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**POTENTIAL OF VETIVER (*VETIVERIA ZIZANIOIDES* (L.) NASH)  
FOR THE USE IN PHYTOREMEDIATION OF  
PETROLEUM HYDROCARBON-CONTAMINATED SOILS IN VENEZUELA**

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# 1 INTRODUCTION

The burden of man-made environmental pollution has increased tremendously over the last century. However, efforts in rectifying contamination are still insufficient (SCHRÖDER *et al.*, 2002). Like many countries, Venezuela faces enormous environmental problems induced by industrial activities. Since the 1920s, the petroleum industry has been the mainstay of the Venezuelan economy. The South American country is now one of the world's top oil producers (HARDY, 1997). However, the long history of oil production has left its mark on the environment. Oil pollution is a major problem affecting the country, especially in areas with oil production and pipelines (WAGNER, 1998). Disposal of oil base wastes and oil spills from well blow-outs and pipeline ruptures are the most common sources of contamination induced by petroleum (REIS, 1996). Environmental awareness has been gradually growing in Venezuela since the 1970s, but only since the enactment of strict environmental laws in the 1990s has the oil industry actually been forced to integrate environmental considerations within its activities. However, a gap still exists between legislative provisions and enforcement (WAGNER, 1998). The research center PDVSA-Intevep (Instituto de Tecnología Venezolano de Petróleo) has the important role of supporting the Venezuelan oil company (PDVSA) in trying to meet the legislative environmental demands (INFANTE *et al.*, 1999b).

Concerning the rehabilitation of oil-contaminated soils and waters, engineering techniques based on physical, chemical and thermal processes are traditionally used (FRICK *et al.*, 1999). However, especially on sites with diffuse, low pollution or in large volumes of water with low contaminant concentrations, help may also be found in promising biological-based techniques such as phytoremediation. This method uses plants to detoxify, restore and purify environmental medium. It is applicable to a broad range of heavy metals and organic pollutants such as petroleum hydrocarbons (PHC) (SCHRÖDER *et al.*, 2002). Particularly in the USA, phytotechniques for the remediation of areas with diffuse pollution have become an important experimental approach over the last 15 years. The commercialization of phytoremediation has recently begun (WATANABE, 1997).

For several years, the Department of Ecology and Environment of PDVSA-Intevep has carried out research regarding biological rehabilitation strategies of oil-contaminated sites such as phytoremediation. The project described in this report was integrated in these investigation activities. The overall goal was to extend the current knowledge of phytoremedial methods for the clean-up of oil-contaminated soil in Venezuela.

In order to develop a functioning phytoremediation system, some design requirements have to be considered, such as the contaminant level, plant selection, irrigation and fertilization (ITRC, 1999).

The idea of this greenhouse study was to test the efficiency of the applied method for the use in phytoremediation of problematic oil pollution. Therefore Boscán, a highly phytotoxic crude oil that is resistant to conventional bioremediation techniques (LEÓN *et al.*, 1998), was chosen as the contaminant agent. Vetiver grass (*Vetiveria zizanioides* (L.) Nash) was selected as the experimental plant. It is a well-known and successfully applied plant species in diverse soil and water conservation practices and was believed to promise a high potential of suitability for phytoremediation. Agronomic applications such as irrigation and fertilization were used to improve the conditions for the plants and their associated microbials in the phytoremediation systems.

The specific objectives of this study were to determine the tolerance of the grass species vetiver to Venezuelan heavy crude oil in soil and to test its potential for stimulating biodegradation processes of PHC. In order to realize these intentions, comparisons of plant growth and soil parameters in different treatments of vegetation and soil were carried out. Moreover, different fertilizer treatments were applied to determine the nutrient concentration with the highest efficiency in supporting plant growth and petroleum degradation. The greenhouse experiment was conducted between May and October 2002 in the area of PDVSA-Intevep in Los Teques, Venezuela. The soil and plant analyses were performed during and after this experimental period. Unfortunately, a general strike in Venezuela started on 2nd December 2002 and lasted 2 months. It heavily affected the oil industry as well as further industrial sectors of the country. Therefore, it was not possible to realize all intended tests. For example, the quality analysis of crude oil in soil (SARA, see 3.4.4) could not be performed.



## 2 OBJECTIVES

The overall goal of the experiment was to extend the current knowledge of phytoremedial methods for the rehabilitation of oil-contaminated soils in Venezuela. In particular, the objectives of the study were to determine the tolerance of the plant species vetiver (*Vetiveria zizanioides* (L.) Nash) to Venezuelan crude oil in soil and to test its potential for stimulating biodegradation processes of PHC.

### **The central questions of the study were:**

- ⇒ Are transplanted vetiver plants capable of surviving in PHC-contaminated soil? How are their growth rates in comparison with plants in uncontaminated soil?
- ⇒ Which fertilizer concentration has the best effect on the development of vetiver plants growing in PHC-contaminated soil ?
- ⇒ What is the maximal decreasing rate of PHC in soil that is attainable when using vetiver as a phytoremedial application during a specific time ?
- ⇒ Does vetiver show a potential for the use in phytoremediation of PHC-contaminated soils in Venezuela ?

### 3 THEORETICAL PRELIMINARY REMARKS

#### 3.1 Petroleum and Environment in Venezuela

As the petroleum industry in Venezuela is the functional context of the study, general background information about it will be provided in this chapter. First, considerable historical events of the country's most important economic sector are presented. While having a critical look at the consequences of the Venezuelan oil exploitation, not only economic benefits but also environmental impacts will be made obvious. Finally, applied rehabilitation technologies of oil-affected soils will be explained as first steps of a growing environmental consciousness in Venezuela.



Figure 1: Crude oil deposits in Venezuela (Available on-line at: <http://www.wrad.org/venezuela.htm>; modified)

The first concessions for natural asphalt and crude petroleum in Venezuela were granted in 1854/1855. Thereafter, the first oil company (“Petrolia del Táchira”) was founded in 1878, being the initial of the Venezuelan petroleum industry (MARTINEZ, 1997). The discovery of the gigantic crude oil field “Mene Grande” at the Maracaibo Lake in 1914 was the key to the global energy markets for Venezuela. At this time, the petroleum resources were mainly exploited by foreign oil companies (PEÑALOZA, 1997). The oil law of 1943 (“Ley de Hidrocarburos”) renewed the petroleum concessions in order to promote the Venezuelan industrialization and thus initiated spectacular socioeconomic transformations in the country. In the 1970s, the idea of nationalizing the petroleum industry became concretely relevant and culminated in the foundation of the national oil company “Pétroleos de Venezuela, S.A.” (PDVSA) in 1976. Since then, modernization and investigation programs have

resulted in an enormous increase of drilling and refinery activities. Nowadays, PDVSA is considered to be one of the biggest and best administrated oil companies in the world (HARDY, 1997).

The Venezuelan crude oil deposits are situated at the Maracaibo Lake in the north-west of the country as well as in the east near the Orinoco river (ESPINOZA *et al.*, 2002) (Figure 1). In the drilling and production processes of crude oil many by-products, such as drill cuttings, drilling fluids and contaminated water, are generated. The most common method of storing these oil base wastes until later treatment is disposing them in reserve pits (Figure 2). Those are high sources for possible oil release (REIS, 1996) Furthermore, accidental oil spills, caused by well blow-outs and pipeline ruptures, are omnipresent risks of pollution by oil (FREEDMAN, 1995) (Figure 3). The areas around drilling, production, transfer and storing sites of crude oil are especially exposed to possible environmental impacts if materials and wastes associated with these activities migrate from releasing points to soil, water and atmosphere (REIS, 1996).

International awareness of environmental impacts started growing in the 1970s. Within this process, Venezuela was one of the first countries in South America setting up a ministry of the environment (MARNR)<sup>1</sup> in 1977. Important legislative steps against nature degradation in Venezuela were the enactment of the environmental law in 1976<sup>2</sup> and of the first penal environmental law in 1992<sup>3</sup> that established strict regulations allowing environmental authorities to undertake drastic actions in contamination cases. A decree concerning the recovery of dangerous material and waste in 1998<sup>4</sup> was significant, too. Consequently, environmental considerations have become an integral part of the petroleum industry. However, a gap exists between legislative provisions and actual enforcement (WAGNER, 1998). Venezuela is still extremely confronted with environmental problems induced by the oil industry, particularly due to technological decisions without environmental considerations in the past (INFANTE *et al.*, 1999b). Sensitive ecosystems and indigenous land overlapping with oil production and pipeline areas are particularly endangered of devastation (WAGNER, 1998).

Intevp<sup>5</sup> is the Research and Technological Support Center of PDVSA. It was founded in 1974 in order to investigate cost-effective and high-quality technologies for the oil business. The investigation of PDVSA-Intevp aims to the application of new technologies in all phases of the Venezuelan oil industry such as exploration, production, manufacturing, transport and marketing (PDVSA, 2003).

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<sup>1</sup> Ministerio del Ambiente y de los Recursos Naturales Renovables

<sup>2</sup> Ley Orgánica del Ambiente. Gaceta Oficial de la República de Venezuela N° 31.004, Caracas, 16.06.1976

<sup>3</sup> Ley Penal del Ambiente. Gaceta Oficial de la República de Venezuela N° 4358, Caracas, 03.01.1992

<sup>4</sup> Normas para el control de la recuperación de materiales peligrosos y el manejo de desechos peligrosos. Gaceta Oficial de la República de Venezuela N° 5.212, Caracas, 12.02.1998

<sup>5</sup> Instituto de Tecnología Venezolano de Petróleo



Figure 2: Reserve pits of drilling wastes in El Tigre, Anzoátegui State, Venezuela (Brandt, 08.09.2001)



Figure 3: Oil spill of a pipeline rupture in El Tigre, Anzoátegui State, Venezuela (Brandt, 08.09.2001)

In 1978, PDVSA-Intevep initiated its environmental activities which continuously grew more important in the 1980s. The constitution of the Department of Ecology and Environment<sup>6</sup> was part of this process. Now, waste disposal and treatment, control of oil spills as well as ecotoxicology are established as investigation assignments of the department (INFANTE *et al.*, 1999b).

Several methods can be used for the rehabilitation of oil-contaminated soils. The “remediation” (*remedium* (Latin): to correct or to remove an evil) techniques are based on the stabilization processes of the contaminant or decontamination of the soil matrix (CUNNINGHAM *et al.*, 1996). Traditionally, engineering techniques based on physical, chemical and thermal processes are used. They can be divided into *ex situ* and *in situ* processes. *Ex situ* methods include excavation and transportation of the polluted soil with final incineration or “landfilling”. *In situ*- rehabilitation methods work on the basis of removing the contaminant from the soil and groundwater without excavating. Examples are “pump-and-treat”, chemical washes and soil vapor extraction (FRICK *et al.*, 1999).

In addition to the expensive engineering techniques, biological-based remediations have become more common since the 1970s (CUNNINGHAM *et al.*, 1996). One treatment worth mentioning is “natural attenuation” which exclusively relies on natural processes in soil and groundwater without human intervention. A related but human influenced method is “bioremediation”. Based on biodegradation processes, toxic petroleum fractions are removed or diminished by microorganisms from the soil medium. An important point is the stimulation of the microbial activity by maintaining optimal conditions within nutrients and other additives, aeration and mixing if necessary. This ecologically safe and cost-effective clean-up technology can be applied both *ex situ* and *in situ* (FRICK *et al.*, 1999).



Figures 4+5: During (on the left side) and after (on the right side) the bioremediation of oil base bottom mud with the Intebios® technology in the Pamatacual reserve pits, Puerto La Cruz Refinery, Anzoátegui State, Venezuela in 1997 (duration: 6 months) (INFANTE *et al.*, 1999a; INFANTE *et al.*, 1999b).

<sup>6</sup> Gerencia de Ecología y Ambiente (TEEA)



Since the early 1990s, research in bioremediation started intensively in the Venezuelan oil industry. Intebios® and Biorize®, both developed at PDVSA-Intevep, are examples for bioremedial technologies for treating any waste generated from perforation, oil production and refinery (INFANTE, 1999) (Figures 4+5).

### 3.2 Phytoremediation

One of the most recent environmental investigation areas of the Department of Ecology and Environment of PDVSA-Intevep is “phytoremediation”. Apart from natural attenuation and bioremediation, it is a further biological-based *in situ*-technology for the rehabilitation of contaminated soil and groundwater. According to CUNNINGHAM *et al.* (1996), phytoremediation (*phyto* (Greek): plant) is defined as the “use of green plants and their associated microbiota, soil amendments and agronomic techniques to remove, contain or render environmental contaminants”. Both inorganic and organic harmful substances can be treated with this method (FRICK *et al.*, 1999).

Naturally occurring processes of degradation, containment and transfer are possible mechanisms in the phytoremediation of PHC, as to be seen in Figure 6.

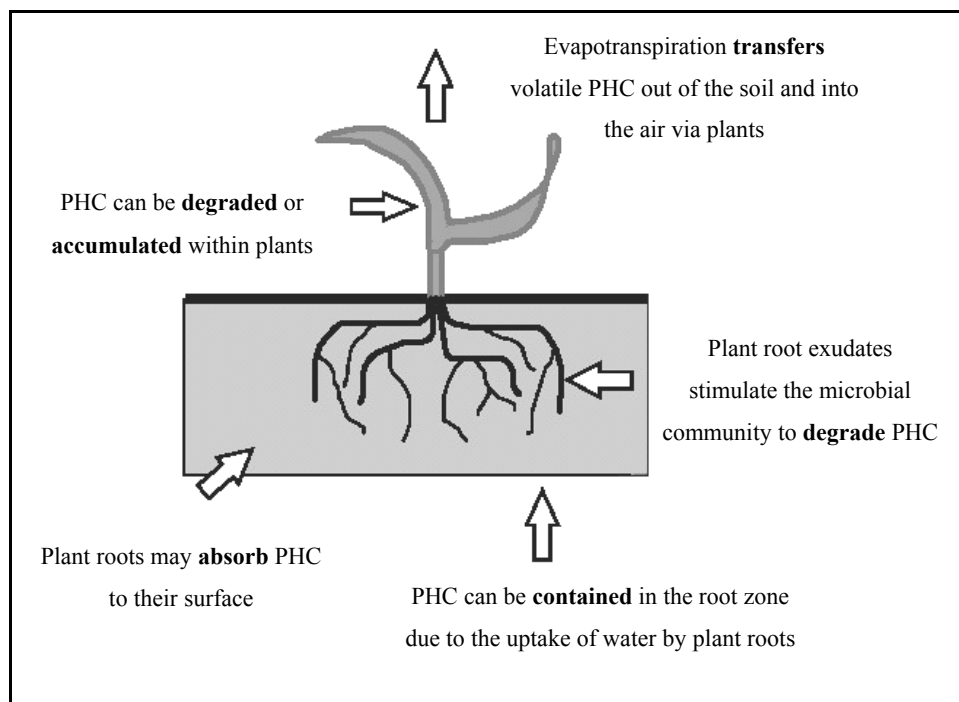


Figure 6: Phytoremediation mechanisms: degradation, containment and transfer of petroleum hydrocarbons (PHC) in soil via interactions with plants and microorganisms (FRICK *et al.*, 1999; modified).

## Degradation

⇒ **Phytodegradation:** By using special enzymes, plants take up, store and biochemically degrade the contaminants to less harmful products. The metabolites are accumulated, turned into new biomass or released and broken down further by microorganisms (ITRC, 1999). Due to the complexity of interactions between plants, microbes and environment, there is always the risk of unforeseen production of toxic metabolites (SICILIANO & GERMIDA, 1998). Only moderately hydrophobic substances (octanol-water partition coefficients,  $\log K_{ow} = 0.5-3.0$ ) are effectively taken up by plants (e.g. BTEX, short-chain aliphatics) (SCHNOOR *et al.*, 1995). In addition, small, low molecular weight compounds are favored (FRICK *et al.*, 1999).

⇒ **Rhizodegradation:** Plants play an indirect role in the rhizodegradation of pollutants. By pumping oxygen to the roots, by decay of organic material and the release of exudates, plants may increase the microbial activity around their roots (SCHNOOR *et al.*, 1995). This plant-induced enhancement of the microbial population is referred to as the “rhizosphere effect” and is believed to result in enhanced biodegradation of toxicants. In specific interactions, plants alter their behavior in the presence of contaminants. In response, they produce specific exudates in order to stimulate microorganisms which degrade the contaminants. Specific interactions, such as those shown in Figure 7, are believed to be survival strategies of plants in contaminated soil.

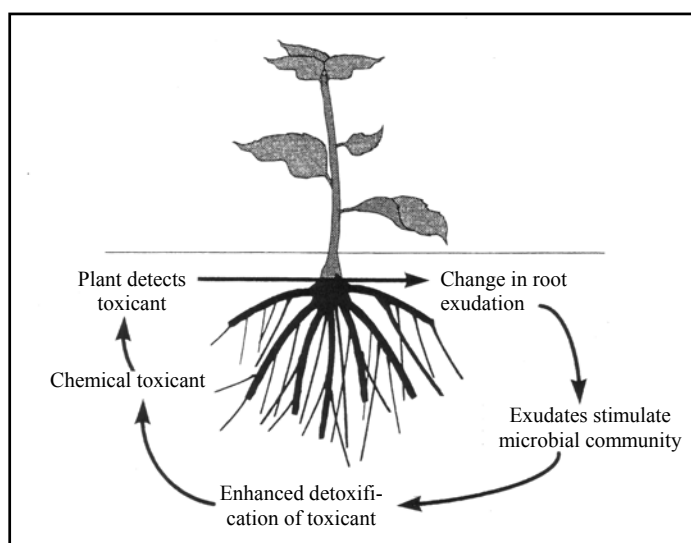


Figure 7: Hypothetical mechanisms in specific interactions (WALTON *et al.*, 1994; modified)

In the case of non-specific interactions, the increase of the microbial community arises from the usual plant metabolites. Generally, environmental factors have a significant effect on the type and quality of root exudates, and the rhizosphere community is closely linked with the root exudate composition (SICILIANO & GERMIDA, 1998). The transformation of contaminants by root enzymes in the rhizosphere is a further degradation process that is induced by plants (SCHNOOR *et al.*, 1995).

