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Research Article

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Screening of oil polluted soil and detection of biologically active agents which are helpful in drug formation

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ABSTRACT

In this present study, an effort has been made to identify the Physical properties (porosity of soil, density of soil, color of soil, texture of soil etc.), Chemical properties (organic matter, soil pH, salinity etc.) and biological properties (presence of microbial community) of polluted soil. Also, presence of some biological active compounds has been obtained from the polluted soil. The separation and identification of organic compounds present in polluted soil was done by using Column chromatography technique and Gas Chromatography-Mass Spectroscopy (GC-MS). GC-MS results demonstrated the presence of Heptane, 3-methyl-, Cyclohexane, 1,1-dimethyl-, Octane, 2-methyl-, Nonane, 3-methyl-, Tetratriacontane, pentacosnae, Octane, 3,6-dimethyl-, Decane, 4-methyl-, Tetradecane, 5-methyl-, Pentadecane, 3-Methyl-, 6-Tridecene, 2,2,4,10,12,12-hexamethyl-7-(3,5,5-trimethylhex, Heptadecane, 2,6,10,15-tetramethyl-, Beta-Sesquiphellandrene. These compounds have been found with anti -bacterial, antimicrobial, antiviral, antifungal properties. The molecular identification of isolated microorganisms (Isolate 1 -5) by 16s RNA sequencing confirmed the presence of *Brevibacillus bortelensis, Rhodococcus rhodhochorus* and three microbes were identified as *Stenotrophomonas acidaminiphilia*. From the results it have been concluded that the researcher has tried to explore the properties of untouched habitat soil of Rajasthan to find out such compounds which have applications in drug formation. Moreover getting such compounds from polluted source mak es it more interesting.

Keywords: Biological agents; GC-MS; Natural sources; Plants; Polluted soil; Therapeutic properties.

INTRODUCTION

The soil is the blanket that covers most of the earth's land surface. Soil acts as an innate sink for air, water, nutrients, minerals, organic matter etc. The minerals and organic matter which stores nutrients, soil water provides nutrients for plants; soil air is the major constituent which provides air to the micro flora and fauna for their metabolic process and releases various additional nutrients in the soil. The presence of different nutrients and minerals in different soil has an essential function for growth of micro flora and fauna. The behavior of soil largely depends upon its structure. Soil is a mixture of various particles like large particles which are coarse, medium-sized particles and small particles having the diameter ranges from 0.02 – 2 mm or more. These particles of various sizes have profound effect on nutrient retention, movement of water, moisture of soil, water holding capacity of soil, organic matter, etc.

The nature has been a source for medicinal products for millennia. Among natural sources soil acts as wealthy source for biological active compounds for pharmaceutical industries. Many useful drugs have been derived from plant and microbial origin which opens the way for researchers to find out much more useful products from these natural origins for the beneficial of all living beings. Nature and natural products have been recognized for their therapeutic potentials since ancient times. Drug discovery with ground-breaking technologies and their successful execution still remains a challenge for scientists. For discovering new and effective drugs researchers relies on natural resources. These natural sources are the ancient wisdom of therapeutic agents for treatment of diseases and the use of modern medicines. Drugs have been discovered from compounds derived from these natural sources (Lahlou, 2007).

Plants serve as a major natural source for extraction of pharmacological products since many years. Wide variety of anti-bacterial, anti-microbial agents has been derived from the medicinal plants till date to cure number of diseases. Innovative drug creation from plants attracts researchers and they have discovered many new technologies for their successful drug findings for indulgence of many higher diseases. In this whole array of discovering novel and potential drug from plants, so many disadvantages also faced. For example loss of plant, loss of species, and loss of medi-

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cal models, which results in the unavailability of natural products which reduces the opportunity of diseases treated by these drugs. The identification of biological active agents isolated from plants is considered as a major task because it has been relied on isolation procedures, extraction processes and purification methods. One other issue is that products or isolates acts differently in synthetic environment than their natural environment which hinder the path of drug discovery (Lahlou, 2013). Because not all natural products are synthesized and having complex structure makes them unfit for industrial applications. As, some products are too sensitive and complex to be synthesized on industrial bases.

Identification and extraction of biologically agents from soil is becoming a new area of research. Soil is a major reservoir for compounds derived from microbial origin. Large variety of microorganisms produce these potent active agents such as low molecular and high molecular weight compounds having various chemical properties for application in several areas of health, food, care, environment contamination control etc.(Karanth) The success of researchers is going on till date to discover new biological active agents from microbial origin. Microorganisms have reservoir to produce compounds having applications in several fields such as pharmacological, medical, agricultural, biological etc. (Smith, 1989; Bulock, 1987; Vandemme, 1948). These compounds have been isolated and identified for the use to treat human and animal diseases. All the components in the soil may have a biological activity in some way directly or indirectly. The biological active agents or compounds refer to as those components which have some physiological effect on other microorganism, plant or animal. Getting these compounds from soil environment is ecologically significant but some other soil factors are also included such as temperature, pH, density, minerals, binding properties, moisture, aeration etc.

In the present investigation, the chemical and physical nature of soil samples, collected from various regions of Mount Abu was studied. The microbial diversity of the soil was also investigated. Physical and chemical properties of the soil help to find out the environmental conditions for the growth of microorganisms. Soil serves as a natural resource for isolating such therapeutic active agents for drug invention particularly from polluted soil.

One of the benefits of this study is the natural resources whether its plants/soils are having biological history. No doubt that the products derived from nature are the strong approach. Revolution came and the idea of structures of the compound, their properties, and their interaction with molecules proved a fortunate thing in the history of drug invention. The use of pure extract by recognizing its chemical and biological properties become a standard format for manufacturing new drugs, novel ideas for known medications and development of natural sources based medications (Patwardhan, 2004). Because of the failure of some drug discovery methods, amendment have been made which lead to the revolutionary and successful leads in key therapeutic such as anti-infections. Nature gives us every possible essential product by interacting with other molecules by their chemical structures. Although, it is surprising but interesting fact that many biological compounds having binding characteristics have been found in the polluted soil. Even polluted natural source have the bank of biol ogical active products. Moreover, by exploring these natural products obtained from polluted area with their structural, chemical databases comprising a large variety of compounds and their interactions with proteins, genes will open the door for new chemical entities through a very simple method for pharmacological world. It must be considerable that the extraction of biological agents having wide therapeutic properties from soil.

