

Oil Spills: Impact on Water Quality and Microbial Community on the Nile River, Egypt

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ABSTRACT

Oil spills accidents in water bodies cause serious environmental hazards and have recently attracted proper attention in Egypt. However, normally, monitoring of water quality during an oil spill accident would focus mainly on the increase of oil and grease content with little attention given to the impact of the spills on microbial community. Nile River water was sampled during some oil spills to assess the effect of these accidents on the water quality and microbial community. Measurements included; pH, EC, TDS, Oil and grease, BOD, COD, total coliform count and fecal coliform count. Additionally, an *in vitro* experiment was conducted to measure the effect of these oil spills on the bacterial community, with following up the total bacterial count and genus identification using API kit technique. Results showed that the pH values, EC and TDS of Nile River weren't affected during the studied oil spills Cases, while oil and grease, BOD and COD tend to increase. The total coliform counts and fecal coliform counts tended to decrease. The bacterial community before being subjected to the oil spill constituted *Arthrobacter*, *Enterobacter*, *E. coli*, *Bacillus* and *Pseudomonas*, while after 14 days of the experiment *Pseudomonas* and *Bacillus* were predominated, which means the predominance of biodegrading bacteria.

Key words: Oil spills, Water, Bacteria, Oil and grease

Introduction

The main and almost exclusive source of fresh water in Egypt is the Nile River. From High Aswan Dam, the Nile flows northward for about 950 km then it divides into Damietta and Rosetta branches, which extend through the Delta to the Mediterranean Sea (Abu Zeid *et al.*, 1991). Along this distance, it estimated that about 52 factories drain directly in the river with or without any kind of treatment of the effluent. Another 134 factories drained in agricultural drains that finally reach the river. The industries are for chemicals, sugar, paper, aluminium, fertilizers, steel weaving, soap, insecticide and many other industries. The effluent varies according to the type of the industry. It contains organic matters, suspended materials, phosphate, heavy metals, oils and many different types of chemicals (El-Moutassem, 1994). Moreover, Egypt depends on the river transportation to transport many of inert materials like sulfur, phosphate, iron, oil and oil products from north to south. Consequently, oil spills in Nile River are sometimes happen as accidents during these activities.

Although oil spills occur in various media, the term is mainly associated with water oil spills (McGenity *et al.*, 2012). Oil spills, especially in freshwater have serious impacts on the environment and living organisms including humans. These negative effects are due to the discharge of various organic compounds that make up crude oil and oil distillate products, the majority of which include various individual hydrocarbons. Other individual compounds that are present in crude oil and oil discharges include (apart from carbon and hydrogen) sulfur, nitrogen and/or oxygen atoms too (Manoli *et al.*, 2000). These organic compounds may affect marine and fresh water life, as well as, humans in various ways; by direct contact with the skin, through inhalation, through ingestion of contaminated water and/or particles, through emitted odors and indirectly by consuming contaminated food (some of the oil hydrocarbons such as PAHs bio-accumulate in fish and other organisms and may concentrate many times more than in water) (EPA, 2013).

Concerning behaves of oil spill in the environment, depending on where and how an oil spill occurs; it will have distinct environmental fate and transport. For example: A water oil spill is usually degraded fast since water is an excellent media for dispersion, emulsifying and microbial degradation processes. If released in the water, oil and oil products tend to accumulate at the surface of the water and float on the water. Small oil droplets may also form which may increase the surface contact with water and also the natural biodegradation of the spilled oil (Das and Chandran, 2011).

The causes of oil spills may be due to accidental spills during storage, handling or transport. Or it may be due to the routine maintenance activities or road runoff. Other causes like burning of fuel and vehicle emissions may be considered (Wetzel and Van Vleet, 2004).

According to the characteristics of the spilled oil, its fate in, and effect on the environment will determined, that was reported by Irwin 1997. Lighter or less dense oil, when spilled in surface water it will accumulate on top of water surface and may disperse over large areas, sometimes oil slicks are formed, this increases the resistance of oil to natural attenuation processes and makes it more persistent in the environment.

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Mixture of volatile compounds (e.g., hydrocarbons), when spilled in water it will start to evaporate when reaches the top of water (in contact with air), consequently, the air above the spill become polluted with various volatile components, and it may travel via wind to other places. Mixture of compounds with different water solubility; some oil individual compounds may dissolve in water (to some extent) such compounds become more bio-available to be taken by water life.

