HPLC Evaluation of PAHS Polluted Soil in Coastal Petroleum Refinery Site Northwestern Suez Gulf, Egypt

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ABSTRACT
Northwestern Gulf of Suez is a well-known region for coastal petroleum refineries. Industrial discharges release organic contaminants which might affect the quality of receiving water and threaten aquatic life. In this work, concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) were measured in the soil in order to evaluate their quality and characteristics. To examine the potential toxicity of sediments by PAHs contaminant, eight composite sediment samples were randomly collected using an American auger around the point 29°57' 33" N and 32°30'40" E in 2012 and covered an area of 2500 m² which represents nearly 1/15 of total plant area (the total area of the plant is approximately 3.250 km²). For the examined samples, the Total Petroleum Hydrocarbons (TPHs) were separated from the soil. A portion of TPH was subjected to column chromatography using silica gel as stationary phase (re-activated at 180°C for 4 h). The first solvent was n-hexane for elution of saturates while the second was benzene to obtain aromatics which are subjected to High Performance Liquid Chromatography (HPLC). The examined sediment samples exhibited diverse concentrations of ΣPAHs equal to 47.23, 18.73, 107.23, 10.36, 16.64, 12.24, 67.11 and 33.84 ppm, respectively.

Key words: Contamination, polyaromatic hydrocarbons, liquid chromatography, TPHs, Suez Gulf

INTRODUCTION
Contamination of soil, water and air with toxic chemicals, especially widespread organic pollutants is one of the major environmental problems facing the world and has been a major problem at the sites in question. Polynuclear aromatic hydrocarbons or Polycyclic Aromatic Hydrocarbons (PAHs) are wide existence organic contaminants (Mai et al., 2001), that consist of two or more fused aromatic rings in a linear or clustered arrangement. They usually contain only carbon (C) and hydrogen (H) atoms, although nitrogen (N), sulfur (S) and oxygen (O) atoms may readily substitute in the benzene ring to form heterocyclic aromatic compounds. They are produced via natural and anthropogenic sources, generated during the incomplete combustion and pyrolysis of solid and liquid fuels derived from industrial activities (Fang et al., 2006; Gan et al., 2009).
Polycyclic aromatic hydrocarbons arouse many concerns because of their high degree of mutagenicity, carcinogenicity and genotoxicity of some high molecular weights PAHs (i.e., those containing four or more rings) when they enter the human body and other living organisms (Wang et al., 2009). Also, PAHs can act as endocrine disruptors (i.e., interfere with hormone production and function). They have effects on liver, lungs, kidneys and nervous system that lead to cancer, immunological, reproductive, fetotoxic and genotoxic diseases (Rushton et al., 2007). Phenanthrene for example, is a known irritant and it photosensitizes skin to light. Pollution by PAHs with concentration of 0.0002 mg L\(^{-1}\) causes dangerous health effects via reproductive difficulties and increases risk of cancer (Paria, 2008).

Polycyclic aromatic hydrocarbons are characterized by their palpable hydrophobic nature. Their low aqueous solubility and high affinity to soil contribute to their persistence in the environment. These species tend to be adsorbed on solid particulates (soils and sediments), especially on the organic fraction of the solids (Rivas, 2006). In aquatic systems, PAHs increase their toxicity with the increasing molecular weight. Although, the rate of uptake from the environment is variable among species, bioaccumulation (air, soil, water, fish species and vegetation all act as sinks accumulate PAHs) tends to be rapid. Biodegradation of PAHs is very slow, resulting in their environmental persistence for long periods of time, where heavily contaminated soils are frequently around industrial plants using petroleum, especially old manufacturing plants. So, contamination of soil and groundwater by PAHs is a major environmental issue in the Northwestern Suez Gulf, Egypt. The major sources of PAHs contamination are old coastal refinery plants, make coastal oil pollution of the Suez Gulf a serious problem.

This study attempts to evaluate the concentration of PAHs polluted sediments and understanding the PAHs dispersion to assess Gulf of Suez pollution and groundwater vulnerability.

**MATERIALS AND METHODS**

**Site characterization:** The studied site included representative costal refinery plant named El Suez Refinery that located Northwestern Suez Gulf, Egypt (Fig. 1). It is a complex refinery with a nameplate distillation capacity of 3250 k t per annum (65,000 barrels day\(^{-1}\)). The refinery that was originally opened in 1921 is operated by the Suez Oil Processing Company, a subsidiary of the Egyptian government-owned Egyptian General Petroleum Corporation (EGPC). Upgrading of this refinery is scheduled to add a 40,000 barrels day\(^{-1}\) hydrocracker unit to crack fuel oil into middle distillates.