MATERIAL AND METHODS

Site location

For the present research work high altitude region of Rajasthan i.e. Mount Abu was choosen because Mount Abu is a hill station in the Aravalli ranges of Sirohi district and is located at highest sea level in Rajasthan. The mountain forms a distinct rocky plateau 22 km long by 9 km wide. The highest peak on the mountain is Guru Shikhar at 1,722 m (5,650 ft) above sea level. Nights are cold and average night temperature is around 4 °C to 12°C. The geographical coordinates of Mount Abu lie $24^0 36' 0''$ N, $72^0 42' 0''$ E. For the present research work, petroleum contaminated soil samples were collected from motor garages of Mount Abu as motor garages assuming to have a high density of petroleum contaminated soil.

Figure 1: The satellite location of Mount Abu (Rajasthan), India

Physical and chemical properties of soil

Pre-treatment of soil samples by drying, crushing and sieving (through various sizes of sieves) were done under laboratory conditions. The determination of pH of the soil, moisture content, bulk density, carbonate and bicarbonate estimation, chloride estimation, heavy

metal detection by Atomic absorption Spectroscopy (AAS) etc. were performed.

Column Chromatography

For separation and identification of soil samples column chromatography and GC-MS (Gas chromatography- Mass spectroscopy) was performed. Column chromatography is a solid-liquid chromatography having stationary phase and mobile phase in solid and liquid form, respectively. In column chromatography the stationary phase is silica gel and mobile phase is solvent. For column chromatography pre activated silica (at 100^0 C for 24 hours) of 60-120 mesh size was filled in column followed by soil sample for column packing. For the separation of aliphatic and aromatic compounds n-hexane and toluene was used as solvent. The flow rate was maintained at 1ml/min. The extract was analyzed by GC-MS (Shimadzu model QP-2010 plus, column-Rtx-5MS, 30 meter x 0.25 mm i.d. x 0.25 um film thickness).

Isolation and screening of microorganisms

Microbial community present in the polluted soil were isolated using enrichment culture plate technique using Bushnell Hass media (Hemalatha and Veeramanikandam, 2011; Subathra *et.al*., 2013). (BH medium was used because soil samples were petroleum contaminated and BH medium refers for isolation of petroleum degraders) 1.0 g of soil sample was weighed and mixed with 100 mL of autoclaved Bushnell Hass media (BH medium). It was supplemented with 1% of 2T engine oil which was served as sole carbon and energy source. These flasks were kept on shaker at 150 rpm for 7 days. After 7 days of incubation 10 ml of enriched media was transferred to freshly prepared BH media and kept on shaker in same conditions as describe. This cycle was repeated for 4 to 5 times. After, every enrichment cycle 1 mL of culture media was diluted to 10^3 to 10^8 fold and these diluted cultures were plated on BH agar plates supplemented with 2T engine oil at 37°C. By culturing and sub-culturing of colonies onto BH agar plate pure culture of bacteria were obtained. The pure microbial strains were kept in 40% glycerol at -4^0C for future use. For molecular identification of selected bacteria 16sRNA sequencing technique was used at Yaazh Xenomics Tamil Nadu, India.

RESULT AND DISCUSSION

Soil is a complex structure having different types of particles of different sizes. Soil holds the water, air, minerals, nutrients, micro-flora-fauna etc. Pore spaces between soil particles are responsible to hold water and air. Soil has its physical, chemical and biological properties. Physical properties includes water holding capacity, movement, fertility, moisture of the soil etc. are inter-related to the structure of the soil. The organic matter, mineral composition, nutrient availability, pH of soil, salinity etc. comprises the chemical properties of the soil. Soil flora and soil fauna are the major biological properties of the soil. These physical, chemical and biological properties of the soil are interconnected. For determination of porosity of soil which is the amount of pore space present in soil the bulk density of soil is calculated (Blake, 1986). There is a complex structure of carbon, organic matter and bulk density which is also studied by (Sakin, 2012). Organic carbon influence many soil properties such as WHC, nutrient turnover which in-turn influence water relation aeration etc. Soil organic matter includes hydrogen, oxygen and nitrogen which are responsible for nutrient cycling. It has been estimated from above discussion that increases in organic matter decreases the bulk density of soil. Increase in organic matter also leads to change in nutrient concentration of soil. Available of nutrients in soil may have important role in variation of bulk density of soil. Porosity, texture and organic matter contents are the factors which affects bulk density (Chandhani, 2013). It has been concluded from above discussion that nutrients, minerals, aeration, carbon, pore space and size etc. all are interconnected and are important part of soil structure. The soil sample PCS1 (Petroleum Contaminated Soil 1) contained gravel with 38.6±0.21% more than PCS2. The percentage of sand is more in PCS1 *i.e.* 35.8 % than PCS 2 with 32.5 %. The higher the percentage of clay the more is the water holding capacity of the soil. In PCS2 the percentage of clay was high with 34.3±0.34 as comparative to PCS1 which was found with 25.6±0.27 Table 1.

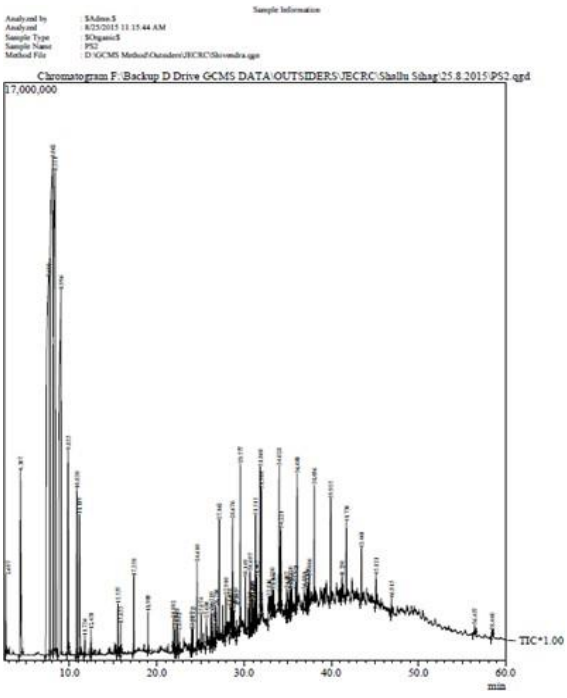
The pH of the polluted as well as normal soil is slightly alkaline in nature as shown in Table 2.

