

GC-MS-ESTIMATION AND EXPOSURE LEVELS OF ENVIRONMENTAL BENZENE IN THE BTEX-MIXTURE OF AIR-POLLUTANTS IN GASOLINE STATIONS AND URBAN ROAD SIDES

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ABSTRACT

The main objectives of the present study are firstly to establish and validate sampling procedure, extraction and to estimate the concentrations of environmental benzene and secondly, to assess the exposure level and potential risk, for the inhalation of environmental benzene pollutant by workers of Gasoline stations and Khartoum inhabitants. A personal air sampling monitors were used to collect air samples from Gasoline Stations and heavy traffic Road Sides at different locations in the Khartoum City, within a circle of a diameter of nearly 16 kilometers. Environmental benzene, as a component of BTEX-mixture of air pollutants, was collected and adsorbed by a charcoal sorbent tube sampler equipped with a pump, extracted by carbon disulphide and then separated from the mixture and estimated by Gas Chromatography/Mass Spectroscopy GC-MS technique. Methods validation gave a correlation coefficient (R^2): 0.9951, while the solvent desorption recovery of benzene from spiked air samples was found to be 81% with a coefficient of variation (CV 0.225 %). The mean concentration of benzene pollutant in the 10 Gasoline Stations was $285.425\mu\text{g}/\text{m}^3$ (0.088 ppm) with a minimum level of prevalence $9.286612\mu\text{g}/\text{m}^3$ (0.0029 ppm) and a maximum level of prevalence of $578.612\mu\text{g}/\text{m}^3$ (0.1780 ppm). The mean concentration of benzene in heavy traffic 10 urban Road Sides was $44.802\mu\text{g}/\text{m}^3$ (0.014 ppm), with a minimum level of prevalence of 20.03 (0.0062 ppm) and a maximum level of prevalence of $74.90\mu\text{g}/\text{m}^3$ (0.0230 ppm). The mean value for both sites have shown, significantly, higher p-value = 0.00. The average exposure levels of environmental benzene in the Gasoline Stations and traffic Road Sides (0.088 ppm \approx 0.09 ppm) and (0.014 ppm), respectively, are both higher by a number of folds than the minimal risk levels (MRLs) acute, chronic and intermediate minimal inhalation. All results have been validated by statistical data analysis.

Keywords: Environmental Benzene, BTEX-pollutants, GC-MS, Gasoline Stations.

INTRODUCTION

The adverse health effects of air pollution became widely acknowledged after severe pollution episodes in Europe, North America and the third World is not abjured. During the last 15 years, however, consistent results, mainly from epidemiological studies, have provided evidence that current air pollutant levels have been associated with adverse long and short-term health effects, including an

increase in mortality (Klea Katsouyann, 2003). And incidence of cardiac arrhythmia (Peters Annett *et al.* 2000; Kristin *et al.* 2007) in addition to its association with heart failure and other cardiovascular causes of death (Hoek Gerarad *et al.* 2001). These effects have better been studied for ambient particle concentrations in the ambient air, but there is substantial evidence concerning gaseous pollutants such as ozone and volatile organic compounds VOC.

Atmospheric benzene, also known as environmental benzene, is one of the components of the VOC mixture of air pollutants and it is also included in the subgroup of air-pollutants, known to environmentalists as BTEX-pollutants. A mixture or cluster of volatile aromatic compounds comprising: benzene, toluene, ethyl benzene and the three isomeric xylenes,

Approximately, 70% of mobile source benzene emissions can be attributed to on-road motor vehicles, with the remainder attributed to non-road mobile sources such as chemicals industries and other human practices (U.S. EPA, 1993b). Some of the benzene in the fuel is emitted from vehicles as unburned or uncombusted fuel.

The predominant sources of total benzene emissions in the atmosphere are gasoline fugitive emissions during transport, fueling service and gasoline motor vehicle exhausts. Mobile sources contribute 85%. Benzene has wide spectrum applications in industry-related stationary sources such as medicinal chemicals, shoes, dyes, detergents, explosives, linoleum, oil, cloth and artificial leather, which constitute 15% of the emissions. Benzene is a solvent for waxes, fats, resins, paints, plastics, and fast drying inks. Other uses are as a raw material in the synthesis of organic compounds such as cyclohexane, styrene, phenol, and rubber (HSDB, 1995). With such wide spectrum of uses and applications, the hazardous effects and its negative health effects of benzene had received much concern by environmentalists and occupational healthcare circles.

