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Possible Uses of Wastewater Sludge to Remediate Hydrocarbon-Contaminated Soil

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1. Introduction

Mexico is one of the most important producers of petroleum in the world. According to the Economist (2009) it was ranked 6th in the world in 2006. Consequently, in areas surrounding drilling sites and during transport contamination occurs frequently. Although autochthonous microorganisms in any given ecosystem are well capable of degrading petroleum (Grant et al., 2007), different techniques, such as phytoremediation, bioaugmentation or biostimulation, have been applied to accelerate removal of hydrocarbons and reduce the residual concentration (Fernández-Luqueho et al., On line). Cultivation of plants in a petroleum contaminated soil or phytoremediation is known to accelerate removal of hydrocarbons from soil, but not always (Barea et al., 2005; Álvarez-Bernal et al., 2007). Bioaugmentation or the application of microorganisms to soil that are capable of degrading petroleum components should normally accelerate removal of hydrocarbons, but their low mobility and survival in soil often hamper dissipation of the contaminants (Bouchez et al., 1999; Teng et al., 2010). Biostimulation or the application of organic wastes to a contaminated soil is the easiest and most forward way to accelerate removal of hydrocarbons from soil (Scullion, 2006; de Lorenzo, 2008).

Urban wastewater was traditionally discarded in rivers contaminating the environment, although that apart from pathogens, the effect on the ecosystems was not excessive. With the onset of the industrial revolution, these practices become less and less sustainable as chemical contamination altered the river ecosystems. Treatment plants were used to treat the wastewater avoiding contamination of the surface water, but generating large amounts of wastewater sludge. This wastewater sludge was often used in agricultural practices, but its large heavy metal content and organic contaminants often limited its use. In Mexico, urban wastewater is generally low in chemical contaminants and heavy metal content, although exceptions do exist, e.g. wastewater generated in the tanneries of Leon contains large amounts of Cr (Contreras et al., 2004). In Mexico, however, wastewater sludge often contains pathogens that restrict its use in agricultural practices (Franco-Hernández et al., 2003). For instance, wastewater sludge obtained from the treatment plant in Lerma contained 30×10⁹ viable eggs of helminthes. Consequently, the sludge can not be applied to arable land, but it can be applied to soil that is not used for agricultural practices, e.g. remediation of contaminated soil (USEPA 1994, 1999). This study reports on the effect wastewater sludge has on the removal of hydrocarbons from soil. Anthracene, phenanthrene or benzo(a)pyrene, recalcitrant polycyclic aromatic hydrocarbon, (PAHs), that are toxic to humans (Cai et al., 2007) were used as models in this study.

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2. Materials and methods

2.1 Sampling sites, collection and characteristics of the different soils used
The soils used in the experiments reported here were collected from different arable lands or from the former lake Texcoco in the State of Mexico, Mexico, (N.L. 19°42’, W.L. 98°49’; 2349 m above sea level). The climate is sub-humid temperate with a mean annual temperature of 14.8 °C and average annual precipitation of 577 mm mainly from June through August (http://www.inegi.gob.mx). The arable soils are generally low in organic matter and N depleted. The area is mainly cultivated with maize and common bean, receiving a minimum amount of inorganic fertilizer without being irrigated (http://www.inegi.gob.mx). The soil of Texcoco is characterized by a high pH and salinity. Details of the arable Acolman soil used in the experiment can be found in Betancur-Galvis et al. (2006) and of the Texcoco soil in Dendooven et al. (2010). Soil was sampled at random by augering the top 0-15 cm soil-layer of three plots of approximately 0.5 ha. The soil from each plot was pooled and as such a total three soil samples was obtained.

2.2 Wastewater sludge
The wastewater sludge used in the experiments reported here was obtained from Reciclagua (Sistema Ecológico de Regeneración de Aguas Residuales Ind., S.A. de C.V.) in Lerma, State of Mexico (Mexico). Details of the wastewater sludge can be found in Franco-Hernández et al. (2003). Briefly, Reciclagua treats wastewater from different sources. Ninety percent of the sewage biosolids were from different industrial origin mainly from textile industries and the rest from households. The waste from each company must comply with the following guidelines: biological oxygen demand (BOD) less than 1000 mg dm\(^{-3}\), lipids content less than 150 mg dm\(^{-3}\), phenol content less than 1 mg dm\(^{-3}\) and not containing organic contaminants. The wastewater is aerobically digested in a reactor and the biosolids obtained after the addition of a flocculant is passed through a belt filter. Ten kg of aerobically digested industrial biosolids were sampled three times aseptically in plastic bags after passing through the belt filter.