**Containment:** By containment, plants may reduce or eliminate the bioavailability of toxic substances (FRICK *et al.*, 1999; ITRC, 1999):

- ⇒ **Phytoextraction:** Accumulation of chemicals in plants is a direct mechanism of containment. As mentioned above, especially moderately hydrophobic substances ( $\log K_{ow} = 0.5-3.0$ ) may be taken up by plants. Besides, the rate of accumulation usually rises with the lipid content of the plant tissue.
- ⇒ **Rhizofiltration:** Adsorption processes on the root surface also belong to the direct containment mechanisms. In particular, hydrophobic chemicals ( $\log K_{ow} > 3.0$ ) are affected.
- ⇒ **Organic pumping:** Due to the transpiration of plants, downward migration of water-soluble chemicals can be reversed. Thus, contaminants may be contained within the root zone and therefore prevented from spreading. The use of plants as “organic pumps” is a further direct containment process.
- ⇒ **Humification:** Incorporation of contaminants into soil organic matter may indirectly be increased by releasing plant enzymes. In addition, decaying plant material may increase the humus content of soil and thus, the potential incorporation of toxicants (CUNNINGHAM *et al.*, 1996).

### **Transfer**

- ⇒ **Phytovolatilization:** After the uptake of contaminants from the soil, plants may transpire volatile contaminants into the atmosphere. After the transfer, the contaminants are often subject to photodegradation (degradation in the atmosphere) (ITRC, 1999). However, subsequent pollution of the atmosphere is a potential risk (FRICK *et al.*, 1999).

Not all plant species can be used for the rehabilitation of contaminated sites. In particular, plants with deep, fibrous roots and fast growth, such as grasses, are useful in phytoremediation. Furthermore, the ability of growing in conditions of stress is an important characteristic (SICILIANO & GERMIDA, 1998). A further advantage is the nitrogen-fixing ability of symbiotic fungi or bacteria as for instance in the case of legumes (SCHNOOR *et al.*, 1995). Using perennials is preferable to using annual plants because a reestablishment on a yearly basis is not required (APRILL & SIMS, 1990). The development of transgenic plant species is one of the most recent advantages in phytoremediation. By genetic engineering, plants can be modified in order to produce specific enzymes for degradation and gain desirable remediation properties. Genetically modified organisms, however, are not always accepted by the public (DIETZ & SCHNOOR, 2001). Besides, the phytoremedial use of genetically modified



species as well as the use of non-native species could create ecological risks. Thus, whenever possible, native species should be applied in phytoremediation. Another advantage of using native plants is the fact that they are pre-adapted to the climatic and soil conditions at the site (FRICK *et al.*, 1999).

A variety of bacteria species are considered to be involved in the degradation of PHC, e.g. *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Mycobacterium* and *Nocardia* (FRICK *et al.*, 1999). Accordingly, WALTON *et al.* (1994) observed that *Pseudomonas* and *Arthrobacter* are present in greater numbers within rhizosphere soil than bulk soil. Soil fungi also play a role in the degradation of PHC, including *Aspergillus*, *Cunninghamella*, *Phanerochaete*, *Saccharomyces*, and *Syncephalastrum* (FRICK *et al.*, 1999).

In comparison to other rehabilitation treatments, phytoremediation could be seen as an intermediate method between natural attenuation and *in situ*-engineering because the processes are based on natural processes, but they are manipulated and enhanced by human intervention. *In situ*-bioremediation is similar to phytoremediation because of its use of microorganisms to degrade contaminants. However, *in situ*-bioremediation does not involve the use of plants. Furthermore, it requires more engineering techniques, e.g. bioventing (pumping air into the soil), than phytoremediation (FRICK *et al.*, 1999).

In many cases, the use of plant-based remediation systems is only the final polishing step after the application of other technologies, because it requires more time to achieve clean-up standards than most of the alternative methods. Besides, it is only effective on sites with shallow contamination within the root zone of plants (SCHNOOR *et al.*, 1995). However, engineering activities often damage highly fragile ecosystems more than the contaminant itself. In particular, *ex situ*-methods are problematic since clean-up operations destroy the soil structure of the sites (LIN & MENDELSSOHN, 1998). In contrast, phytoremediation is considered to be a less environmentally destructive, more aesthetically pleasing and solar powered treatment system. Besides, it is more cost-effective than most of the alternatives (CUNNINGHAM *et al.*, 1996). New remediation developments are hybrid-technologies combining phytoremediation and engineering techniques (CUNNINGHAM & OW, 1996).

However, detailed knowledge of the physiological and molecular mechanisms of phytoremediation have only begun to emerge. Therefore, a more systematic approach concerning the optimization of remediation processes as well as the selection of plants is required to improve this promising technology for the rehabilitation of contaminated soils (SCHRÖDER *et al.*, 2002).

### 3.3 Vetiver

In the phytoremediation studies, vetiver grass (*Vetiveria zizanioides* (L.) Nash) was chosen as experimental plant (Figure 7). It is one of 11 species of the genus *Vetiveria* with the following taxonomic position:

**Family:** Poaceae (Gramineae)  
**Subfamily:** Panicoidae (Andropogonidae)  
**Tribe:** Andropogoneae  
**Subtribe:** Sorghinae

All species of *Vetiveria* were originally distributed in the Old World tropics. *V. zizanioides* is believed to be native to northern India, where it has usually been found growing wildly or traditionally cultivated in low, damp wetlands (NRC, 1993).

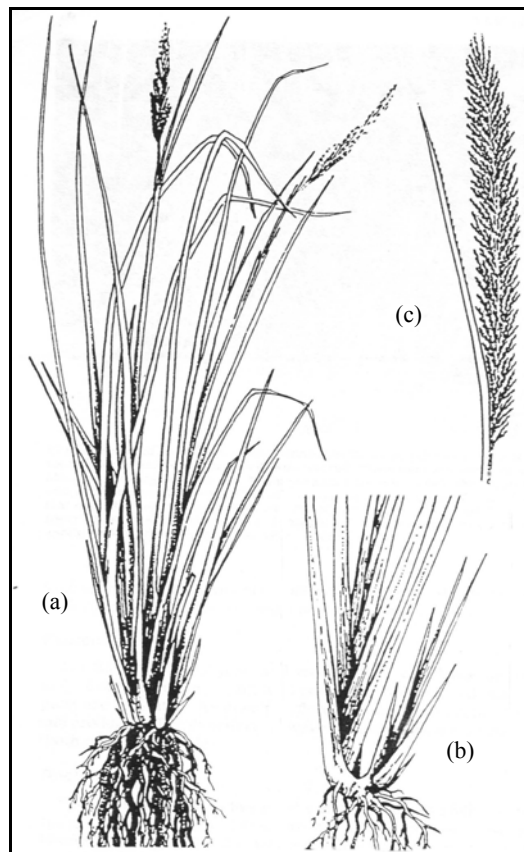


Figure 7: The vetiver plant (*Vetiveria zizanioides* (L.) Nash) is a densely tufted, perennial grass, characterized by a stiff and erect stem with leaf blades up to 75 cm long and 8 cm wide (WORLD BANK, 1993). The figure shows culms (a), root stock (b) and panicle (c)<sup>7</sup> (NRC, 1993)

<sup>7</sup> Only the North Indian type of *V. zizanioides* is flowering, the South Indian type is nonflowering (see 3.3.1).

On the basis of morphological, physiological and genetic characteristics, this chapter will explain why vetiver was selected as a phytoremedial plant for crude oil-contaminated soils. In addition, the current applications of this useful multipurpose species will be presented with special attention to the use in phytoremediation.

### 3.3.1 Characteristics

The distinguishing morphological feature of vetiver is the massive, finely structured root system. Under optimal conditions, the species may grow very fast, reaching up to 4 m of rooting depth in the first year. Showing an extraordinary power of penetration, the vetiver root is able to go through difficult soils, including asphaltic ground. As the plant has neither stolons nor rhizoms, vetiver is easy to control. Usually, the root system grows straight down without competing with neighboring vegetation. Therefore, it is possible for this plant to establish itself in cultivated land (GREENFIELD, 2000). According to NANAKORN *et al.* (2000) (cited in CHOMCHALOW, 2000), the roots densely bind together like an underground curtain or wall, enabling the plant to retain water and moisture and therefore creating a favorable environment to a diversity of microorganisms in the soil. The root characteristics were the main reason for studying the potential of vetiver as a plant applied in phytoremediation.

The extraordinary tolerance to a wide range of environmental stresses was another important reason for the usage of *V. zizanioides* in phytoremediation studies:

- ⇒ Relating to climatic factors, vetiver can not only withstand temperature ranges between -14 and 55 °C, but also prolonged drought, fire, flood and submergence. Once affected by adverse harmful conditions, the plant usually recuperates immediately (GREENFIELD, 2000).
- ⇒ The species shows tolerance to various extreme levels of edaphic factors. By researching the vetiver's growth in adverse soil conditions, TRUONG & BAKER (1997) proved its tolerance to a wide range of soil pH (3.3 to 9.5) and high levels of sodicity (33% exchangeable Na), magnesicity (20 Cmol/kg Mg) and salinity (47.5 mS/cm). Furthermore, they demonstrated that vetiver grass resists very high levels of heavy metals in the soil such as aluminium (68%-87% Al sat.), arsenic (100-250 ppm), cadmium (20 ppm Cd), copper (50-100 ppm Cu), chromium (200-600 ppm Cr), nickel (50-100 ppm Ni) and manganese (Mn > 578 ppm).

Vetiver belongs to the group of plants with the specialized C<sub>4</sub>-photosynthesis. Thus, the species is able to survive and compete under dry conditions with minimum annual rainfall of about 200 mm. For this

reason, however, it is difficult to establish vetiver under shady conditions, as it requires open sunlight (NRC, 1993), an important fact to know when designing a phytoremediation experiment with vetiver.

There are 2 types of *V. zizanioides*: A wild original form from North India that flowers regularly, producing fertile seeds, and a cultivar form from South India. Usually nonflowering, nonseeding and believed to be absolutely sterile, the propagation of the South Indian type occurs vegetatively by division of roots. Thus, its cultivation is easy to control and does not represent an ecological risk. Only the South Indian type can be used in conservation practice, including phytoremediation (NRC, 1993).

### 3.3.2 The Vetiver System

According to CHOMCHALOW (2000) “the Vetiver System (VS), originally known as the Vetiver Grass Technology (VGT), is a low-cost, simple technology, employing live vetiver plants for soil and water conservation and environmental protection”. The VS is a very practical, inexpensive, environmentally friendly and very effective method of soil erosion and sediment control, water conservation, land stabilization and rehabilitation. Nowadays, the VS is applied as a conservation technology all over the subtropical and tropical world (Figure 8).

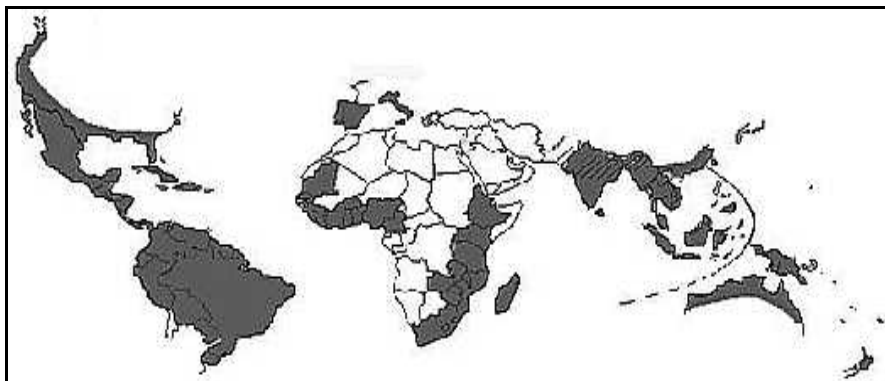


Figure 8: Known distribution areas of active programs of the Vetiver System  
(Available on-line at: [http://www.vetiver.org/TVN\\_worldvetmap.gif](http://www.vetiver.org/TVN_worldvetmap.gif))

For thousands of years, vetiver grass has been used for the benefit of man. In particular the aromatic essential oil content, which can be extracted from the vetiver's roots, has been used as a fragrance, medicine or insect-repellent. Nowadays, the world-wide interest of vetiver's use mostly aims at the application in environmental conservation (GRIMSHAW, 1990). Agricultural practices of soil and water conservation, such as soil erosion control, soil moisture conservation, biological pest control and trapping of agrochemicals or nutrients, are sectors where the VS can be applied. Moreover, it can be used in bioengineering activities where plants are used for slope and embankment stabilization (CHOMCHALOW, 2000). Also in Venezuela, the use of the VS in erosion control has already been successfully tested and promoted in a 2-years project (RODRÍGUEZ, 2000).

Since the mid 1990s, the interest in using the VS in phytoremediation of soils has been growing. Several investigation results demonstrate the successful application, as illustrated by the following examples:

A significant amount of successful work was undertaken in Australia and South Africa using vetiver for the rehabilitation of gold, platinum, coal and other mines (TRUONG, 1999). Investigations in China demonstrated positive effects of *V. zizanioides* in purifying urban garbage leachate of landfills (XIA *et al.*, 1997) and efficiently removing phosphorus and nitrogen from eutrophic water (ZHENG *et al.*, 1997). In Thailand, the effect of vetiver in decontamination of pesticides in order to prevent their accumulation in crops or other parts of the agroecosystem was successfully proven in a preliminary study by PINTHONG *et al.* (1996).

Publications about the successful use of vetiver in phytoremediation of oil-contaminated soils are not yet available, but a growing interest in this aspect is noticeable. According to EGBUCHE (2001), a pilot project in oil-producing areas of the Niger Delta, Nigeria, has been started. In combination with *Hibiscus* and an adapted consortium of oil-metabolizing microorganisms, the VS is used to support the ecosystem restorations from oil spill.

TRUONG (2002)<sup>8</sup> gave further evidence that studying the potential of vetiver in phytoremediation of PHC-contaminated soils can be useful. On the Vetiver Network Discussion Board he reported that on a preliminary trial for phytoremediation on petroleum contamination at a small mine in Queensland, Australia, an establishment of vetiver at fully saturated concentration was possible. However, the plants died later on soil contaminated with diesel, engine oil and hydraulic fluid, only growing well when it was mixed with unpolluted soil. In contrast, drilling fluid had no effect on vetiver growth, he further explained.

### 3.4 Petroleum Hydrocarbons

A basic knowledge of the petroleum chemistry is essential for applying successful phytoremediation to petroleum-contaminated soil. In this chapter, crude oil, the constituents of PHC and their behavior are described. Finally, chemical effects of oil on the environment are summarized and short definitions of important parameters concerning the analysis of crude oil are given.

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<sup>8</sup> Dr. P. Truong: principal soil conservationist with Queensland's Department of Natural Resources (DNR) in Australia, co-ordinator of the regional Pacific Rim Vetiver Network and publisher of several papers of the Vetiver's use in phytoremediation projects.

### 3.4.1 Crude Oil

Petroleum and its derivatives are mixtures of gaseous, liquid and solid hydrocarbons. The components of naturally-occurring reservoirs of crude oil are therefore named petroleum hydrocarbons (PHC). These sedimentary rock deposits are new formations made of organic residues of ancient organisms and developed under heat, pressure and reduced anaerobic conditions over geological time periods. According to the origin's content of different elements, all types of crude petroleum contain small quantities of sulfur, nitrogen, oxygen and traces of metals, particularly vanadium, nickel, iron and copper. Therefore, each crude oil has its individual chemical composition depending upon its origin and location (COLE, 1994; US-EPA, 2000). Petroleum crude oils can be broadly divided into paraffinic, asphaltic and mixed crude oils (TODD *et al.*, 1999) They are the mineral source for a lot of refinery products such as petroleum gas, gasoline, naphthas, kerosene, fuel oils, lubricating oils, coke, waxes and asphalt (US-EPA, 2000).

### 3.4.2 General Petroleum Chemistry

The principal constituents of PHC are the elements hydrogen (10-14%) and carbon (83-87%). Occurring in varied structural configurations, PHC can be broadly divided into 2 families: aliphatics (fatty) and aromatics (fragrant) (Figure 9).