The pH of PCS1 and PCS2 was 7.8 and 7.6 respectively. There was not a healthy amount of moisture in both contaminated and normal soil; however PCS1 holds more moisture with 0.23% as compared to PCS2 (0.04%). The water holding capacity of the PCS 2 was more than PCS 1.The higher the bulk density lowers the porosity of the soil, as in PCS 1 has higher bulk density resulting in lower porosity of the soil. The contaminated soil and the normal soil were obtained with high chloride amount with 8.6 mg/100 g, 18 mg/100 g, 15.6 mg/100 g, and 17 mg/ gm of PCS1, NS1, PCS2 and NS2 respectively. Carbonate was found higher in the PCS2 with 0.62 mg/100 gm. The petroleum contaminated soil samples and the normal soils sample were also having the macro and micronutrients like zinc, copper, phosphate, carbon potassium etc. These soil samples were traces with the heavy metals like Iron and Manganese were traced during the Atomic absorption spectroscopy in all contaminated and normal soil samples. Lead, chromium and nickel were traced only in PCS1. The petroleum contaminated soil samples were highly polluted with the petroleum hydrocarbons range from carbon atom no 1 to carbon no atom 50 (C_1-C_{50}). NSO compounds (Nitrogen, Sulphur, and Oxygen) are also obtained. Chromatograms of all soil samples are

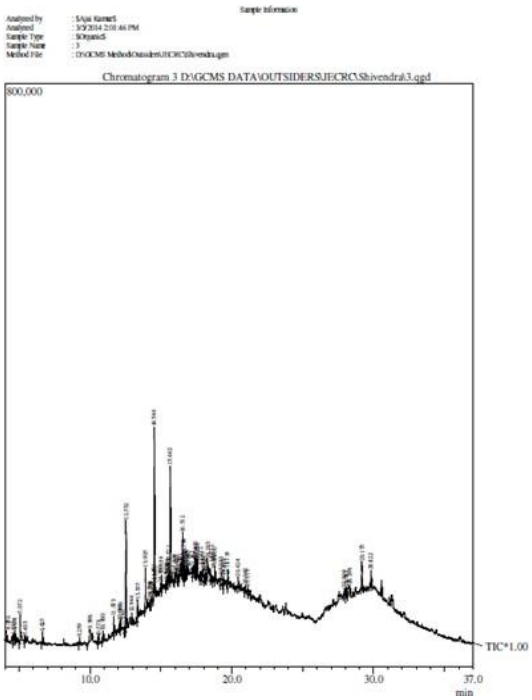
Table 1: Texture of the collected petroleum contaminated soil and normal soil samples

S. No.	Sample No.	Gravel (%)	Sand (%)	Clay (%)
1	PCS 1	38.6±0.21	35.8±0.41	25.6±0.27
2	NS 1	30±0.29	16.2±0.21	53.8±0.45
3	PCS 2	33.2±0.32	32.5±0.21	34.3±0.34
4	NS 2	41±0.43	15.5±0.22	26.9±0.56

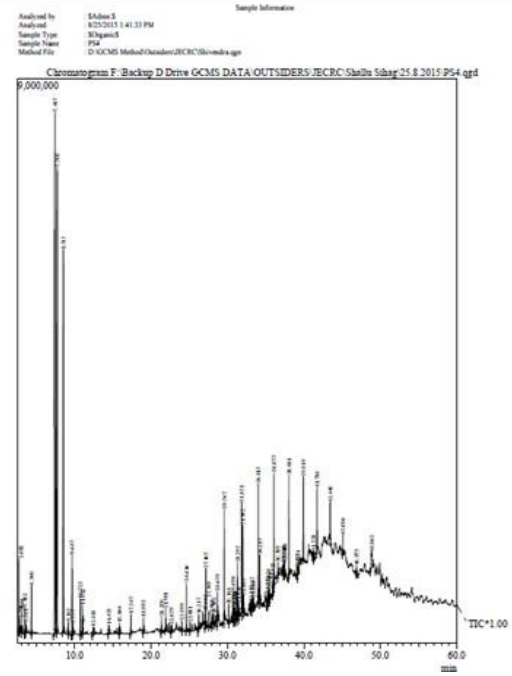
Footnotes- PCS= Petroleum contaminated soil, NS= normal soil



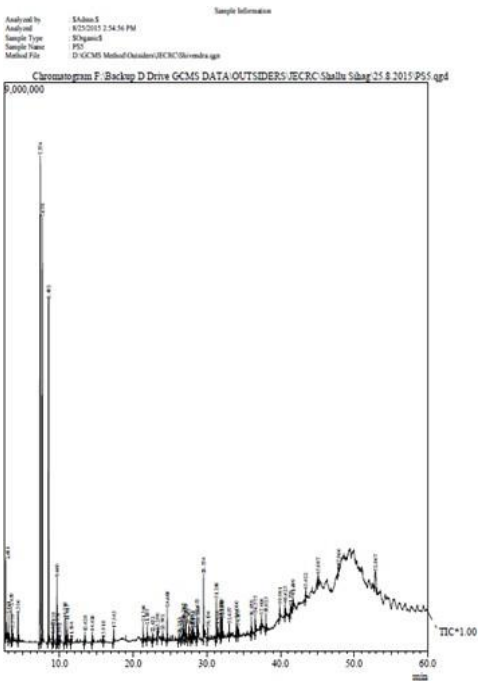
Chromatogram of Soil sample PCS1



Chromatogram of Soil sample PCS2



Chromatogram of Soil sample PCS3



Chromatogram of Soil sample PCS4

Figure 2: Chromatograms of all soil samples (PCS1, PCS2, PCS3 and PCS4)

Table 2: Physico-chemical properties of the collected petroleum contaminated and normal soil samples