2.3 Aerobic incubation experiment, soil characterization and determination of PAHs
All the reported data were obtained from aerobic incubation experiments. The details of the experimental design and the methods used to characterize the soil can be found in each of the mentioned manuscripts. The amounts of PAHs added to soil varied although they were generally high so as to facilitate the study of the dynamics and the possible effects of the treatments.

2.4 Extraction of PAHs from soil
The amounts of Anthra, Phen and BaP in soil were measured as described by Song et al. (1995). A sample of 1.5 g of soil was weighted into a 15 ml Pyrex tube and 10 ml acetone was added, shaked in vortex and sonicated for 20 min. The PAHs extracted with acetone were separated from the soil by centrifugation at 13700 × g for 15 min, the supernatant was added to 20 ml glass flasks and the acetone used to extract PAHs was left to evaporate. The same procedure was repeated twice more and the extracts were added to a 20 ml flask. The extracts were passed through a 0.45 μm syringe filter, the filtered extracts were concentrated to 1 ml and then analyzed by GC.
3. Results and discussion

3.1 Characteristics of the wastewater sludge and vermicompost

The pH of the sludge sampled at different times ranged from 6.4 to 8.1, while the most important nutrients, such as NH$_4^+$, ranged from 221 to 702 mg N kg$^{-1}$ soil and extractable P from 11 to 600 mg P kg$^{-1}$ dry sludge (Table 1). The high total N content, which ranged from 28 to 42 g kg$^{-1}$ dry sludge, will provide more mineral N upon mineralization of organic N, when the wastewater sludge is added to soil (Castillo et al., 2010).

| Characteristics                  | A    | B     | C     | D     | E     |
|----------------------------------|------|-------|-------|-------|-------|
| pH$_{H_2O}$                      | 7.1  | 6     | 7.5   | 6.4   | 8.1   |
| Conductivity (mS m$^{-1}$)       | 2.6  | NM    | 5.7   | 5.7   | 7.9   |
| Organic carbon (g kg$^{-1}$)     | 499  | NM    | 350   | 509   | 288   |
| Inorganic C (g kg$^{-1}$)        | 3.9  | NM    | NM    | NM    | NM    |
| Total N (g kg$^{-1}$)            | 41   | NM    | 33    | 28    | 42    |
| Total P (mg kg$^{-1}$)           | 5.1  | NM    | 6.8   | 1.7   | NM    |
| NH$_4^+$ (mg kg$^{-1}$)          | 221  | 3071  | 702   | 500   | 13000 |
| NO$_3^-$ (mg kg$^{-1}$)          | 29   | NM    | NM    | 86    | 122   |
| NO$_2^-$ (mg kg$^{-1}$)          | 41   | NM    | NM    | 8     | 8     |
| Extractable PO$_4^{3-}$ (mg kg$^{-1}$) | 11   | 400   | 112   | 600   | NM    |
| Cation exchange capacity (cmol$_c$ kg$^{-1}$) | 1.6  | NM    | 1.4   | NM    | NM    |
| Cl$^-$ (g kg$^{-1}$)             | 1.67 | NM    | NM    | NM    | NM    |
| Ash (kg$^{-1}$)                  | 327  | NM    | NM    | NM    | NM    |
| Na$^+$ (mg kg)                   | ND   | NM    | 4792  | NM    | NM    |
| Water content (g kg$^{-1}$)      | 820  | 660   | 805   | 793   | 847   |

A: Franco-Hernández et al. (2003) B: Betancur-Galvis et al. (2006), C: Contreras-Ramos et al. (2007), D: Fernandez-Luqueno et al. (2008), E: Lopez-Valdez et al. (2010). * mean of four replicates, b NM: Not measured. All values are on a dry matter base.

Table 1. Physicochemical characteristics of the wastewater sludge.

Heavy metal concentrations in the wastewater sludge are generally low (Franco-Hernández et al., 2003) making this wastewater sludge of excellent quality (USEPA, 1994) (Table 2). Additionally, concentrations of toxic organic compounds are also low (Reciclagua, Personal communication).

