Proposed Pathways for Phytodegradation of Phenanthrene and Pyrene in Maize (*Zea Mays* L.) Using GC-Ms Analysis

Mahdieh Houshani
University of Tabriz
Seyed Yahya Salehi-Lisar (y_salehi@tabrizu.ac.ir)
University of Tabriz
Rouhollah Motafakkerazad
University of Tabriz
Ali Movafeghi
University of Tabriz

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are widespread organic pollutants which are persistent in the environment. Biodegradation of PAHs is one of the major mechanisms for their removal from environment. However, unlike microorganisms such as fungi and bacteria, the degradation pathways of organic pollutants in plant systems are not completely clear. This paper displays the possible pathways for the degradation of phenanthrene and pyrene (as two abundant PAHs in the environment) in maize plant. Maize plants were treated by phenanthrene and pyrene and after 7, 14, and 21 days, a number of intermediate compounds were identified using gas chromatography–mass spectroscopy (GC–MS) analysis. The obtained results showed that although maize plant can metabolize both compounds, but the degradation rate of phenanthrene was faster and higher than that of pyrene. The degradation of phenanthrene occurred mainly in the second week, whereas the degradation of pyrene was slower and mostly happened after the third week. Intriguingly, the degradation of both compounds was primarily observed in the roots. The number of identified intermediate compounds was different in the shoot and root and depends on the type of contaminant and treatment time. The most outstanding identified intermediates were quinones, dihydrodiols, phthalate and phenolic compounds which were formed through the cleavage of phenanthrene and pyrene. Accordingly, the probable degradation pathways of phenanthrene and pyrene in maize plants were proposed.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds encompassing multiple aromatic rings. Polycyclic aromatic hydrocarbons are known as widespread potent toxic environmental pollutants with carcinogenic properties (Dean et al. 2001; Sharma et al. 2014; Yang et al. 2014). Owing to their increasing concentrations in the environment, they attract a great deal of attention in the recent years (Aina et al. 2006; Parish et al. 2005; Chen et al. 2014; Sazakli et al. 2015). Because of problems in conventional chemical and physical techniques, the removal of pollutants from contaminated environment through biological resources seems to be more outstanding as ecofriendly and cost effective alternatives (Parrish et al. 2005). Among bioremediation subdivisions, phytoremediation employs plants to remove and decompose organic as well as inorganic pollutants from contaminated soil, sediments and water resources (Kabra et al. 2011; Al-Thakair and Malik 2016). A number of grasses and leguminous species are potent candidates for phytodegradation of organic pollutions (Fan et al. 2008; Luke et al. 2013, Houshani and Salehi-Lisar 2020).

The degradation pathways of organic pollutants in microorganisms such as fungi and bacteria were deeply investigated (Cerniglia 1992; Haritash and Kaushik 2009; Seo et al. 2009; Al-Thukair and Malik 2016). Bacterial degradation of PAHs is generally started with the aromatic ring cleavage by dioxygenases and production of cis-dihydrodioles. Consequently, they are dehydrogenated to form pyrocatechol, and subsequently ketones, quinines, aldehydes, phenols, and carboxylic acids (Takacova et al. 2014). For example, cis-4,5-pyrene dihydrodiol, 4-5-phenanthrene dicarboxylic acid, 1-hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, phthalic acid, and protocatechuic acid were detected as

intermediate compounds through the catabolism of pyrene (Rehmann et al. 1998, Haritash and Kaushik 2009). However, there is a deep gap of information regarding the degradation and detoxification of PAHs by higher plants. Hence, the main objectives of the present research were: 1) to assess the ability of maize plants to degrade phenanthrene and pyrene as two prevalent PAHs in the environment, 2) to identify the intermediate compounds resulting from their biodegradation by gas chromatography-mass spectroscopy (GC-MS) analysis, and 3) to propose the possible phytodegradation pathways. Our findings shed some light on the possible metabolic pathways of PAHs in higher plants.

Materials And Methods

Experimental design and treatments

The experiment was conducted as pot culture of plants under controlled conditions using a completely randomized design (CRD) with three replications. Phenanthrene (≥97%, Merck, Germany) and pyrene (≥96%, Merck, Germany) were added to sterile perlite in a precise concentration (75 mg/kg) after dissolving in ethanol. Treated perlite was used for plant cultivation after the evaporation of ethanol for 72 h.

Plant culture and sampling

The seeds of maize (*Zea mays* L.) were obtained from the Research and Education Center for Agriculture and Natural Resources of East Azerbaijan (Tabriz, Iran) and stored at 4°C until cultivation. The seeds were selected on the basis of their vigor and uniformity, disinfected using 1% (v/v) sodium-hypochlorite solution for 5 minutes, and sufficiently washed using sterile distilled water. The sterilized seeds were planted in untreated (control) and PAHs contained perlite. After 3 days, all germinated seeds were transferred to growth chambers under controlled conditions (25-30°C, 16/8 h light/dark photoperiod and relative humidity of 60%). The water content of the pots was adjusted to 100% of field capacity every two days by using sterile distilled water. After 4 and 10 days, the water of pots was replaced with 50% and 100% Hoagland solution, respectively. Finally, the plants were harvested after 7, 14, and 21 days, divided into roots and shoots, washed with distilled water, dried on towel paper and kept at -20 ^{o}C until analysis. The samples were examined by GC-MS (Agilent technologies, Palo Alto, Canada) for determining the possible biodegradation intermediate compounds of PAHs.