S. No.	Test Name / Sample name	PCS 1	NS 1	PCS 2	NS 2
1	pH	7.8 ± 0.1	8 ± 0.2	7.6 ±0.1	7.3 ± 0.1
2	Electric Conductivity µs cm^{-1}	0.6 ± 0.002	0.34 ± 0.002	0.68 ± 0.02	0.72 ± 0.01
3	Moisture content %	0.23±0.40	0.07±0.53	0.04±0.05	0.61±.041
4	WHC %	54.34±0.31	59.13±0.32	58.11±0.09	53.14±0.34
5	Bulk density g mL^{-1}	0.88±0.03	0.92±0.12	0.72±0.04	0.67±0.01
6	Porosity %	65.34±0.12	66.29±0.31	09.32±0.10	0.57±0.12
7	Organic matter %	6.34	6.12	1.32	1.24
8	C/N	121.31±0.67	97.32±0.52	1.02±0.01	1.35±0.1
9	Chloride mg *per*100g	8.6±0.03	18±0.08	15.6±2.0	17±1.9
10	Carbonate mg *per* 100g	0.21±0.01	0.34±0.04	0.62±0.03	0.68±0.03
11	Bicarbonate mg *per* 100 g	1.67±0.35	1.21±0.15	1.9±0.20	2.12±3.9
12	Zinc mg kg^{-1}	0.19±0.01	0.24±0.01	0.32±0.03	0.3±0.02
13	Copper mg kg^{-1}	1.6±0.12	0.84±0.21	1.64±0.06	0.98±0.12
14	Phosphate	42±0.09	36±0.03	37±0.06	40±0.04
15	Carbon	0.14±0.07	0.3±0.02	0.19±0.03	0.21±0.02
16	Potassium mg kg^{-1}	206±0.01	198±0.04	188±0.01	194±0.03
17	Iron mg kg^{-1}	2.62±0.08	1.62±0.21	1.64±0.14	1.68±0.21
18	Cadmium mg kg^{-1}	-	-	Not traceable	-
19	Nickel mg kg^{-1}	138.06	-	-	-
20	Lead mg kg^{-1}	12.78	-	Not traceable	Not traceable
21	Chromium mg kg^{-1}	21.8	-	-	Not traceable
22	Manganese mg kg^{-1}	2.2±0.03	0.9±0.02	1.24±0.01	0.74±0.01

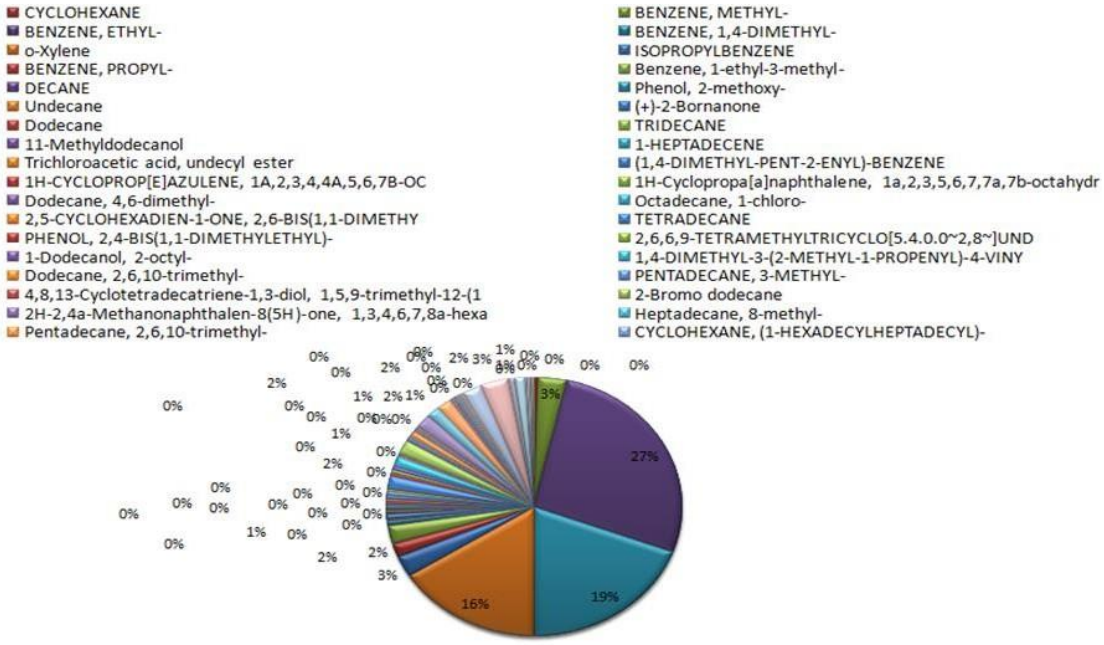


Figure 3: Pie diagrammatically representation of compounds present in polluted soil PCS1

shown in Figure 2 and pie-diagrammatically representations of all compounds present in soil samples are-shown in Figure 3 to Figure.5.

The phylogenetic analysis of *Brevibacillus bortelensis, Rhodococcus rhodochorus, Stenotrophomonas acidaminiphilia* are shown in Figure no.6 to 10.

The present paper also talks about and looks at how natural products have been delivered fruitful results for the pharmaceutical industry for medication disclosure and advancement. It's likewise offers a broad overview to use polluted natural source to be utilized as a part of pharmaceutical industry for medication revelation. The evaluation of bioactive compounds in hexane extraction (Table no.3) exposed the existence of 1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl which is a compound having biological activities such as antiviral, anti-inflammatory, anti-microbial and antifungal activities (Chaturvedi,2011).

Table 3- Biological active agents with their molecular formula, molecular weight, boiling point, structure and applications identified in chromatographic and GC-MS

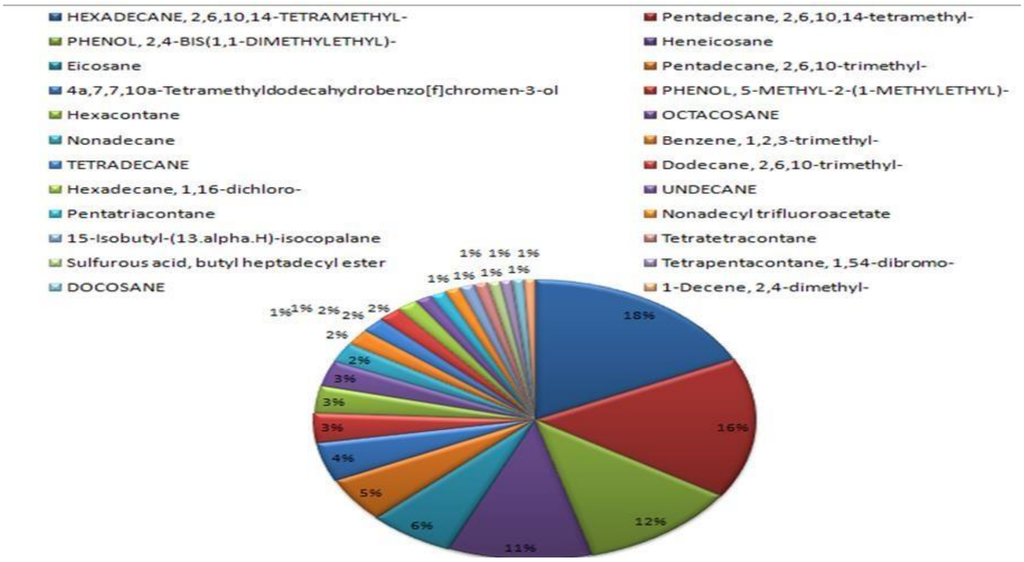


Figure 4: Pie diagrammatically representation of compounds present in polluted soil PCS2

