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Pigments and Moisture Contents in *Phragmites australis* (Cav.) Trin. Ex Steudel, Would Be Engines for Monitoring Biodegradation of Petroleum Contaminants in Constructed Wetlands

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Abstract: Phytoremediation is becoming increasingly important as a quasi-natural method for the remediation of contaminated soils, using biocenoses of plants and micro-organisms for wastewater treatment. Phytoremediation of petroleum hydrocarbons is an attractive technology mainly because of plant ability to transform organic pollutants to CO_2 and H_2O removing environmental toxicity. Constructed wetlands (CWs) have been used to treat groundwater petroleum contaminants. Plants have shown the capacity to absorb, uptake and convert organic contaminants to less toxic metabolites. In a pilot-scale subsurface flow constructed wetlands, planted with the reed *Phragmites australis***,** in a contaminated site with petroleum pollutants. Gravel beds of the CWs were supplied with activated carbon and electron acceptor, separately, to enhance the biodegradation process. Chlorophyll pigments are measured as an indication of plants productivity and biomass. The active role of *Phragmites* and its roots associated microorganisms were overviewed, taking the advantages of the estimated pigments and moisture contents analyses of leaves and stems. The aboveground vegetations would reflect that the contaminants could be efficiently degraded, if provided with suitable concentrations and suitable energy sources, without damaging the plants. Potential of plant-microbial interactions for *in-situ* bioremediation of hydrocarbon-contaminated soils, maintaining healthy plants would encourage the reuse of the plants for further remediation of other contaminants.

Key words: Phytoremediation, constructed wetlands, microorganisms, chlorophylls, Phragmites.

INTRODUCTION

Phytoremediation, the use of plants and their associated microorganisms for the *in situ* treatment of contaminated soils, is a steadily emerging technology with potential for the effective and inexpensive cleanup of a broad range of organic and inorganic wastes.

Phytoremediation takes advantage of the natural processes of plants, i.e. water and chemical uptake, metabolism within the plant, exudates release into the soil that leads to contaminant loss (WSRC, 2004). In addition, plants with aerenchyma such as reed (*Phragmites australis*) can release oxygen into the rhizosphere and are used for rhizoremediation (Muratova *et al*., 2003).

Petroleum wastes are documented to naturally degrade in natural wetland environments (Wemple & Hendricks, 2000). The microbial community associated with the plant rhizosphere creates an environment conducive to degradation of many volatile organic compounds (Schnoor *et al*., 1995; Pardue *et al*., 2000). Subsurface flow constructed wetlands (CWs) have been used to treat petroleum wastewaters (Knight *et al*., 1999). Field trials have shown successful removal of petroleum hydrocarbon contaminants by *in situ* remediation (Cunnigham *et al*. 2001). Aromatic hydrocarbons, BTEX (benzene, toluene, ethyl benzene, trimethylbenzene) and MTBE (methyl tert-butyl ether) are groundwater contaminants at a former refinery site in Leuna (Germany). Pilot scale subsurface flow constructed wetlands, planted with *Phragmites australis* (Cav.), were setup, for remediation researches in these regionally contaminated aquifers. Gravel beds CWs were provided with activated carbon and iron (Fe III), in separate beds, for treatment enhancement in the phytoremediation process. In a first attempt, estimation of the aboveground vegetation pigments and moisture contents, in the current CWs, were evaluated. The aim of the present investigation is to elucidate the possible contaminants flux, to the pigments and moisture contents of the green mass of *Phragmites australis* in the different treatment beds of the CWs. Monitoring pigments of the macrophytes could be of importance, as they may display sensitivity towards the contaminants. Chlorophylls could be engines in the phytoremediation of other contaminants.

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The aboveground pigments and moisture might reflect the direct or indirect role of *Phragmites* in the degradation process. Plants interaction and cooperation with roots microorganism, its tolerance to the petroleum contaminants are overviewed.