Extraction and quantification of PAHs

All steps of extraction were conducted in an ice bath. 1 g of each sample was homogenized with 3 ml of 2-propanol and then 7 ml petroleum ether was added to homogenates and mixed. The homogenates were passed through four-layer clean cheesecloth and then transferred into a decanter and shacked for 20 s after addition of distilled water (20 ml) and saturated sodium sulfate solution (2 ml). The aqueous lower phase was removed and the upper phase (petroleum ether) was washed again with distilled water (10 ml)

and saturated sodium sulfate solution (1 ml). The upper phase (petroleum ether) containing PAHs was transferred into sealed falcon tubes and kept at -20 ^{o}C until analyses (Parrish et al. 2006).

GC-MS analysis

The petroleum ether of the samples were evaporated and replaced by methanol (Merck, Germany, HPLC grade). GC-MS analysis of the methanolic extract of maize was performed by an Agilent 6890 GC system and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a HP-5MS (5% diphenyl 95% dimethyl poly siloxane) fused to a capillary column (30 cm × 0.25 mm ID × 0.25 µmdf). An electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1 ml min^{-1}. Temperature program started at 60°C and after one minute it reached to 290°C with an increase rate of 15°C/min and remained at 290°C for 10 min. The chromatograms were obtained by using NIST software to identify the desired compounds.

Results

3.1. Degradation of PAHs

3.2. Biodegradation intermediates of phenanthrene

Detected intermediate compounds of phenanthrene biodegradation in the shoot and root of maize on days 7, 14, and 21 have been presented in Table 1. In shoots, 3 compounds were identified on day 7 including 1, 3 dimethyl-4-azaphenanthrene (6.97%), 2,4-cyclohexadien-1-one, 3,5-bis (1,1-dimethylethyl) 4-hydroxy (4.35%), and 4-methoxy-6-methyl 5-nitroisobenzofuran-1,3-dione (1.24%). In comparison, 12 compounds were identified on day 14 and the most common compounds were 1, 2-benzenediol, 3, 5-bis (1, 1-dimethylethyl) (10.75%), and dibenzoxazabicycloundecane, 1H-2, 6, 10-(epoxymethy) (8.86%). Furthermore, the amount of 1,3 dimethyl-4-azaphenanthrene was increased from 6.97–9.74% on the 14th day compared to the 7th day. The number of identified compounds on day 21 was decreased in comparison with days 7 and 14 and only two compounds were recognized. However, the content of 3-quinolinecarboxylic acid was enhanced from 0.55% on day 14 to 1.22% on day 21.

Table 1
The identified compounds in the shoot of maize plant treated with phenanthrene (75 ppm) for 7, 14, and 21 days.

NO	Compound	Retention time (min)	Kovats index (KI)	Amount of identified compounds (%)		
				7th day	14th day	21th day
1	1,3 dimethyl-4-azaphenanthrene	6.97	1753	6.97	9.74	-
2	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl) 4-hydroxy	16.84	1543	4.35	-	-
3	4-methoxy-6-methyl 5-nitroisobenzofuran-1,3-dione	17.03	2141	1.24	-	-
4	4-Methyl-2-trimethylsilyloxy-acetophenone	15.01	1440	-	0.31	-
5	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilane	15.04	1354	-	0.31	0.55
6	trimethyl[4-(1,1,3,3,-tetramethylbutyl) phenoxy] silane	15.06	1631	-	0.63	-
7	pyrrolo[2,3-b]dibenzofuran	15.40	775	-	0.55	-
8	3-Quinolinecarboxylic acid	15.45	1695	-	0.55	1.22
9	Propanamide, N-(4-methoxyphenyl)-2,2-dimethyl	15.62	1696	-	0.17	-
10	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	16.62	1775	-	10.75	-
11	1-Methyl-3-phenylindole	16.69	1805	-	3.98	-
12	(R)-2-[5-hydroxymethyl-8- methyl-3,4-dihydro[4,3-e	16.99	1631	-	3.51	-
13	Dibenzoxazabicycloundecane; 1H-2,6,10-(Epoxymethy)	17.35	992	-	8.86	-
14	Acetamide, N-[4-(trimethylsilyl)phenyl]	19.88	1600	-	1.36	-

The biodegradation intermediates of phenanthrene in roots have been presented in Table 2. Generally, the number of identified compounds in all days was more than those of shoots. On the 7th day, seven compounds were identified including 2, 4-a-epoxymethanophenanthrene-9-ol-8-acetic acid (3.92%), 2, 4-dimethyl benzo[h]quinoline (1.4%) and phenaleno [3, 2-f] quinolin-7-one (1.20%). On day 14, the number of compounds was increased to 16, among which 13 compounds were new in comparison with day 7. The highest quantities belonged to 7-methoxycoumarin (1.72%), 1, 2-benzenediol, 3, 5-bis (1, 1-dimethylethyl) (1.48%) and 3,4-dimethoxy-2-nitro-benzaldehyde (1.04%). Although the number of identified compounds was decreased on day 21, 6 new compounds were recognized. Among the new

compounds the presence of phthalate intermediates were remarkable (2.49%). Also, the content of 1,3 dimethyl-4-azaphenanthrene was increased from 0.84% on day 14 to 1.18% on day 21.