The direct exposure to oil spills may leads to a variety of human health threats starting from minor illness to cancers. Human beings also may affected indirectly by oil spills through consumption of contaminated food or water, especially in the case of consumption of fish that was in contact or in an oil spill polluted environment, the fish lives in a polluted environment, will keep adsorbing in its body some oil components, without excretion, which may reach concentrations several orders of magnitude higher than those of the surrounding waters. Through consumption of such polluted fish meat, humans may become seriously exposed to higher concentrations of oil components than in the surrounding environment or as compared to ingestion of the polluted water or bathing in the polluted water (Maskaoui and zhong, 2009).

Historically, the two worst spills took place in the oceanic shores of the United States; Exxon Valdez spill in 1989 and British Petrol Deepwater Horizon spill in the Gulf of Mexico 2010. A lot of studies have been carried out in both Cases to evaluate their effect on the environment. Atlas and Hazen, 2011 had studied the environmental impact of both spills, they have reported that the former accident was a tanker spill where some of 41.5 million liters of heavy oil of North Slope had been leaked near island shores which led to formation of cobble or rocky shorelines. The second was a well leak where some of 779 million liters of light Louisiana oil have been spilled in the Gulf of Mexico which led to formation of a deep sea cloud of fine droplets of low concentration oil, the efforts continued for 84 days to stop the leak. The increase in petroleum hydrocarbons was also reported, which consequently leads to increase of COD and BOD. On the other hand, the total count of viable bacteria before spills was $1 - 5 \times 10^3$ CFU/ml while it reached $1 - 5 \times 10^5$ CFU/ml during spills. The shift in the community of microbial eukaryotes was also reported by Bik et al, (2012) in the second Case, the community has been predominated with fungi during the spill. Biodegrading bacteria also dominate the bacterial community during both spills (Atlas and Hazen, 2011)

The present work aimed to survey the quality of Nile River water during some of accidental oil spills, and studying the effect of these oil spills on the community of bacteria using an *in vitro* experiment.

Materials and Methods

1- Cases study; studying the effect of oil spills on water quality of Nile River in some accidental oil spills cases had happened in the last few years

Many accidental oil spills cases had happened in the Nile River through the last few years, three of them have been selected to study the effect of such oil spills on Nile water quality. The study was carried, in each case, by measuring the following parameters; pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), oil and grease, total coilform count (TC) and fecal coliform count (FC), all the parameters have been carried out according to (APHA, 2012). To perform the study, water samples have been collected from un-polluted sites and from polluted sites during a spill.

The selected accidental oil spills cases in the Nile River were outlined in Figure (1) and described as follows;

- 1.1 Helwan case; in July, 2008, large quantities of mazut leaked into the Nile River, after a main oil pipeline of the Helwan Cement Company ruptured, near the districts of Manial and Helwan, resulting in highly polluting the water and causing three water refineries to suspend operations. The spill, which covered an area of two kilometers and was spotted at different locations, resulted of a leak when the National Petroleum Pipelines attempted to separate an unused pipeline from one currently in use.
- 1.2 Aswan case; in Sept, 2010, a barge sank in the Nile River in southern Egypt at near Aswan and caused an oil spill, threatening the city's water supply. The accident caused a leak of some 100 ton of fuel in the river, and the slick was up to 2 km long.
- 1.3 Naj Hammadi case; in Oct, 2012, a heavy oil spill had happen in upper Egypt, at Naj Hammadi sugar factory due to a leakage from the factory waste pipe. A slick of about 15 m diameter was floating on the surface of water, traveling with water flow to



Fig. 1. Cases outline map.

downstream governorates, that led to a total of 18 water stations were shut down in the neighboring governorates of Assiut and Qena, as reports of oil leaks have surfaced in several governorates (Al-Masry Al-Youm, 1/11/2012).

2-Studying the effect of oil spills on the microbial community of Nile River water using in vitro batch experiment

A batch experiment was designed using four 3 L glass pools, each containing 2 L of Nile River water brought from the Nile River at Al-Kanater city. Different concentrations of oil were added to get the final concentration of oil and grease in the pools as follow; 0, 400, 100 and 3 mg/l, representing the control (unpolluted site), case 1 (resembling Helwan case), case 2 (resembling Aswan case) and case 3 (resembling Naj Hammadi case), respectively. To conduct the experiment in approximately under natural conditions, the set was incubated at room temperature with shaking at 90 rpm for two week (the moderate oil spill time in Nile River in the three studied cases). The following parameters were also measured pH, EC, TDS, BOD, COD and Oil and grease, at zero time and at the end of the experiment while total bacterial count (TBC), total coilform count and fecal coliform count, were measured each two days to follow up the deviation expected to occur due to the oil contamination.