MATERIAL AND METHODS

Pilot-scale gravel beds subsurface flow constructed wetlands (SSFCWs) were setup, for phytoremediation of groundwater contaminated with the petroleum hydrocarbons BTEX and MTBE. Their concentrations in the inflow point accounted for 5mg/l and 20mg/l, respectively. The distances 0.9, 2.5 and 4.1 m, from the inflow point of contaminants, were chosen for sampling groundwater as well as aboveground parts.

Two gravel beds CWs were used as control i.e. without treatment agents and two treatment beds were supplied with activated carbon (carbon-based adsorption materials). Another two beds were treated with Iron (III), a typical electron acceptor utilized by microorganisms, for treatment enhancement and provides a potential alternative to oxygen addition for the bioremediation of petroleum-contaminated aquifers. All beds of the CWs were planted with the reed grass *Phragmites australis* which is one of the plants with a demonstrated potential to tolerate petroleum hydrocarbons, i.e. the ability of a plant to grow in hydrocarbon contaminated soil.

Samples of leaves and stems were taken in August and September 2007 for pigments and moisture contents. Leaves and shoots of three plants, at every given distance, were cut and kept in polyethylene bags and transferred quickly to the lab for pigments analyses following Lichtenthaler and Wellburn (1985). Samples were grinded and dried with liquid nitrogen in mortar with pestle. Five ml of extracting agent (250 ml of acetone with 1.25 ml of concentrate ammonia solution) was added into grinded samples and incubated in 20 min^{-1} shaker for 15 minutes. Centrifugation of the solution was done at cooling condition (4ºC, 10,000 rpm, and 10 minutes). Supernatants were then measured for light absorption with spectrophotometer at wavelengths of 470, 647 and 664 nanometers. A series of equations for calculating pigment composition in samples (chlorophyll a, chlorophyll b and carotenoids) as following:

Chlorophyll a (mg/g) = $12.25\ A_{664} - 2.79\ A_{647}$
Chlorophyll b (mg/g) = $21.50\ A_{647} - 5.10\ A_{664}$
Total carotenoids (mg/g) = $1000\ A_{470} - 1.82$ *chlorophyll a* - 85.02 *chlorophyll b*/198
Where: A_{470}, A_{647}, and A_{664} are the absorbance of the supernatant, at 470, 647 and 664 nm, respectively.

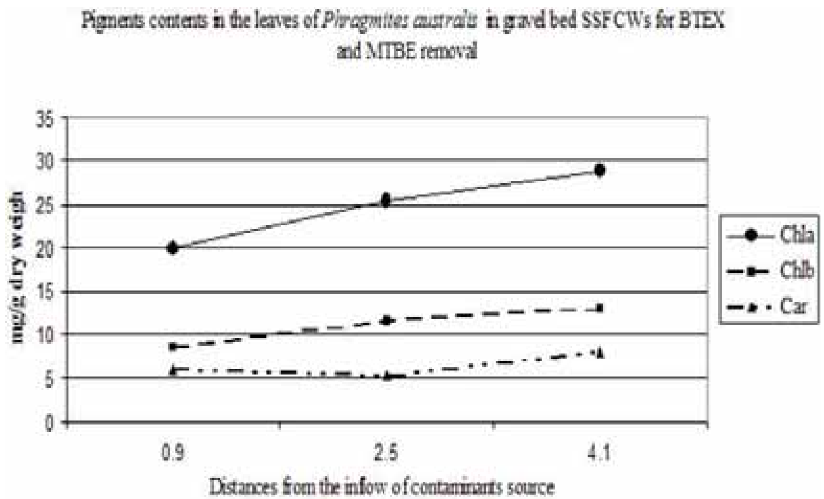
RESULTS AND DISCUSSION

The values of chlorophyll *a* and *b* in the leaves of *Phragmites australis* showed increasing trends in the gravel bed CWs (control) as well as in the carbon treated beds, as shown in figure (1), in August samples, along distances from the inflow point of contaminants source towards the outflow side. Such increases indicate that, leaves performed efficient photosynthetic activity, within healthy chloroplasts, during the degradation of BTEX and MTBE. Although data on the concentrations of the degraded contaminants in the outflow point are not available to support the present research, but literatures admit their efficient biodegradation in the rhizospheres of natural as well as constructed wetlands.

