobile phase of methanol, acetonitrile and water (40: 40: 20) was used to elute phenols at flow rate of 0.5 mL/min. Injection volume was 20µL and detection of wavelength was280 nm. All other parameters were optimized for analyzing priority phenols. The run was 45 min, flow rate optimized as 1 ml/min, column temperature was kept at 25°C, injection volume maintained at 10µL and UV detection at 245nm. pH was adjusted to 4.5 with phosphoric acid.

Sampling, Sample Pretreatment and Standard Preparation: Water samples i.e., tap water; bottled water and drain water were collected from different sources of furniture market. The water supplied to the study area was through the city pipe lines after disinfection by chlorination. Sterilized glass bottles were used to collect water samples. After storing the water samples in ice box the samples were transferred to the laboratories for further physicochemical and analytical analysis. To carry analytical analysis, samples were first filtered through 0.45µm cellulose filter. Then acidified with HCL to make pH<2 and lastly refrigerated at 4°C until analysis. Standard were prepared by first dissolving each of the selected phenol (Phenol, catechol and p-cresol) in the methanol solution of 10ml then filtration of each standard filtered via 0.45µm filter for HPLC analysis.

Qualitative and Quantitative Analyses: Qualitative analysis was done to determine the presence of priority phenols in water. For this purpose, peak of maximum height was selected in phenols (Phenol, catechol and *p*-cresol) in standard chromatogram. Existence of each individual standard was confirmed by comparing the retention time of that selected peak in the standard chromatogram, with the sample chromatogram.

Quantitative analysis was carried out to calculate the amount of selected priority phenols in water samples. For this purpose following formulas formulated by chromacademy were used:

$$\text{Response factor} = \frac{\text{Peak area of the standard}}{\text{Standard amount (used in 1 ml of solvent)}}$$

$$\text{Amount of standard in the sample} = \frac{\text{Peak area of sample (sample peak)}}{\text{Response factor of detected priority phenol}}$$

RESULTS AND DISCUSSION

The purpose of the present study was the determination and quantification of selected priority phenols (Phenol, catechol and *p*-cresol) and other organic contaminants in water (Tap water, bottled water and drain water) and soil samples. Priority phenols consist of a number of substituted phenolic compounds. These compounds are assigned by U.S EPA as priority phenols because of their toxic effect in the minute quantity. They are found to be carcinogenic, mutagenic, endocrine disruptors etc. [14]. That's why phenol, *p*-cresol and catechol (Priority phenols) were selected in this study to determine their concentration in a furniture market of Lahore. The analytical instrument used to carry out this study was high liquid chromatography. The presence of priority phenols was confirmed by comparing the retention time of standard chromatogram with the sample chromatogram. The comparison disclosed the presence of priority phenols i.e. phenol, catechol, *p*-cresol and methanol in water samples (Tap water, bottled water and drain water) and soil samples. Phenol, *p*-cresol and methanol were observed in different samples whereas; catechol was not detected in any water or soil sample. In order to check the quality of water it was important to check the physical parameters of water i.e. (pH, turbidity, TSS and color). pH was measured by pH meter. It is an important indicator of water quality but it doesn't tell us about the water quality as a whole so there are other parameters which should be done in order to check the water quality. Turbidity is directly proportional to total suspended solids. Turbidity of water samples was measured by using turbidity meter. Total suspended solids (TSS) are also an important indicator to ensure water quality. According to the results, pH of all water samples were within the limit of WHO standards [15] (6.5-8.5 for drinking water and 6-10 for liquid effluents) [16].According to the study results turbidity of bottled water samples was within the limit i.e. 1 NTU while in four tap water samples T1(1.10), T2 (2.51), T3(2.16) and T5 (10.28) the value of TSS was exceeding the limits of standard i.e., (1NTU for drinking water) On the other hand, four drain water samples showed elevated levels of turbidity which was 7.65 in D4, 6.51 in D1, 5.21 in D3 while6.17 in D5,. In all water samples total dissolved solids were within the range set by international standards i.e., less than 150mg/L (Table 2).

The elevated levels of turbidity in most of the drain and tap water samples depicts higher amount of total solids because of high organic waste that was observed during the field survey. During analytical analysis of water samples phenol was detected and quantified in five of the tap water samples and in all drain water samples.