On the other hand, the microbial community during the fourteen days of the experiment was inventoried and identified, using microscopic characterization and API kit technology, to detect the dominated bacterial isolates during oil spills.

Results

1-Effect of oil spills on water quality of Nile River in some accidental oil spills cases had happened in the last few years

The water assessment chemical, physical and microbiological parameters have been chosen to study the effect of three different oil spills cases happen in the Nile River in the last few years were summarized in table (1).

Table 1. Water quality assessment in the Nile River in three different oil spills cases

| Parameter | Unit | Helwan case 2008 | | Aswanc 2010 | | Naj Hammadi case 2012 | | Low 48/ article 60 |
|----------------|------------|--------------------------|-----------------------|-------------------------|----------------------|-------------------------------|----------------------------|--------------------------|
| | | Helwan normal Nile water | Helwan polluted water | Aswan normal Nile water | Aswan polluted water | Naj Hammadi normal Nile water | Naj Hammadi polluted water | |
| Oil & grease | mg/l | 0.3 | 426 | 0.07 | 109 | 0.09 | 2.92 | 0.1 |
| pH | | 7.76 | 7.68 | 7.51 | 7.65 | 7.62 | 7.96 | 7 - 8.5 |
| EC | mmhos/cm | 0.366 | 0.394 | 0.286 | 2.89 | 0.299 | 0.311 | |
| TDS | mg/l | 234 | 252 | 225 | 238 | 222 | 227 | 500 |
| BOD | mg/l | 15 | 130 | 5 | 76 | 4 | 30 | 6 |
| COD | mg/l | 18 | 550 | 7 | 230 | 7 | 98 | 10 |
| Total Coliform | CFU/100 ml | 8000 | 5000 | 900 | 600 | 2800 | 1600 | |
| Fecal Coliform | CFU/100 ml | 1000 | 600 | 20 | 8 | 500 | 200 | |

The oil and grease in Naj Hammadi oil spill case was 2.92 mg/l while it was 109 mg/l in Aswan case, and reach 426 mg/l in Helwan case. That might be due the type and volume of the spill. As mentioned before in Helwan case the main oil pipeline of Helwan Cement Company ruptured, so a heavy spill had happen, while in Aswan case it was an accident during transportation of oil through the Nile causing a leak of some 100 ton of fuel in the river, and the slick was up to 2 km long, but in Naj Hammadi case it was just a leakage from the pipe waste of the sugar factory, and the slick was 15 m. As noted from the given results in table (1) the pH, EC and TDS didn't affected by the oil spills in all cases, while BOD and COD values were obviously increased, due to the carbon compounds of oil (Figures 2 & 3). On the other hand, the microbiological parameters tested in the study cases have been negatively affected by the oil spills where the total coliform and fecal coilform counts were 8000 and 1000 CFU/100 ml and become 5000 and 100 CFU/100 ml, respectively, in Helwan. They were 900 and 20 CFU/ 100 ml and become 600 and 8 CFU/ 100 ml, respectively, in Aswan, while in Naj Hammadi they were 2800 and 500 CFU/ 100ml and become 1600 and 200 CFU/ 100 ml, respectively, (Figures 4 & 5).

2-Effect of oil spills on the microbial community of Nile River water

The effect of oil spills on water quality of Nile River during the spills was monitored using *in vitro* experiment; the results were summarized in table (2). The pH, EC and TDS values didn't affected through the fourteen days the experiment, while there was an obvious decrease in BOD and COD values in range of 15 – 30%. The oil and grease values showed also a considerable decrease in range of 20% in all cases. The decreasing in BOD, COD and oil and grease values may be due to the normal volatilization of oil spills during the experiment, nevertheless, the role of degrading bacteria could not be neglected.