In general, BTEX compounds are relatively easy to remediate (Committee on *In situ* Bioremediation *et al.*, 1993) and easy degradable in natural wetlands (Wemple and Hendricks, 2000). Geyer *et al.* (2005) has suggested that different microbial communities were involved in the biodegradation of benzene and toluene of an aquifer heavily contaminated with pollutant. This is supported by Al Feider and Vogt (2007), who indicated the presence of microorganisms with genetic potential degrading benzene and toluene in groundwater systems.

Concerning the CWs beds, in the present pilot-scale site, activated carbon would improve removal capacity of BTEX as mentioned by Daifullah and Girgis (2003), supporting probably, active carbon transport for efficient photosynthesis in addition to the atmospheric carbon contribution. However, the fate of accumulated aromatic hydrocarbons is not clear as mentioned by Patalakh and Kozar (2001). They realized that, BTEX degradation occurred through spontaneous metabolic transformation into some aromatic amino acids, which are not harmful for plants. It is uncertain, thus, that BTEX compounds are taken up by plants and accumulate to levels in the foliage that can create an ecotoxicological hazard if plants can increase volatility (Ferro *et al*., 1997). Consequently, the level of degradation of BTEX in leaves could not be predicted, as it depends on the way of pollutant drawing in the metabolism.

On the other hand, although the physicochemical properties of benzene would predict extensive plant uptake, rapid biodegradation could prevent this uptake from occurring (Ferro *et al*., 1997). This could add another reason that the plants leaves functions were going well.



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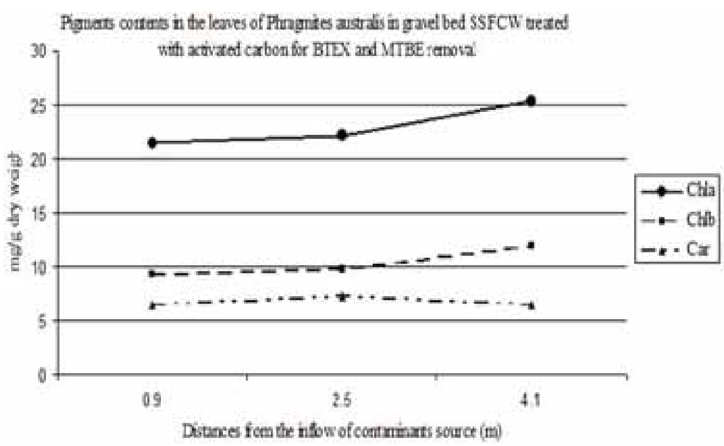

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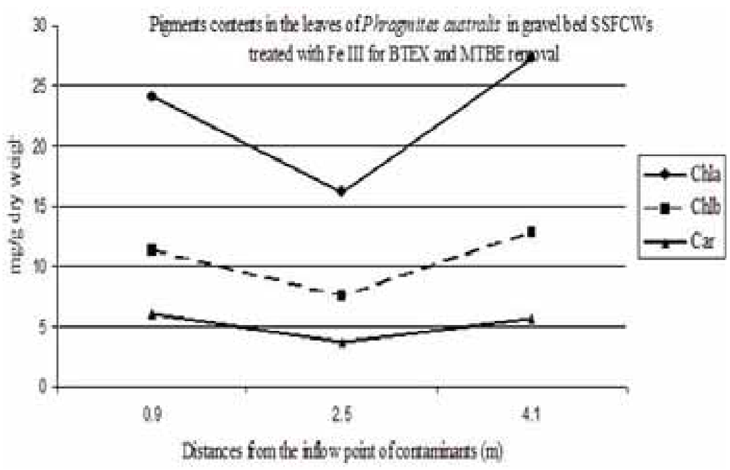

