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Isotopic Abundance Analysis of Biofield Treated Benzene, Toluene and *p*-Xylene Using Gas Chromatography-Mass Spectrometry (GC-MS)

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Abstract

Benzene, toluene and *p*-xylene are derivatives of benzene, generally produced from crude petroleum and have numerous applications in industry. The aim of the present study was to evaluate the impact of biofield treatment on isotopic abundance of these benzene derivatives by gas chromatography-mass spectrometry (GC-MS). Benzene, toluene and *p*-xylene samples were divided into two parts: control and treatment. Control part was remained as untreated and treatment part was subjected to Mr. Trivedi's biofield treatment. Control and treated samples were characterized using GC-MS. GC-MS data revealed that isotopic abundance ratio of $^{13}C/^{12}C$ or $^{2}H/^{1}H$ (PM+1/PM) of treated samples were significantly increased from un-substituted to substituted benzene rings (where, PM- primary molecule, PM+1- isotopic molecule either for $^{13}C/^{12}C$ and/or $^{2}H/^{1}H$). The isotopic abundance ratio of $^{13}C/^{12}C$ or $^{2}H/^{1}H$ (PM+1/PM) in benzene was decreased significantly by 42.14% as compared to control. However, the isotopic abundance ratio of (PM+1/PM) in treated toluene and *p*-xylene was significantly increased up to 531.61% and 134.34% respectively as compared to their respective control. Thus, overall data suggest that biofield treatment has significantly altered the isotopic abundance ratio of (PM+1/PM) in a different way for un-substituted and substituted benzenes.

Keywords: Biofield treatment; Benzene; Gas Chromatography-Mass Spectrometry; Toluene; *p*-xylene

Abbreviations

GC-MS: Gas chromatography-Mass spectrometry; PM: Primary molecule; PM+1: Isotopic molecule either for $^{13}C/^{12}C$ or $^{2}H/^{1}H$

Introduction

Benzene, toluene and *p*-xylene isomers are nonpolar organic liquid, volatile, aromatic and the most important constituents of gasoline (Figure 1). Benzene, toluene and *p*-xylene are one of the important sources of energy being utilized with petrol/gasoline for vehicle fuel [1]. All three compounds are produced during the process of making gasoline and other fuels from crude oil as well as in making coke from coal. Benzene have been used for long time in manufacturing plastics, detergents, pesticides, and other chemicals such as ethyl benzene, cyclohexane, nitrobenzene, chlorobenzenes and maleic anhydride [2,3]. Toluene has numerous commercial and industrial applications and was used as a solvent in paints, lacquers, thinners, glues, correction fluid, nail polish remover, and in printing and leather tanning processes. *p*-xylene has been used in adhesives industry, paint industry, and as manufacturing solvent in rubber industries [4]. Molecule wise production, air contamination, World Health Organization (WHO) permissible limit, and percentage use of these compounds in petroleum products are shown in Table 1. Besides their importance, benzene has acute toxicity than the other components, toluene and *p*-xylene. Because of their low water solubility, acute toxicity and genotoxicity, benzene derivatives were classified as priority pollutants by the US Environmental Protection Agency [5]. The evidence for carcinogenicity of benzene in humans was evaluated by the International Agency for Research on Cancer (IARC) in 1982. Benzene, toluene and *p*-xylene can cause damage to the haematopoietic system, including pancytopenia [6]. While toluene and *p*-xylene have no direct effect on human, it was believed that cancer risks associated with toluene or *p*-xylene because of benzene impurities [7]. The prime sources of air and aquifer contamination by these molecules were due to the sequences of accidental gasoline spills, evaporation from petroleum refineries and leakage from service station tanks [8]. Benzene and naphthalene have less frequently degraded chemically than substituted aromatics. These substituted and non-substituted hydrocarbons have degraded by catabolically active bacteria or gasoline-degrading microorganisms for the purposes of *in situ* aquifer bioremediation, suggesting co-oxidative or syntrophism processes [9,10].