Table 2. Water quality assessment of Nile River *in vitro* experiment

| | Oil & grease (mg/l) | | pH | | EC (mg/l) | | TDS (mg/l) | | BOD (mg/l) | | COD (mg/l) | |
|---------|---------------------|--------|-------|------|-----------|-------|------------|-----|------------|-----|------------|-----|
| | start | end | start | end | start | end | start | end | start | end | start | end |
| Control | 0.0036 | 0.0034 | 7.65 | 7.68 | 0.312 | 0.309 | 258 | 255 | 13 | 10 | 9 | 7 |
| Case 1 | 400 | 336.7 | 7.76 | 7.72 | 0.329 | 0.313 | 282 | 276 | 150 | 110 | 500 | 360 |
| Case 2 | 100 | 79.6 | 7.67 | 7.71 | 0.301 | 0.315 | 272 | 269 | 78 | 66 | 200 | 149 |
| Case 3 | 3 | 2.27 | 7.71 | 7.77 | 0.322 | 0.318 | 263 | 261 | 30 | 24 | 70 | 56 |

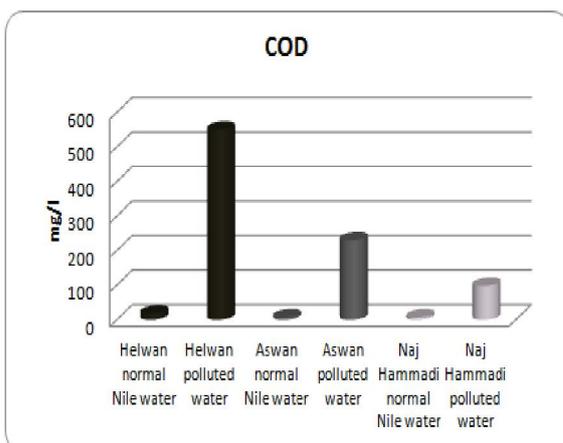


Fig. 2. COD high values due to oil spills

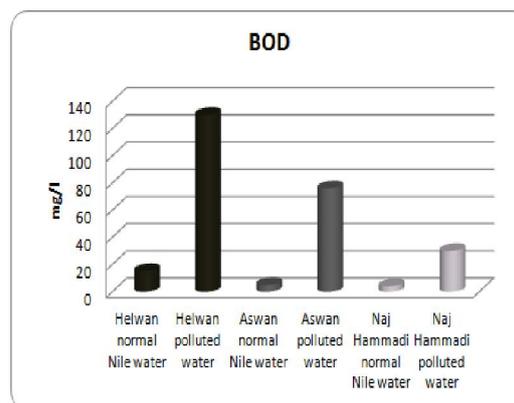


Fig. 3. BOD high values due to oil spills

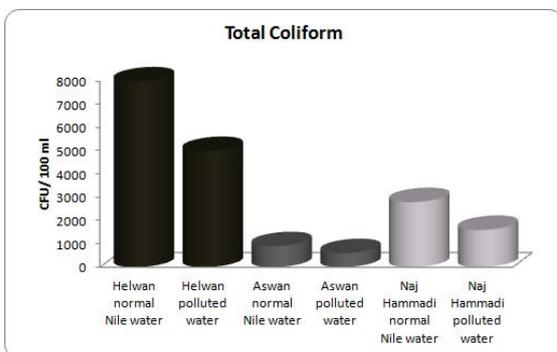


Fig. 4. Effect of oil spills on total coliform count

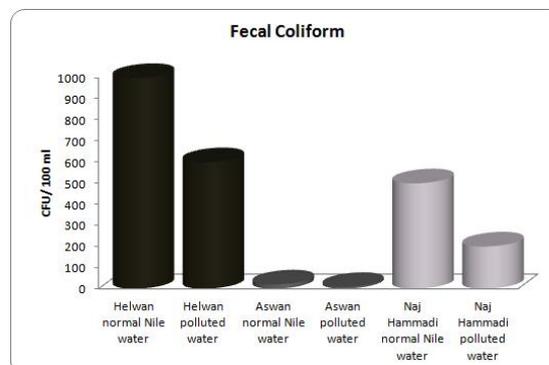


Fig. 5. Effect of oil spills on fecal coliform count

The effect of oil spills on total coliform and fecal coliform counts were illustrated in figures (6 & 7). It was also noted that their counts have been significantly decreased and reached zeros at the end of the experiment. Actually the control in both total and fecal showed gradual decreasing in counts of both of them with time, but in case 1, where the oil and grease value was 400 mg/l, the total coliform and fecal coliform counts were sharply come down in the next day (99% of counts lost) and completely disappeared in day 7. The effect of oil spills on total and fecal coliform counts in case 2, where the oil and grease value was 100 mg/l, was slightly decreased as the counts of both bacterial groups decreased by 75% and completely disappeared in day 10. In case 3, where the oil and grease value was 3 mg/l, the counts of both groups decreased by 60%, and completely disappeared at the end of the experiment. In general, we can conclude that; the more the oil spill volume, the more the negative effect on total coliform and fecal coliform groups of bacteria.