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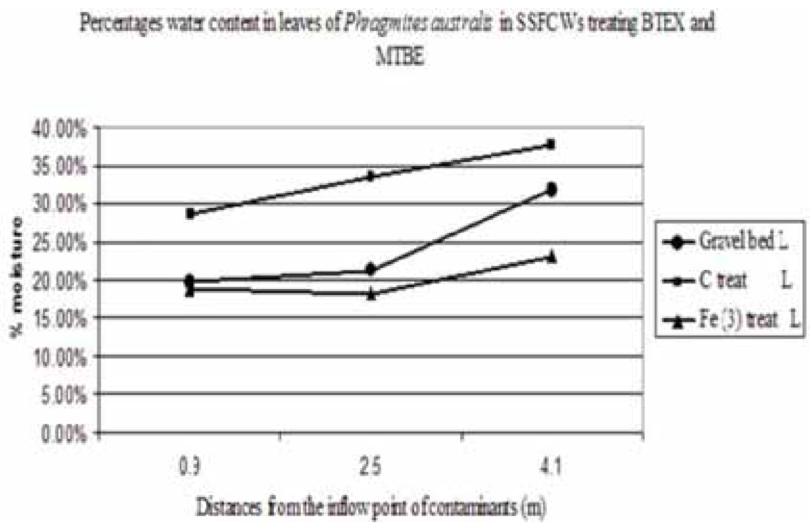

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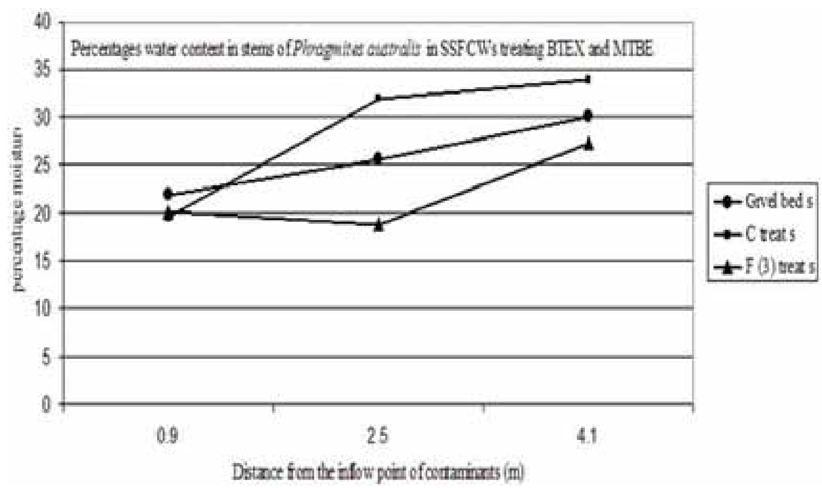


Fig. 1(e):

Car: total carotenoids, C: activated carbon beds, F (3): Fe III, S: stem, L: leaf

Fig. 1: Average values of pigments and moisture contents in leaves and stems of *Phragmites australis* in SSFCWs in gravel beds, treated with activated carbon and Fe III for the removal of BTEX and MTBE of contaminated aquifers at Leuna (Germany) in August 2007.

Natural attenuation could have been another pathway of BTEX major contaminants that caused their decrease, as revealed by field results of Kao *et al*. (2007). Moreover, degradation of BTEX compounds under Fe (III)-reducing conditions has the potential to be an effective natural attenuation process (Martinez, 2006). This also, might prove that contaminants were not translocated up to the aboveground plants parts.