Rate of chemical reaction depends on the mass of the nucleus, and isotopic substitutions slightly affect the partitioning of energy within molecules. These deviations from perfect chemical equivalence are termed isotope effects. Absolute abundances of isotopes are commonly reported in terms of atom percent.

For example, ^{13}C, atom percent $^{13}C=[^{13}C/(^{12}C + ^{13}C)]100$

Various applications of isotope abundance study includes (a) the distribution of contaminant sources of any molecule on a native, regional, and global scale, (b) the identification and quantification of alteration reactions and (c) the characterization of elementary reaction mechanisms that govern product formation [11].

The stability of benzene derivatives could be enhanced by Mr. Trivedi's unique biofield treatment which is already known to alter the physical, and structural properties of various living and non-living substances [12]. Scientists have postulated that it is due to the flow of bioelectricity in the human body. When an electrical signal passes through any material, a magnetic field is generated in the surrounding space [13]. Human has the ability to harness energy from environment/universe and can transmit into any object (living or non-living) around the globe. The object(s) always receive the energy and responded into useful way that is called biofield energy. This process is known as biofield treatment. Mr. Trivedi's unique biofield treatment is also called as The

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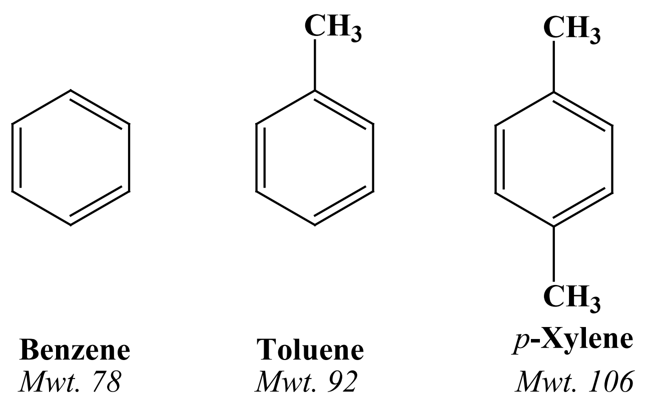

Figure 1: Structure of benzene, toluene and *p*-xylene.

Trivedi Effect®, which known to alter the physical, structural and atomic properties in various metals [14-16] and ceramics [17,18] in material science. Additionally, biofield treatment has been studied in the field of microbiology [19,12], biotechnology [20,21], and agriculture [22-24]. We have reported that biofield treatment has substantial altered the atomic, structural and physical properties in silicon carbides [25] and carbon allotropes [26]. Based on the outstanding results achieved by biofield treatment on metals and ceramics, an attempt was made to evaluate the effect of biofield treatment on isotopic abundance ratio of either $^{13}C/^{12}C$ or $^{2}H/^{1}H$ (*i.e.*, PM+1/PM) in treated samples of benzene, toluene and *p*-xylene as compared to control.

Experimental

Benzene was procured from Qualigens Fine Chemicals, India. Toluene was procured from Merck India and *p*-xylene was procured from Research lab, India.

Biofield treatment modalities

Benzene derivatives (Benzene, Toluene and *p*-xylene) were taken in this experiment for biofield treatment. The compounds were divided into two parts named as control and treated. No treatment was given to this set. The second set (T1, T2, and T3) of benzene derivatives was handed over to Mr. Trivedi for biofield energy treatment under standard laboratory conditions. Mr. Trivedi provided the biofield treatment through his energy transmission process to second sets of samples without touching the samples. After treatment, the treated samples were stored at standard conditions for GC-MS analysis as per the standard protocol. An optimum precautionary measure was taken throughout the experiment. The experimental results in treated groups were analysed and compared with the untreated (control) set.