The total bacterial counts were also monitored through the experiment, which surprisingly showed an explosion of numbers. The total count of bacteria in the control gradually decreased with time due to the consumption of nutrients, where the count was 230 CFU/ml at the beginning of the test and became 100 CFU/ml at the end. The total count of viable bacteria was gradually increased during the 14 days of the experiment where it was 230 CFU/ml at the beginning of the experiment and became 15200, 11300 and 7900 CFU/ml at the end, in case 1 (oil and grease was 400 mg/l), case 2 (oil and grease was 100 mg/l) and case 3 (oil and grease was 3 mg/l), respectively (Figure 8). The identification of bacterial isolates in each community indicated the gradually predominance of biodegrading bacteria as the community continue to subjected to the oil spill conditions. The sensitive bacteria gradually disappeared while the survived ones gradually predominated and proliferated, as the counts of viable bacteria shifted up at the end of the experiment

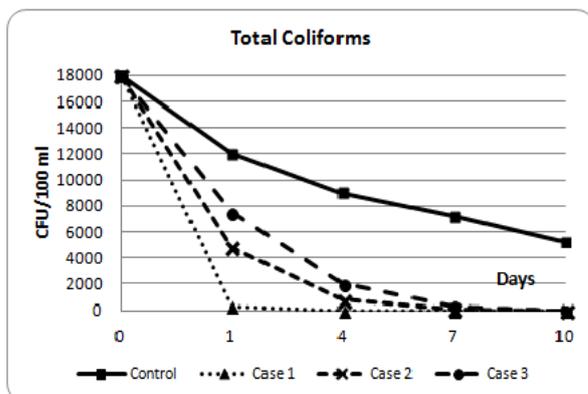


Fig. 6. Decreasing of TC counts with spill volume

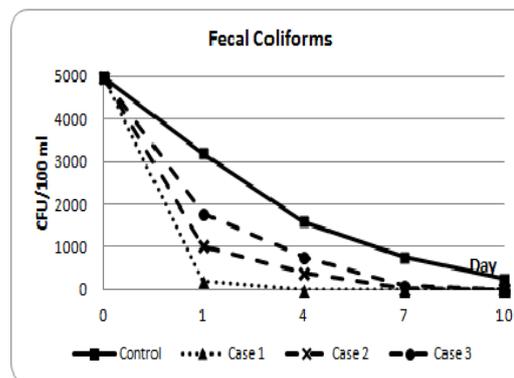


Fig. 7. Decreasing of FC counts with spill volume

As indicated in figure 9, the community of bacteria was including *Arthrobacter*, *Enterobacter*, *E. coli*, *Bacillus*, and *Pseudomonas*. As the oil spill rich 3 mg/l (case 3), the percentage of *E. coli* and *Enterobacter* in the community decreased, while *Arthrobacter*, *Pseudomonas* and *Bacillus* increased. As the oil spill volume reach 400 mg/l (case 1), all other bacterial genera disappeared except *Pseudomonas* which constitute the whole community. As it is well known, *Pseudomonas* is a group of bacteria, which can survive the severe conditions of heavily contamination with oil spill and also they are well known as an aromatic compound bio-degrader (Krieg and Holt, 1986).