As for MTBE, it is principally biologically degradable as well. Martienssen (2006), in his investigation, demonstrated significant MTBE degradation at the current contaminated site in the pilot-scale constructed wetlands at Leuna (Germany), detected through identification of intermediate by-products. Plants may serve as efficient conduits to withdraw MTBE from the wet subsurface, releasing it to the atmosphere (Davis and Erickson, 2004).

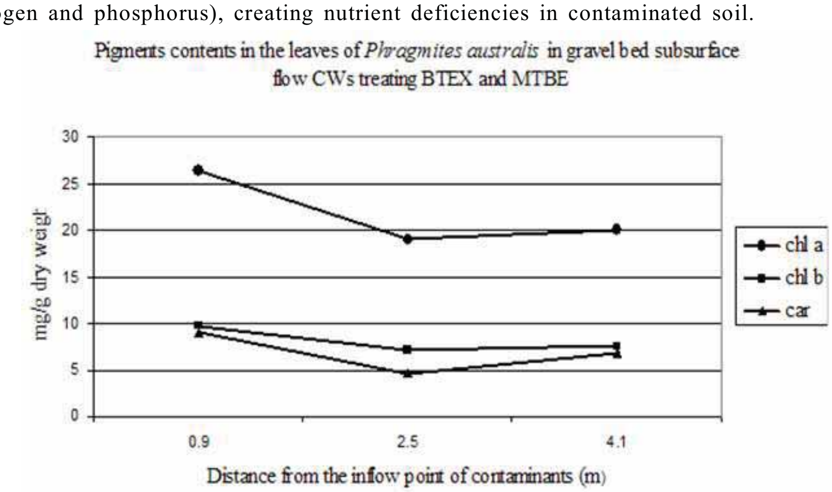
Also, the presence of suitable electron acceptor (Fe III), efficiently ensure sufficient MTBE degradation (Martienssen *et al.* 2003). In addition, MTBE mass loss could be attributed to dilution and dispersion (Fiorenza, 2003). Nevertheless, Lovley *et al.* (1989) mentioned that microorganisms can completely oxidize all of its aromatic substrates to carbon dioxide with Fe III as the sole electron acceptor. But the pigments decreases estimated in the middle of the system (2.5m), accompanied by moisture decreases in stems and leaves of *Phragmites* could be, probably, due to the depression of CO2 assimilation of the leaves induced by water stress. Unfavorable conditions for growth also lowered electron transport system (ETS) activity in *Phragmites australi*s (Cav.) Trin. ex Steud. (Urbanc-Berčič and Gaberščik, 2001).

According to Log Kow, BTEX (1.8 to 3.2) / MTBE (1.0 to 1.3) are sufficiently soluble to enter with the transpiration water. Therefore, Patalakh and Kozar (2001) mentioned that, evapotranspiration enhances BTEX transport to the root zone, where robust populations of BTEX-degrading microorganisms and enhancing also, oxygen transport, thereby reducing its levels in the groundwater.

Generally, more hydrophobic compounds (like petroleum hydrocarbons) are more strongly bound to root surfaces or partition into root solids, resulting in less translocation within the plant (Briggs *et al*., 1982; Schnoor *et al*., 1995; Cunningham *et al*., 1997).

The increasing moisture contents in leaves and stems in August samples at 4.1 m, might be attributed to that, during any degradation processes pathways, microbes living in the rhizosphere, can promote plant health by stimulating root growth (regulators), enhancing water and mineral uptake (Pilon-Smits, 2005; Kuiper *et al*., 2004). On the other hand, *Phragmites* has, also, an important role preventing the spread of petroleum hydrocarbons in soil and groundwater, by taking these contaminants (to a small degree), adsorbing them onto their roots, or keeping them near root zone by water uptake.

Leaves chlorophylls *a* and *b* samples of September, showed decreasing trends in the gravel (control) as well as Fe III treated beds (Fig. 2). This could be probably due to insufficient nutrients supply, as Xu and Johnson (1997) have shown that petroleum hydrocarbons can significantly reduce the availability of plant nutrients in soil. When soil microorganisms degrade the hydrocarbons, they use up or immobilize available nutrients (nitrogen and phosphorus), creating nutrient deficiencies in contaminated soil.