Table 2
The identified compounds in the roots of maize plants treated with phenanthrene (75 ppm) for 7, 14, and 21 days.

NO	Compounds	Retention time (min)	Kovats index (KI)	Amount of identified compounds during time (%)		
				7th day	14th day	21th day
1	Phenaleno[3,2-f] quinolin-7-one	9.39	1165	1.2	-	-
2	2,4-a-Epoxymethanophenanthren-9-ol-8-acetic acid	12.11	3145	3.92	-	-
3	2,4-dimethyl benzo[h] quinoline	16.49	2003	1.4	0.4	0.39
4	2,3-dihydro-6-nitro-1,4-Phthalazinedione	16.74	1889	0.5	-	-
5	4-Methyl-2-trimethylsilyloxy-acetophenone	16.79	1440	0.1	0.79	-
6	Silicic acid, diethyl bis(trimethylsilyl) ester	16.87	1049	0.28	-	-
7	(+-)5-(1-Acetoxy-1-methylethyl)-2-methyl-2-cyclohexen	15.70	1354	1.94	0.92	-
8	3,4-Dimethoxy-2-nitro-benzaldehyde	7.18	1756	-	1.04	-
9	7-methoxycoumarin	10.86	1732	-	1.72	-
10	Benz(1,4) oxathiino(2,3-c) pyridine	11.69	1908	-	0.42	-
11	1-Methyl-5-phenylsulfanyl-1H-pyrazole-4-carbonitrile	11.75	1964	-	0.29	-
12	1,2-Benzenediamine, 4-(4-aminophenoxy)	11.85	1304	-	0.29	-
13	1,3-dimethyl-4-azaphenanthrene	15.18	1753	-	0.84	1.18
14	Indolizine, 2-(4-methylphenyl)	15.51	1767	-	0.08	-
15	Benzene 1, 2-bis(trimethylsilyl)	15.77	1124	-	0.07	-
16	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilan	16.65	1354	-	3.26	-
17	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	17.19	1775	-	1.48	0.43
18	N-ethyl-1,3-dithioisoindoline;1H-Isoindole	17.41	1192	-	0.45	-
19	5-Methyl-2-phenylindolizine	17.68	1743	-	1.09	-
20	6-Methyl-2-phenylindole	19.01	1951	-	0.08	-

NO	Compounds	Retention time (min)	Kovats index (KI)	Amount of identified compounds during time (%)		
				7th day	14th day	21th day
21	1,6-Dimethyldecahydronaphthalene	15.20	1223	-	-	0.64
22	1,2-dihydro-2-oxocinchoninic acid methyl ester	25.53	1758	-	-	0.44
23	4-Acetyl-6-methoxy-2(1H)-quinolinone	25.80	1826	-	-	0.38
24	Benzopyrido (2,1A)	25.82	1596	-	-	0.38
25	Benzeneacetic acid .α. 3 4-tris (trimethylsilyl)oxy - trimethylsilyl ester	25.97	2152	-	-	1.37
26	1,2-Benzenedicarboxylic acid, diisooctyl ester (phthalate)	32.51	2525	-	-	2.49

3.3. Biodegradation of pyrene

Different compounds were identified in the shoots of maize plants treated with pyrene for 7, 14, and 21 days (Table 3). 10 compounds were identified on the 7th day and the most important compounds among which were 1,3-dimethyl-4-azaphenanthrene (3%), 1,2-Benzenediol, 3,5-bis (1,1-dimethylethyl) (0.28%), 5-methyl-2-trimethylsilyloxy-acetophenone (4.46%), dibenzoxazabicycloundecane; 1H-2,6,10-epoxymethy (5.21%), and 3-quinolinecarboxylic acid (1.37%). The highest amount was belonged to 2,4,6-cycloheptatrien-1-one and 3,5-bis-trimethylsilan (3.48%). 10 compounds were identified on the 14th day, with 4 new ones. In addition, the content of some compounds was increased on day 14 in comparison with day 7. For example, 1,2-benzenediol, 3,5-bis (1,1-dimethylethyl) reached from 0.28–1.51% and 3-quinolinecarboxylic acid increased from 1.37–3.24%. On day 21, 23 compounds were identified which were more than those of days 7 and 14. The most abundant compounds were curcumin (2.05%), zingiberene (8.77%), bisabolene (2.80%), β-sesquiphellandrene (3.21%) β-thujone (0.54%), and coumarin-3-carboxamide (0.44%). Additionally, nitrogen-containing compounds such as benzo (b) carbazole and phthalate were also notably identified on day 21.