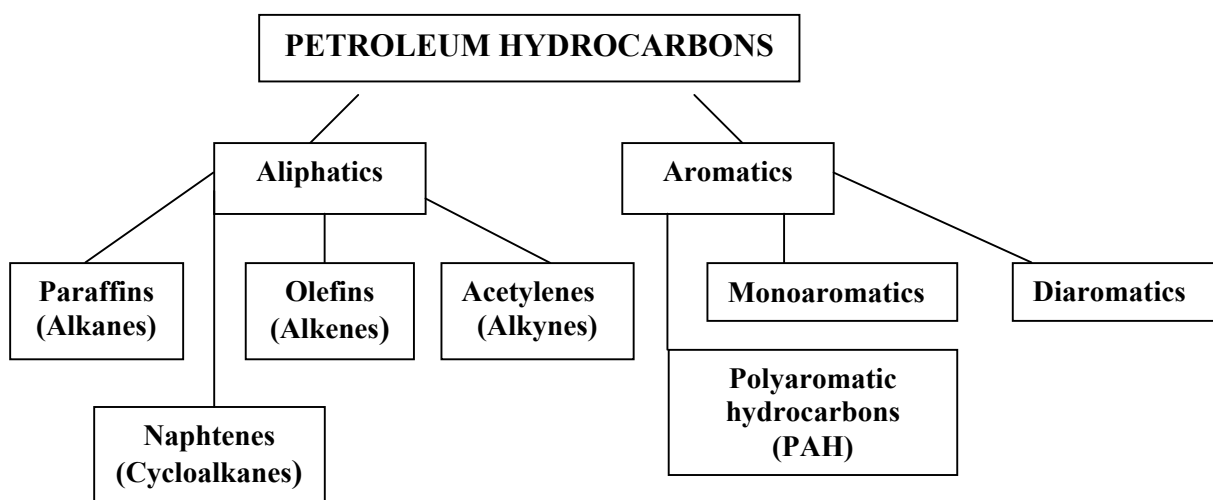


Figure 9: Petroleum hydrocarbon structural relationships (POTTER & SIMMONS, 1998; modified)

#### Aliphatics

The aliphatics are further divided into 4 classes: paraffins (alkanes), olefins (alkenes), acetylenes (alkynes) and naphthenes (cycloalkanes) (Figure 9). Paraffins, olefins and acetylenes are hydrocarbons built with straight or branched chains. Paraffins are saturated, whereas olefins and acetylenes are

unsaturated hydrocarbons due to the presence of double and triple bonds, respectively. Naphthenes are saturated hydrocarbons with one or more rings that can be combined with one or more paraffinic side chains. Only the saturated aliphatics such as paraffins and naphthenes play an important role in the quality analysis of crude oil (REIS, 1996).

### **Aromatics**

The structural components of aromatic hydrocarbon molecules are one or more 6-membered carbon rings (benzene) that demonstrate high chemical stability due to double bonds. The rings are often linked with substituted naphthenic rings or paraffinic side chains. The family is divided into monoaromatics, diaromatics and polyaromatic hydrocarbons (Figure 9). Monoaromatics have one ring such as benzene, toluene, ethylbenzene and xylene, collectively known as BTEX. Diaromatics have 2 fused benzene rings. Polyaromatic hydrocarbons (PAH) are condensed aromatic ring structures with more than 2 benzene rings. Aromatics are important constituents in the quality analysis of crude oil (REIS, 1996; POTTER & SIMMONS, 1998).

### **Other Hydrocarbon Components**

Furthermore, asphaltenes and resins are important in the quality analysis of crude oil, too. Both are defined as the polar fraction of petroleum (DIALLO *et al.*, 2000). Asphaltenes are large polyaromatic hydrocarbons which contain short aliphatic chains and polar atoms such as sulfur, oxygen, nitrogen and various metals (NALWAYA *et al.*, 1999).

### **Behavior of Petroleum Hydrocarbons**

Aliphatics are nonpolar or only slightly polar molecules, whereas aromatics demonstrate moderate polarity. Consequently, aromatics tend to be more water soluble than aliphatics, whereas aliphatics tend to be more volatile (POTTER & SIMMONS, 1998). Within the aromatics, BTEX are the predominant leachable components from petroleum (SALANITRO *et al.*, 1997). Low molecular weight-compounds are generally more soluble and volatile than high molecular ones. Typically, PHC have a variety of isomers, the number of which increases as the number of carbons increases, resulting in a high chemical complexity (POTTER & SIMMONS, 1998).

Asphaltenes are called “heavy organics”. They are proved to be the most polar fraction of crude oil. Therefore, asphaltenes are insoluble in light *n*-alkanes but soluble in aromatic solvents. Despite the high polarity, asphaltenes may become stabilized in non-polar fractions of crude oil (NALWAYA *et al.*, 1999). The key parameters that control the stability of asphaltenes in crude oil are the ratio of

aromatics to saturates and that of resins to asphaltenes (DIALLO *et al.*, 2000). The traditional theory of asphaltene stability in crude oil states that in the presence of resins, which are neutral polar components, asphaltenes adsorb them on their surface, forming a neutral or slightly polar resin layer. Asphaltene particles are thus dispersed in crude oil with a highly polar core of asphaltene polyaromatics and a slightly polar shell of resins. However, asphaltene particles can be destabilized by a variety of factors such as changes in temperature and pressure or changes of component ratios in the crude oil. The consequences are asphaltene aggregation, flocculation and precipitation (NALWAYA *et al.*, 1999).

### 3.4.3 Environmental Effects of Petroleum

When petroleum migrates from releasing points to soil, water or atmosphere, it can impact the environment. The intensity of these impacts depends on the toxicity of the oil which is determined by the oil type and concentration as well as by the resistance of the biotic community that is affected. In general, the effect of toxic substances on organisms, populations and communities ranges from temporary stress to lethality (REIS, 1996). The impact of oils on plants that have been observed include the oil-trapping ability of vegetation, the yellowing and death of oiled leaves, a reduction of germination and annual species, differing susceptibilities and recovery rates of perennials, competitive advantages to some species and growth stimulation. Chronic pollution may completely eliminate vegetation (BAKER, 1970). However, it is impossible to generalize the effects of PHC on different types of ecosystems (REIS, 1996). Furthermore, a prediction of the effects of oil is difficult because petroleum rapidly changes its location and composition due to physical, chemical and biological processes after its transfer to an environmental medium. These changes are collectively mentioned as weathering. The main weathering processes of PHC are dissolution in water, volatilization and biodegradation. Photodegradation (in the atmosphere) can also be important in the case of spills on land or water surfaces. In general, those compounds which are more water soluble and volatile are lost most rapidly from contaminated sites (POTTER & SIMMONS, 1998). Thus, the toxicity of the remaining oil usually decreases but the released components keep staying risky on other sites until further degradation (REIS, 1996).

### 3.4.4 Definitions of Target Parameters

#### Total Oil and Grease

The determination of total oil and grease (TOG) is a gravimetric method which measures the **quantity** of all material with similar physical characteristics that is extracted by the used solvent. These substances include hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related



biogenic material. The results strictly depend on the used method (GREENBERG *et al.*, 1992; WEISMAN, 1998).

### **Total Petroleum Hydrocarbons**

The determination of total petroleum hydrocarbons (TPH) is a gravimetric method, too. The **quantity** of all material that is extracted by the used solvent is measured. The same extraction process as for the determination of TOG is used but, moreover, a silica gel fractionation step is included to remove polar compounds. TPH include the PHC-compounds: paraffins, naphthenes and aromatics (US-EPA, 2001; WEISMAN, 1998).

### **SARA**

The common practice in the petroleum industry for analyzing the **quality** of crude oil is to divide it into 4 chemically distinct fractions: saturates, aromatics, resins, and asphaltenes (SARA) (DIALLO *et al.*, 2000).

## 4 MATERIAL AND METHODS

### 4.1 Greenhouse

#### 4.1.1 Experimental Design

In the greenhouse, the following treatments were applied:

- ⇒ **Treatment A:** PHC-contaminated soil, plants, medium fertilizer level (F2)
- ⇒ **Treatment B:** PHC-contaminated soil, plants, high fertilizer level (F3)
- ⇒ **Treatment C:** PHC-contaminated soil, no plants, medium fertilizer level (F2)
- ⇒ **Treatment D:** PHC-contaminated soil, no plants, high fertilizer level (F3)
- ⇒ **Treatment E:** Uncontaminated soil, plants, low fertilizer level (F1)

Due to the potential risk of an increased mortality of plants in contaminated soil, the treatments A and B (PHC, plants) were replicated 4 times per sampling whereas the treatments C and D (PHC, no plants) only had 2 replicates per sampling. Treatment E (no PHC, plants) was replicated 3 times per sampling.

Altogether, a number of 45 greenhouse pots was used. Starting with the date of the transplantation of vetiver to the greenhouse pots, the experiment lasted 6 months. Destructive samplings were taken at 2-month intervals.

#### 4.1.2 Soil

The soil used for the experiment was taken from the experimental station's area of INIA CIAE <sup>9</sup>, situated in Sta. Barbara / Maturín, Monagas State, Venezuela. Representing a typical sandy Oxisol (Soil Taxonomy USA) of the Eastern Venezuelan savanna, the soil had a loamy-sandy texture, slightly acid pH as well as very low contents of organic matter and nutrients (Table 1).

Dry leaves of the tree genera *Hieronyma* Allemao, *Eucalyptus* L'Hér, *Fraxinus* L., *Otholobium* C.H. Stirt. and others were collected in the area of PDVSA-Intevep in Los Teques, Miranda State, Venezuela. These were added to the soil in order to improve water retention and aeration.

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<sup>9</sup> Instituto Nacional de Investigaciones Agrícolas / Centro de Investigaciones Agrícolas del Estado Monagas, Venezuela

Table 1: Physical and chemical characteristics of soil (PDVSA-INTEVEP, 2001); modified)

Sand [%]	Silt [%]	Clay [%]	Texture <sup>10</sup>	pH (H <sub>2</sub> O) <sup>11</sup>	Organic matter [%]	N [%]	P [ppm]	K [ppm]	Ca [ppm]	Mg [ppm]	Al [meq/100 g of soil]
87.9	6.0	5.4	Loamy sand	6.9	0.9	0.04	4	21	81	17	0.1

“Boscán” was used as PHC agent in this study. This crude oil type is produced in the “Boscán field”, located 40 km south-west of Maracaibo, Zulia State, Venezuela. It is classified as a heavy, naphthenic, low API gravity crude oil (ROSALES & VILLALOBOS, 2001) (Table 2). The API (American Petroleum Institute) gravity is a degree for the heaviness of crude oil, indicating the proportion of large, carbon-rich molecules (PETROLEUM COMMUNITY FOUNDATION, 2003).

Table 2: Physicochemical properties of Boscán crude oil (LEÓN *et al.*, 1998; modified)

API gravity [°]	Pour Point (F)	Sulfur [%]	Saturates [%]	Aromatics [%]	Resins and Asphaltenes [%]
10	60	5.6	13.4	40.0	46.6

What was the reason for choosing Boscán as PHC-agent in the phytoremediation study? Due to high concentrations of aromatics, resins and asphaltenes, Boscán is expected to be highly resistant to conventional bioremediation techniques. Thus, further investigation for enhancing the biodegradation rates of heavy crude oils is necessarily required (LEÓN *et al.*, 1998). This study intended to follow this research assignment, thus finding out if the method used was applicable on contaminated sites by crude oils with special emphasis on heavy, low API gravity ones.

The first step in processing the growth medium for the greenhouse experiment was to pass the air-dried soil samples through a 0.6 cm-sieve to remove large particles and to homogenize them. Furthermore, dry leaves were manually crumbled.

### **Treatments A-D (Contaminated Soil)**

To facilitate equal distribution of the crude oil, the soil medium was prepared in small units. Each unit was made of 6.90 kg soil, 0.23 kg leaves (3% of dry soil weight), 0.38 kg crude oil (5% of dry soil weight) and 1 liter of water, all manually mixed until the compound was homogenous. After joining 2

<sup>10</sup> According to the texture triangle of the USDA (United States Department of Agriculture) [Reprinted by ROWELL, 1997]

<sup>11</sup> pH (H<sub>2</sub>O) was the only soil parameter of the table which was analyzed in this study. The other soil parameters of the table were taken from the mentioned source.

portions, the treated soil was packed into plastic bags inside of PVC tubes (50 cm in length, 20 cm in diameter) with perforated acrylic fiber plates at the bottom.

### **Treatment E (Uncontaminated Soil)**

The growth medium, made of 14.55 kg soil, 0.45 kg leaves (3% of dry soil weight) and 2 liters of water, was manually mixed and packed into greenhouse pots as in treatments A-D.

The soil of each pot had a total weight of 17 kg. The medium was allowed to equilibrate in the greenhouse for 6 to 14 days before introducing the plants.

### **4.1.3 Plants**

The used vetiver plants (*Vetiveria zizanioides* (L.) Nash) were genotype clones and had been cultivated in Maracay, Aragua State, Venezuela<sup>12</sup>.



Figure 10: Greenhouse (Brandt, 29.10.2002)

At first, the tillers of about 25 vetiver plants were separated. After cutting them to the same size (shoot: 20 cm, root: 10 cm), the tillers were stored in water for 2 days to improve their rooting ability (RODRÍGUEZ, 1997). Then, 2 vetiver tillers were transplanted in each pot of the treatments A, B and E. All experimental pots were placed in a half-open, sunny greenhouse situated in the area of PDVSA-Intevep in Los Teques, Miranda State, Venezuela (Figures 11, 12) (Appendix Table 7).

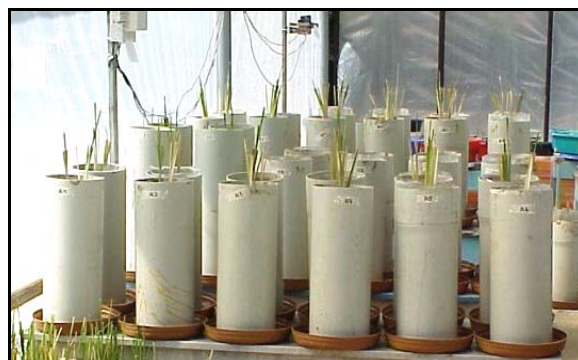


Figure 11: Experimental greenhouse pots (Brandt, 22.05.2002)

<sup>12</sup> Bioambientes, S.R.L., El Limón / Maracay, Aragua State, Venezuela

#### 4.1.4 Climatic Growing Conditions

In order to know about the climatic conditions throughout the growing time of the experimental plants, temperature and humidity were automatically measured every 30 minutes inside the greenhouse by a meteorological station<sup>13</sup> during the whole experiment.

##### Temperature

Over the period of measurement, changes of monthly average temperatures were small and maintained their value around 19.6 °C. Conversely, throughout the single days big variations of temperature were observed. Absolute minimum temperatures of approximately 11 °C were measured at night, whereas absolute maximum temperatures, typically recorded in the early afternoon, were usually between 34 and 41 °C. The highest temperature of all the period (47 °C) was measured in July (Table 3).

Table 3: Temperature in greenhouse, measured in °C from May to October 2002

Temperature [°C]							
	May	June	July	August	September	October	Total average
<b>Monthly average</b>	20.2	19.2	19.2	19.4	19.9	19.8	19.6
<b>Minimum</b>	14.4	11.7	9.3	10.3	9.9	8.9	10.8
<b>Maximum</b>	39.6	41.3	47.0	38.4	34.0	33.7	39.0

##### Humidity

Throughout the period of measurement, humidity had monthly averages of approximately 64%. Maximum humidity between 82 and 90% was measured at night, whilst minimum humidity of approximately 32% was generally determined in the early afternoon (Table 4).

Table 4: Humidity in greenhouse, measured in % from May to October 2002

Humidity [%]							
	May	June	July	August	September	October	Total average
<b>Monthly average</b>	63.9	66.2	63.2	63.7	62.8	61.3	63.5
<b>Minimum</b>	30.0	32.0	28.0	33.0	35.0	32.0	31.7
<b>Maximum</b>	82.0	89.0	86.0	85.0	90.0	84.0	86.0

<sup>13</sup> Health Weather Link, Davis Instruments' Product # 7874, Hayward, California, USA, 1997

## 4.1.5 Plant Maintenance

### 4.1.5.1 Irrigation

The availability of water and oxygen are important factors for phytoremediation of contaminated soils. Low moisture levels result in a loss of microbial activity and dehydration of plants. Conversely, too much moisture limits gas exchange with the consequence of anoxic zones, where aerobic degradation processes are reduced (FRICK *et al.*, 1999).

#### Water-Holding Capacity

A medium soil moisture content was decided to be optimal and was defined as 50 to 60% of the water-holding capacity of soil. In comparison to other phytoremediation experiments, BOSSERT & BARTHA (1985) determined 50% water-holding capacity in a 8.4% oil-contaminated sludge (sandy loam) as suitable for maintaining aerobic conditions in the medium. CHAINEAU *et al.* (1997) maintained soil moisture to 65%. The water-holding capacity depends on the texture, organic matter content, colloids and density of soil (KUNTZE *et al.*, 1994).

For the determination of the water-holding capacity, contaminated and uncontaminated soil samples in plastic tubes with perforated bottoms were used. The soil columns imitated their corresponding greenhouse pots in composition and density but were smaller (approximately 10 cm in length and 7.5 cm in diameter). Each type of soil medium was replicated twice. The samples were put on a sieve and water was slowly added for 10 minutes in order to fill all soil pores. After excess moisture had drained freely away within 3 days, the soil samples were completely put on plates, weighed and put into an oven at 105 °C for 48 hours. After cooling down to room temperature in a desiccator, the samples were weighed again. The water-holding capacity in % of soil dry weight was calculated with the formula:

$$\text{Water - holding capacity [\%]} = \frac{\text{Humid soil sample [g]} - \text{Dry soil sample [g]}}{\text{Dry soil sample [g]}} \times 100$$

(SCHLICHTING *et al.*, 1995; modified)

#### Irrigation Rates

Water was added to all greenhouse pots before planting to achieve a medium soil moisture. The soil media differed in their water-holding capacities which was greater in uncontaminated than in contaminated soil (Appendix Table 6). However, each pot of all treatments got the same water

quantity, which was 17% of the dry soil weight of a greenhouse pot. This water amount corresponded to 60% of the water-holding capacity of contaminated soil and 53% of uncontaminated soil. After planting, the evapotranspiration was continuously creating soil-moisture deficits. Thus, water had to be added 3 times a week in order to maintain the soil at near medium moisture conditions.