GC-MS spectroscopy

The gas chromatography-mass spectroscopy (GC-MS) analysis was performed on Perkin Elmer/auto system XL with Turbo mass, USA, having detection limit up to 1 picogram. For GC-MS analysis the treated sample was further divided into three parts as T1, T2 and T3. The GC-MS data was obtained in the form of % abundance vs. mass to charge ratio (*m/z*), which is known as mass spectrum. The isotopic abundance ratio of $^{13}C/^{12}C$ or $^{2}H/^{1}H$ (PM+1/PM) was expressed by its deviation in treated samples as compared to the control. The percentage change in PM+1/PM isotopic ratio was calculated on a percentage scale. The values PM+1/PM of treated samples were calculated from the following formula:

$$\text{Isotopic abundance ratio (\%)} = \frac{R_{\text{Treated}} - R_{\text{control}}}{R_{\text{control}}} \times 100 \qquad (1)$$

Where, R_{Treated} and R_{Control} are the ratio of intensity at PM+1 to PM in mass spectra of treated and control samples respectively.

Results and Discussion

GC-MS Spectroscopy

The mass spectra of control and treated samples of benzene, toluene and *p*-xylene are shown in (Figures 2-10).

GC-MS spectra of benzene: Base peak was observed at *m/z*=78 in control and treated samples (T1 and T2) (Figure 2), whereas in T3 sample, the most intense peak was found at *m/z*=77 (Figure 3). The intensity ratio of PM+1 and PM peaks are presented in (Table 2). Three major peaks at *m/z*=50, 39 and 26 were observed in control benzene sample due to degradation of benzene to C_4H_2, C_3H_3 and C_2H_2 ions. Peak at *m/z*=50 in control was observed after leaving an ethylene fragment from benzene. Benzene after breaking into two equal parts showed peak at *m/z*=39 in control. Finally, acetylene was produced in benzene fragmentation which was seen at *m/z*=26 in control. More importantly the treated benzene samples were fragmented in to four major peaks at *m/z*=63, 50, 39 and 26. Three peaks (except peak at *m/z*=63) were same for both treated and control samples of benzene. This peak at *m/z*=63 was responsible for leaving one methyl group after ring opening (due to C_5H_3) [27].

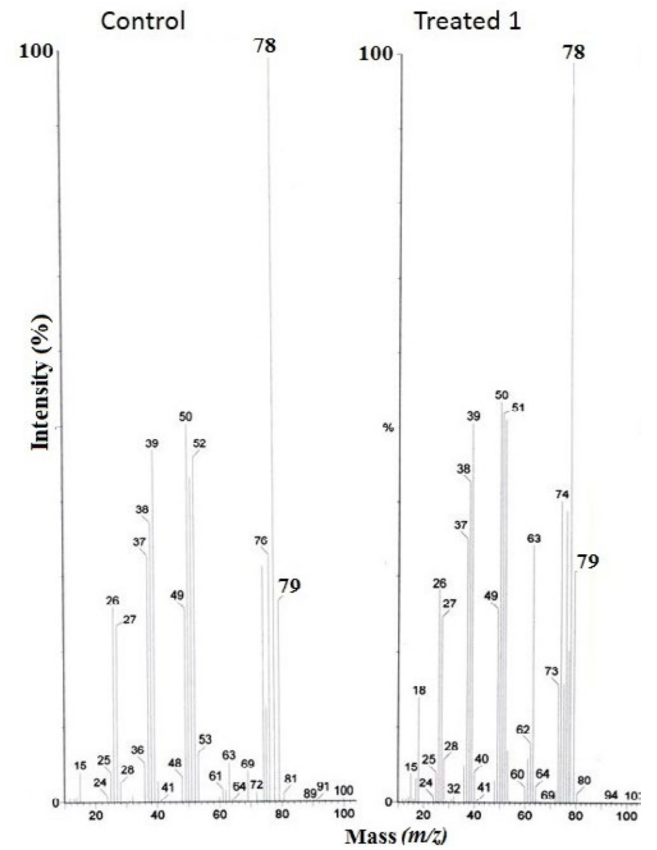

Figure 2: GC-MS spectra of control and treated (C and T1) samples of benzene.