The atmospheric lifetime and half-life of benzene are estimated to be 12 days and 8 days, respectively. The risk assessments reviewed as of April 1996, benzene was the major contributor to the overall cancer risk in 85 of the approximately 550 risk assessments reporting a total cancer risk. Benzene was also the major contributor to the overall cancer risk in 16 of the approximately 130 risk assessments reporting a total cancer risk equal to or greater than 10 in 1 million, and contributed to the total cancer risk in 92 of these risk assessments (OEHHA, 1996a).

For non-cancer health effects brief inhalation exposure to high concentrations of benzene can cause central nervous system depression. Acute effects include central nervous system symptoms of nausea, tremors, drowsiness, dizziness, headache, intoxication, and unconsciousness. Benzene vapors are mildly irritating to the eyes and respiratory tract. Increased incidences of leukemia, especially acute myelogenous leukemia and its variants including erythroleukemia and myelomonocytic leukemia have been observed in humans occupationally exposed to benzene (ARB, 1984a). A retrospective mortality study in China in 1989 has provided supporting evidence that benzene exposure is associated with cancers in humans (Yin, 1989). Animal cancer bioassays show benzene causes leukemia and a variety of other cancers including cancers of the lymphoid system, skin, ovary, oral cavity, lip, tongue, lung, mammary gland, and two secretors organs unique to rodents, the Zymbal and perpetual glands (ARB, 1984a).

The U.S. EPA classified benzene in Group A-human carcinogen based on sufficient epidemiological evidence, and determined an inhalation potency value of 8.3×10^{-6} (microgram per cubic meter)⁻¹. The U.S. EPA estimates that if an individual were to breathe air containing benzene at $0.1 \mu\text{g}/\text{m}^3$, over a lifetime, that person would theoretically have no more than a 1 in 1 million increased chance of developing cancer (U.S. EPA, 1994a). The International Agency for Research on Cancer (IARC) classified benzene in Group 1: Human Carcinogen based on sufficient evidence in humans (IARC, 1987a).

The agency for toxic substances and disease registry (ATSDR 1991, 2010), categorized benzene inhalation as the major cause to, acutely, affect the immune system Table (1).

Table 1: Minimal Risk Levels (MRLs), Agency for Toxic Substances and Disease Registry (ATSDR, 1991, 2010)

Compounds	Acute	Chronic	Intermediate	End Point
	Inhalation	Inhalation	Inhalation	(acute) (chronic) (Intermediate)
Benzene	0.009ppm	0.003ppm	0.006ppm	Immune System for all types
Toluene	1.000ppm	0.800ppm	-	Neurol. System for all types
Ethylbenzene	5.000ppm	0.060ppm	2.000ppm	Neurol. Renal Neurol
Xylenes	2.000ppm	0.050ppm	0.600ppm	Neurol. System for all types

For duration, Acute = 1 to 14 days. Intermediate = 15 to 364 days and Chronic = 1 year

Although the assessment of toxic and probably carcinogenic, VOCs (BTEXs)-air-pollutants has become a vital global issue of air quality and of crucial concern to environmentalists and occupational health professionals and researchers, yet no research investigations have been conducted prior to this current study in the Sudan or the region. The number of exposed people to these carcinogenic atmospheric pollutants, in Khartoum City, is unaccountable. The population of the City, during the last two decades was tripled and hence the number of vehicles and fueling gasoline stations in addition to crowding of vehicles in the City streets has also increased. A number of conferences, international seminars and workshops had been organized to discuss the alarming rate of cancer cases in the Sudan, yet no remarks of the causes of these cancer cases had been mentioned. The present work describes, in one hand, a selective method of sampling, an efficient extraction procedure and, on the other, adopting a sensitive GC/MS-method of analysis estimating only the concentration level of benzene-air pollutant within the BTEX-mixture collected from Gasoline Stations and heavy traffic Road sides. Moreover the exposure levels to workers and populations in nearby residential areas have been monitored and, accordingly, the exposure levels of environmental benzene has been assessed and reported.