Before each irrigation process, the soil was tested for its moisture deficit. Therefore, water was slowly added to one of the pots with contaminated soil, until fluid was observed coming out of the bottom holes, supposing that maximal 80% of the water-holding capacity of soil was achieved. The same quantity of water was added then to all the other pots of contaminated soil. Uncontaminated soil got the same amount only during the first month of growing. In the case that percolated water with oil was collected in the plates below the greenhouse pots, it was included in the irrigation water in order to avoid loss of crude oil.

Transpiration rates, water uptake and deficits of soil moisture increased according to the growth of the plants. Therefore, irrigation rates of all planted pots (treatments A, B, E) had to be continuously raised. From the 2nd month onward, uncontaminated units (treatment E), having higher biomass production and transpiration rates, were irrigated twice as much as the contaminated pots (treatments A, B) after testing the soil moisture deficit as described before. Pots without plants (treatments C, D) had a constant irrigation rate throughout the experiment.

In summary, all greenhouse pots were given about 100 ml of water per irrigation at the beginning of the experiment. Then, irrigation rates gradually increased to maximal 400 ml of water for the contaminated pots (treatments A, B) and maximal 800 ml for the uncontaminated pots (treatment E). The unplanted pots (treatments C, D) were always irrigated with 100 ml of water.

#### **4.1.5.2 Fertilization**

According to the recommendation of FRICK *et al.* (1999), an enhanced effectiveness of phytoremediation efforts is possible by application of high fertilization amounts. In PHC-contaminated soils, the ratio of C to N is greatly increased because petroleum is rich in carbon but poor in nitrogen and other nutrients. In consequence, available nutrients are quickly used up, or they are immobilized as a result of PHC-degradation by microbial populations that are capable to use the rich source of petroleum carbon. Stress symptoms of plants in oil-polluted soils, comparable to those of extreme nutrient deficiency, can often be observed. Adding adequate amounts of nutrients results in reduced competition among plants and microorganisms, and therefore improves the vitality of plants, microbial activity and PHC-degradation.

### **Fertilization Rates**

The basic nutrients nitrogen (N), phosphorus (P) and potassium (K) were given and 3 different fertilization levels were used. Treatment E received a low concentration of fertilizers in order to avoid overfertilizing effects (GISI *et al.*, 1997) but to support basic nutrition for a good development of plants in uncontaminated soils. The contaminated soil pots (treatments A-D) were fertilized with medium and high nutrition levels.

Fertilization rates were chosen, based on a C to N to P ratio of 100:2:0.2 (HUTCHINSON *et al.*, 2001) and adjusted according to the advice of PDVSA-Intevep (2001), working with the same type of soil and crude oil concentrations (643 mg N/kg, N-P-K 30-15-15). Furthermore, experiment data of WALWORTH *et al.* (1999) (500 mg N/kg, no P and K) and YATEEM *et al.* (1999) (675 mg N/kg, N-P-K 15-15-15) were considered.

Thus, the calculation of fertilizers for the PHC-polluted soils resulted in 360 mg N/kg (F2: treatments A, C) and 720 mg N/kg (F3: treatments B, D). The fertilization rate for the uncontaminated soils (F1: treatment E) was 120 mg N/kg. In each case, phosphorus and potassium amounted to the half of nitrogen. The fertilizers were composed of urea, potassium nitrate and urea phosphate. The total fertilizer amount of each greenhouse pot was divided in 4 parts and, integrated into irrigation water, added as a solution to the plants (Appendix Table 7).

### **Modifications**

In order to get information concerning the effects of the fertilization, a weekly observation of the vitality of the plants was started after the first fertilizer application (a quarter of the total fertilizer amount). Treatment B in contaminated soil, which was fertilized with a high level of fertilizers (F3), demonstrated a high mortality rate of the transplants (> 30%). In comparison, the mortality rate of treatment A in contaminated soil, which was fertilized with a medium level of fertilizers (F2), was lower (< 10%). The plants growing in uncontaminated soil and fertilized with low fertilization rates (F1) indicated total vitality (Figure 12).

Due to these observations, the high fertilization rate (F3) in treatment B had to be drastically reduced. Moreover, the medium fertilization rate (F2) was reduced in order to prevent negative effects with the following applications. The modified fertilization rates were 220 mg N/kg (F2: treatments A, C) and 300 mg N/kg (F3: treatments B, D) applied in 4 parts (considering that the first part had already been given!). In each case, phosphorus and potassium amounted to half of nitrogen (Appendix Table 8). After the 2nd fertilizer application with modified concentrations, the mortality rate of treatment B in



contaminated soil (F3) stabilized at a level of approximately 40%, whilst the mortality of transplants of treatment A in contaminated soil (F2) first increased and then leveled out at approximately 25% (Figure 12).

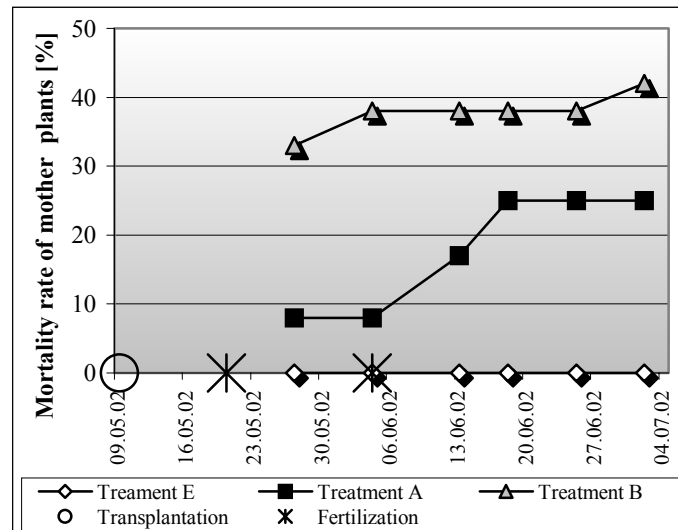


Figure 12: Mortality rate of vetiver transplants in % during the first 2 months of the experiment (09.05.–04.07.2002). Treatment A: PHC-contaminated soil + medium fertilizer level (F2), Treatment B: PHC-contaminated soil + high fertilizer level (F3), Treatment E: Uncontaminated soil + low fertilizer level (F1). (Appendix Table 9)

#### 4.1.6 Sampling

In the course of the experiment, which lasted 6 months, 3 destructive samplings were taken at 2 month-intervals (Appendix Table 7).

##### 4.1.6.1 Plants

After removing the whole soil column from the greenhouse pot, the roots were carefully separated from the soil. Afterwards, the soil was precisely checked in order to pick out all detached root segments. The shoots were cut off the root systems, and the tillers were separated. The number of tillers, their lengths, and any special observations were determined. All shoot material coming from the same mother plant (transplant) was jointly collected. Then, the clump diameters were measured. Before carefully rinsing the roots with water in a sieve, soil and leave particles were removed from the root surface. Finally, the roots were stored in isopropyl alcohol (50%) and kept refrigerated at 4 °C until further handling.

#### 4.1.6.2 Soil

The soil of each root was carefully mixed after picking out all plant material. Various portions were taken and mixed again to achieve homogeneity. Finally, a soil sample (approximately 400 g) was taken and stored at a temperature of 4 °C.

## 4.2 Laboratory

### 4.2.1 Plant

#### 4.2.1.1 Root Surface

Due to the work being very time-consuming, the analysis of the root surface was only carried out with material of the last sampling (at 6 months of the experiment). One root sample of each treatment with plants (treatments A, B, E) was selected and approximately 3 g of representative material was analyzed respectively. After rinsing the roots with water to clean them from the isopropyl alcohol where they had been stored in, they were stained for 5 minutes in a crystal violet solution (1 g/100 ml water) at 50 °C to make the root segments better visible. Then, the roots were rinsed with water in a strainer until most of the excess stain was removed. The samples were then stored in a 50% isopropyl alcohol solution until further analysis (EWING & KASPAR, 1998).

The samples were thoroughly rinsed with water before scanning. The roots were dispersed on a thin, transparent plastic tray without touching or overlapping each other. The tray was filled with a 0.01 M NaOH solution to prevent the stain from bleeding and to ensure good spreading of the segments. A white sheet was used as a background. The roots were scanned in grayscale color mode with a resolution of 600 dpi (scanner type: CanonScan N670U). About 20 images of each root sample were made in uncompressed tagged tiff-file format (EWING & KASPAR, 1998).

The scanning images were analyzed with Win/Mac Rhizo 2002c, Régent Instruments Inc. Firstly, root diameter classes were determined. Particularly fine roots which are defined as roots < 2 mm in diameter (FINNERN *et al.*, 1994) were important to specify. The thinner root diameters are, the more surface area per weight can be determined. Thus, 8 diameter classes for fine roots with ranges of 0.25 mm and only one class for thicker roots  $\geq 2$  mm in diameter were defined. Root segments were classified by the program according to their diameters and then manually corrected under precise examination. The length was automatically measured, and the surface area was calculated by the

program, assuming the roots had round cross-sections (REGENT INSTRUMENTS INC., 2002) (Figure 13).

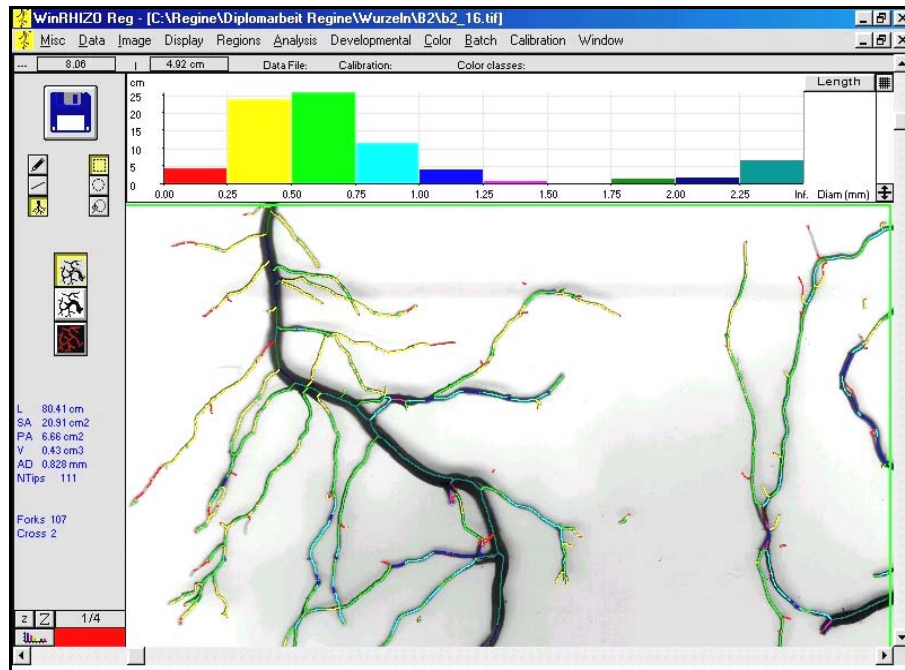


Figure 13: Example of a scanning image analysis of root segments (Win/Mac Rhizo 2002c, Régent Instruments Inc.). Classification of roots according to their diameters, automatical measurement of root length and calculation of surface area.

#### 4.2.1.2 Biomass

Both shoots and roots, including the scanned material, were dried at 60 °C for at least 3 days, and immediately weighed after taking them out of the oven.

### 4.2.2 Soil

#### 4.2.2.1 Soil Reaction

A 1:1 suspension of air-dried soil in distilled water (20 g soil and 20 mL distilled water) was put in a plastic beaker, stirred in a reciprocating shaker for one hour and centrifuged for 20 minutes. Of each soil sample, 2 replicates were prepared. The soil  $\text{pH}_{\text{H}_2\text{O}}$  was measured with a METROHM 691 pH-meter. The data was noted after stabilization of the value (SUMNER, 2000).

#### 4.2.2.2 Total Oil and Grease

The analyses of total oil and grease (TOG) were based on the US-EPA<sup>14</sup> methods 9071B (US-EPA, 1998) and 3540C (US-EPA, 1996), including modifications by Pétroleos de Venezuela, S.A. (PDVSA). 20 g of soil sample were acidified with concentrated hydrochloric acid (HCl) to pH 2 in order to improve the extraction. By thoroughly mixing with magnesium sulfate monohydrate ( $\text{MgSO}_4$ ), the sample was chemically dried to avoid creation of emulsions. 2 replicates were prepared of each sample. After putting the mixture into an extraction thimble, it was covered with glass wool and put in a Soxhlet extraction apparatus (Figure 15). Methylene chloride ( $\text{CHCl}_2$ ) was used as solvent. The extraction lasted approximately 10 hours to ensure maximal completeness. Afterwards, the solvent was separated from the extract in an evaporator at 45 °C. Before redissolving the oil and grease extract in  $\text{CHCl}_2$  and passing through filter paper with about 1 g of  $\text{MgSO}_4$ , the dry weights of the used extraction flasks were determined. After a final solvent evaporation, the flask with the extract was dried at 40 °C for about 2 days to eliminate traces of the solvent. Before weighing the flask with oil and grease content on an analytical balance, it was cooled down to room temperature in a desiccator. The weighing process was repeated until the weight was constant.

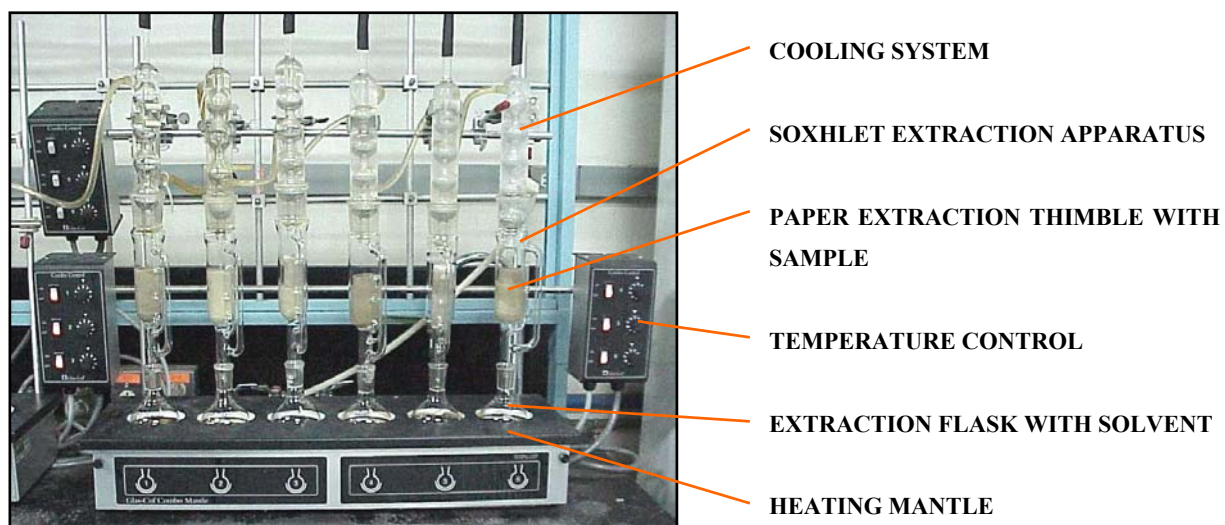


Figure 14: Soxhlet apparatus for the extraction of total oil and grease (Brandt, 23.09.2002).

#### Determination of the Dry Soil Fraction

A soil sample of 20 g was dried at 105 °C for 24 hours and weighed after cooling down to room temperature in a desiccator. Each sample was replicated twice. The dry soil fraction was calculated:

<sup>14</sup> United States Environmental Protection Agency, Office of Research and Development, Washington, DC 20460

$$\text{Dry soil fraction} = \frac{\text{Dry soil sample [g]}}{\text{Humid soil sample [g]}}$$

### Calculation of the TOG Content

$$\text{TOG as \% of dry soil} = \frac{\text{Gain in weight of flask [g]} \times 100}{\text{Wet soil sample [g]} \times \text{Dry soil fraction}}$$

## 4.3 Statistical Analysis

The statistical data analyses were conducted using the Superior Performance Software System (SPSS) 8.0 for Windows. Concerning the plant and soil data of independent samples, the hypothesis was investigating differences, with the null hypothesis being that there was no difference between the means of different treatments at the same time. The statistical evaluation of the differences of the means makes the assumption that the data is continuous, approximately normally distributed and that the variances of each group are homogeneous. In order to test for normal distribution, the Kolmogorov-Smirnov test was used. The data was checked concerning the homogeneity of variance by using the Levene test. Then, the differences in plant and soil data between the different treatments were statistically analyzed by the One-way analysis of variance (ANOVA), *post hoc*-least significant difference (LSD)-test. In the case of determining that the data did not fulfil the assumptions, the nonparametric equivalent of the One-way ANOVA, the Kruskal-Wallis test, which is based on the comparison of medians, was used. Significant differences were reported at the 0.05 significance level ( $p < 0.05$ ). Asterisks with the treatment symbols (e.g. <sup>\*</sup>A), which are written next to the data in the tables (in Appendix), indicate to which treatment/s the data is significantly different. Moreover, the correlation of the variables “root biomass” and “TOG decrease” in the contaminated treatments with plants were evaluated. As a degree of the intensity of the bivariate correlation, the Pearson’s correlation coefficient for normal-distributed variables was used (BÜHL & ZÖFEL, 1999; DYTAM, 1999).