The wastewater sludge can be classified as a class “B” wastewater sludge (Franco-Hernández et al., 2003) considering its pathogen content (USEPA, 1994) (Table 3). One of the problems of the wastewater sludge was its large number of eggs of Helminthes detected. Generally, the number of pathogens is one of the main limitations in the use of this kind of sludge in agricultural practices. Addition of lime to pH 12, which is a simply and unexpensive treatment, strongly reduced the number of pathogens. However, even with liming, the sludge can be applied to soil that is not used for agricultural practices, e.g. remediation of contaminated soil. Another possible disadvantage is the large EC or salt content, which ranges from 2.6 to 7.9 dS m$^{-1}$. Consequently long-term application of the wastewater sludge to arable land might inhibit plant growth (Mer et al., 2000). The concentrations of Na$^+$ are also high and might inhibit microbial activity and plant growth upon frequent application (Finocchiaro & Kremer, 2010).
Table 2. Concentration of heavy metals in the biosolids and USEPA norms (1994) for excellent and acceptable biosolids.

| Metal | USEPA | A: Franco-Hernandez et al. (2003), B: Contreras-Ramos et al. (2005). |
|-------|-------|------------------------------------------------------------------|
| Pb    | 19 a  | 300  |
| Mn    | 13    | NG   |
| Ni    | 63    | NG   |
| Co    | 63    | NG   |
| Cu    | 29    | 1500 |
| Cr    | 298   | 1200 |
| Zn    | 162   | 2800 |
| Cd    | 8     | 39   |
| Ag    | ND    | 85   |

Table 3. Microorganisms in the wastewater sludge and maximum allowed limits of them (USEPA, 1994).

| Microorganism | USEPA (1994) maximum acceptable limits |
|---------------|----------------------------------------|
|               | Class A | Class B |
| Fungi (CFU g⁻¹ dry biosolids) | 950 b | NM |
| Total coliforms (CFU g⁻¹ dry biosolids) | 66×10³ | 2×10⁶ |
| Faecal coliforms (CFU g⁻¹ dry biosolids) | 1200 | NM d |
| Shigella spp. (CFU g⁻¹ dry biosolids) | ND | < 10⁰⁰ |
| Salmonella spp. (CFU g⁻¹ dry biosolids) | 250 | 2 |
| Viable eggs of Helminthes (eggs kg⁻¹ dry biosolids) | 30×10³ | < 10×10³ |

3.2 Dynamics of polycyclic aromatic hydrocarbons in soil

In all of the experiments done, abiotic factors had only a small effect on the concentrations of phenathrene, anthracene or benzo(a)pyrene in soil (Table 4). On average, 81% of the Anthra added to soil was extracted from soil immediately. For BaP the mean amount extracted from soil immediately was 78% and for Phen 73%. Similar results were reported by Song et al. (2002). They found recoveries of 93% for Anthra, 74% for Phen, and 71% for BaP from soil with 98% sand. The amount of Anthr that was not extractable from sterilized soil between day 0 and the end of the experiment, i.e. varying between 70 and 112 days, was on the average 5%, while it was 4% for BaP and Phen. Consequently, the sequestration of the studied PAHs was low in the agricultural soil. Some authors reported an increased sequestration and a decreasing
### Table 4: Percentage of anthracene, phenanthrene and benzo(a)pyrene removed from the soil due to abiotic processes, i.e. the amount that was not extractable (Ext) and sequestered (Seq), and the amount removed biologically (Bio) from the Acolman and Texcoco soil.

| References                          | Anthracene | Benzo(a)pyrene | Phenanthrene |
|-------------------------------------|------------|----------------|--------------|
|                                     | Ext a      | Seq b          | Bio c        | Ext | Seq | Biol |
|                                     | Acolman soil                                                                 |
| Contreras-Ramos et al. (2006)       | 14         | 11             | 18           | 7   | 8   | 11   | 27  | 11  | 50  |
| Betancur-Galvis et al. (2006)       | 28         | 0              | 39           | 35  | 5   | 31   | 17  | 6   | 38  |
| Alvarez-Bernal et al. (2006)        | 31         | 5              | 63           | 31  | 5   | 46   | 33  | 0   | 66  |
| Rivera-Espinoza and Dendooven (2007)| ND         | 4              | 25           | 39  | 0   | 58   | ND  | 1   | 36  |
| Contreras-Ramos et al. (2006)       | 0          | 6              | 35           | 0   | 2   | 14   | 25  | 2   | 70  |
| Fernandez-Luqueño et al. (2008)    | 24         | ND             | ND           | ND  | ND  | ND   | 32  | ND  | ND  |
| Mean                                | 19         | 5              | 36           | 22  | 4   | 32   | 27  | 4   | 52  |
|                                     | Texcoco soil                                                                 |
| Betancur et al. (2006)              | 18         | 8              | 12           | 26  | 10  | 4    | 5   | 16  | 18  |