The quaternary sediments of sand and gravel represent the main deposits in the Northwestern Gulf of Suez below the limestone plateau. The thickness of the quaternary bearing layer is gradually increasing toward the Gulf of Suez with the down throw of the step faults and varying from 62-240 m. The dry layer is composed of gravelly sand clay and its thickness is ranging from 4-63 m from Southeast to Northwest, respectively, the water level in this section varying from 0.0+3.10 m (Usama, 2001). The climatic conditions prevailing in the area are typical of arid provinces of North Africa, i.e., a hot summer and a warm rainy winter with an average temperature of 30°C throughout the year and an annual precipitation that ranges between 4.1 and 0.1 mm.

**Sample collection:** To compare the potential toxicity of sediments with different contaminant characteristics, field sampling has been oriented to collect a representative soil sample over space according to Abdel-Moghny et al. (2012) and Zawrah et al. (2014). Five deep soil samples with a weight ranging from 250-300 g are collected from the one point with sum equal to 40 samples.
These random samples were collected around the point 29°57' 33" N and 32°30' 40" E in 2012, using an American auger from different locations covered 2500 m² (total area of the plant approximately 3.250 km²) at a depth of 70 cm (Fig. 2). The samples were mixed, homogenized, air-dried and stored in closed plastic bags at room temperature (25°C) in the laboratory until use (Dong et al., 2010). The soil samples consist of gravel (18.72%), sand (76.18%), silt and clay (5.10%).

**Sample treatment and analysis**

**Organic hydrocarbons extraction:** In our previous work (Zawrah et al., 2014), the Total Petroleum Hydrocarbons (TPHs) were extracted from sediment samples in a soxhlet using benzene/methanol (70:30 vol/vol) according to El Nemr et al. (2004), El-Sikaily et al. (2002), Mostafa et al. (2009), Aboul-Kassim and Simoneit (1995, 1996), UNEP/IOC/FAO/IAEA (1991), UNEP/IOC/IAEA (1992) and Zaghdan et al. (2005) and presented. The extraction has been
continued until the solvent in the arm of the soxhlet was colorless. Total petroleum hydrocarbons was recovered by distillation and placed in a weighted beaker until it gives a constant weight, TPH was calculated by differences in weight and their values were in the range of 2.44-4.78 wt%.

**HPLC analysis:** A portion of TPH is subjected to column chromatography using silica gel as stationary phase (re-activated at 180°C for 4 h). The first solvent is n-hexane for elution of saturates then using benzene to obtain aromatics which are subjected to HPLC.

The studied HPLC instrument model Agilent 1200 series equipped with photodiode array detector is used for identification and quantification of 16 PAHs present in extracted oil using gradient method. The used column is SUPELCOSIL LC-PAH (15 cm×4.6 mm, 5 µm), sample size 2.0 µL, mobile phase (Acetonitril: Water) HPLC grade (gradient program), mobile phase program 50:50% (v/v) for 1 min, 100% Ace at 20 min, for 15 min, then 50:50% again at 35 min, flow rate 1.0 mL min⁻¹. The identification of PAHs peaks is established by a chromatographic reference mixture of 16 PAHs standards of known composition listed by USEPA (1998) shown in Fig. 3, the data is illustrated in Table 1.

**Methods of statistical analysis:** The methods of statistical analysis were calculated by converting to matrix random XYZ and Y error by Origin 6.1 Program.

**RESULTS AND DISCUSSIONS**

The soil samples are analyzed for sixteen of the most important PAHs. The results presented in Table 1 and shown in Fig. 4a reveal that naphthalene and acenaphthene are not detected among the PAHs except in sample 2 with concentrations of 1.64 and 1.67 ppm, respectively. Also, acenaphthylene is not detected among the PAHs except in samples 2 and 4 with concentrations equal to 4.99 and 0.08 ppm, respectively. Phenanthrene is not detected among the
Table 1: High performance liquid chromatography analysis of detected polycyclic aromatic hydrocarbons in ppm for the examined samples