## 5 RESULTS

### 5.1 Plant Growth Analysis

#### 5.1.1 Biomass

Biomass is defined as material of organisms per area (SCHAEFER, 1992). Total biomass, reported as dry weight per greenhouse pot, increased in all treatments over the course of experiment. As expected, the yields were lower in contaminated soil (treatments A, B) than in uncontaminated soil (treatment E) (Figure16), and the differences were significant at  $p < 0.05$  during the whole experiment.



Figure 16: Vetiver plants in contaminated soil (Treatment A [T<sub>A</sub>]: medium fertilizer level, Treatment B [T<sub>B</sub>]: high fertilizer level) and in uncontaminated soil (Treatment E [T<sub>E</sub>]: low fertilizer level) after 6 months of growing. The height of pots differ a little, but they contain the same quantity of soil medium in the same density. (Brandt, 23.10.2002)

After 2 months of the experiment, significant differences ( $p < 0.05$ ) in total biomass between the 2 fertilizer treatments in contaminated soil were determined (Figures 17, 18). Until the 4th month, plant growth in contaminated soil was still more rapid in the medium level of fertilizers (relative increase of total biomass [biomass / initial biomass] in treatment A: 7 times) than in the high level of fertilizers (relative increase of total biomass in treatment B: 6 times) but the differences were not significant anymore. Later on, development of plants in treatment B accelerated and insignificantly surpassed the total biomass production of treatment A (Table 5). Thus, there was no significant difference in final total biomass of contaminated soils between the medium (treatment A: 95 g) and the high level

(treatment B: 100 g) of fertilizers. Compared to the control (treatment E: 177 g), total biomass of contaminated soils yielded at 53% (treatment A) and 56% (treatment B) respectively. (Figure 19).



Figure 17: Vetiver plants in contaminated soil after 2 months of growing (Treatment A: medium fertilizer level). On the left side: transplant in good condition, on the right side: dead transplant, both demonstrating ability of tillering. (Brandt, 04.07.2002)



Figure 18: Vetiver plants in contaminated soil after 2 months of growing (Treatment B: high fertilizer level). Despite of being in bad conditions, both transplants demonstrated the ability of vegetative reproduction. (Brandt, 04.07.2002)

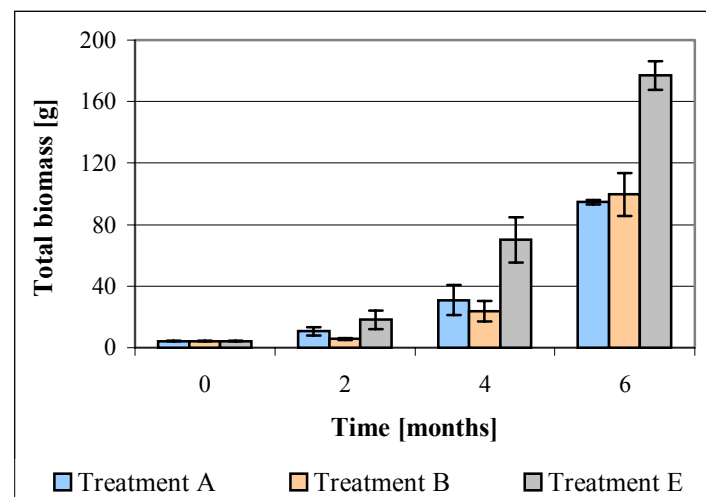


Figure 19: Total biomass as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash in grams of dry weight per greenhouse pot. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 13)



Table 5: Relative increase of total biomass [ $\text{biomass}_n / \text{initial biomass}$ ,  $n = 2, 4, 6$  months]. Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 13)

Treatment	Relative increase of total biomass		
	2	4	6
	[months]		
A	2.5	7.2	22.2
B	1.3	5.6	23.3
E	4.2	16.4	41.5

In order to compare aboveground and underground plant material, total biomass was separated into shoot and root biomass. In contaminated soil, there was no significant difference in final shoot biomass between the medium and the high fertilizer level (treatment A, B: 47 g in each case), both reaching 58% of the control dry weight (treatment E: 81 g) from which they differed significantly at  $p < 0.05$  (Figure 20).

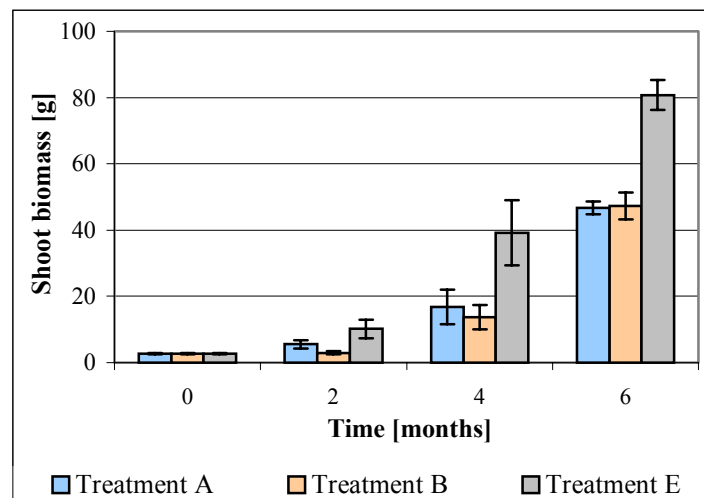


Figure 20: Biomass of shoots as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash in grams of dry weight per greenhouse pot. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 11)

However, concerning final root biomass, differences between the fertilizer applications in contaminated soils were insignificantly. The medium level of fertilizers (treatment A) demonstrated smaller final yields with 47 g than the high fertilizer level (treatment B) with 51 g. In comparison, root biomass was 49% (treatment A) and 54% (treatment B) of the control dry weight (treatment E: 94 g). The differences between final root biomass in uncontaminated and both treatments in contaminated soil were significant ( $p < 0.05$ ) (Figures 21-24).



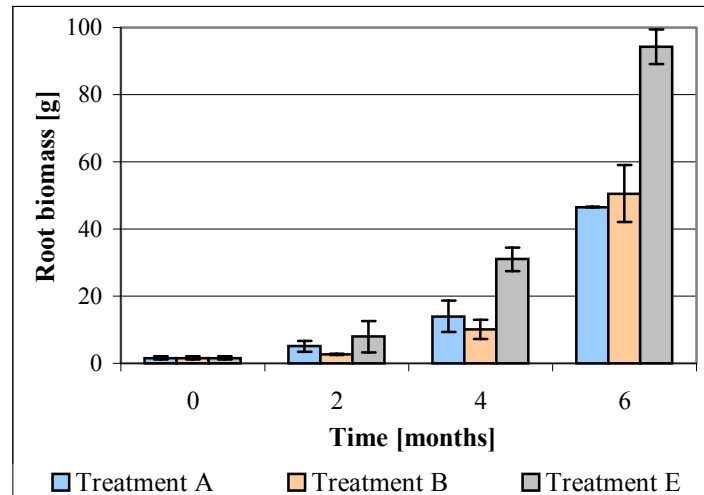


Figure 21: Biomass of roots as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash in grams of dry weight per greenhouse pot. Values are means  $\pm$  standard deviation (SD); Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 12)



Figure 22: Vetiver roots in contaminated soil after 6 months of growing (Treatment A: medium fertilizer level). Root length: 62 cm. (Brandt, 25.10.2002)



Figure 23: Vetiver roots in contaminated soil after 6 months of growing (Treatment B: high fertilizer level). Root length: 56 cm. (Brandt, 30.10.2002)



Figure 24: Vetiver roots in uncontaminated soil after 6 months of growing (Treatment E: low fertilizer level). Root length: 48 cm. Due to a lack of space in the pot, the roots are grown together. (Brandt, 23.10.2002)

### 5.1.2 Shoot-Root Ratio

Initially, shoots had almost double the weight of roots (shoot-root ratio: 1.7) due to the plant cuttings before transplanting. However, throughout the course of development, a growing trend in favor of the root biomass was observed in all treatments (Figure 25).

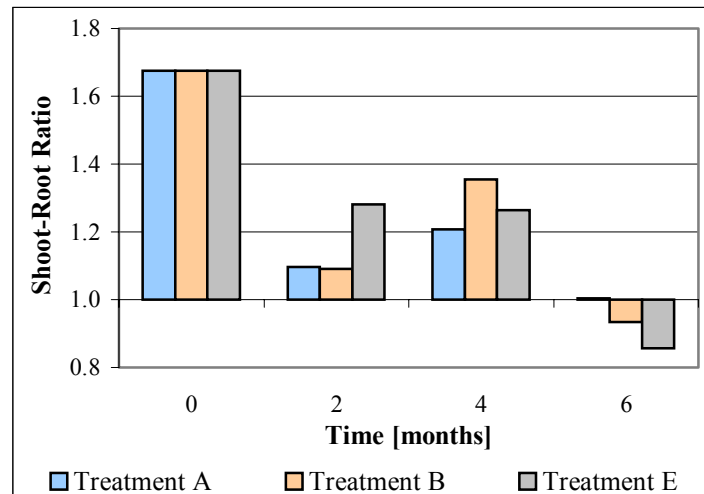


Figure 25: Shoot-root ratio as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash. Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 14)

In contaminated soil (treatments A, B), the shoot-root ratio fluctuated slightly, whilst the ratios in uncontaminated soil (treatment E) steadily changed. Until the 4th month, more shoot than root biomass was determined in all treatments, thus the shoot-root ratio was  $> 1$ . However, in the 6th month of the experiment, the ratios changed in the high fertilizer level of contaminated soil (treatment B) as well as in the control (treatment E), both having a shoot-root ratio of 0.9. Shoot and root material of the medium level of fertilizers in contaminated soil (treatment A) had identical final dry weights and a shoot-root ratio of 1 (Figure 25).

### 5.1.3 Number of Tillers

The experiment started with 2 plants per greenhouse pot. Throughout time of growing, the number of tillers increased by vegetative reproduction in all treatments.

In the 2nd month, the contaminated soil treatments significantly differed ( $p < 0.05$ ) in the average number of tillers per greenhouse pot, with more tillers in the medium (treatment A: 7 tillers per pot) than in the high level of fertilizers (treatment B: 3 tillers per pot). The largest number was found in the

uncontaminated soil (treatment E: 8 tillers per pot), and the difference to treatment B was significant at  $p < 0.05$ . In the 4th month, the largest number of tillers was still determined in uncontaminated soil (treatment E), but differences to the treatments in contaminated soil were not significant. At the end of the studies, larger numbers of tillers were detected in the PHC-soil (treatment A: 44 tillers per pot, treatment B: 54 tillers per pot) than in uncontaminated soil (treatment E: 38 tillers per pot). The differences of treatment B to treatment A and E were significant ( $p < 0.05$ ) (Figure 26).

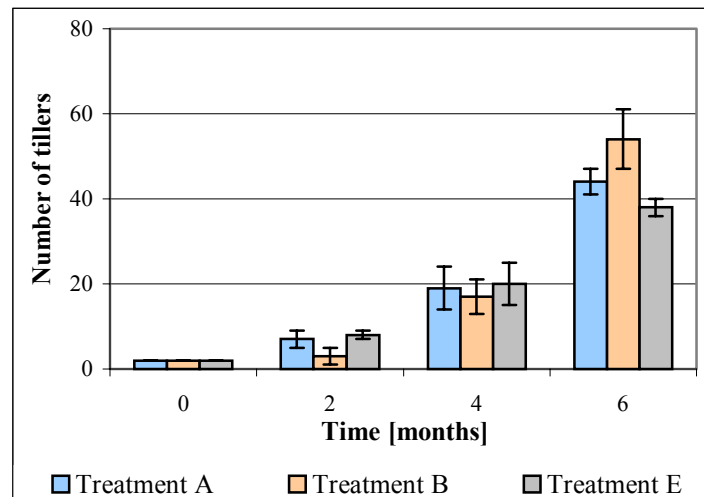


Figure 26: Number of tillers per greenhouse pot as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 15)

#### 5.1.4 Height of Tillers

At the beginning of the greenhouse studies, all plants had a height of 15 cm. As the experiment progressed, plant height increased in all treatments. However, differences between contaminated and uncontaminated soil were clearly evident throughout the study. Generally, tillers in contaminated soil were slower and shorter growing than those of uncontaminated soil.

Average height in contaminated soil reached its maximum at 4 months. Insignificantly higher results were found in the medium level of fertilizers (treatment A: 39 cm) than in the high level (treatment B: 36 cm). In comparison, the control (treatment E) average height already reached its maximum of 56 cm within 2 months of growing (Figure 27). Absolute maximums of plant height were determined at 76 cm for the high (treatment B) and 80 cm for the medium fertilizer level (treatment A) in contaminated soils and 120 cm in uncontaminated soil (treatment E) (Figure 28).

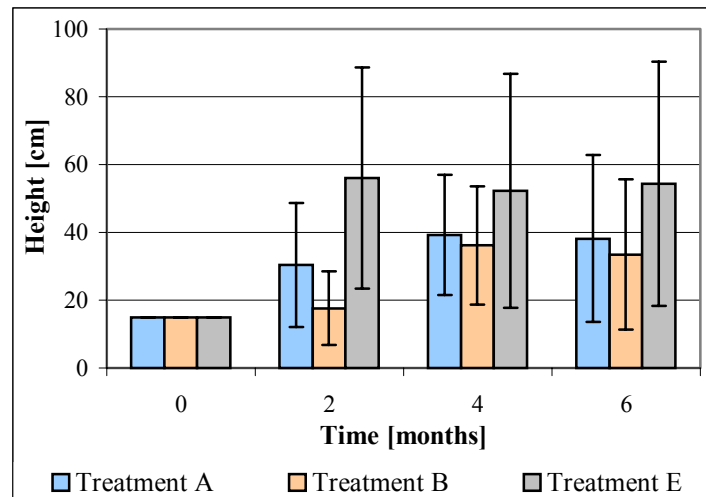


Figure 27: Height of tillers as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash in cm. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Tables 16-18)

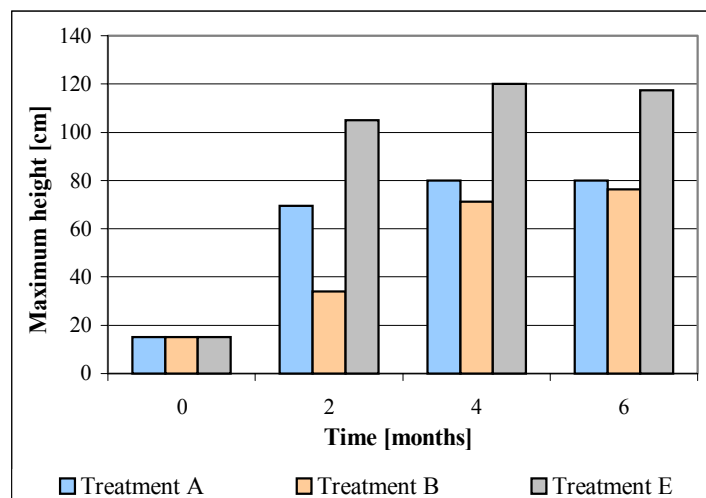


Figure 28: Maximum height of tillers as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash in cm. Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 16-18)

Furthermore, an evaluation based on height classes was made. After observing the plant height characteristics, a classification in 4 classes was decided to be useful. 3 classes with constant ranges of 29.9 cm for tillers with small and medium height and one open class for large tillers were created. According to Figure 29, plant development in height of the medium fertilizer level (treatment A), with 45% of tillers above 30 cm, was clearly advanced at 2 months compared to the high level of fertilizers

(treatment B) with only 10% of tillers above 30 cm. However, plant height of treatment B increased in the following months, and differences became smaller between both treatments. However, the medium fertilizer level (treatment A) maintained an advanced height development compared to the high fertilizer level (treatment B) until the end of the study (A: 21% of tillers, B: 12% of tillers above 60 cm). The classification made the differences in height development between the uncontaminated and the contaminated soil clearly evident. Already in the 2nd month, 17% of the tillers of the uncontaminated soil (treatment E) had a height of above 89 cm. By contrast, tillers of contaminated soils (treatments A, B) never reached this height. From the 2nd month onwards, the proportional distribution of tillers among the height classes in the uncontaminated soil (treatment E) did not change.

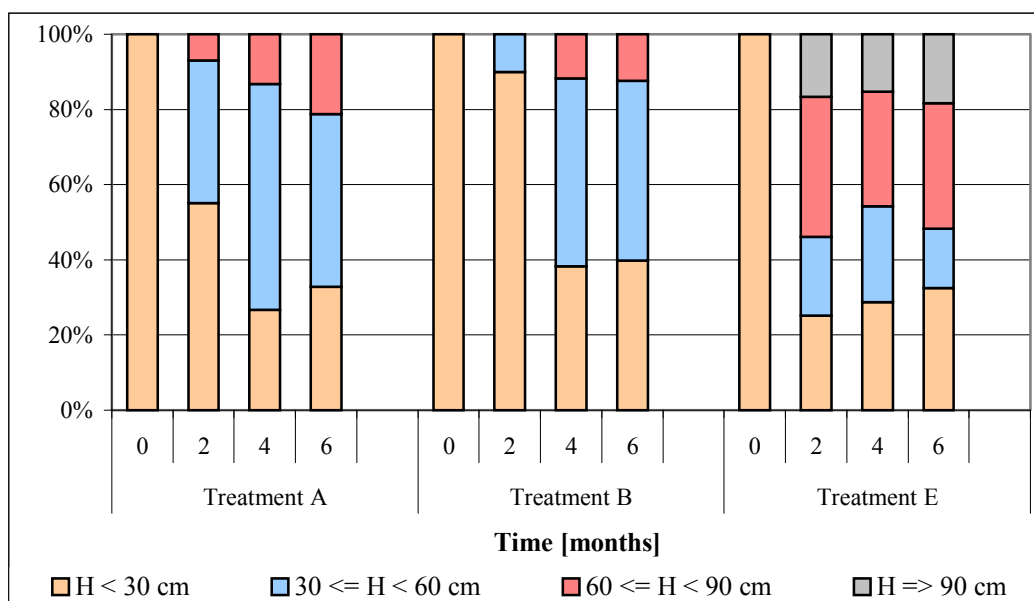


Figure 29: Classification of tillers due to their heights as a function of treatment and time for *Vetiveria zizanioides* (L.) Nash. 4 classes: 3 classes with constant ranges of 29.9 cm and one open class. Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 19)

Summarizing all final results, plant height in contaminated soils averaged 63% for the high fertilizer level (treatment B) and 69% for the medium fertilizer level (treatment A) of the height recorded in uncontaminated soil (treatment E).