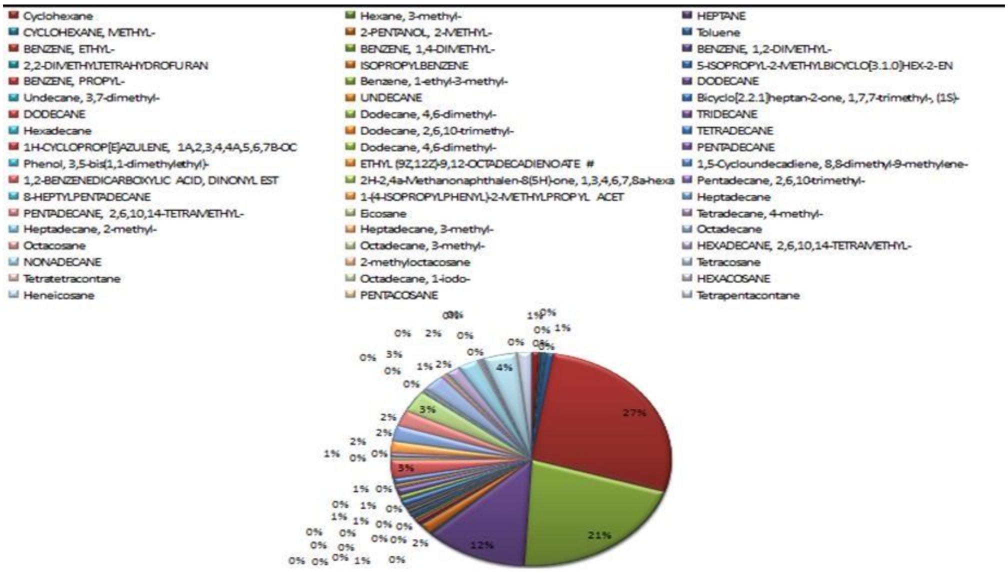


Figure 5: Pie diagrammatically representation of compounds present in polluted soil PCS3

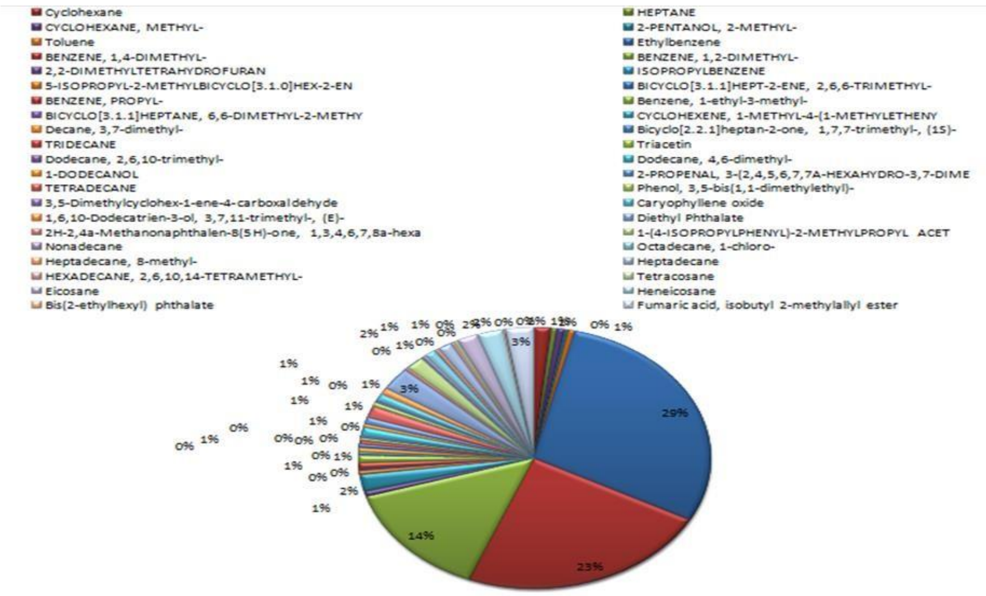


Figure 6: Pie diagrammatically representation of compounds present in polluted soil PCS4