*a* Ext: Difference between the amount of PAHs added to soil and extracted immediately after expressed as a percentage of the total amount added, *b* Seq: Difference between the amount of PAHs added to the sterilized soil and extracted at the end of the incubation expressed as a percentage of the total amount added, *c* Biol: Difference between the amount of PAHs added to the unsterilized soil and extracted at the end of the incubation expressed as a percentage of the total amount added.

3.2 The effect of wastewater sludge on removal of anthracene, BaP and phenanthrene from soil

Application of sewage sludge accelerated and reduced the final concentrations of PAHs in soil. In the agricultural soil 39% of the Anthra and 38% of the Phen was removed after 112 days, but 54% and 73%, respectively, when wastewater sludge was added (Table 4). The effect of wastewater sludge on the removal of BaP in the agricultural soil was smaller. Thirty one % of BaP was removed from soil and 35% when wastewater sludge was added after 112 days. The extractability of PAHs, with aging of contaminated soil (Nam and Alexander, 2001). Northcott and Jones (1999) found that extraction of BaP decreased 17% after 525 days aging. Most of the PAHs that was not extractable from soil was biologically removed. Approximately 36% of the Anthra added was biologically removed, 32% of BaP and 52% of Phen. It is well known that soil microorganisms can remove hydrocarbons from soil and numerous bacteria and fungi have been reported that can degrade PAHs (Fernández-Luqueño et al., On line).
application of wastewater sludge had an even larger effect on the removal of PAHs from the Texcoco soil. The biological removal of Anthra increased approximately 3.5 times, BaP 6 times and Phen 3 times in the Texcoco soil when added with wastewater sludge.

Different factors in the sludge might have contributed to the accelerated removal of PAHs from an agricultural soil. First, sludge is rich in N and P, which are important nutrients to sustain microbial activity. The agricultural soil of Acolman is N depleted, which can inhibit microbial activity and thus removal of PAHs from soil (Betancur-Galvis et al., 2006). In the Acolman soil, application of an equal amount of inorganic N and P as was applied with the sludge resulted in a similar removal of PAHs from soil (Table 4). As such, the N and P in sewage sludge stimulated removal of PAHs from soil. However, in the alkaline saline soil of Texcoco, the removal of PAHs from soil amended with sludge was higher than when applied with inorganic N and P. The removal of Anthra was 31% when inorganic N+P was added and 43% when sludge was added. The effect of the sludge was less outspoken with BaP, but larger for Phen as 32% was abiotic removed when inorganic N+P was added, but 52% when sludge was added. The pH in the alkaline saline Texcoco soil is high so it can be argued that changes in pH due to the application of the sludge accounted for the higher removal of the PAHs from the soil. However, adjusting the pH in the soil amended with sludge to the same pH as in the unamended soil did not affect removal of PAHs from soil (Fernández-Luqueño et al., 2008).

Another factor that might have contributed to the accelerated removal of the PAHs when sludge was added to soil were the microorganisms in the sludge. Survival of microorganisms added to soil is normally low as competition for resources, i.e. C substrate, is strong and autochthonous microorganisms are better adapted to soil conditions. However, in the Texcoco soil microorganisms added with the sludge might contribute to the removal of PAHs from soil. For instance, in soil amended with 1200 mg Phen kg$^{-1}$, 109 mg was extracted when sludge was added, 218 mg in the unamended soil and 316 mg in soil amended with sterilized sludge (LSD=195 mg). The micronutrients in the wastewater sludge might also have stimulated microbial activity and thus removal of PAHs from soil.

Application of wastewater sludge often accelerates removal of PAHs from soil, but not always, even when using the same soil. Rivera-Espinoza et al. (2006) added wastewater sludge to soil contaminated with anthracene, benzo(a)pyrene and phenanthrene and found no significant effect on their removal.

4. Conclusion

It was found that application of wastewater sludge stimulated removal of PAHs from soil, but not always. The nutrients in the sewage sludge are important for this increased removal, although the microorganisms in the sludge might contribute to the increased dissipation especially in an alkaline saline soil. Additionally, the organic material in the sludge will improve the soil structure and aeration, thereby further improving the removal of contaminants from soil.

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