| Components          | Acronym | Molecular formula | No. of rings | Concentration of PAHs in samples (ppm) |
|---------------------|---------|-------------------|--------------|----------------------------------------|
|                     |         |                   | S (1) | S (2)* | S (3) | S (4) | S (5) | S (6) | S (7) | S (8) |
| Naphthalene         | Nap     | C_{10}H_{8}       | 2     | 0.00   | 1.6   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| Acenaphthylene      | Acy     | C_{12}H_{8}       | 3     | 0.00   | 4.99  | 0.00   | 0.08   | 0.00   | 0.00   | 0.00   |
| Acenaphthene        | Ace     | C_{12}H_{10}      | 3     | 0.00   | 1.67  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| Fluorene            | Flu     | C_{16}H_{10}      | 3     | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| Phenanthrene        | Phe     | C_{18}H_{10}      | 3     | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| Anthracene          | Ant     | C_{14}H_{10}      | 3     | 4.37   | 0.00  | 9.68   | 1.07   | 1.28   | 0.00   | 22.87  | 0.00   |
| Fluoranthene        | Flr     | C_{16}H_{10}      | 4     | 0.00   | 0.19  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   | 0.90   | 0.00   |
| Pyrene              | Pyr     | C_{16}H_{10}      | 4     | 7.01   | 1.04  | 10.45  | 0.00   | 0.00   | 0.00   | 0.00   | 27.33  | 0.00   |
| Benzo(a)Anthracene  | B[a]A   | C_{18}H_{12}      | 5     | 1.91   | 0.31  | 2.84   | 0.07   | 0.00   | 0.00   | 0.00   | 2.96   | 1.09   |
| Chrysene            | Chr     | C_{18}H_{12}      | 4     | 0.00   | 0.44  | 2.06   | 8.14   | 0.00   | 0.00   | 0.00   | 6.72   | 0.00   |
| Benzo(b)Fluoranthene| B[b]F   | C_{18}H_{12}      | 5     | 9.85   | 2.48  | 7.12   | 0.31   | 0.00   | 1.10   | 0.65   | 3.46   |
| Benzo(k)Fluoranthene| B[k]F   | C_{18}H_{12}      | 5     | 0.00   | 1.00  | 25.29  | 0.00   | 0.00   | 1.93   | 0.85   | 8.05   |
| Benzo(a)Pyrene      | B[a]P   | C_{20}H_{12}      | 5     | 12.15  | 1.99  | 22.58  | 0.30   | 2.39   | 5.29   | 1.40   | 9.25   |
| Dibenzo(a,b)Anthracene| D[ab]A | C_{22}H_{12}      | 6     | 2.85   | 1.32  | 11.74  | 0.00   | 3.07   | 3.92   | 1.30   | 4.70   |
| Benzo(g,h,i)Perylene| D[ghi]P | C_{22}H_{12}      | 6     | 3.39   | 1.26  | 4.52   | 0.37   | 2.64   | 5.29   | 2.11   | 4.06   |
| Indeno(1,2,3-cd)Pyrene| I[cd]P | C_{22}H_{12}      | 6     | 5.68   | 0.40  | 10.97  | 0.00   | 6.37   | 0.00   | 0.00   | 3.23   |

Total PAHs: 47.23, Standard deviation: 4.01, Standard error: 1.07

PAHS: Polycyclic aromatic hydrocarbons, *: The value of Nap that found in S(2) suggested that this composite contains recently polluted sediments

Fig. 4(a-b): PAHs concentrations in (a) Different contaminated samples and (b) Statistical error
PAHs except in sample 5 with concentration of 0.88 ppm, whereas fluorene is not detected among the PAHs in any samples. Only sample 7 that shows high concentrations of Anthracene 3 rings PAHs equal to 22.87 ppm, also samples 1, 3, 4 and 5 show different concentrations of Anthracene equal to 4.37, 9.68, 1.07 and 1.28 ppm, respectively. Whereas, in both samples 6 and 8 the 2 and 3 rings PAHs disappear. The average concentrations of 2 and 3 rings PAHs in dry soil are 6.06 ppm in all samples, the analysis error shown in Fig. 4b.

For heavier PAHs (with more than 3 rings), concentrations are 33.11 ppm from the average concentrations of total PAHs in dry soil that are 39.17 ppm. This means that the concentrations of heavier PAHs in the samples exceeded more than five times PAHs with 2 and 3 rings as shown in Fig. 5. These are attributed to the fact that PAHs vary widely in molecular structure, the most common of which range from 2-6 fused rings. Also, physicochemical properties variation such as water solubility that plays effective role due to shallowness of water level as mentioned previously (“Site characterization” section). Water solubility of PAHs ranges from 31 mg L\(^{-1}\) for naphthalene (2 rings) to 290 µg L\(^{-1}\) for benzo(g,h,i)perylene (6 rings) as mentioned by Sims and Overcash (1983).

As mentioned by Khalladi et al. (2009), the solubility of PAHs depends on the number of rings and the age of contamination. Generally, heavier PAHs have extremely low solubility in water, where the 2- and 3-ring PAHs are of special concern because they are water soluble and are transported with the groundwater over significant distances. As the sediments are predominantly coarse sand in this region (see section 2.1), which do not have a high adsorptive capacity (Law, 1981; Zanardi et al., 1999). This point of view matches with Chang et al. (2000), they found that PAHs were eliminated in the presence of water by 30-80%.