1. Material and methods

1.1 Sampling locations and air sampling system

Thirty air samples were collected from 10 Gasoline Stations and another thirty air samples were collected from 10 heavy traffic Road Sides in Khartoum City. The sampling locations extended, randomly, over a circle of a diameter of 12 kilometers. Sampling was performed towards the end Autumn season 2010, where the prevailing climatic conditions, comparatively to other seasons, were mild. The average day temperature was in the range of 36-40°C during mornings and evenings and 45-52°C during noon (midday). The humidity was 10% and the wind direction was south to north. Air samples were collected during the three periods of the day: morning from 9-10 am, afternoon from 1-2 pm and evening from 6-7 pm. (10 samples per period per day).

The sampling system consisted of absorbent tube (Skc. Inc) activated charcoal tube attached with low flow sampling pump (I.S. version, CAELLA CEL, Regent House, Wolseley Road Kempston Bedford MK 42 7JY). The sampling flow rate was specified as approximately 200 ml/min. The air sampler was erected vertically 130 cm high from the ground (near the breathing zone). After a sample was collected, the sorbent tube was stored at 4 °C in refrigerator prior to GC-MS. analysis.

1.2 Quality Control Analyses

1.2.1 Preparation of working standards

Stock BTEX standard solution (2 mg/ml) was prepared in carbon disulphide. A portion of the standard solution (50µL) was diluted to get a concentration of 20 µg/mL as a primary solution. Three working standards were prepared from this primary solution: 1.0ng/ µL, 2.50ng/ µL and 5.0 0ng/ µL, respectively.

1.2.2 Calibration of the GC-MS instrument

The quality control program has included, firstly the calibration of the GC-MS instrument, which consists of preparing three benzene standard samples of concentrations 1.00 ng/L; 2.5.00 ng/L and 5.00 ng/L were made and injected, separately, into the GC-MS-instrument. The retention time was recorded to be 3.463 min and m/z to be 78. The resulting calibration curves had shown that the calibration coefficient (R^2) was found to be 0.99517, the RSD 15% and the limits of detection (LODs) for benzene was 0.0293 ng/µl (6.02469µg/m³).

Percentage Recovery of benzene from BTEX-spiked samples

Pollutant free air-free-samples were spiked with 4.00 ng/µL of standard BTEX-standard mixture. The BTEX mixture was then desorbed from the charcoal tube as described above and then the % recovery of benzene was then determined from the resulting GC-MS-chromatogram of the BTEX-mixture. Four trials on spiked air samples were performed to yield (3.6301; 3.6501; 3.6401 and 3.6301 ng/L with a mean of 3.6401 and % recovery of 81% and a coefficient of variation (CV) of 0.225.

1.3 Desorption (extraction of BTEX-pollutants GC-MS analysis

The plastic-end-caps of the charcoal tube were removed and the two sections (front and back) of the activated charcoal were, carefully, transferred into two vials using carbon disulfide (2 ml) as a solvent. After ultrasonication for, approximately, 30 min, the vials were stabilized for 1 hr and placed in a fridge prior to GC-Ms-analysis.

1.4 GC-MS analysis of the naturally polluted BTEX-samples

Appropriate aliquots of the BTEX-extracts or field blanks were separately being analyzed. Aliquots were injected, according to manufacturer instructions, into the gas chromatograph /mass spectrometer (GC-MS) [Schmatzu-GC-MS Q2010, SERIALNO. C70264101271SA P/N 225-10040-34], for analysis. Two types of capillary columns have been tested for their resolution efficiency: the DB-5-nonpolar column (30 m X 0.25mm X 0.25 μ m) and RTX-50-medium polar column (30m X 0.25mm X 0.25 μ m). The GC/MS-Operating Conditions were as follows: Injection temperature was 180 $^{\circ}$ C, Split ratio 20:1. Column pressure 47.7, Total Flow 24 ml/min, Column Flow 1ml, Purge Flow 3ml/min. The oven temperature program: Initial: 35 $^{\circ}$ C initial (hold time 2 min); raised to 60 $^{\circ}$ C at a rate of 5 $^{\circ}$ C /min (hold time 1 min); raised to 75 $^{\circ}$ C at a rate 5 $^{\circ}$ C / min (hold time 1min) and finally raised to a constant temperature of 220 $^{\circ}$ C at a rate of 20 $^{\circ}$ C /min.