### 5.1.5 Clump Diameter

At the beginning of the experiment, the average clump diameter of transplanted individuals was 0.9 cm. Whilst growing, the plants continuously produced tillers and clump diameter increased in all treatments.

Within 2 months of development, the average clump diameter of the medium fertilizer level (treatment A) in contaminated soil was 1.9 cm, which was significantly higher ( $p < 0.05$ ) than that in the high fertilizer level (treatment B: 1.4 cm). But in the following months, treatment B accelerated the diameter increase. In the 4th month, both treatments in contaminated soil had the same average clump diameter (3.0 cm). In the 6th month, treatment B demonstrated with 4.5 cm an insignificantly higher value than treatment A with 4.3 cm. In uncontaminated soil (treatment E), the average clump diameter was higher than in contaminated soil from the 2nd month (2.4 cm) until the end of the experiment (5.2 cm). But the differences to treatment A and B were only significant until the 4th month ( $p < 0.05$ ) (Figure 30).

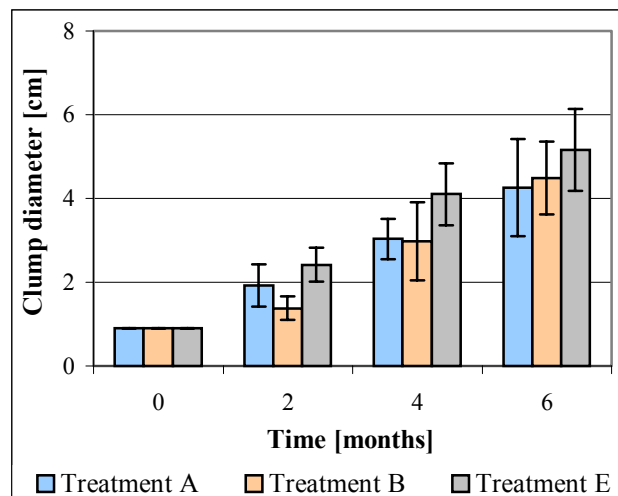


Figure 30: Clump diameter of plants as a function of time and soil treatment for *Vetiveria zizanioides* (L.) Nash in cm. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + high fertilizer level (F3); Treatment E: uncontaminated soil + low fertilizer level (F1). Treatments C and D not analyzed (no plants). (Appendix Table 20)

### 5.1.6 Root Surface

Comparative computer analyses illustrated great differences in root structure between contaminated and uncontaminated soil. Undoubtedly, roots growing in uncontaminated soil were finer than those growing in contaminated soil. However, the results only had exemplary function and could not be statistically evaluated because only one pot from each soil treatment was examined.

Measurements of the root diameters resulted in an average of 0.78 mm for the medium (treatment A) and 0.87 mm for the high level of fertilizers (treatment B) in contaminated soil, whereas in uncontaminated soil (treatment E) an average diameter of 0.46 mm was observed (Appendix Table 21). 83% of the root surface in the medium (treatment A) and 80% in the high fertilizer level (treatment B) of contaminated soil belonged to fine roots with a diameter smaller than 2 mm. In

comparison, in uncontaminated soil almost the total root surface (97%) consisted of fine roots. When specifying fine root areas, it was conspicuous that there were more roots with diameters smaller than 0.5 mm in uncontaminated soil (treatment E: 42% of root surface) than in contaminated soil (treatment A: 15%, treatment B: 11% of root surface). Between 1.5 and 2.0 mm, the opposite was true, with 16% (treatment A) and 11% (treatment B) of root surface in contaminated soil and only 6% in uncontaminated soil (treatment E) (Figures 31-33).

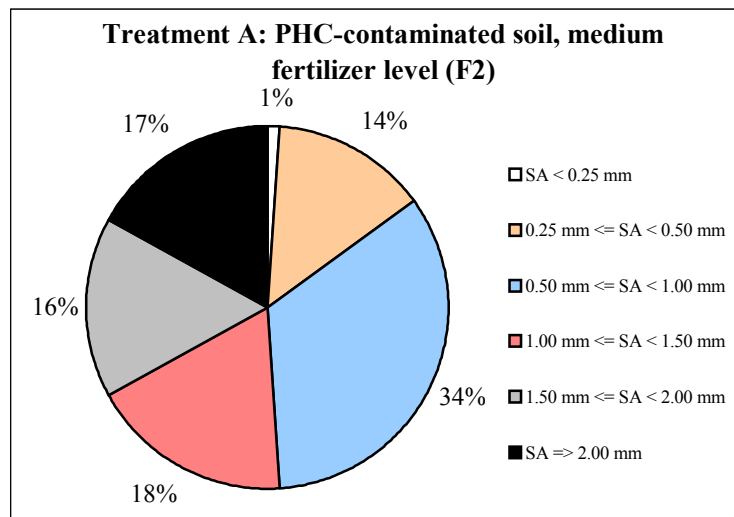


Figure 31: Root surface area (SA) of *Vetiveria zizanioides* (L.) Nash at 6 months in %, classified within diameter classes in mm of the respective root segments. Analysis with Win/Mac Rhizo 2002c Régent Instruments Inc. A representative root sample of one exemplary pot was analyzed (unit: A6, sample dry weight: 3.5 g). In favor of a better clearness of the diagram, 2 classes were grouped to one except for the first 2 classes (SA < 0.25 mm; 0.25 mm <= SA < 0.50 mm). Treatment A: PHC-contaminated soil + medium fertilizer level (F2) (Appendix Table 22)

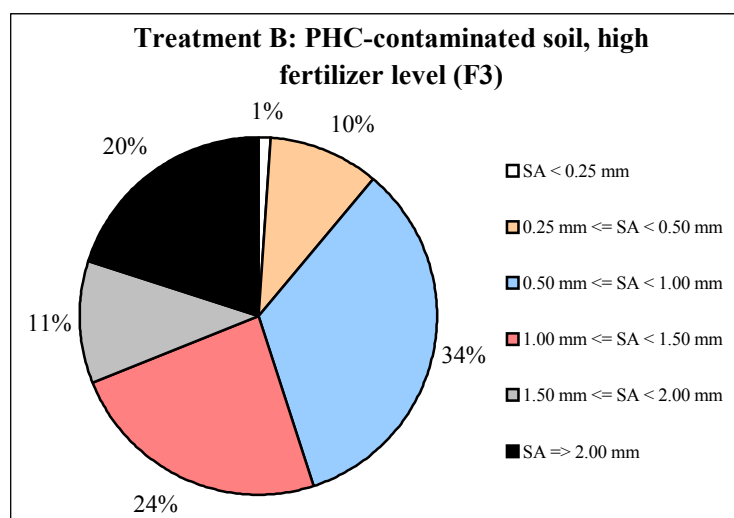


Figure 32: Root surface area (SA) of *Vetiveria zizanioides* (L.) Nash at 6 months in %, classified within diameter classes in mm of the respective root segments. Analysis with Win/Mac Rhizo 2002c Régent Instruments Inc. A representative root sample of one exemplary pot was analyzed (unit: B2, sample dry weight: 3.1 g). In favor of a better clearness of the diagram, 2 classes were grouped to one except for the first 2 classes (SA < 0.25 mm; 0.25 mm <= SA < 0.50 mm). Treatment B: PHC-contaminated soil + high fertilizer level (F3) (Appendix Table 22)



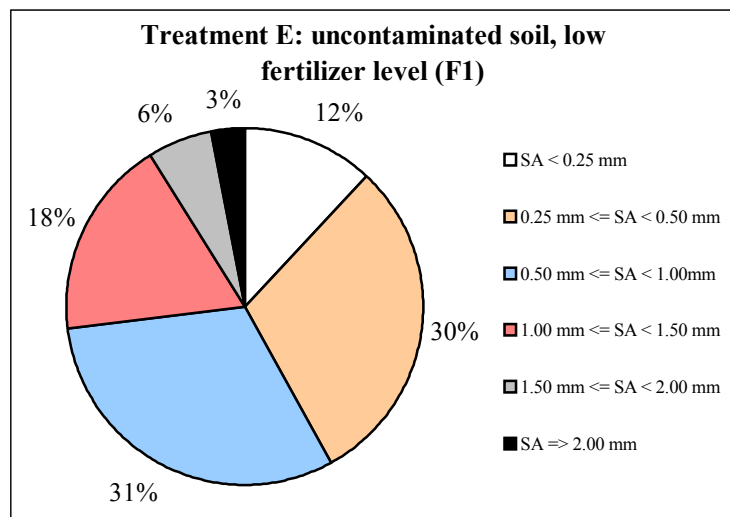


Figure 33: Root surface area (SA) of *Vetiveria zizanioides* (L.) Nash at 6 months in %, classified within diameter classes in mm of the respective root segments. Analysis with Win/Mac Rhizo 2002c Régent Instruments Inc. A representative root sample of one exemplary pot was analyzed (unit: E5, sample dry weight: 2.3 g). In favor of a better clearness of the diagram, 2 classes were grouped to one except for the first 2 classes (SA < 0.25 mm; 0.25 mm <= SA < 0.50 mm). Treatment E: uncontaminated soil + low fertilizer level (F1). (Appendix Table 22)

The total length of roots, projected to the whole respective root system of a greenhouse pot, was 776 m for the medium fertilizer level (treatment A) and 676 m for the high fertilizer level (treatment B) in contaminated soil, and 3618 m for uncontaminated soil (treatment E). Projections of total root surface to the whole root resulted in 1.92 m<sup>2</sup> for the medium fertilizer level (treatment A) and 1.86 m<sup>2</sup> for the high fertilizer level (treatment B) in contaminated soil, and 4.90 m<sup>2</sup> for uncontaminated soil (treatment E) (Appendix Table 21).

### 5.1.7 Vitality of Plants

In the first 2 months, the mortality rates of plants, which had been transplanted in contaminated soils, were 25% for the medium fertilizer level (treatment A) and 40% for the high fertilizer level (treatment B). In contrast, all transplanted plants in uncontaminated soil (treatment E) survived and were in good conditions (Figure 13). However, even affected plants in contaminated soils could regenerate by tillering. Only 8% of the transplants in the medium fertilizer level (treatment A) and 21% in the high fertilizer level (treatment B) died without having been reproduced (Appendix Table 9).

The mortality rates of tillers were very small in all treatments (treatment A: 2%, treatment B: 2%, treatment E: 4%) (Appendix Table 10). Despite reduced biomass and heights, the tillers in contaminated soils did not exhibit signs of toxicity on their shoots. However, one of the greenhouse pots of the medium fertilizer level in contaminated soil (treatment A) was infested with mites (order: Acari). They caused local red discoloration on culms which had no negative effects on the biomass



production compared to the other pots. Generally, tillers in uncontaminated soil demonstrated more frequently dry culms and leaf tips than plants in contaminated soils did.

## 5.2 Analysis of Growth Medium

### 5.2.1 Soil Reaction

Regarding to the soil pH, there were significant differences between all contaminated and the uncontaminated treatments at 2, 4 and 6 months of growing. In the contaminated treatments (A-D), soil was medium to slightly acid (pH: 5.7–6.3). In the uncontaminated medium (treatment E), the soil demonstrated almost neutrality (pH: 6.7–6.9) over the period of the experiment (terms according to FINNERN *et al.*, 1994).

The average pH in contaminated soil for the medium fertilizer level was 5.9 with plants (treatment A) and 6.0 without plants (treatment C). For the high fertilizer level, the average pH was determined as 6.0 with plants (treatment B) and 6.2 without plants (treatment D) (Appendix Table 25). Acidity showed a trend of being higher in the medium than in the high fertilizer level of contaminated soils and increased in the presence of plants. In uncontaminated soil (treatment E), the average pH had a value of 6.9. In all treatments, initial pH values were higher than final ones. None of the observed acidification processes had continuous courses (Figure 34).

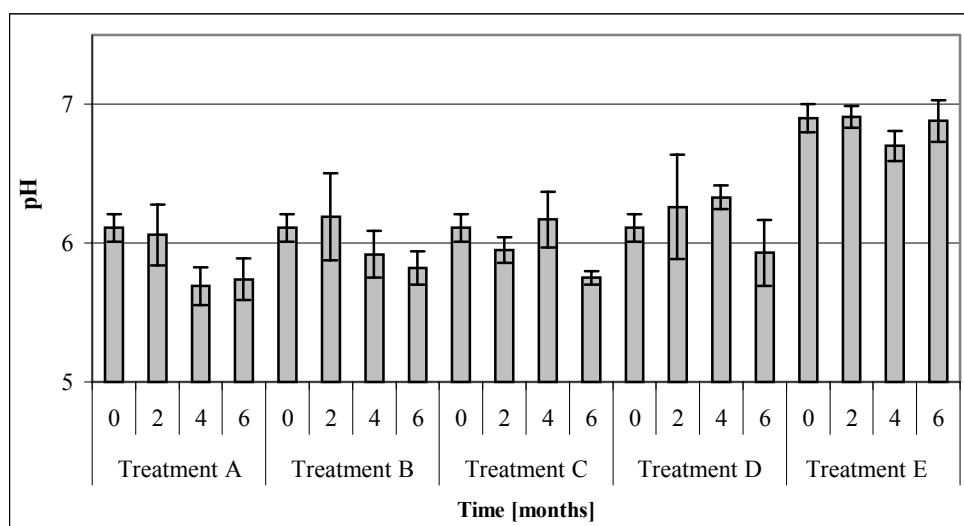


Figure 34: Soil reaction (pH) as a function of time and soil treatment. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + plants (*Vetiveria zizanioides*) + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + plants (*Vetiveria zizanioides*) + high fertilizer level (F3); Treatment C: PHC-contaminated soil + medium fertilizer level (F2) (no plants); Treatment D: PHC-contaminated soil + high fertilizer level (F3) (no plants); Treatment E: uncontaminated soil + plants (*Vetiveria zizanioides*) + low fertilizer level (F1). (Appendix Tables 23, 24)

### 5.2.2 Total Oil and Grease

The initial total oil and grease (TOG) content was 5.1% of the total soil dry weight. A decrease of TOG was detected in all crude oil contaminated treatments over the course of experiment.

Concerning treatments with plants in contaminated soil, similar amounts of TOG decrease were found in the different fertilizer levels. In both treatments, TOG constantly decreased until the 4th month of growing, in the medium fertilizer level (treatment A) at a rate of 18%, and in the high fertilizer level (treatment B) at a rate of 19%. However, in both treatments further decrease could not be observed. On the contrary, even higher TOG contents were detected after 6 months compared to after 4 months of the experiment. Final TOG contents were 4.3% of the total soil dry weight in the medium fertilizer level (treatment A), and 4.5% of the total soil dry weight in the high fertilizer level (treatment B). The differences were insignificant (Figure 35, Appendix Table 28).

In contaminated soil without plants, the TOG decrease demonstrated irregular courses in both fertilizer treatments. In the medium fertilizer level (treatment C), the maximal TOG decreasing rate of 13% was found at the end of the experiment. The maximal decreasing rate of 14% in the high fertilizer level (treatment D) was determined after 2 months of the experiment. Final TOG contents were 4.4% of total soil dry weight in the medium fertilizer level (treatment C), and 4.5% of total soil dry weight in the high fertilizer level (treatment D) (Figure 35, Appendix Table 28).

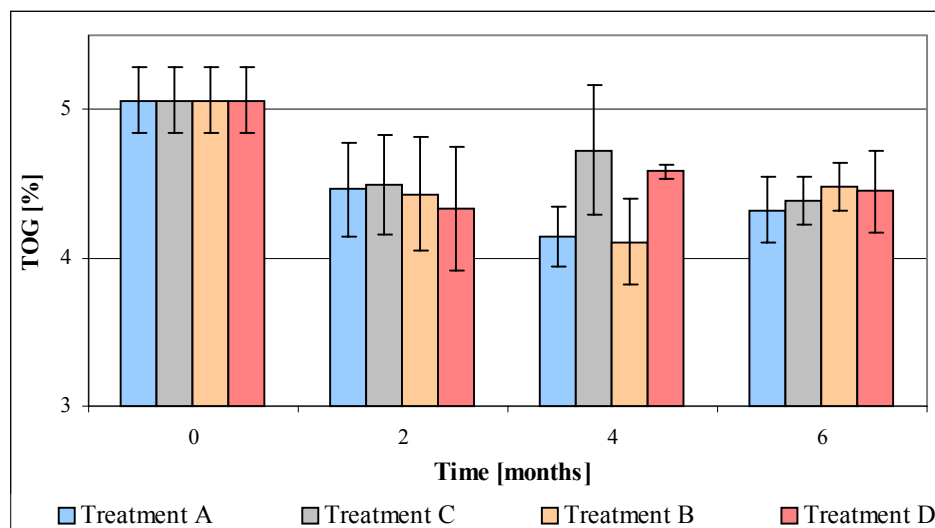


Figure 35: Total oil and grease (TOG) as a function of time and treatment in % of soil dry weight. Values are means  $\pm$  standard deviation (SD). Treatment A: PHC-contaminated soil + plants (*Vetiveria zizanioides*) + medium fertilizer level (F2); Treatment B: PHC-contaminated soil + plants (*Vetiveria zizanioides*) + high fertilizer level (F3); Treatment C: PHC-contaminated soil + medium fertilizer level (F2) (no plants; control of treatment A); Treatment D: PHC-contaminated soil + high fertilizer level (F3) (no plants; control of treatment B); Treatment E not analyzed (uncontaminated soil). (Appendix Tables 26, 27)

Comparing TOG decreases between treatments with and without plants within each fertilizer level, there were not found significant differences in the 2nd and in the 6th month, neither in the medium (treatments A, C) nor in the high fertilizer level (treatments B, D). Only in the 4th month were found significant differences between plants and unplanted controls concerning the medium (treatments A, C) and the high fertilizer treatment (treatments B, D).