Table 3
The recognized compounds in the shoots of maize plants treated with pyrene (75 ppm) for 7, 14, and 21 days.

NO	compounds	Retention time (min)	Kovats index (KI)	Amount of identified compounds during time (%)		
				7th day	14th day	21th day
1	1,3-dimethyl-4-azaphenanthrene	15.17	1753	0.3	0.2	-
2	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	17.12	1775	0.28	1.51	0.5
3	5-Methyl-2-trimethylsilyloxy-acetophenone	16.10	1440	4.46	-	-
4	(R)-2-[5-hydroxymethyl-8-methyl-3,4-dihydro[4,3-e	16.41	1578	7.08	-	-
5	Trimethyl[4-(1,1,3,3,-tetramethylbutyl) phenoxy]silan	16.51	1631	2.08	-	-
6	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilan	16.63	1543	3.48	1.32	-
7	Dibenzoxazabicycloundecane; 1H-2,6,10-(Epoxymethy)	16.77	922	5.21	1.24	-
8	3-Quinolinecarboxylic acid	17.18	1695	1.37	3.24	-
9	N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline	17.35	992	5.64	-	-
10	4-Methyl-2-trimethylsilyloxy-acetophenone	17.08	1440	1.69	1.17	-
11	Thymol	28.77	1322	-	0.2	0.36
12	Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2, 8-dimethyl	15.01	1811	-	0.1	-
13	2,3-dimethyl-4-azaphenanthrene	17.48	2034	-	1.34	-
14	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy] silan	19.16	1640	-	3.76	-
15	B-Thujone	11.02	2929	-	-	0.54
16	1-Propylheptylcyclohexane	14.03	1123	-	-	1.15
17	4H-Furazano[3,4-b]1,4-diazepin-5(6H)-one, 7-methyl	15.10	1592	-	-	0.41
18	curcumin	22.58	1673	-	-	2.05
19	Zingiberene	22.91	1472	-	-	8.77

NO	compounds	Retention time (min)	Kovats index (KI)	Amount of identified compounds during time (%)		
				7th day	14th day	21th day
20	Bisabolene	23.26	1495	-	-	2.80
21	β-Sesquiphellandrene	23.62	1509	-	-	3.21
22	trans-3,4-Dimethyl-2-phenyltetrahydro-1,4-thiazine	24.15	1543	-	-	0.45
23	Coumarin-3-carboxamide	25.13	1669	-	-	0.44
24	Methyl 1,2-dihydro-2-oxoquinoline-4-carboxylate	25.53	1374	-	-	0.24
25	5H-Benzo[b]carbazole	25.80	1826	-	-	0.90
26	Benzo(b)carbazole	26.08	2067	-	-	0.64
27	Acetyl-6-methoxy-2(1H)-quinolinone	26.18	1826	-	-	0.66
28	1-Methyl-5-phenylsulfanyl-1H-pyrazole-4-carbonitrile	27.14	1964	-	-	0.16
29	1,2-Benzenediamine, 4-(4-aminophenoxy	27.44	1304	-	-	0.16
30	N-Methylcyclohexylamino	27.73	947	-	-	0.43
31	2-(N-Methylpyrrolyl) thienoate	28.34	1660	-	-	0.34
32	1,2-dihydroanthra[1,2-d]thiazole-2,6,11- trione	28.87	2504	-	-	0.36
33	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)	30.86	1457	-	-	0.26
34	Di-(2-ethylhexyl)phthalate (1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl)	32.50	2509	-	-	3.31
35	mono-(2-ethylhexyl) phthalate	32.50	2152	-	-	3.31

The intermediates of pyrene biodegradation in roots have been presented in Table 4. 18 compounds were identified on day 7 which were more than those in shoots. The quantity of some compounds including methyl 1,2-dihydro-2-oxoquinoline-4-carboxylate (1.94%), 3,11-diheptyloxybenzo[c] benzo [a] phenanthrene (0.05%), 6H-phenanthro[9,8-gh]quinolin-6-one (0.36%), 2,4-dimethyl benzo [h] quinoline (0.04%), anthracene-9,10-diethyl-9,10-dihydro (0.07%), 1,2-benzenediol, 3,5-bis (1,1-dimethylethyl) (0.45%), and 1,3-dimethyl-4-azaphenanthrene (0.04%) were considerable. The number of identified compounds on day 14 was 13 and from which 6-2,3-dimethyl-6-formyl-7-methoxyindole (2.25%), isobutanoyl-7-

methoxycoumarin (2%), 1,2-benzenediol, 3,5-bis (1,1-dimethylethyl) (1.66%), and 3-quinolinecarboxylic acid (0.06%) were more abundant. In addition, naphthoquinone (0.07%) as two-ring PAHs compounds and anthracene- 9-ethyl-9,10-dihydro-10-t-butyl (0.11%) as three ring PAHs compounds were also detected on day 14. 16 compounds were detected on day 21 from which 15 compounds were new. The most important ones were 1,5-dimethyldecahydronaphthalene (0.4%), thymol (0.33%), 2 dimethylisopropylsilyloxynaphthalene (0.57%), 4-acetyl-6-methoxy-2(1H)-quinolinone (0.86%), and 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3 (0.42%). The increasing quantity of some constitutes such as 1, 3-dimethyl-4-azaphenanthrene (from 0.04 to 0.58%) and 2, 4-dimethyl benzo[h]quinoline (from 0.04 to 0.42%) was also observed.