RESULTS AND DISCUSSION

Samples obtained from these two collection sites, the Gasoline Stations and Road Sides, are referred to as near and far air sample, based from their distance from the source of emission. Such protocol of sampling designations is aimed revealing the concentration levels of the benzene pollutant in the ambient air of the City and also represent a measure of exposure levels to which the Gasoline Station workers are subjected to. The far air samples concentration levels of benzene represent a measure of exposure levels of the pedestrians, traffic police and drivers of vehicles as well as the exposure levels to which the inhabitants of the nearby residential areas are exposed to. Table (2) represents the mean levels of benzene pollutant in air samples (285.425 μ g/m³) (0.088ppm) collected inside the 10 Gasoline Stations and the mean levels of benzene (44.802 μ g/m³) (0.014ppm) collected on the 10 roads sides, at least 200 meters far from the Gasoline Stations.

Table 2: The Mean Concentration Levels of BTEX-Pollutants in the Ten Gasoline Stations and on the Road Sides

Gasoline stations Mean (μ g/m ³)	Benzene	SD*	Road sides Mean (μ g/m ³)	Benzene	SD
1	9.286	4.350	I.	25.433	18.576
2	100.108	31.234	II.	63.995	25.644
3	307.433	77.781	III.	40.337	16.446
4	430.746	160.69	IV.	39.934	19.306
5	578.612	101.086	V.	74.90	61.900
6	217.766	52.888	VI.	20.030	12.726
7	514.555	256.501	VII.	31.605	19.946
8	90.545	26.813	VIII.	29.343	15.811
9	332.744	135.875	IX.	71.572	67.607
10	272.455	237.540	X.	50.875	20.529
Mean of Gasoline stations	285.425 μ g/m ³ (0.088ppm)	177.657	Mean of Roads sides	44.802 μ g/m ³ (0.014ppm)	18.637

Mean = the average value for the levels of benzene at three periods (morning, noon and evening)
SD = the standard deviation

From the practical results obtained (Table 2), the following observations and findings were revealed: It has been found that the average concentration level of Benzene in air samples collected inside the Gasoline Stations is 285.425 μ g/m³ (0.088 ppm), which is 6 times higher than that monitored for the Road Sides 44.802 μ g/m³ (0.014 ppm), to confirm this observation the statistical analysis T-test was used to compare the mean benzene levels in the gasoline stations and road sides. Benzene exposure levels among the gasoline station (mean = 285.425 μ g/m³) were significantly higher ($p < 0.000$) than those among the roads sides (mean = 44.802 μ g/m³) as shown in (Table 3).

Table 3: The mean concentration levels of the benzene pollutant in the 10 Gasoline Stations and the 10 Road Sides

Site	Number of samples	Mean $\mu\text{g}/\text{m}^3$	Standard Deviation	Standard Error Mean
Gasoline Station	30	185.4249	88.0881	7.64301
Road Sides	30	44.8021	39.06577	4.13240

P-Value = 0.000

This extremely high prevalence of benzene pollutant is, presumably, due to the fact that, the former is very near to the source of emission, which is a constant and stable source, while the latter, beside it is, comparatively, far from the source of emission, it is dependent on the magnitude of the traffic on the street and it an open site is also dependent on the prevailing climatic factors: wind direction, wind speed, presence of particulate matter and dust, temperature and humidity. Such site is considered a moving and unstable source.

It could be noticed from Table 2, that the average standard deviation was high (177.650) and (18.637) for both the Gasoline Stations and Road Sides, respectively. The environmental factors, mentioned earlier, are presumably; differ in magnitude during the mornings, noon or evenings. Accordingly, the magnitude of such climatic factors is unpredictable and, in some times, also immeasurable, in addition to the fact that they are difficult to control.

It could also be observed from Table (2) that air samples collected from the Gasoline Station (1) ($9.286\mu\text{g}/\text{m}^3$) have, exceptionally, shown profoundly low levels of benzene. This was attributed to the reason that on that during the day of sampling the wind speed, at that site, was high and the atmosphere was, tremendously, contaminated with particulate matters such as soil, sand, and dust particles, which have minimized the aggregation of benzene to the BTEX-mixture in one hand, and on the other greater quantity of benzene molecules were adsorbed to the particulate particles, accordingly decreasing the concentration of the BTEX level in the air.

Air samples were collected at three periods of the day (morning, noon and evening) from gasoline stations and roads sides, the results were compared and validated using one way ANOVA statistical analysis. The results have shown that the concentration levels of benzene in gasoline stations, were insignificantly different between each period of the day ($p = 0.956$), Table 4.