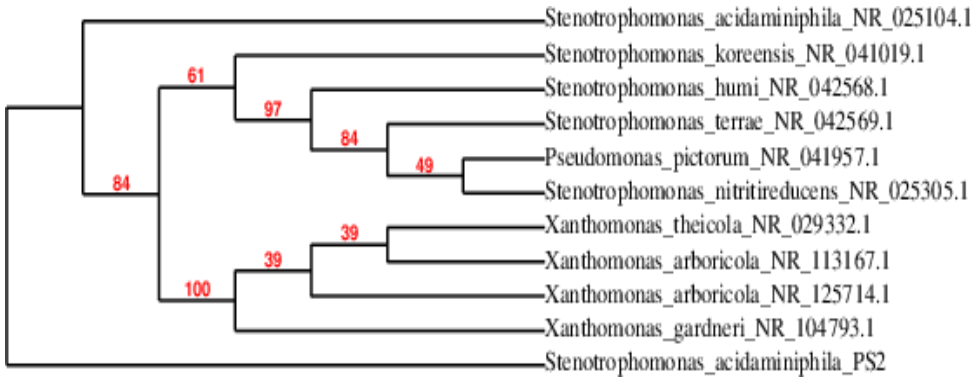


Figure 7: The phylogenetic tree of isolate (1) Stenotrophomonas acidaminiphila

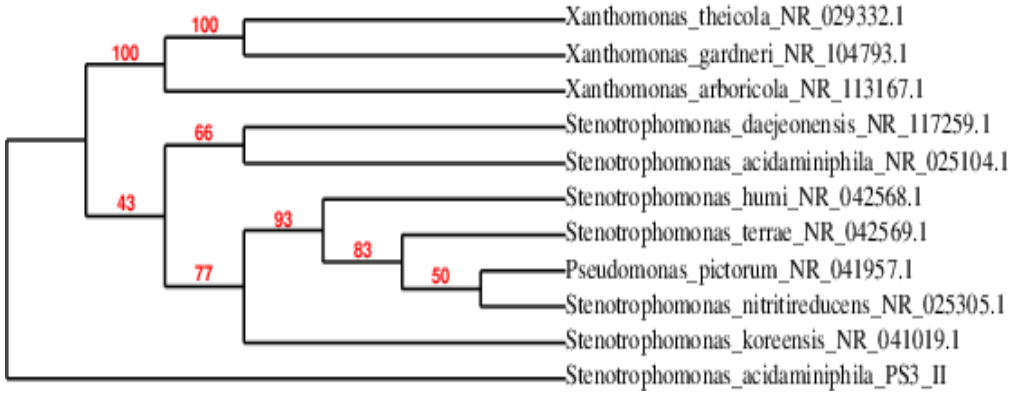


Figure 8: The phylogenetic tree of isolate (2) Stenotrophomonas acidaminiphila

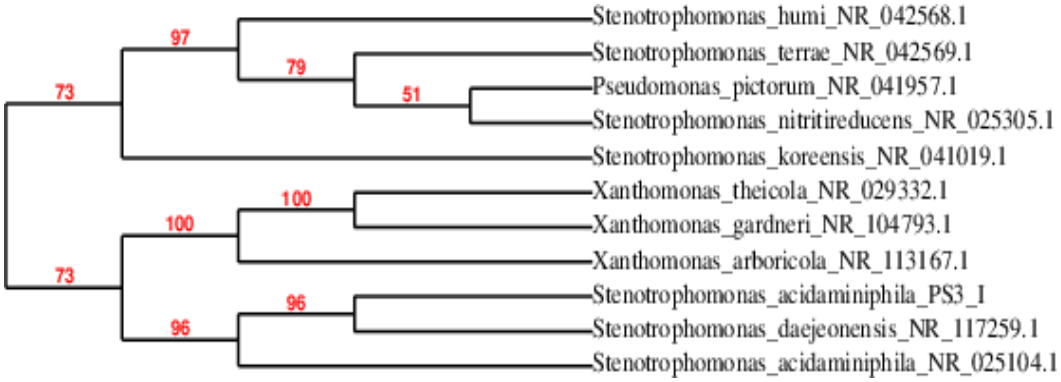


Figure 9: The phylogenetic tree of isolate (3) Stenotrophomonas acidaminiphila

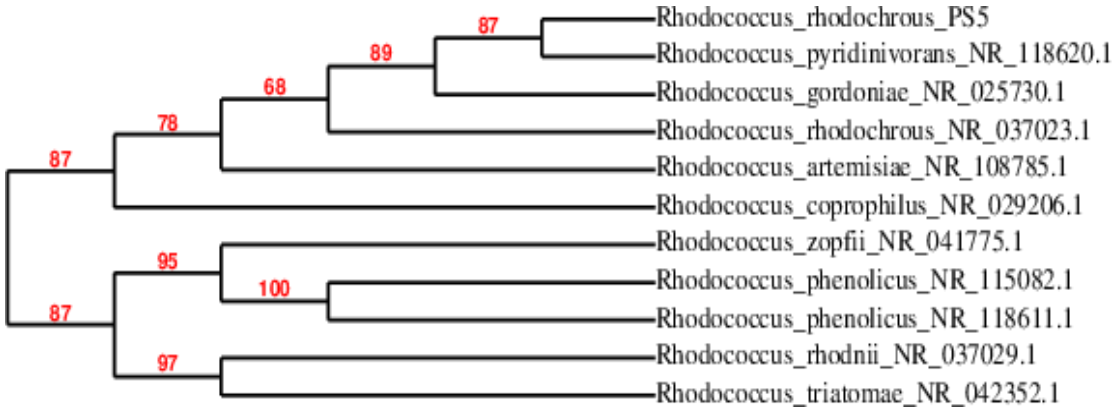
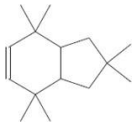
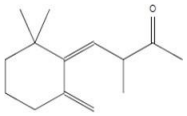
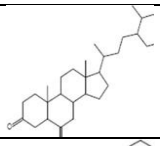
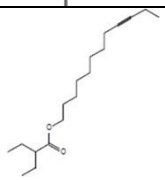
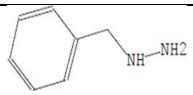
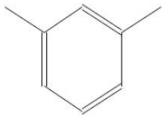
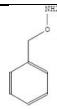
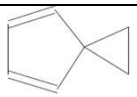
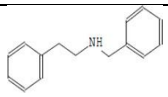
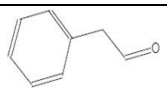
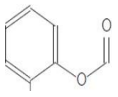
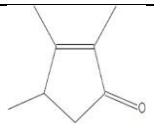
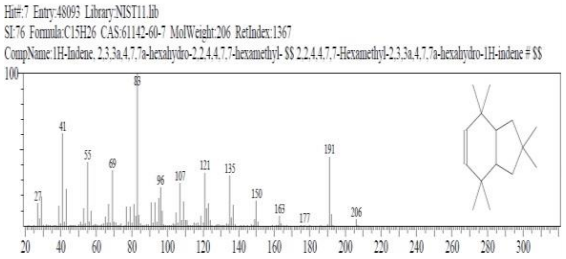


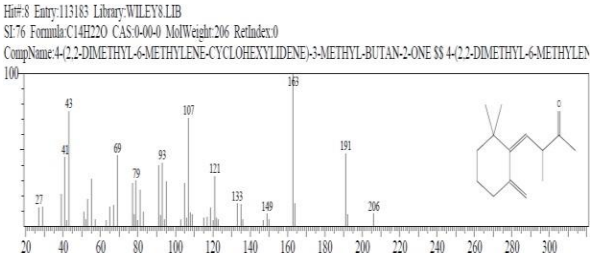
Figure 10: Phylogenetic tree of isolate (4) Rhodococcus rhodochrous

Table 3: Biological active agents with their molecular formula, molecular weight, boiling point, structure and applications identified in chromatographic and GC-MS analysis of polluted soil