Table 4
Identified compounds in the roots of maize plants treated by pyrene (75 ppm) for 7, 14, and 21 days.

NO	Compounds	Retention time (min)	Kovats index (KI)	Amount of identified compounds during time (%)		
				7th day	14th day	21th day
1	2(1H)-Naphthalenone, octahydro-4a-methyl	7.12	1449	0.24	-	-
2	Benzaldehyde, 3,4-dimethoxy-2-nitro	7.18	1756	0.35	-	-
3	3,11-diheptyloxybenzo[c]benzo[a]phenanthrene	8.07	2400	0.05	-	-
4	1,8-dimethoxy-9,10-anthracenedion	9.01	2074	0.26	-	-
5	6H-phenanthro[9,8-gh]quinolin	9.07	1996	0.36	-	-
6	7-Methyl-7H- Dibenzo[b,g]carbazole	9.23	2586	0.32	-	-
7	Methyl 1,2-dihydro-2-oxoquinoline-4-carboxylate	10.68	1758	1.94	-	-
8	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro	14.88	2470	0.43	-	
9	2-Ethylacridine	14.91	1989	0.04	-	0.85
10	1,3-dimethyl-4-azaphenanthrene	14.97	1753	0.04	-	0.58
11	2,4-dimethyl benzo[h]quinoline	15.53	2003	0.04	-	0.42
12	Anthracene, 9,10-diethyl-9,10-dihydro	15.80	2049	0.07	-	-
13	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	15.86	2223	0.11	-	-
14	(-+) 5-(1-Acetoxy-1-methylethyl)-2-methyl-2cyclohexen	15.03	1354	0.16	0.12	-
15	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	15.99	1775	0.45	1.66	-
16	2,3-dimethyl-4-azaphenanthren	16.43	2034	0.58	0.07	-
17	1,4-Phthalazinedione, 2,3-dihydro-6-nitro	17.34	1889	1.46	-	-
18	1H-Indole, 1-methyl-2-phenyl	16.43	1805	0.4	-	-
19	2,3-dimethyl-6-formyl-7-methoxyindole	10.70	1705	-	2.25	
20	6-Isobutanoyl-7-methoxycoumarin	10.86	1732	-	2	-
21	Anthracene, 9-ethyl-9,10-dihydro-10-t-butyl	14.79	2283	-	0.11	-

NO	Compounds	Retention time (min)	Kovats index (KI)	Amount of identified compounds during time (%)		
				7th day	14th day	21th day
22	5-Methyl-2-trimethylsilyloxy-acetophenone	14.79	1440	-	0.11	-
23	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl	14.85	1908	-	0.04	-
24	5-Nitrobenzofuran 2- carboxylic acid	14.91	1413	-	0.06	-
25	3-chloro-1,4-naphthoquinone	15.08	1961	-	0.07	-
26	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	15.73	2233	-	0.06	-
27	3-Quinolinecarboxylic acid	16.77	1695	-	0.06	-
28	N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline	17.19	1775	-	0.55	-
29	Silicic acid, diethyl bis(trimethylsilyl) ester	8.15	1049	-	-	0.22
30	Octahydro-1,4- naphthalenedione	12.25	1517	-	-	0.20
31	5-Methyl-2-phenylindolizine	15.80	1743		-	0.32
32	1,5-Dimethyldecahydronaphthalene	15.94	1299	-	-	0.40
33	2-Methyl-6,7-methylenedioxy-4[1H]quinolone	25.58	1899	-	-	0.38
34	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3	25.70	2823	-	-	0.42
35	4-Acetyl-6-methoxy-2(1H)-quinolinone	25.84	1826	-	-	0.86
36	11H-Benzo(A)carbazole	26.06	2437	-	-	0.86
37	2-Dimethylisopropylsilyloxynaphthalene	26.17	1664	-	-	0.55
38	pyrrolo[2,1-b][1,3]benzothiazin-9-one	27.07	1741	-	-	0.57
39	2-Methyl-7-phenylindole	31.06	1951	-	-	0.52
40	Thymol	31.70	1322	-	-	0.33
41	Benzeneacetic acid .α. 3 4-tris (trimethylsilyl)oxytrimethylsilyl ester	31.85	2152	-	-	2.3

The proposed degradation pathway for phenanthrene and pyrene

Proposed degradation pathway of phenanthrene

The GC-MS analysis results and detected intermediates were used to propose a possible pathway for biodegradation of phenanthrene and pyrene in maize plant. The suggested pathway for degradation of phenanthrene was including 3 major steps (Figure 1). A) Rings cleavage of phenanthrene to form 2,4-dimethyl benzo[h]quinoline and benzopyrido compounds, B) Addition of methyl and methoxy groups and production of 1, 5- dimethyl decahydronaphthalene, phthalate, and 7-methoxycoumarin, and C) Further cleavage of intermediates to simpler structures such as phenol derivations.