Table 4: The benzene Concentration Levels in the Gasoline Stations at three Periods of the Day

Durations	Sample No.	Mean $\mu\text{g}/\text{m}^3$	Standard Deviation	Standard Error
Morning	10	271.3856	224.46775	70.98295
Afternoon	10	302.4629	283.44567	89.63339
Evening	10	282.4264	190.66936	60.29495
Total	30	285.4249	228.08817	41.64301

P = 0.956

But the concentrations levels of benzene on roads sides Was significantly different between each period ($p = 0.027$), and was highest at mornings and lowest in evenings, as shown in (Table 5).

Table 5: Benzene Concentration Levels in the Roads Sides at three Periods of the day

Durations	Sample No.	Mean $\mu\text{g}/\text{m}^3$	Standard Deviation	Standard Error
Morning	10	70.7958	54.39627	17.20161
Afternoon	10	35.4285	18.42293	5.82584
Evening	10	28.1821	21.53825	6.81099
Total	30	44.8021	39.06577	7.13240

p-value = 0.027

It is worth mentioning that the Gasoline Station is a continuous source of emission, which is a constant and stable source. Comparatively, Road Sides location is, comparatively, far from the source of emission, depending on the traffic density and the prevailing climatic factors. Such site is considered a moving and unstable source.

Environmental Benzene Prevalence in Air and Exposure Levels

The average levels of prevalence of environmental benzene in the Gasoline Stations air have been found to be $285.425\mu\text{g}/\text{m}^3$ ($0.088\text{ ppm}\approx 0.09\text{ ppm}$). The value obtained in the present study is 10 times higher than the MRLs acute minimal inhalation (0.009 ppm), 30 times higher than chronic (0.003 ppm) and 15 times higher than intermediate inhalation value (0.006 ppm). Level of exposure is alarming and may affect the immune system of the gasoline station workers. Moreover, longer periods of exposure (average live time) may lead to leukemia cases among these subjects.

On the other hand the average levels of prevalence of environmental benzene in locations far from the gasoline stations and beside the 10 road sides was found to be $44.802\mu\text{g}/\text{m}^3$ (0.014 ppm) this value is 1.5 higher than the MRLs acute inhalation value of 0.009 ppm , 4 times higher than the chronic 0.003 ppm and 2 times higher than the MRLs intermediate inhalation value (Table (1) and Table (2)). It is evident that the value of benzene pollutant within the road sides is extremely higher than the MRLs by several folds, which would definitely affect the health of the pedestrians, residents of the nearby community and even the drivers of vehicles.

Chronic human exposure to benzene may result in adverse hematological effects, characterized by a variety of blood dyscrasias, including leucopenia, thrombocytopenia, pancytopenia and anemia (ARB, 1984a). Benzene is also considered to be a human carcinogen and is well established as a cause of leukemia (JARC, 1982). There are many case reports, case series and epidemiological studies associating exposure to benzene with leukemia in humans. However, very few of these studies have provided quantitative information about the relative risk of leukemia under specified conditions of exposure (Aksoy et al, 1972, 1974; Askoy, 1980; Thorpe 1974. The results of a U.S. National Institute of Occupational Safety and Health (NIOSH) study of male workers exposed to benzene during the manufacture of rubber hydrochloride have been published on three occasions. The findings were essentially the same in each of the three published studies: there was a three-fold increase in the risk of death from lympho-haematopoietic cancers and an almost six-fold increase for leukemia cases. Among workers employed for 5 years, 5 had died from leukemia, compared to 0.23 expected, indicating a 21-fold increase in risk.

Based on the fact that the current reported work is the first endeavor of investigation of volatile organic compounds in the Sudan. Accordingly, many practical laboratory parameters have now been, set up, and confidently, been established and tested as to produce reliable results. Laboratory procedures and research roads have now been paved to extend research in atmospheric pollution.

CONCLUSIONS

The identification and determination of the levels of benzene-pollutant in the environment provides a description of the current environmental situation, in respect, to one of the most important group of carcinogenic volatile aromatic hydrocarbons species. The work provides the appropriate sampling and estimation techniques that enable young environmentalists with the required laboratory skills to monitor air pollution in African capital cities. The workers in gasoline stations were found to be the mostly exposed **subjects** to extremely alarming levels of the benzene pollutants through all durations of the day. On the other hand, pedestrians, drivers of vehicles and residents of the nearby community to the traffic roads are also exposed to a high concentrations of benzene pollutant at only the morning period compared with noon and evening.

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