Concerning the bivariate correlation of TOG decrease and root biomass, the statistical analyses resulted in very small Pearson coefficients of TOG decrease and root biomass in the medium and the high fertilizer treatment (Treatment A: 0.05 and Treatment B: -0.19). Thus, the bivariate correlation between the parameters was insignificant in both treatments (Appendix Table 29).

## 6 DISCUSSION

The greenhouse experiment was realized to determine the tolerance of the plant species vetiver (*Vetiveria zizanioides* (L.) Nash) to a Venezuelan heavy crude oil in soil. Moreover, the potential of the grass for stimulating biodegradation processes of petroleum hydrocarbons (PHC) in loamy savanna sand was tested.

### **Survival and Growth Rates of Vetiver Transplants in PHC-Contaminated Soil**

Vetiver transplants suffered under the influence of crude oil-contamination. After 2 months of growing, they showed a rate of mortality at 33% whereas in uncontaminated soil all transplants were in good condition. The effects of PHC on plants were attributed to phytotoxicity. The level of phytotoxicity strictly depends on the type and constituents of oil. Generally, the toxicity increases along the series: paraffins – olefins - naphthenes – aromatics (BAKER, 1970). In this study, a native heavy crude oil (Boscán) with 40% aromatics was used. Due to the high aromatic content, the oil was considered as medium to highly toxic to plants. In phytotoxic tests of CHAÎNEAU *et al.* (1997 and 2000), a fuel oil with 30% aromatics was classified as slightly phytotoxic. Not only the type of oil but also the amount is important to consider in the case of phytotoxicity (BAKER, 1970). The applied crude oil concentration of 5% (oil/soil) is a comparatively high level tolerated by plants. In general, a level of 1% of oil and grease in soil is acknowledged as a practical threshold above which PHC become detrimental to plant life (REIS, 1996). However, different plants can tolerate different concentrations of PHC. For instance, the perennial forage crop alfalfa (*Medicago sativa* L.) demonstrated significant reduction in biomass even at 2% (oil/soil) (WILTSE *et al.*, 1998), oat (*Avena sativa* L.) did not exhibit symptoms of toxicity up to 3% (oil/soil) (SCHWENDINGER, 1968, cited by FRICK *et al.*, 1999) whereas RADWAN *et al.* (1998) reported that the cultivated crop plant *Vicia faba* L. could tolerate up to 10% crude oil in soil.

Possibly, the bad conditions of plants in contaminated soil could have resulted from the uptake of toxic PHC components. According to CUNNINGHAM *et al.* (1996), PHC-absorption by plant is not a significant mechanism. Investigations in maize (*Zea mays* L.) also indicated that PHC translocation into the plant is a minor process (CHAÎNEAU *et al.*, 1997). However, LIN & MENDELSSOHN (1998) demonstrated the ability of PHC uptake in the case of salt marsh plants. WILTSE *et al.* (1998) observed leaf burn in alfalfa growing in crude oil-contaminated soil and suggested that a PHC component was translocated and volatilized through the plant. However, leaf burn was only detected in the first 3 months and gradually disappeared with the progress of the experiment, apparently due to a dissipation of the responsible component. In this study, vetiver transplants in contaminated soil showed similar

symptoms as alfalfa did in the mentioned experiment of WILTSE *et al.* (1998). Uptake of toxic hydrocarbons and their accumulation or volatilization might be a possible cause for the general bad condition. Once in the plant, PHC may damage cell membranes. A reduction of the transpiration rate and photosynthesis and an increase of the respiration rate are often observed, but effects of oil on plants vary depending on epidermal and cellular mechanisms of the species (BAKER, 1970). However, only physiological research may clear the toxic mechanisms of crude oil to vetiver plants.

The toxicity of oil generally decreases with the progress of weathering (BAKER, 1970; WILTSE *et al.* 1998). Vetiver was transplanted 6 to 14 days after preparing the oil-contaminated growth medium. Due to the short time, the weathering process of oil was in its beginning when contacting the transplants. However, within a few days the toxicity may reduce, as demonstrated by COWELL (1969) (cited in LIN & MENDELSSOHN, 1998). He reported that the damage of marsh population was more intensive from oil that reached the vegetation within minutes of the spill than from oil that was at sea for 8 days. The most toxic, low molecular weight hydrocarbons are also the most volatile, which rapidly evaporate. Only higher molecular weight hydrocarbons may remain on site and thus, toxicity may decrease already within only a few days (REIS, 1996). In an experiment by CHAÎNEAU *et al.* (2000), 18% of the initial fuel oil-concentration in soil was volatilized at room temperature during only a few days.

Small size particles (silt and clay) and organic matter in soil may adsorb PHC. Thus, the bioavailability of oil constituents can be reduced, resulting in less toxicity to plants (FRICK *et al.*, 1999). The bioavailability of crude oil components was likely to be high over the course of the experiment. Firstly, the adsorption of PHC to soil particles was considered to be negligible due to the low concentrations of clay (5%) and silt (6%). Secondly, the adsorption of PHC to soil organic matter was assumed to be only slight as well due to the low natural organic content in soil (< 1%). Additionally, 3% of dry leaves were distributed in the medium. However, at the beginning of the experiment leaves were not decomposed. Decomposition rates of organic matter, resulting in polymeric humic fractions with a high adsorption and incorporation potential of PHC components (CUNNINGHAM *et al.*, 1996), depend on the properties of material and the environmental conditions. In general, foliage of deciduous trees, as used in the experiment, are decomposed faster than foliage of coniferous trees but slower than herbs and grasses (GISI *et al.*, 1997). In the experiment, temperature, moisture and pH might have been optimal for microbial activity but the contaminants possibly affected the decomposition processes. Besides, a significant handicap for decomposition was the lack of macro- and mesofauna species such as earthworms, arthropods and enchytraeid worms. In healthy soil ecosystems, organic matter is fragmented and dispersed by their activity, thus enhancing the decomposition activity (BRADY & WEIL, 1999). Under these circumstances, the experimental period

of 6 months was expected to be too short for significantly increasing the humus content in soil by decomposition of the leaves and therefore decreasing the bioavailability of PHC.

In summary, the usage of 5% Boscán crude oil in loamy sand was believed to induce a high level of phytotoxicity. It is possible that the potential herbicidal effects were slightly reduced due to volatilization of the most toxic components within the 2 weeks before transplanting. The high mortality rate of transplants was possibly the effect of an uptake of phytotoxic constituents.

However, vetiver is considered as a highly tolerant species to adverse soil conditions (see 3.3.1). In this study, on average 86% of the vetiver transplants, growing in contaminated soil, were able to produce tillers. Compared to uncontaminated soil, the tiller production ability of transplants was reduced by only 14% despite of the high mortality. Many of the transplants, which reproduced, died later but their tillers could survive and establish in contaminated soil. Thus, the survival strategy of vetiver was asexual reproduction. The tillers in contaminated soil had a low mortality rate (2%) which was even lower than in uncontaminated soil (4%). Moreover, they did not show signs of toxicity on their shoots. But their root structures were changed, compared to samples in uncontaminated soil. Toxic effects of crude oil were believed to be responsible for the reduction of secondary and tertiary branches of the root systems. However, a difference in health conditions between mother plants (transplants) and reproduced tillers was obvious. In part, a rising dissipation of the most toxic oil constituents throughout the course of the experiment which offered better soil conditions to the tillers than to their mother plants, could be an explanation. Another cause might be that vetiver, generally regarded as a tough plant species, is rather weak as propagating material during its early stage of growth (CHOMCHALOW, 2000). Thus, despite of having the same genetic background, the transplants, which needed to acclimatize to the new environment, seemed to be more sensitive to toxic effects than the adapted tillers of their mother plants, which were growing under the polluted soil conditions. In order to increase the chance of survival, YATEEM *et al.* (1999) generally recommend in phytoremediation projects the use of seeds rather than transferring a whole plant. Besides, transplanting is more labor-intensive than seeding (FRICK *et al.*, 1999). However, in the case of vetiver, propagation by seeds is not possible. The non-fertile type of *Vetiveria zizanioides* (South Indian type) (see 3.3.1) usually applied in conservation practice has the great advantage that it does not spread as a weed from seed, and will grow only if planted vegetatively. If it produces seeds, these are not viable. The seeds of the other type of *V. zizanioides* (North Indian type) are fertile but pose a great risk of the species becoming invasive (GREENFIELD, 2000).

In the presence of PHC, the development and growth of tillers was significantly reduced during all periods of the experiment. Compared to uncontaminated soil, reductions of total biomass averaged at around 50%, and plant heights were reduced by approximately 40% in contaminated soil. For instance,

CHAÎNEAU *et al.* (1997) recorded a growth inhibition of 30% in the case of maize on a 1.2% oil-contaminated medium after 110 days. Mortality of plants and reductions in height and biomass are typical reactions caused by oil-contamination (LIN & MENDELSSOHN, 1998). However, growth and vitality of vetiver plants in contaminated soil varied in accordance with the fertilization. Thus, strictly speaking, results of different fertilizer rates in contaminated soils can not be summarized but have to be separately compared to the results in uncontaminated soil. Direct comparisons of results in contaminated and uncontaminated soils are also problematic because the fertilizer rates in uncontaminated soil were much lower than the rates applied in contaminated soil. Nevertheless, comparisons are helpful to show growth trends. In the following section, the differences in growth between the fertilizer levels applied in contaminated soil are described.

### **Effects of Fertilizers on the Development of Vetiver in PHC-contaminated Soil**

Fertilizers increase plant growth in oil-polluted soils in the case of nutrient deficiency (LIN & MENDELSSOHN, 1998; HUTCHINSON *et al.*, 2001). In the 2nd month, vetiver analyses came to significantly higher results for the medium than for the high fertilizer rate in all plant growth parameters (biomass, height, tiller production, clump diameter). Therefore, the medium level of fertilization<sup>15</sup> was more adequate to compensate lack of nitrogen, phosphorus and potassium (NPK) in soil than the high fertilizer level was. Moreover, the high fertilizer rate seems to have affected plant conditions. The reason is that a constant rise of fertilization does not result in a proportional growth of biomass. Growth is always limited by minimum factors which can not be compensated by an improvement of other factors. With rising fertilization, growth increases as well, but then continuously slows down showing a logarithmic course ("Law of Mitscherlich"). Overfertilizing usually leads to yield depressions (GISI *et al.*, 1997). At least in the case of the high fertilizer level, growth reductions and irreversible damage of transplants were considered to be the result of a sum of phytotoxic and overfertilization effects. In the medium fertilizer rate, the accelerated mortality rate of transplants after the 2nd fertilizer application could be an indication of excessive fertilizers, too, although this can not be proved. Therefore, the reduction of vitality and growth in the medium fertilizer level was considered to be mainly caused by phytotoxicity. In the first 2 months, the tillering rates were almost identical in uncontaminated soil and in the medium fertilizer level under contaminated conditions but lower in the high fertilizer level in contaminated soil. Therefore, overfertilization and not phytotoxicity might be the main factor responsible for the reduction of tiller production in contaminated soil at the beginning of the experiment.

Fertilizer applications increase the salinity of the soil solution and therefore decrease the water potential of the soil. For this reason, a nitrogen amount of 2000 mg/kg soil H<sub>2</sub>O should not be

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<sup>15</sup> Until then, 60% of the total fertilization amounts had been applied to both treatments in contaminated soil (Appendix Table 8).

exceeded. Effects of multiple salts are additive. Optimal water management is very important in highly fertilized soils (WALWORTH *et al.*, 1999). In the experiment, fertilizer application and moisture contents were aspired to be in balance. However, at the beginning of the experiment, when plants were establishing themselves in the soil, the percolation rate of irrigation water was observed to be very high, permanently causing water losses and moisture gradients which were rising from the top to the bottom of the soil columns. The transplants' power of resistance were still small and moreover might have been reduced by the toxic effects of the oil. Besides, the root systems were underdeveloped and the water uptake potential might have been reduced. In addition, PHC may also reduce the availability of water (SCHWENDINGER, 1968, cited in FRICK *et al.*, 1999). Therefore, temporary droughts in the soil surface, which could have been supported by unfavorable ratios of high fertilizer concentrations and water shortages, might have caused dehydration of the plants which had exceeded their resistance, therefore resulting in a high mortality of transplants.

However, a visible recovery of plant growth in the high fertilizer level was generally noticeable from the 2nd month onward, mainly as a result of accelerated and successful tiller production<sup>16</sup>. The growth rates in the medium fertilizer level maintained a continuous course during the experiment. Thus, in the 6th month growth in both fertilizer treatments was on the same level with a small but insignificant trend of better growth in the high fertilization. Thus, the efficiency of the applied fertilizers in the high fertilization level increased throughout the course of plant development. At the end of the study, the tiller production was even most effective in the high fertilization rate of contaminated soils. Thus, overfertilization seemed to be no problem anymore.

Based on the results of the plant growth analysis in different fertilization treatments, the following application of fertilizers was concluded to be useful in the case of vetiver growing in oil-polluted soils under tropical climate conditions. For a period of 6 months, a total NPK-concentration, as in the medium fertilization level (Appendix Table 8), is considered to be adequate. To regulate the availability and to avoid losses of nutrients, it is important to split the total fertilizer amounts. 4 to 6 applications with small doses at the beginning and higher doses in accordance to the plant growth are believed to be optimal. The transplants should receive the first fertilizer dosis after an adaptation period to the soil of approximately 2 weeks. At the same time, particularly in the first weeks, special attention should be paid to daily irrigation of the plants in order to avoid drought in the root zones. Maintaining an adequate but not excessive quantity of available nutrients is not an easy task. Analyzing collected leachates in addition to plant growth studies might be helpful to identify optimal fertilizer rates and avoid losses of nutrients in a phytoremediation system.

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<sup>16</sup> Until then, 80% of the total fertilization amounts had been applied to both treatments in contaminated soil (Appendix Table 8).



The soil was medium to slightly acid in all treatments of contaminated soil, whereas in uncontaminated soil pH was almost neutral. Urea and other ammonium fertilizers have the potential of acidifying soil due to microbial nitrification processes (GISI *et al.*, 1997). Indeed, a trend of acidification with applied urea was detectable in all contaminated and uncontaminated treatments. Besides, in contaminated treatments which were fertilized with higher fertilizer rates than uncontaminated soil, the pH decrease was also greater. However, trends of higher average pHs in the high fertilizer level than in the medium were contradictory to the assumption of an acidification process according to the applied urea amount. Thus, urea probably had an influence on the pH value of the soil but was not the only factor responsible. The production of organic acids as intermediates of PHC degradation can also decrease the pH in contaminated soil (LAWLOR *et al.*, 1997). Indeed, pH decrease was more accelerated in contaminated than in uncontaminated soil. Furthermore, acidification increased in the presence of plants. Firstly, plants release protons and organic acids. And secondly, microbial activity is enhanced in the rhizosphere of plants, resulting in increased carbonic acid concentrations in soil (GISI *et al.*, 1997).

High nitrogen and phosphorus concentrations are generally recommended for phytoremediation of contaminated soils (see 4.1.5.2). However, the potential of several negative effects should always be considered when using fertilizers. Whether by depressing the water potential, changing the soil reaction or by diverse interactions with other factors, oversupply of fertilizers can affect the conditions of plants. In addition, excessive fertilization, applied in the field, involves ecological risks with lasting effects. In particular, oversupply of nitrogen is problematic. In the form of nitrate, it can easily move through leaching from soil to groundwater. Furthermore, nitrate and certain other nitrogen containing compounds have the potential to cause toxic effects. Potassium is also easily lost by leaching, much more so than phosphorus. However, all nutrients which are added to aquatic systems contribute to the problem of eutrophication. The risk of eutrophication can be reduced by using oilophilic fertilizers, which are not soluble in water and stay with the oil. This alternative to conventional fertilizers was successfully tested in a bioremediation project of crude oil-contaminated beach soils from the 1989 Exxon Valdez oil spill in Alaska (BRADY & WEIL, 1999).