Table 2: Results of the physical parameters of Water Samples

Sr. No.	Water Sample	pH of sample	Turbidity (NTU) Of sample	Amount of TSS in (mg/L) Of sample
Tap Water				
1	T1	6.16	1.10	100
2	T2	6.05	2.51	100
3	T3	6.26	2.16	100
4	T4	6.22	0.34	100
5	T5	6.08	10.28	50
6	T6	6.18	0.09	50
pH standard 6.6-8.5 Turbidity standard 1.0NTU, TSS 150mg/L				
Drain Water				
11	D1	6.40	6.51	200
12	D2	6.28	3.71	100
13	D3	6.11	5.21	50
14	D4	6.59	7.65	200
15	D5	6.07	6.17	100
pH standard 6-10 Turbidity standard 5NTU, TSS 150mg/L				
Bottled Water				
16	B1	6.10	0.91	100
Standard Values: pH standard 6.6-8.5 Turbidity standard 1.0NTU TSS 150mg/L				

The reason for such a high concentration of phenol in the tap water samples was wood treatment facilities in the study area. Resins, wood adhesives and paints used in the furniture manufacturing processes are considered to be the major source of phenol. Phenol has the capability to contaminate ground water through infiltration and dumping and this may be the reason of presence of phenol in tap water samples as well. Presence of phenol in water samples is alarming as inhalation of phenol causes respiratory diseases while chronic effects include gastrointestinal effects including anorexia, liver and kidney effects. In addition to this, chronic ingestion may lead to ulcers, abdominal pain, nausea and vomiting causing diarrhea and even severe headache. Chronic effect of phenol on the skin includes necrosis and dermal inflammation [17]. Quantification analysis of showed the presence of *p*-cresol in various water samples in comparatively higher concentration. In tap water samples, the detected concentration of phenol in T3, T4, T5 and T6 tap water sample was 1.91, 1.90, 1.91 and 1.90 ppm. Whereas, phenol was detected in all drain water samples in concentrations of 1.89 (D1), 1.86 (D2), 1.88 (D3) and 1.89 ppm (in both D4 and D5 sample). In soil phenol was detected in only one sample which was S1 in concentration of 1.89 ppm. Moreover, *p*-Cresol was also found in about all tap water samples i.e., T3, T4, T5 and T6 in concentration of 2.17, 2.16, 2.17 and 2.16ppm respectively, in drain water samples i.e., D1, D2, D3, D4 and D5 in concentration of2.14, 2.13, 2.13, 2.14 and 2.14ppm and in one soil sample S1 in concentration of 2.14ppm out of ten collected soil samples.

Table 3: Results of HPLC Quantitative Analysis.

Sample Code	Phenol (ppm)	p-Cresol (ppm)	Catechol (ppm)
Tap Water			
T3	1.91	2.17	□
T4	1.90	2.16	□
T5	1.91	2.17	□
T6	1.90	2.16	□
Drain Water			
D1	1.89	2.14	□
D2	1.86	2.13	□
D3	1.88	2.13	□
D4	1.89	2.13	□
D5	1.89	2.14	□
Soil Sample			
S1	1.89	2.14	□

The presence of *p*-cresol in tap water and drain water indicated ground water contamination because of discharge of effluents from wood processing units either to soil or directly to drains. Another reason of the detection of priority phenols in water was domestic waste that may contain home-care products like breathe fresheners, lotions etc. These products also contain small amounts of priority phenol. The detected phenols are associated with health impacts like anemia, hypertension by increasing the heartbeat rate, paralysis, vomiting, cramps, irritation in mouth and throat, headache, drowsiness etc. It also damages human organs like kidneys, liver, CNS [18]. EU Directive 2455/2001/EC sets a maximum concentration of 0.5 μgL^{-1} for phenols in drinking water and according to them their individual concentration should not exceed 0.1 μgL^{-1} but our detected values were far exceeding these standard values. Thus proper and strict regulations should thus be imposed to ban the use of priority phenols in the manufacturing of various products to save people from their adverse impacts.

CONCLUSIONS

The present study was conducted to access the presence of priority phenols (Phenol, catechol and *p*-cresol) in water (Tap water, bottled water and drain water) and soil samples from Furniture market area. The results of this study depicts that the priority phenols presence depend on the activities going around in the catchment area of water and soil. Phenol was detected in four tap water samples above the threshold limit by the EU Directive 2455/2001/EC standards (0.5 μgL^{-1} in drinking water). It was also detected and quantified in one drain and one of the soil samples. Moreover, *p*-cresol was detected in tap water samples, drain water samples and in soil samples. Catechol was not present in any of the samples. The presence of phenol and *p*-cresol in water samples and soil samples was due to different activities in the catchment area of water. Throughout the year, these compounds were used by furniture industry as adhesives and other requirements. Extensive use of these compounds in furniture making leads to possible groundwater pollution. Proper and strict regulations should be imposed to ban the use of priority phenols in the manufacturing of various products to save people from their adverse impacts.

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