Isotopic abundance ratio of $^{13}C/^{12}C$, or $^{2}H/^{1}H$ (PM+1/PM) in

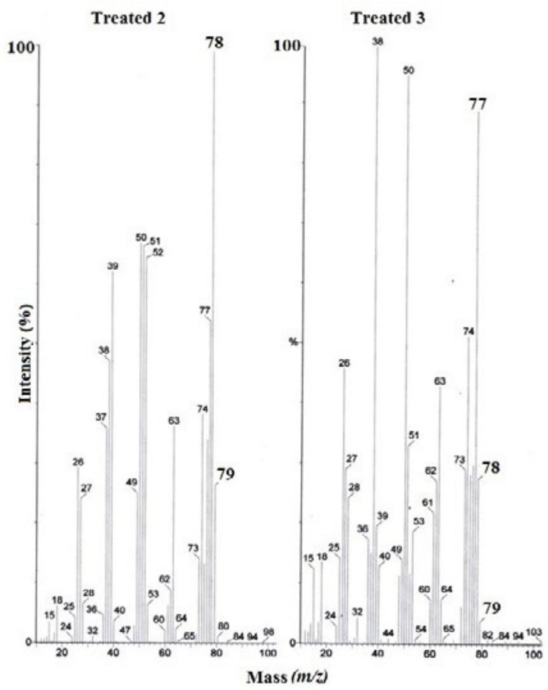

Figure 3: GC-MS spectra of treated (T2 and T3) samples of benzene.

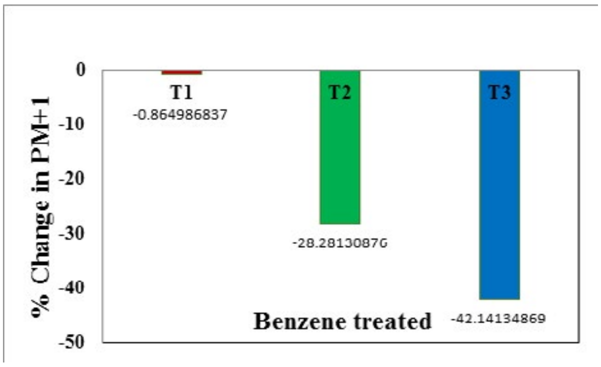

Figure 4: Percent change in isotopic abundance ratio of (PM+1/PM) in treated samples of benzene (T1, T2 and T3).

control and treated benzene was calculated and presented in the (Figure 4). In the bar diagram, the isotopic abundance ratio of PM+1/PM of treated benzene ring was decreased up to 42.14% after biofield treatment as compared to control. In mass spectrum, number of stable fragments were increased by one (3→4 peaks from control to treated) in treated sample of benzene as compared to control. The decreased isotopic abundance ratio of PM+1/PM in treated benzene (Figure 4) samples may be due to decrease μ (reduced mass) and binding energy in molecules with lighter isotopes.

GC-MS spectra of toluene: Molecular ion peak was observed at *m/z*=92 in both control and as well as treated samples with different intensity ratio (Figures 5-6). The intensity ratio of PM peak and PM+1 peak are given in (Table 3). Other than the base peak, four major peaks at *m/z*=63, 51, 39, and 26 were observed for both control and treated samples of toluene due to C_5H_3, C_4H_2, C_3H_3 and C_2H_2 ions, respectively. Peak at *m/z*=63 was observed due to fragmentation of toluene to C_5H_3 and ethylene. Peak at *m/z*=51 was observed after leaving an ethylene and methyl fragment during the process of toluene fragmentation. One low intensity peak at *m/z*=77 was observed due to the formation of benzyl ion (C_6H_5) which was further degraded to propa-1,2-diene radicals and acetylene, responsible for the intense peak at *m/z*=39 and *m/z*=26 and respectively. Fragmentation pattern and number of fragmented peaks were same for control and treated toluene samples. Isotopic abundance ratio of $^{13}C/^{12}C$, or $^{2}H/^{1}H$ (PM+1/PM) of control and treated toluene was calculated and presented as bar diagram in the Figure 7. In the bar diagram, the isotopic abundance ratio of PM+1/PM of treated toluene was increased significantly up to 531.61% (in T1) after biofield treatment as compared to control. Atoms taking part in chemical bonds with higher isotopic number might have higher binding energy with increased μ (reduced mass) and *vice versa*. Thus the increased isotopic abundance ratio of PM+1/PM in toluene might increase the reduced mass and binding energy after biofield treatment that may enhance the stability of toluene significantly [28].