Proposed degradation pathway for pyrene

Based on the obtained results, the suggested pathway for pyrene degradation was consisted of 4 main steps (Figure 2). A) Cleavage of rings and formation of compounds such as 1,2-dihydroanthra[1,2-d] thiazole-2,6,11- trione, 5H-benzo[b]carbazole, and methyl 1,2-dihydro-2-oxoquinoline-4-carboxylate, B) Desulphonation and deamination of produced compounds and producing some intermediates such as H1-pyrol 2,5dion dihydro 1,3,5,trimethyl phenil, coumarin-3-carboxamide, and zingiberene, C) Get methyl, methoxy, and silis and formation of intermediates such as curcumin, thymol, and phthalate, and finally D) Additional cleavage to simpler structures such as phenol.

Discussion

PAHs can be degraded through different biological, chemical and photochemical processes in the environment resulting in a variety of intermediate compounds. However, there are only a few comprehensive report of PAHs degradation in plants (Vyas et al. 1994; Cajthaml et al. 2001; Takacova et al. 2014; He and Chi 2016). According to the obtained results from GC-MS analyses, maize plant can make several chemical changes on the PAHs for their degradation resulting in production of different by-products. These chemical modifications include hydroxylation, methoxylation, glycosylation, isomerization and oligomerization.

Overall, our results showed that phenanthrene degradation was faster than that of pyrene. The phenanthrene degradation occurred in the second week whereas the degradation of pyrene was happened at a much slower rate after the third week. These findings were consistent with our previous results indicating that the concentration of pyrene was decreased in the shoots and increased in the roots over time (Houshani et al. 2019). On the other hand, the phenanthrene concentration was decreased in the shoots and roots over time and the higher concentrations were detected in 7-day-old plants. In addition, the results demonstrated that main parts of degradation were occurred in the roots in comparison with the shoots (Houshani et al. 2019). The lower degradation rate of pyrene might be due to its higher molecular weight and organic carbon partition coefficient (Carmichael and Pfaender 1997; Juhasz and Naidu 2000; Tabak et al. 2003; Lee et al. 2008; Cheema et al. 2010). Hence, pyrene may not be transported into the cells for degradation by the catabolic enzymes (Fewsom 1988; Juhasz and Naidu 2000). What's more, plants can play a role in the degradation of PAHs through releasing some enzymes

such as dehydrogenase, nitroreductase, peroxidase, and laccase from roots to the rhizosphere. A number of these enzymes have also the ability to transform organic contaminants by catalyzing chemical reactions in soil (Cunningham and Ow 1996; Haritash and Kaushik 2009; Lawal 2017). Similar results have been previously proposed by Lee et al. (2008) and Cheema et al. (2010).

A generalized degradation pathway of PAHs has been suggested in different microorganisms such as fungi and bacteria. It was indicated that phthalic acid and its derivations were produced through the PAHs degradation by white rot fungi and bacteria (Bumpus 1989; Ye et al. 1996; Kotterman et al. 1998; Kim et al. 2005; Seo et al. 2009) and were ultimately converted to highly polar metabolites, water-soluble compounds and carbon-di-oxide (Cerniglia 1992; Machado et al. 2000; Takacova et al. 2014). The detection of phthalic acid in this study showed that maize plant is able to degrade the excess phenanthrene and produce phthalic acid like bacteria. In addition, the obtained results indicated that intermediates such as dihydrodiols, quinolinone, and phthalate has been undergone more cleavage to produce simpler metabolites such as phenol, zingiberene, coumarin-3-carboxamide, curcumin, and 1, 2 benzenediol. Although some of these compounds are naturally existed in plants as the secondary metabolites, but they were not detected in the control maize plants. Therefore, they can be produced by the catabolic routes of phenanthrene and pyrene or it can be produced by the plant secondary metabolism in response to PAHs. Bacteria generally begin the degradation of PAHs through attacking to the aromatic rings with dioxygenases leading to the formation of cis-dihydrodiole which is consequently dehydrogenated to pyrocatechol as a main intermediate (Takacova et al. 2014). Similarly, *Mycobacterium* spp. could metabolize the pyrene up to 60% within 8 days at 20 °C (Rehmann et al. 1998; Haritash and Kaushik 2009). As a result, cis-4,5-pyrene dihydrodiol, 4-5-phenanthrene dicarboxylic acid, 1-hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, phthalic acid and protocatechuic acid were reported as degradation products (Haritash and Kaushik 2009).