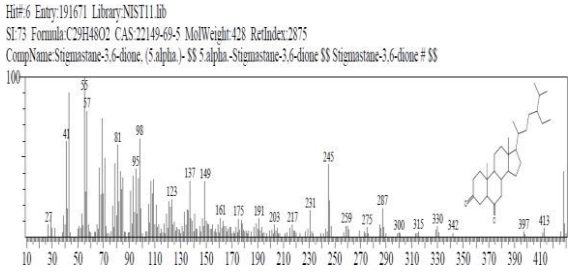
S. No.	Compound name	Molecular Formula	Molecular Weight	Boiling Point	Structure	Application
1	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl	$C_{15}H_{26}$	206	Not found		Biological applications
2	4-(2,2-Dimethyl-6-Methylene-Cyclohexylidene)-3-Methyl-Butan-2-One	$C_{14}H_{22}O$	206	-		Antifungal activity
3	Stigmastane-3, 6-dione, (5.alpha.)-	$C_{29}H_{48}O_2$	428	-		antimicrobial
4	2-Ethylbutyric acid, dodec-9-ynyl ester	$C_{18}H_{32}O_2$	280	-		Flavour and fragrance applications
5	Benzyl hydrazine	$C_7H_{10}N_2$	122	265.4 °C at 760 mmHg		Pharmaceutical applications
6	M-Xylene	C_8H_{10}	106	138-139℃		Paint and varnishes remover activity
7	O-Benzylhydroxyl-amine	C_7H_9NO	123			Biological and pharmacological applications
8	Spiro[2,4]Hepta-4,6-Diene	C_7H_8	92	128.7 ^{0}C at 760 mmHg		Organic synthesis
9	*N*-Benzyl-2-Phenethylamine	$C_{15}H_{17}N$	211	327-328^{0}C 750mm Hg(lit.)		Synthesis of dithiocarbamates
10	Benzene-acetaldehyde	C_8H_8O	120	195^{0}C		Antibacterial activity
11	Formic Acid, 2-Methoxyphenyl Ester	$C_8H_8O_3$	152	100.8 °C (213.4 °F; 373.9 K		Antifungal property
12	2-Cyclopenten-1-One, 2,3,4-Trimethyl	$C_8H_{12}O$	124	183.7°C at 760 mmHg		Pharmacological activity



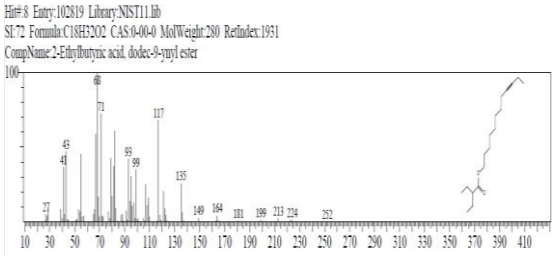
(a) 1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4 ,4,7,7-hexamethyl



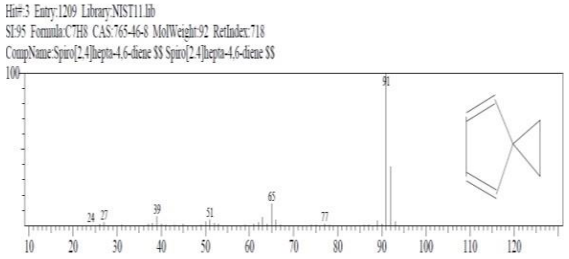
(b) 4-(2,2-Dimethyl-6-Methylene-Cyclohexylidene)- 3-Methyl-Butan-2-One



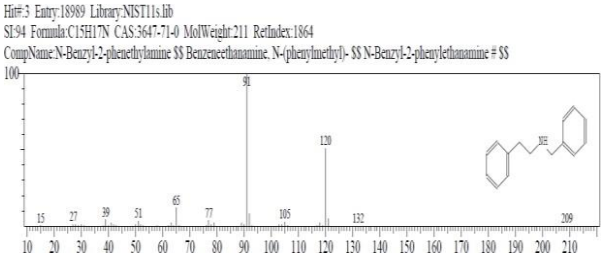
(c) Stigmastane-3,6-dione, (5.alpha.)-



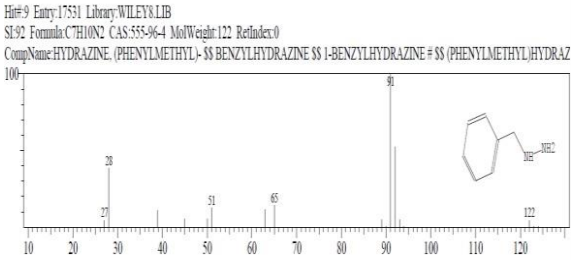
(d) 2-Ethylbutyric acid, dodec-9-ynyl ester



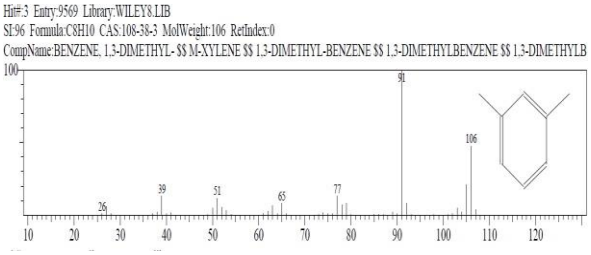
(e) Spiro[2,4]hepta-2.6 diene



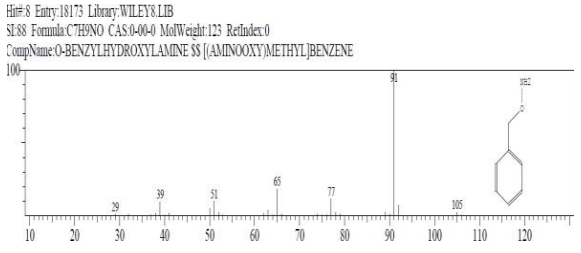
(f) n-Benzyl-2-phenethylamine



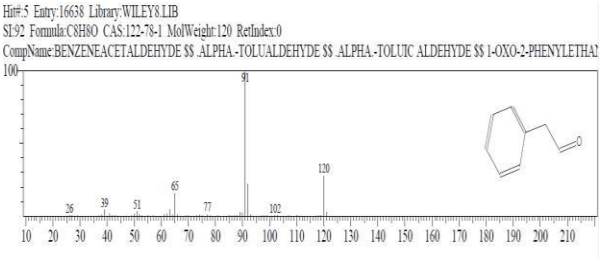
(g) Benzylhydrazine



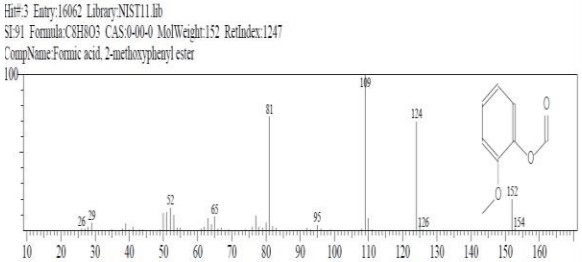
(h) M-Xylene



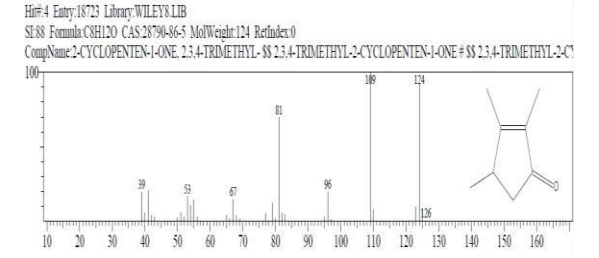
(i) O-Benzylhydroxylamine



(j) Benzeneacetaldehyde



(k) Formic acid, 2-methoxyphenyl ester



(l) 2-Cyclopenten-1-One, 2, 3, 4-Trimethyl

Figure 11: a-l: Molecular formula, molecular weight, Name of therapeutic compounds (a-l) present in oil polluted soil identified by GC-MS library