GC-MS spectra of p-xylene: Molecular ion peak was observed at *m/z*=106 in both control and treated samples with different intensity ratio (Figures 8-9). The intensity ratio of PM+1 and PM peaks are given in (Table 4). Other than the molecular ion peak (at *m/z*=106), six major peaks at *m/z*=91, 77, 63, 51, 39, and 26 were observed in both control and treated samples of *p*-xylene in the mass spectrum due to C_7H_7, C_6H_5, C_5H_3, C_4H_2, C_3H_3 and C_2H_2 ions, respectively. We have already discussed about the origin of all six peaks following the degradation pattern of benzene and toluene for both control and treated in toluene.

Compound (M.wt.)	Mixed with petrol (%)	Environment (ppb)	WHO permissible limit (ppb)	World production (million tons)
Benzene (78)	1-2	0.6-106	10	8-10
Toluene (92)	5-8	6-350	700	5-10
***p*-xylene (106)**	7-10	0.6-178	500	10-15

Table 1: Benzene, toluene and *p*-xylene compounds with production, utility and pollution data.

Parameter	Control	T1	T2	T3
Peak Intensity at *m/z*=78 (PM)	100	100	100	26
Peak Intensity at *m/z*=79 (PM+1)	26.59	26.36	19	4
Ratio of peak intensity (PM+1/PM) at *m/z*=79 to *m/z*= 78	0.27	0.26	0.19	0.15

Table 2: GC-MS isotopic abundance analysis result of benzene.

Parameter	Control	T1	T2	T3
Peak Intensity at *m/z*=92 (PM)	60.45	21.80	71.41	72.94
Peak Intensity at *m/z*=93 (PM+1)	8.32	17.24	10.33	14.84
Ratio of peak intensity (PM+1/PM) at *m/z*=92 to *m/z*=93	0.13	0.79	0.14	0.20

Table 3: GC-MS isotopic abundance analysis result of toluene.

Parameter	Control	T1	T2	T3
Peak Intensity at *m/z*=106 (PM)	78.74	76.63	80.36	81.87
Peak Intensity at *m/z*=107 (PM+1)	12.15	27.71	15.51	22.64
Ratio of peak intensity (PM+1/PM) at *m/z*=107 to *m/z*=106	0.15	0.36	0.19	0.27

Table 4: GC-MS isotopic abundance analysis result of *p*-xylene.

Isotopes Bonds	Isotope type	Reduced mass ($m_A m_B/(m_A + m_B)$)
^{12}C-^{12}C	Lighter	6.00
^{13}C-^{12}C	Heavier	6.26
^{1}H-^{12}C	Lighter	0.923
^{1}H-^{13}C	Heavier	0.929
^{2}H-^{13}C	Lighter	1.04
^{13}C-^{13}C	Heavier	6.5
^{2}H-^{12}C	Lighter	1.71

Table 5: Possible isotopic and non-isotopic bonds and calculated values of μ in benzene, toluene and *p*-xylene. m_A: Mass of atom A; m_B: Mass of atom B; Here, A may be C or H and so on.

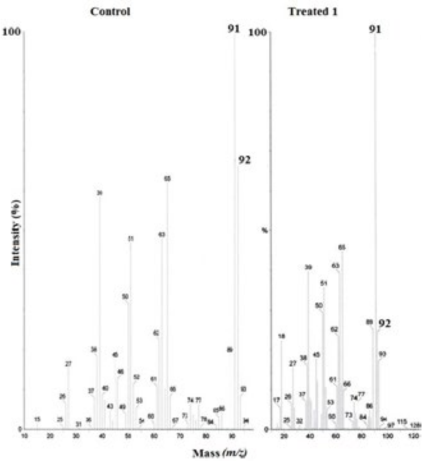

Figure 5: GC-MS spectra of control and treated (C and T1) samples of toluene.