In this study, some intermediate compounds such as quinones, dihydrodiols, and hydroxyl- PAHs were produced through PAHs degradation in maize plants. Such intermediates were also observed in bacteria, fungi, and algae. For example, wood-rotting fungi have the ability to degrade PAHs by excreting extracellular enzymes and lignin in wood. Intriguingly, these enzymes are not very specific and can also transform organic pollutants such as PAHs to quinones (Cajthaml et al. 2002). In this process, some wood-rotting fungi may cleave the rings and finally produce carbon dioxide and water. In comparison, some other fungi produce quinone derivatives as end products (Bumpus 1989; Juhasz and Naidu 2000; Haritash and Kaushik 2009). Fungi can oxidize PAHs by cytochrome P-450 system to form phenols and trans-dihydrodiols which can be removed from the organism. Consistently, marine algae have the ability for transformation of Benzo [*a*] pyrene (BaP) as PAHs to dihydrodiols and quinones in a period of 5-6 days (Warshawsky et al. 1995). It was reported that *Selenastrum capricornutum*, a freshwater green alga, can metabolize BaP to cis-dihydrodiols by using a dioxygenase enzyme system (Warshawsky et al. 1988). Taken all together, our results showed that some degradation pathways of PAHs in maize were comparable with those of bacteria, fungi, and algae. Accordingly, similar intermediate compounds such

as quinones, dihydrodiols, phthalate, and phenolic compounds were produced through catabolism of phenanthrene and pyrene in maize plants.

Conclusion

Heretofore, researches in the field of biodegradation processes of environmental organic pollutants have mainly focused on microorganisms such as bacteria and fungi. The current study was conducted to provide more information of such processes in a plant system. The obtained results emphasized that maize plant had potential for degradation of phenanthrene and pyrene. The plants showed higher ability for the degradation of phenanthrene compared to pyrene. Obviously, the detection of a number of intermediate compounds produced by the cleavage of phenanthrene and pyrene proposed a possible catabolic pathway of pyrene and phenanthrene in maize plant.