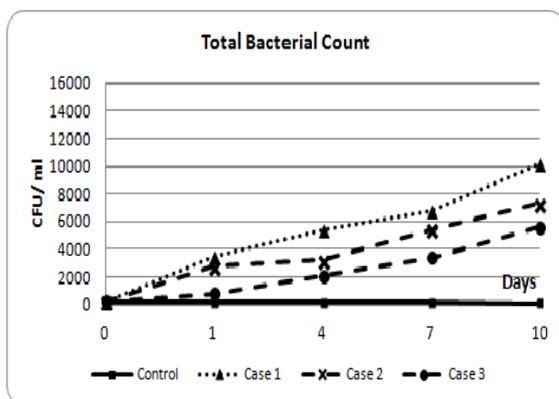


Fig. 8. Increasing TBC with spill volume

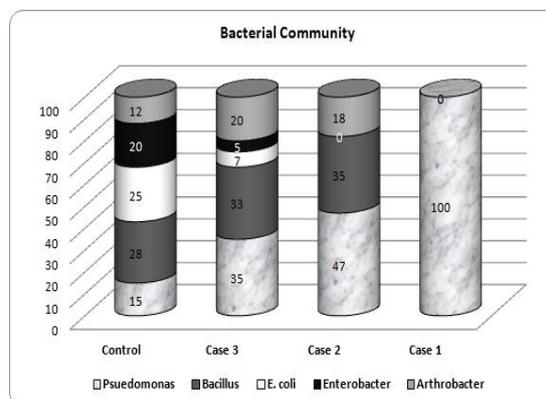


Fig. 9: Bacterial community dominated with *Bacillus* and *Pseudomonas* due to oil spills

Discussion

The analyses of Nile River water in some of accidental oil spills cases which had happened in the last few years; Helwan 2008, Aswan 2010 and Naj Hammadi 2012, indicated that the pH, EC and TDS didn't affected by the oil spills in all cases, while BOD and COD values were obviously increased, due to the hydrocarbons high content of oil. The total coliform and fecal coliform counts were decreased with increasing

oil spill volume, which may be due to the high sensitivity of these groups of bacteria to stress conditions (APHA, 2012). The *in vitro* experiment using the same concentration of oil and grease found in each study case, with conducting the experiment for 14 days indicated the same idea, as mentioned above. On the other hand, the counts of viable bacteria were gradually increased during the experiment while total coliform and fecal coliform decreased. These findings are in accordance with what reported by (Atlas and Hazen, 2011) where they found that the total bacterial count in the Gulf of Mexico before oil spill was $1 - 5 \times 10^3$ CFU/ ml and during the spill it become $1 - 5 \times 10^5$ CFU/ ml.

The bacterial community during the 14 days of the experiment tended toward the known well survived isolates as *Pseudomonas* and *Bacillus*, while other genera as *Arthrobacter*, *E. coli* and *Enterobacter* could not survive the high petroleum carbon content. *Arthrobacter* is growing well in mineral salt media (Sneath *et al.*, 1986) so its percentage in the community start to increase from 12% in the control to 20% in case 1 (oil and grease was 3 mg/ l), but its percentage in the community starts to decrease and finally disappeared as the concentration reached 400 mg/l (case 1). *Pseudomonas* has been known as a bacterium that has a remarkable ability to degrade a wide range of organic pollutants, including polycyclic aromatic hydrocarbons, halogenated derivatives, and recalcitrant organic residues (Johnson *et al.*, 1996). A large number of *Pseudomonas* strains capable of degrading polycyclic aromatic hydrocarbons have been isolated from different water resources (Johnson *et al.*, 1996; Kiyohere *et al.*, 1992). *P. citronellolis* is one such hydrocarbon-degrading bacterial strain, which metabolizes citronellol (Fall *et al.*, 1979). *P. protegens* and *P. fluorescens* strain were isolated from Nile River showed high ability to biodegrade petroleum oil (Jahin *et al.*, 2014). On the other hand, *Bacillus* had been reported by many authors to survive severe conditions and included in degradation of many complex organic matters; Ijah and Ukpi, 1992 had isolated *Bacillus* strain from oil spilled soil showed its capability to biodegrade crude oil. A pure culture of *Bacillus cereus* have been used to biodegrade oily wastewater by 91% through 5 days in Malaysia (Bujang *et al.*, 2013), also *B. cereus* was previously isolated from the Nile River expressed its ability to uptake PHE at 100 mg/l (Jahin *et al.*, 2014).

Conclusion

The impact of oil spills on the quality of Nile River water was evaluated during three oil spills cases have been occurred in the Nile River in the last few years. The study cases were; Helwan case 2008, Aswan case 2010 and Naj Hammadi case 2012. The pH, EC and TDS of water during the spills weren't affected, while oil and grease, COD and BOD values increased. The counts of coliform and fecal coliform bacteria were negatively impacted. The *in vitro* experiment indicated that the total count of viable bacteria increased during the spill, as well as, with the spill volume. The bacterial community before being subjected to the oil spill constituted *Arthrobacter*, *Enterobacter*, *E. coli*, *Bacillus* and *Pseudomonas*, while after 14 days of the experiment *Pseudomonas* and *Bacillus* were predominated, which means the predominance of biodegrading bacteria.

Recommendations

- The transportation of oil and oil products through the Nile River should be prohibited.
- Bioremediation of oil spills using microorganisms might be considered.

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