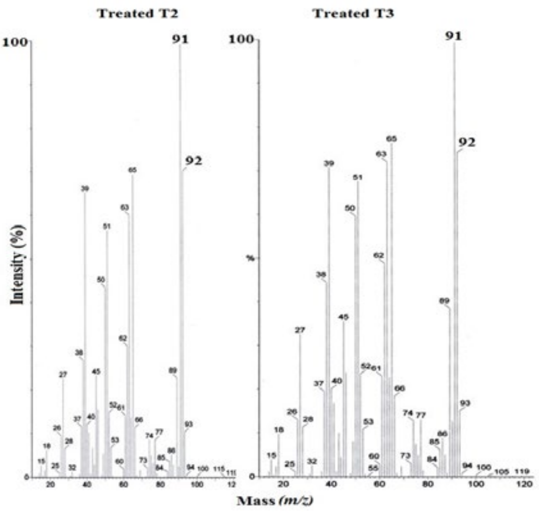

Figure 6: GC-MS spectra of treated (T2 and T3) samples of toluene.

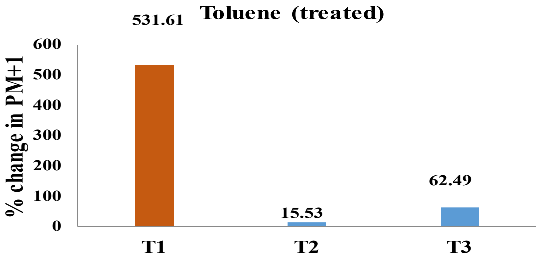

Figure 7: Percent change in isotopic abundance ratio of (PM+1/PM) in treated samples of toluene (T1, T2 and T3).

Fragmentation pattern and number fragmented peaks were same (total 7 peaks) for control and treated *p*-xylene samples. Isotopic abundance ratio of $^{13}C/^{12}C$, or $^{2}H/^{1}H$ (PM+1/PM) of control and treated *p*-xylene were calculated and presented in the Figure 10. In the bar diagram, the isotopic abundance ratio of PM+1/PM of treated *p*-xylene was increased up to 134.34% (for T1) after biofield treatment as compared to control. Increased isotopic abundance ratio of PM+1/PM in treated samples of *p*-xylene may increase binding energy of respective bonds which might increase chemical stability of *p*-xylene. This suggested that, ^{12}C and ^{1}H atoms in treated toluene and *p*-xylene probably transformed into ^{13}C and ^{2}H, respectively by capturing one neutron thereby increased the ratio. The inter-conversion of ^{13}C and ^{12}C can be possible if a nuclear level reaction including the neutron and proton occurr after biofield treatment. Thus, it is assumed that biofield treatment possibly induced the nuclear level reactions, which may lead to alter the $^{13}C/^{12}C$ in treated benzene, toluene and *p*-xylene samples. Possibly, in benzene, toluene and *p*-xylene molecules, various bonds might present such as ^{12}C-^{12}C, ^{1}H-^{12}C, ^{13}C-^{12}C, ^{2}H-^{12}C, ^{1}H-^{13}C, ^{2}H-^{13}C and ^{13}C-^{13}C. Reduced mass is calculated and presented in (Table 5). It is seen from the table that μ (reduced mass) of normal ^{12}C-^{12}C and ^{1}H-^{12}C bond was 6 and 0.923, respectively. It showed that reduced mass is increased in case of heavier isotope (*i.e.*, ^{13}C-^{13}C=6.5, ^{2}H-^{13}C=1.04). It may enhance the bond strength, stability, and binding energy of toluene and *p*-xylene molecules [29].