Declarations

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Figures

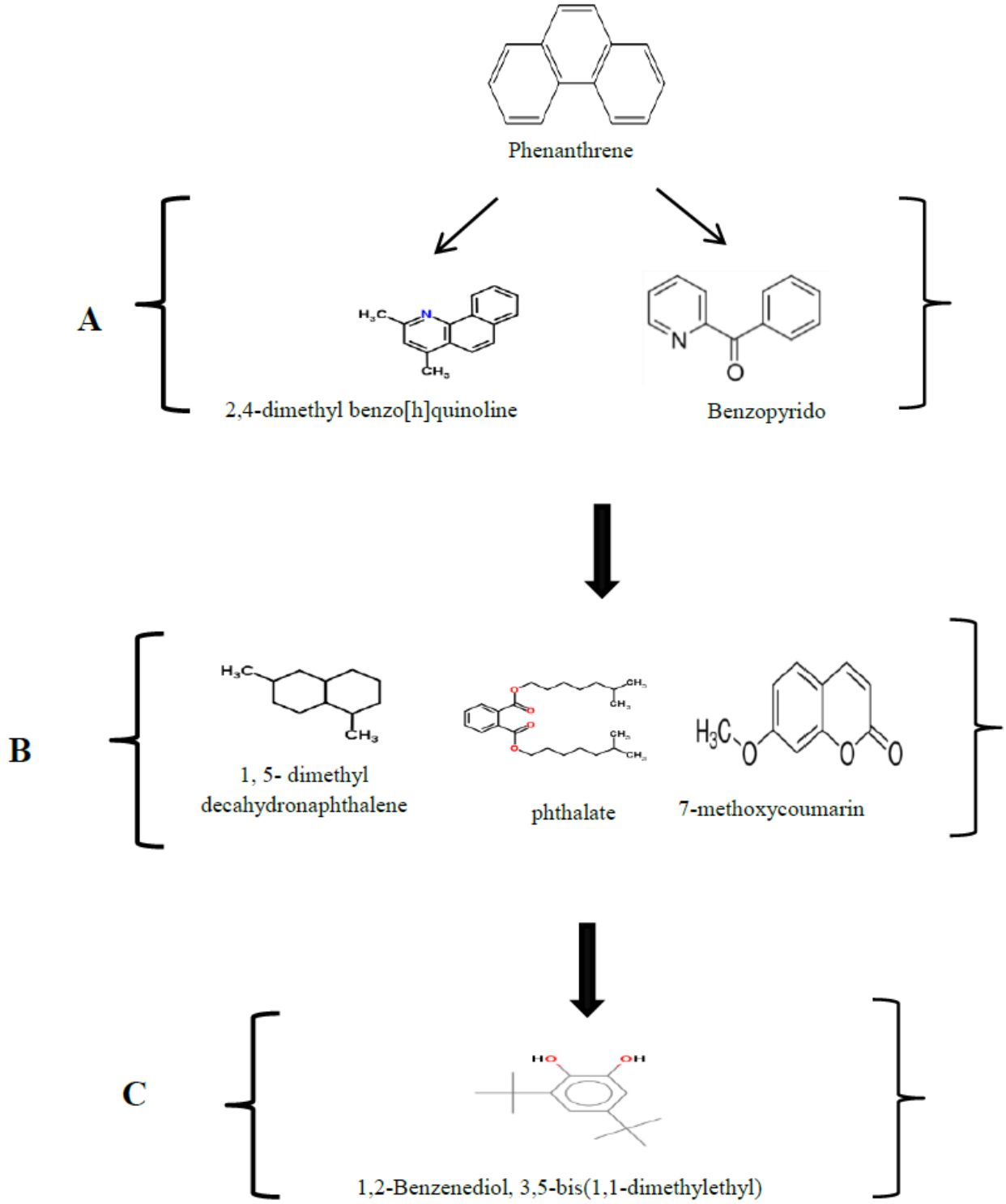

Figure 1

The proposed pathway of phenanthrene degradation by Zea mays.

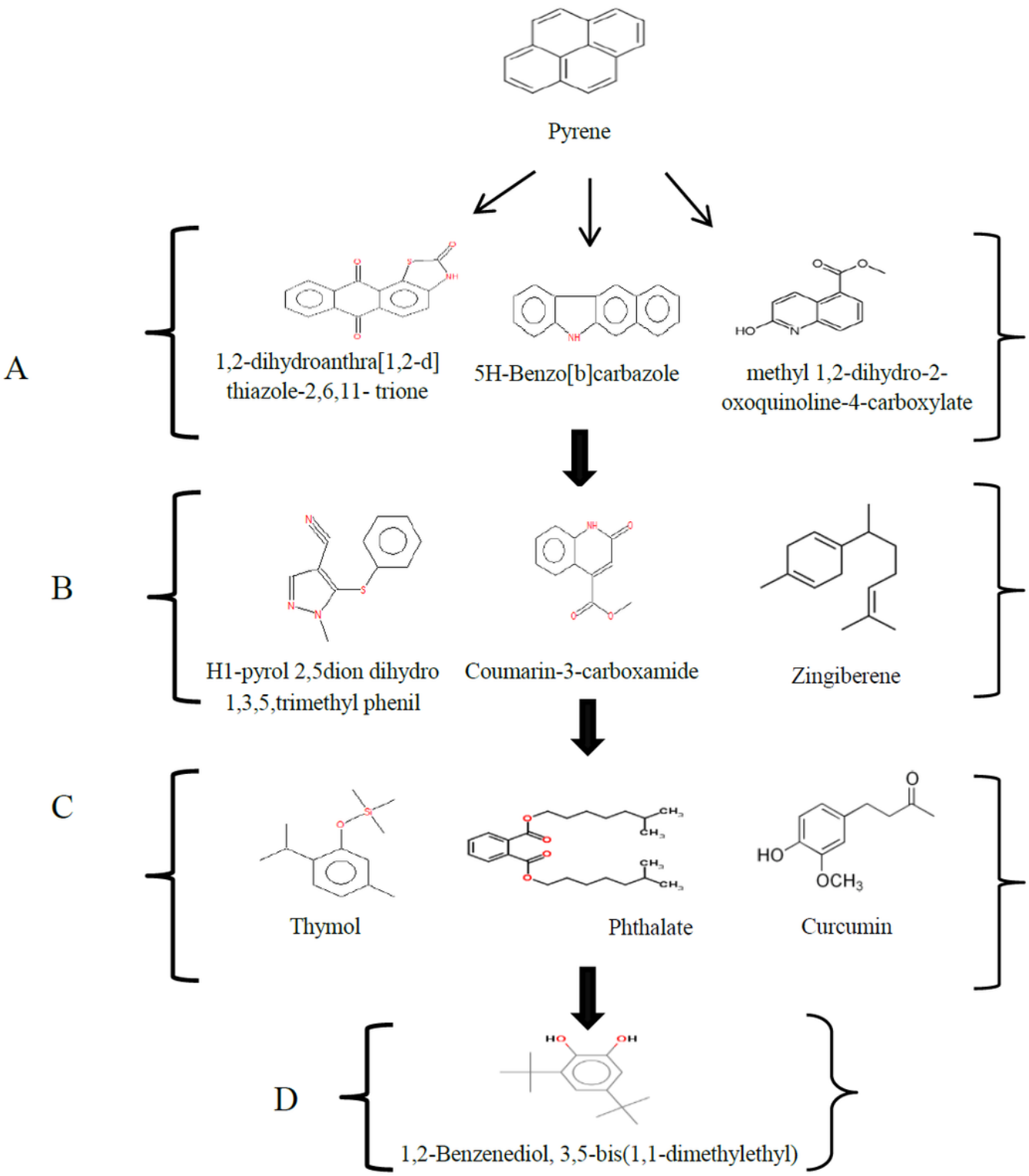

Figure 2

The proposed pathway of pyrene degradation by Zea mays.