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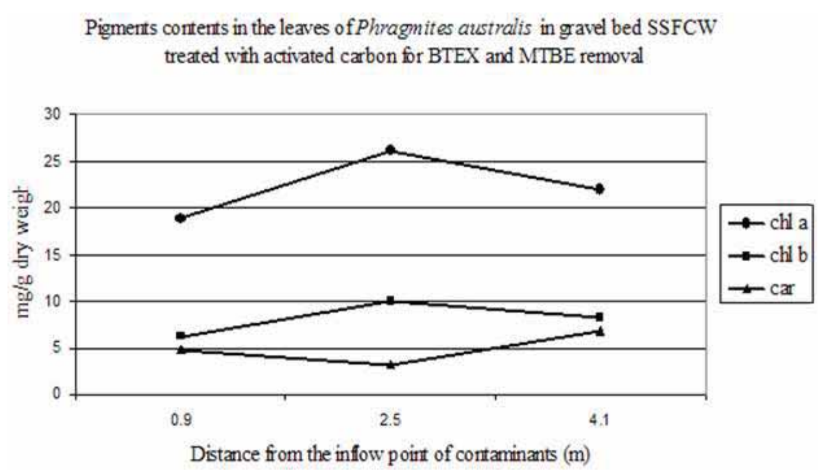

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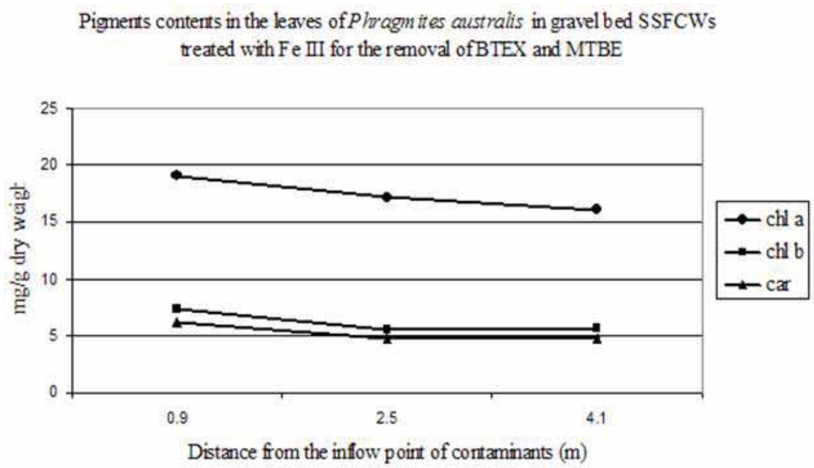

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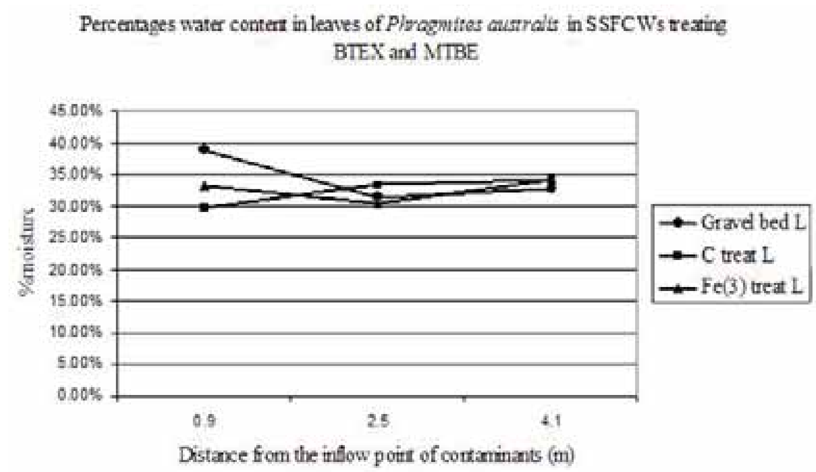