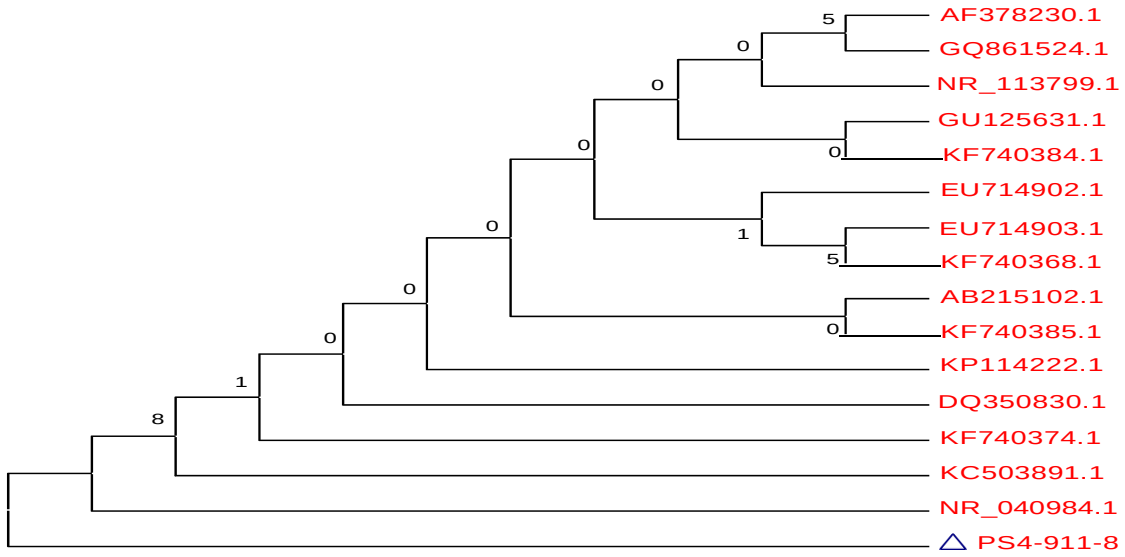


Figure 12: The phylogenetic tree of isolate (5) Brevibacillus bortelensis

analysis of polluted soil-4-(2,2-Dimethyl-6-Methylene-Cyclohexylidene)-3-Methyl-Butan-2-One has been found with antifungal activity (Mahoud, 2013). Stigmastane-3, 6-dione, (5.alpha.) has in vitro antimicrobial activity (Zhao, 2005). 2-Ethylbutyric acid, dodec-9-ynyl ester has been known for their flavour and fragrance. It acts as perfuming agents (website link). Spiro [2, 4] hepta-4, 6-diene is a compound having applications in organic synthesis (Menchikov, 2016). *N*-Benzyl-2-phenethylamine has been used in synthesis of dithiocarbamates (DTCs) (Lo 1996; Reinprecht1996; Castro 1997; Verma 1997). These DTCs have variety of applications such as it has antiviral activity (Ramesh, 1996), antibacterial, anti-microbial (Safak, 1990), anti-tumour agents (Aragones, 1996). Benzyl hydrazine acts as raw material for various applications in pharmaceuticals, organic synthesis, agrochemicals and dyestuff etc. It has many industrial applications such as it can be used in preparation of polymerization catalysts, in power plants, in rocket fuels, in gas used in air bags etc. (website link). m-xylene is known for its applications in paint and varnish removers. It also has been found to use in production of isophthalic acid of agriculture chemicals (website link). O-hydroxylamine and its derivatives have their potential applications in biological and pharmacological agents due to their vast used as synthetic intermediates (Emami, 2012). Its derivatives have been reported to have antibacterial (Foroumadi, 2006), antifungal (Emami 2001; Emami, 2002; Emami 2005), herbicidal and antitumor activities (Rajabalian, 2007; Saban, 2009). O-substituted derivatives of hydroxylamine used mainly in preparation of pesticides and crop protection agents. Also, it can serve as intermediates for the production of many pharmaceutical compounds and fine chemicals of various drugs (Schumann, 1964). Benzeneacetaldehyde is a compound known for its antibacterial activity (Ahmadi, 2015). It also posses antioxidant activity (Alizadeh, 2014), Formic acid, 2-methoxyphenyl ester have antifungal activity (Ghorab, 2000), 2-Cyclopenten-1-One, 2,3,4-Trimethyl have been found with pharmacological activities such as analgesic, anti-bacterial, anti-diabetic etc. properties (Bastin, 2011). The details of these biological active agents (such as molecular formula, molecular weight, chemical structure etc.) from GC-MS library are shown in Figure. no.11.

CONCLUSION

In the present study, the physical, chemical and biological properties of polluted soil were studied. The microbial community delivers the successful results for the production of such compounds which are having a huge level of pharmacological and industrial applications. A new lead has been discovered for the pharmaceuticals to use the untouched polluted habi tat for producing secondary metabolites having vast application in the field of medication. New ways to deal with enhance and quicken the joint drug disclosure and improvement procedures are relied upon to happen chiefly from development in medication target elucidation and lead structure revelation. Its might be extricated from tissues of earthbound plants, marine life forms or microorganism maturation soups. A rough (untreated) separate from any of these sources regularly contains novel, fundamentally assorted concoction mixes.

It has been observed and it should be considered that the soil is the huge bank for biological agents. It's likewise offers a broad overview of the most current systems being utilized as a part of pharmaceutical explore today for medication revelation. The spot of normal items in the restorative arms stockpile was thoroughly talked about and investigated. A characteristic item is a concoction compound or substance delivered by a living life form found in nature that as a rule has a pharmacological or organic action for use in pharmaceutical drug revelation and medication plan. Future research

should focus on more revelation of the chemical structures and the mechanism of action to facilitate efficient uses of important soil sources in biological applications.

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