The contour map in Fig. 6 illustrates the heterogeneous distribution of PAHs in the representative sampling area. Because of the extreme hydrophobicity of the 4-6 rings (inherent molecular recalcitrance) and low solubility of these organic contaminants in water, sorption in the soil matrix is very high. This means 4-6 rings are considered as long-term source of contaminant. Normally PAH is presented in much higher concentrations in soil phase than in aqueous phase. PAH fate in soils is also variable, ranging from biodegradable to recalcitrant. Bioavailability and mineralization of hydrophobic organic contaminated soil declined with increasing contact time (aging phenomena). Certain PAHs are also classified as Persistent Organic Pollutants (POPs) and Persistent Bioaccumulative Chemicals (PBCs). Shuttleworth and Cerniglia (1995) reported a
Fig. 6(a-b): Concentration map of PAHs in soil (ppm) around the point 29°57’ 33” N and 32°30’ 40” E in 2012

phenanthrene half-life (3 rings) in soil and sediment of 16-126 days but benzo[a]-pyrene (5 rings) ranges from 229 to over 1400 days.

Whereas, prediction of such contaminants fate in soils and sub-soils are the key issue for risk assessment and evaluate the feasibility of treatment technologies (Ncibi et al., 2007). Figure 6 exhibits the distribution of PAHs over sandy gravel quaternary sediments with area equal 2500 m². It is known that these soils are most vulnerable. Sediments are harboring PAHs hazardous pollutants that can directly influence water quality, therefore creating very stressful conditions for aquatic life. Because of seepage velocity in vulnerable sandy soils ranges from 13.2-2.8 m h⁻¹, pollutants leaks are the most critical problem (Halmemies et al., 2003). The pollution of groundwater can be only a question of hours.

Since, PAHs are organic micro-pollutants and strongly adsorbed in soils, especially onto terrestrial colloids, resulting in long-lasting environmental effects. As, many organic compounds
have low solubility in water, heavier PAHs (4-6 rings sparingly soluble in water) may leach from the soil for a longer period of time and thus ultimately become a continuous source of the soil and groundwater contamination. So, their size allows them to circulate within a large fraction of the porous medium. The sorption of PAHs onto soils is broadly influenced by the solubility of the different PAHs and their organic fraction.

Other potential factors exerting some influence on the adsorption stage; like temperature, salinity or the presence of dissolved organic matters are in fact variables which influence the solubility of the aromatic compounds. The adsorption of PAHs is favored by increasing salinity or decreasing in temperature while the presence of humic substances has no appreciable influence on the adsorption extent. Adsorption process is more complex to detect in this site due to high salinity which related to the Suez Gulf water. This causes increasing PAHs adsorption. Moreover, this region is characterized by its warm water, warm rainy winter and hot summer months, which cause increasing diffusion of PAHs. This clarification is compatible with Ibrahim (2004), who detected high concentrations of PAHs ranging from 3487.25-5516.52 μg kg\(^{-1}\) wet weight in different stations far away from 10-60 km Southern oil refineries. He indicated that the degree of PAHs contamination in the Northwestern Gulf of Suez seemed to increase Northward. Likely, the industrial activities in the northern region, which comprise oil refineries and petrochemical industries are one of the principal anthropogenic sources, they include oil spills and leakage. El Nemr et al. (2005) studied the sources and distribution of PAHs along Suez Gulf. They indicated that the anthropogenic hydrocarbon inputs from petroleum refinery discharges were more apparent at this site. This may reflect the existence of acute PAHs contamination in the sediments due to massive and continuous inputs of these pollutants to the region.

CONCLUSION

The scientific community has begun noticing the deterioration of sediment quality at an alarming rate worldwide. The PAHs contaminated sediments, especially from industrial facilities often function as a long term pollution source for surface and ground waters. Due to sediments frequently release the persistent micropollutants back to the pore water, cause hazardous because of the complexity of environmental systems that contain wide variations. Therefore, the detection, identification and quantification of PAHs in soils are of considerable interests in environmental analyses.

This study explains the consequences for risk assessment which includes site characterization and ecological risk quantification. Using traditional analytical methods for PAHs, these techniques require sampling, cleanup, HPLC separation and detection. The data reveals that the ΣPAHs equal to 47.23, 18.73, 107.23, 10.36, 16.64, 12.24, 67.11 and 33.84 ppm for the eight composite samples, respectively. The majority of PAHs in these samples is heavy fractions that consist of 4 and 6 rings. Whereas, PAHs that consist of 2 and 3 rings represent only a small percentage or has been disappearing in some samples.

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