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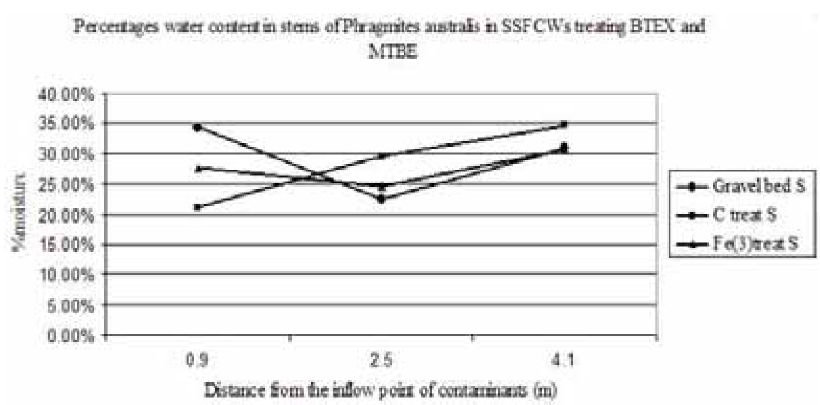

Fig 2(e):

Fig.2: Average values of pigments and moisture contents in leaves and stems of *Phragmites australis* in SSF CWs in gravel beds, treated with activated carbon and Fe III for the removal of BTEX and MTBE of con at Leuna (Germany) in September 2007.

As the main micro-nutrients, nitrogen and phosphorus are used directly for building the microbial cell structure which can limit the biological degradation. However, plants might respond to chemical stress in the soil by changing the composition of root exudates controlling, in turn, the metabolic activities of rhizosphere microorganisms (Chaudhry *et al*., 2005). Some organic compounds in root exudates may serve as carbon and nitrogen sources for the growth and long-term survival of microorganisms that are capable of degrading organic pollutants (Pilon-Smits, 2005; Salt *et al*., 1998; Anderson *et al*., 1993). However, the plants nutrition supply could be maintained through the death of microorganisms, recycling, thus, nutrients for plants consumption.

Plants tolerance might explain the slight increases in plants pigments (chls *a* and *b*) in the carbon treatment beds at 2.5 m distance from the inflow source.

The decreases in chlorophyll contents in September samples probably, also, due to that winter weather prevailed. The short sunshine periods along the daytime could have an effect on the photosynthetic process then, and on chlorophyll contents.

Water contents of the leaves and stems of *Phragmites* in September samples were increasing with increasing distances from the inflow of the contaminants, only in Fe III treated gravel beds. While in the control and activated carbon beds, moisture contents in the leaves and stems of *Phragmites* were lower at distances far from the inflow contaminant source. Reducing conditions in the rhizosphere of the plant might result in various stress symptoms, like internal water stress as indicated by Kramer (1940).

Total carotenoids values didn't exhibit pronounced changes compared with the estimated chlorophylls.

Conclusion:

Interactions between plant systems and contaminants that can facilitate phytoremediation are complex and complicated and need more investigations. Pigments and moisture contents of *Phragmites* showed that the plant leaves contributed, probably to the bioremediation process through transpiration. The exposure concentrations of the petroleum contaminants at Leuna site probably were optimum that did not, severely, affect the pigments and water contents of the reed in the CWs. Chlorophylls and water contents could be an indicator of the phytoremediation of petroleum hazardous contamination, but to a low extent. Therefore, long term monitoring pigments of the macrophytes is important, as they may display sensitivity towards the contaminants. Chlorophylls as well as moistures of leaves and stems of the wetland plants could be, probably, engines in the phytoremediation of other contaminants. Application to variety of contaminants, accompanied by more monitoring issues in plants, are recommended.

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