Conclusions

In summary, the biofield treatment has significantly changed the isotopic ratio of (PM+1/PM) in benzene, toluene and *p*-xylene. Benzene molecule with lower isotopic ratio of (PM+1/PM), might have lower stability due to the decreased μ (reduced mass) and binding energy in molecules with lighter isotopes. In case of toluene and *p*-xylene, the isotopic abundance ratio of (PM+1/PM) treated samples increased significantly as compared to control. The increased isotopic abundance may increase the binding energy of the chemical bonds in toluene and *p*-xylene, with increased μ (reduced mass). The observed fragmentation pattern and number of fragmented peaks in mass spectra were same for control and treated toluene and *p*-xylene samples. These nuclear level transformations of $^{12}C \rightarrow ^{13}C$ or $^{1}H \rightarrow ^{2}H$, were observed which probably induced through biofield treatment. Benzene was less frequently

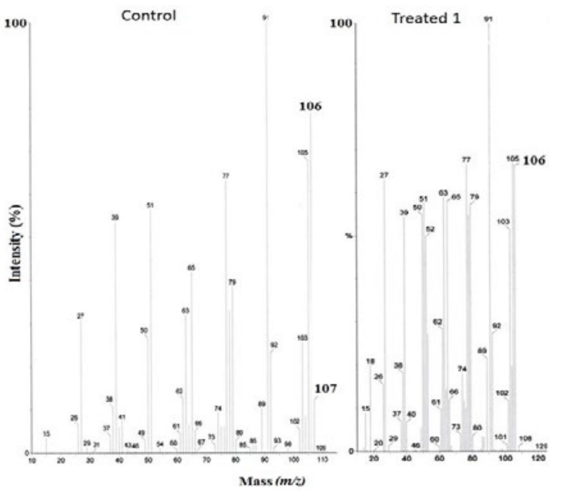

Figure 8: GC-MS spectra of control and treated (C and T1) samples of *p*-xylene.

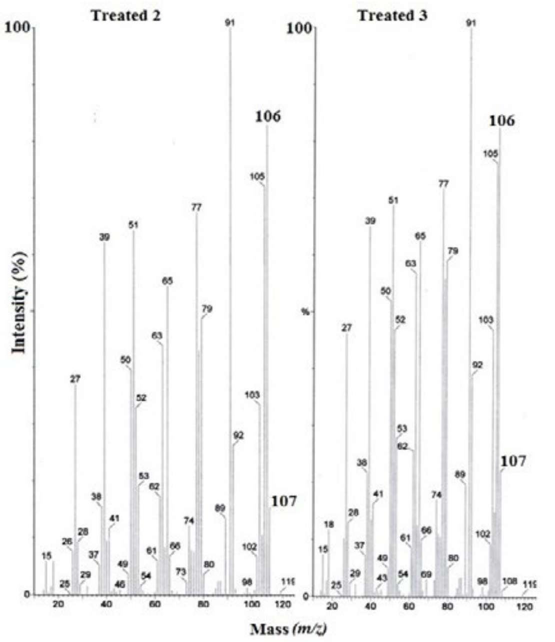

Figure 9: GC-MS spectrum of treated (T2 and T3) samples of *p*-xylene.

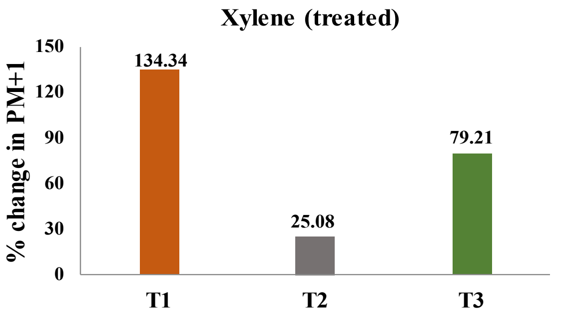

Figure 10: Percent change in isotopic abundance ratio of (PM+1/PM) in treated samples of *p*-xylene (T1, T2 and T3).

degraded bio-chemically than substituted aromatics *i.e.*, toluene and *p*-xylene. The decreased isotopic ratio of (PM+1/PM) of benzene after biofield treatment might reduce its stability which could be helpful for biodegradation of benzene in bioremediation of benzene contaminated aquifers. On the contrary increased isotopic ratio of (PM+1/PM) in toluene and *p*-xylene may increase the stability of toluene and *p*-xylene may be useful as ingredient of gasoline.

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