### **Decreasing Rates of PHC in Soil in the Presence of Vetiver**

Previous results on phytoremediation research indicate that the use of plants has the potential to be an effective method for the clean-up of PHC from soils. The effectiveness of phytoremediation is greatly dependent on environmental conditions (FRICK *et al.*, 1999). Adequate quantities of nutrients, water and oxygen facilitate phytoremediation. Besides, higher temperatures enhance degradation processes (Wright *et al.* 1997, cited in FRICK *et al.*, 1999). For example, the biodegradation of kerosene in a contaminated sandy loam soil reached its maximum rate when the temperature was above 20 °C (Dibble and Bartha, 1979, cited in FRICK *et al.*, 1999). Abundant organic matter or clay in soil may

restrict oil degradation processes due to their binding abilities of contaminants (CUNNINGHAM *et al.*, 1996). In this study, the aim was to maintain optimal nutrient, water and oxygen conditions. However, lateral gas exchange might have been impeded by the plastic bags around the soil media. Due to insufficient perforation, water accumulation at the bottom of the pots was often observed, especially in the first months when the roots did not yet occupy the whole soil volume. Reduced gas exchange and water accumulation can result in a lack of oxygen which affects microbial activity (BRADY & WEIL, 1999) and thus, the microbial degradation of PHC. The temperature averaged 20 °C with fluctuations between 10 and 40 °C and was believed to be optimal for biodegradation processes. Organic matter and clay contents were low in soil. Therefore, bioavailability of the contaminants was not believed to be reduced.

Plants stimulate degradation by several mechanisms of plant-soil interaction: improvement of the physical and chemical properties of contaminated soil, increase of microbial activity and increase of contact between root-associated microorganisms and contaminants in soil (APRILL & SIMS, 1990). Both plants and microorganisms accomplish degradation, either independently or through joint interactions, such as the rhizosphere effect. Up to now, the degradation of PHC compounds by microorganisms in the rhizosphere of plants has been considered as the primary mechanism for oil loss. Direct degradation of PHC by plants has also been suggested (FRICK *et al.*, 1999). Thus, differences in the decreasing rates of oil constituents between vegetated and unvegetated soil were assumed in the vetiver study. However, the differences in total oil and grease (TOG) (see 3.4.4) decrease between vegetated and unvegetated systems were not significant after 6 months (medium fertilization: 15% with plants, 13% without plants; high fertilization: 11% with plants and 12% without plants). Thus, the presence of plants did not result in higher biodegradation rates of PHC. Similarly, in the study of HUTCHINSON *et al.* (2001), differences between treatments with and without plants were not detected after half a year. This was partly attributed to the soil preparation which exposed large contaminant surface areas and enhanced favorable bioremediation conditions such as aeration and nutrient availability. Thus, the biodegradation by microorganisms was rapid in all pots during the first months and probably masked any effects caused by the vegetation. Small roots and shoots had small influences on soil. Later on, the effects of soil processing disappeared with increasing lack of exposed contaminant surfaces and oxygen in unvegetated systems. In vegetated soil, root penetration supported the reduction of aggregate sizes and thus, contaminated surfaces were continually exposed. Besides, roots created macropores which facilitated the transport of gases and liquids, and microbial activity increased in their rhizosphere. Then, biodegradability in vegetated systems became significantly higher than in unvegetated soils. Probably for the same reasons, in this experiment the importance of vetiver for biodegradability of PHC was also low in the first 6 months. Perhaps, it would have increased if the study had been continued. Thus, only long-term experiments with a minimum duration of one year are considered to be useful to evaluate the suitability of vetiver

in phytoremediation of oil-polluted soils. However, even after a one-year period of growing no significant decrease of oil was detectable in the presence of vetiver in an experiment of CUNNINGHAM *et al.* (1996).

As already mentioned, after 6 months the decrease rates of TOG were 15% in the medium and 11% in the high fertilizer level, both in the presence of vetiver. They can be considered as rather small in comparison to other experiments. A phytoremediation study of alfalfa (*Medicago sativa* L.) resulted in degradation rates of 28% after 6 months of growth (WILTSE *et al.*, 1998). YATEEM *et al.* (1999) reported a reduction in total petroleum hydrocarbons (TPH) (see 3.4.4) of 37%, 36% and 24% in vegetated soil (*Vicia faba* L., *Medicago sativa* L., *Lolium perenne* L.) after 7 months. Experiments with bermuda grass (*Cynodon dactylon* (L.) Pers.) and tall fescue (*Festuca arundinacea* Schreb.) resulted in an average of 49% TPH degradation within half a year (HUTCHINSON *et al.*, 2001). However, the results are not directly comparable because different parameters were used (TOG and TPH). Whereas TOG include polar compounds such as asphaltenes and resins, those are excluded in TPH (US-EPA, 2001). Consistent uses of standard methods and precise definitions of target parameters as TPH would be helpful in order to compare results of different studies within the phytoremediation research.

Interestingly, the final decreasing rates of all treatments were approximately the same as the rates which were found after 2 months of growing. It can be supposed that PHC was degraded mostly in the first 2 months. Thus, only the volatile and rapidly biodegradable PHC fraction might have been reduced. Especially aliphatic, low molecular weight compounds tend to be volatile and more than aromatics do (see 3.4.2). The effect of volatilization on reducing initial oil concentrations in soil can be significant. For instance, CHAÎNEAU *et al.* (2000) reported a decrease of 18% by volatilization. PHC-degradations from the 2nd to the 6th month of the study were negligible. Thus, degradation processes of aromatics and polars, which are regarded as the most resistant components in PHC to biodegradability (LEÓN *et al.*, 1998), seemed to be improbable. However, only quality analyses (SARA, see 3.4.4), which were not possible to realize in this study, can determine the biodegradability of the individual components of the applied crude oil.

High levels of fertilizers in the presence of *Juncus roemerianus* Scheele reduced residual oil concentrations in soil more than low levels of fertilizers (LIN & MENDELSSOHN, 1999). In this study, a significant response in degradation to the difference in fertilizer concentrations could not be detected. Thus, the medium fertilizer level seemed to be sufficient to meet the demands of plants and microorganisms on NPK. Higher nutrient levels were not necessary in order to increase PHC degradations.

The TOG decrease in soil demonstrated a discontinuous course in all treatments, and for this a method error was possibly the responsible factor. Perhaps, manual mixing of the large soil amounts of each greenhouse pot could not guarantee homogeneous compounds. Especially firm crude oil aggregates in the soil were not reduced in size by the mixing process and thus, heterogeneity was increased. Therefore, if possible, mechanical mixers should be used to facilitate homogenizing processes of soil media, as successfully applied in the experiments of APRILL & SIMS (1990) and HUTCHINSON *et al.*, (2001). Furthermore, mincing soil samples before analyzing the TOG content would be advisable to minimize aggregate sizes and optimize extraction processes, therefore reducing variations in TOG between replicates of the same soil sample. If mechanical mixing and mincing are not possible, the number of replicates per soil sample should be increased.

A correlation between root biomass and TOG decrease during the period of 6 months could not be proved in the medium fertilizer level or in the high fertilizer level of contaminated soil ( $r = 0,05$  and  $-0.19$  respectively). Thus, the root biomass had no effect on the TOG decrease. However, the root structure is considered just as important as the root biomass concerning degradation processes (WILTSE *et al.*, 1998). Especially, fibrous root systems with large surface areas, creating large rhizosphere volumes, are favorable for the use in phytoremediation (see 3.2). As already mentioned, the root surface and rhizosphere volume of vetiver were reduced in contaminated soil. An impairment of the root surface area by PHC in soil was also detected in the case of switchgrass (*Panicum virgatum* L.) (BANKS *et al.*, 2000). Reduced root surfaces result in smaller rhizosphere volumes, thus effects of plant on PHC-degradation might be small as well. Thus, the impact on the root surface of vetiver by PHC was probably a responsible factor for not finding a successful influence on degradation by the species.

According to SIRIPIN (2000), vetiver studies revealed in a high soil microbial biodiversity in the rhizosphere of the plant. Among others, associations with nitrogen-fixing bacteria, phosphate-solubilizing microbes and mycorrhizal fungi were found which may explain the survival ability of vetiver under adverse conditions. However, soil conditions can affect microbial populations. An interesting observation in this study was the apparent reduction of the typical aroma of vetiver roots (see 3.3.2), which were growing in crude oil-contaminated soils. This probably indicated a metabolism change of vetiver under the toxic effects. Root exudates control the quality and quantity of microbial populations in the rhizosphere (GISI *et al.*, 1997). Thus, if the vetiver metabolism changes, this may also have dramatical effects on the microorganisms. In the reverse case, microorganisms also have a big influence on the conditions of plants (GISI *et al.*, 1997). Plant-microbe-interactions demonstrate a very high complexity. Microbial investigations of the rhizosphere soil could not be realized in this study but whenever possible, they should be included in phytoremedial investigations of plant species.

## 7 CONCLUSIONS AND RECOMMENDATIONS

### **Are transplanted vetiver plants capable of surviving in PHC-contaminated soil? How are their growth rates in comparison with plants in uncontaminated soil?**

Vetiver transplants were affected by the used heavy crude oil which was considered to be medium to highly phytotoxic and highly bioavailable in loamy sand. However, the plant's reproducing ability was not much diminished. Moreover, it demonstrated an improvement in the course of the experiment which resulted even in a higher tiller production in contaminated than in uncontaminated soil after 6 months. This can be interpreted as a survival strategy of the vetiver plant, growing under unfavorable conditions, or as a response to the higher fertilization rates, applied in contaminated soil. The produced tillers in contaminated soil did not show signs of toxicity on their shoots but their root structures were changed due to a reduction of secondary and tertiary branches. Growth was reduced at averaged 50% and height at averaged 40% in contaminated soil. Vetiver proved a capability of surviving in PHC-contaminated soil and therefore, was considered as a tolerant plant species to crude oil in soil.

### **Which fertilizer concentration has the best effect on the development of vetiver plants growing in PHC-contaminated soil ?**

During the first 2 months, the medium fertilizer level seemed to be adequate to compensate lacks of nitrogen, phosphorus and potassium in soil. The high fertilizer level affected, additionally to the phytotoxic effects of crude oil, the vitality of transplants, the tiller production and growth of tillers. Overfertilization was supposed to be the reason. However, a recovery of growth in the high fertilizer level was observed in the course of time. At the end, biomass and plant height in both fertilizer treatments were on the same level. Therefore, low applications of fertilizers at the beginning and increasing fertilizer rates with plant development should be useful to support the development of vetiver plants in contaminated soil. The total fertilizer concentration should be orientated according to the medium level of fertilizers applied in this study.

### **What is the maximal decreasing rate of PHC in soil that is attainable when using vetiver as a phytoremedial application during a specific time ?**

The presence of vetiver plants did not result in higher PHC biodegradation rates in soil. After 6 months, the TOG content decreased at 15% in the medium and at 11% in the high fertilizer level, and the differences to unplanted controls were not significant. Interestingly, the final decreasing rates were not higher than the decreasing rates which were detected at 2 months. Probably, mostly the volatile

aliphatic and less the aromatic and polar fractions of the crude oil were degraded. A correlation between root biomass and TOG decrease was not found, supporting the detection that vetiver had no influence on the PHC degradation in soil.

### **Does vetiver show a potential for the use in phytoremediation of PHC-contaminated soils in Venezuela ?**

The study demonstrated that vetiver is not qualified for the use in phyto- or rhizodegradation (see 3.2) to reduce heavy crude oil at a concentration of 5% in sand. However, more research of the species under the influence of different oil conditions and agronomic performance for prolonged periods is necessary in order to safely prove or disprove the vetiver's potential to enhance petrochemical biodegradation. Nevertheless, it seems doubtful whether further research of vetiver in phyto- or rhizodegradation is useful. Neither in this study nor in a study of CUNNINGHAM *et al.* (1996) a significant decrease of PHC in the presence of vetiver was detectable. Therefore, an investigation of other promising plant species seems to be more advisable.

However, vetiver might be useful for alternative applications on oil-contaminated sites in Venezuela. An effective application of vetiver plants on oil-contaminated soils might be their use as organic pumps (see 3.2) to reduce the loss of contaminants with draining water. Especially during the rainy season in Venezuela, which lasts from April/May to October/November (SNOW, 1976), water percolation and the risk of PHC downward migration from contaminated sites can be increased. Furthermore, the water-holding capacity of the soil is reduced in the presence of oil-contamination (see 4.1.5.1), due to the hydrophobic properties of crude oil (CHAÎNEAU *et al.*, 1997) and thus, supporting water draining effects. On the other hand, hydrophobic PHC-constituents have low leaching potentials (APRILL & SIMS, 1990). A study of prairie grasses by APRILL & Sims (1990) demonstrated that water loss due to percolation was significantly higher in unvegetated than in vegetated systems, and differences increased with the development of the plants. Plants removed a lot of water from soil and therefore, reduced the risk of percolation and downward movement of dissolved contaminants. Similarly in this study, vegetated pots with vetiver also consumed a lot more irrigation water than unvegetated pots did. Transpiration is the force of the "organic pump mechanism" (APRILL & Sims (1990). It is caused by gradients of water potential between plants and atmosphere and therefore increases under low humidity and high temperatures (NULTSCH, 1991). The organic pump-mechanism might be very effective in the tropical climate in Venezuela, where mean annual values in temperature are between 18 and 32 °C (in elevations < 1000m) (SNOW, 1976).

The use of vetiver in order to control soil erosion on oil-contaminated sites in Venezuela seems also to be a promising use. Vegetation covering generally improves soil stabilization, thus preventing the

distribution of contaminated soil particles by water and wind (FRICK *et al.*, 1999). Vetiver is a highly qualified plant for erosion practices in tropical and subtropical regions. Its use in erosion control has already been successfully tested (see 3.3.2) and promoted in Venezuela (YÉPEZ, 2002)<sup>17</sup>.

Furthermore, vetiver plants can be beneficial for initiating the revegetation of PHC-contaminated sites. As a tolerant species to oil, it can ameliorate polluted soil to allow other, less hardy species, to get established. An experiment of CUNNINGHAM *et al.* (1996) indicated that vetiver survived in a 3% TPH-contaminated clay, which was initially extremely phytotoxic to a variety of plants. After a one-year period of growing, several crop species could be cultivated together with vetiver, even though a significant decrease of the contaminants was not detectable. Apparently, plant-produced physicochemical effects were sufficient to reduce the toxic environmental effects and improve the soil as a growth medium. In this experiment, vetiver survived even in a 5% TOG-contaminated soil and therefore, is highly promising for amelioration.

Stabilization mechanisms by using vetiver or other plants can reduce the bioavailability and distribution of oil constituents in soil (FRICK *et al.*, 1999). However, risks of spreading and affecting other organisms and the environment remain. Thus, contaminant stabilization is only recommendable at shallow contaminated sites with slightly toxic components. In the case of highly toxic substances in high concentrations, remediation methods which remove the pollutants seem to be preferable. Anyway, the tolerance of vetiver can be exceeded in the presence of extreme contamination. Furthermore, unfavorable side effects possibly may occur. For example, if PHC are taken up by plants, the potential of biomagnification (food chain contamination) must be carefully considered (CUNNINGHAM *et al.*, 1996). Furthermore, the volatilization of toxic oil compounds can be enhanced if plants translocate and transpire them through stems and leaves, as demonstrated by WILTSE *et al.* (1998). This may reduce the amount of contaminants available in soil, but may implicate subsequent contamination of the atmosphere (FRICK *et al.*, 1999).

Evaluating the extent of contamination and choosing adequate remediation methods demand a high degree of responsibility. Having a good knowledge of the interactions between contaminants, plants, microorganisms and the environment is the basis for decisions in soil rehabilitation. Information gaps should be closed by further research. As a result, the efficiency of remediation technologies can be improved, therefore preventing threats to human health and damage to the ecosystem induced by contamination.

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## 8 SUMMARY

Venezuela is one of the largest oil-producers in the world. The exploitation of oil impacts upon the environment, namely by drilling and production processes, refinery activities and accidental oil spills. Since the enactment of strict environmental laws, especially in the early 1990s, research in preventing and recuperating oil pollution became an integral part of the petroleum industry activities in Venezuela. For the rehabilitation of oil-contaminated sites, engineering techniques based on physical and chemical mechanisms are conventionally used but a growing interest in biological-based methods is noticeable in Venezuela and all over the world. Phytoremediation represents a promising alternative technology. This method is based on the use of plants and their associated microorganisms to remove or contain organic or inorganic contaminants present in soil or water.

Within the research on phytoremediation in Intevep, the Research and Technological Support Center of the national oil company PDVSA, a greenhouse experiment was conducted. Vetiver grass (*Vetiveria zizanioides* (L.) Nash) was chosen as the experimental plant. The 6-month study aimed to determine the tolerance of vetiver to a Venezuelan heavy crude oil in soil, which is considered to be highly phytotoxic. Additionally, the potential for stimulating biodegradation processes of PHC was tested. The methods used were based on comparisons in plant growth and soil parameters between different treatments.

Vetiver transplants suffered under the influence of crude oil. However, most of them demonstrated the ability of asexual reproduction. At the beginning, tiller production was higher in uncontaminated soil but later on was more successful under oil-polluted soil conditions. With increasing numbers of tillers in contaminated soil, differences in clump diameter between the treatments decreased and lost their significance after 6 months of growing. Despite significantly reduced biomass and heights during all phases of the experiment, the tillers did not exhibit signs of toxicity on their shoots in the presence of contaminants. However, under the influence of contaminants, the root structure was changed and the surface area reduced. As to the different fertilizations in contaminated soil, growth was more successful in the medium than in the high fertilizer level at the beginning, but in the course of the experiment, plant growth achieved a similar development level. In summary, vetiver was found to be a tolerant species concerning the toxic effects of crude oil in soil. Concerning the total oil and grease content in soil, the degradation rates were considered as rather small in all treatments. No significant increase in biodegradation in the presence of vetiver plants was detected. Thus, vetiver was deemed to be unsuitable for facilitating biodegradation of crude oil in soil.



However, vetiver is a beneficial plant in soil and water conservation practice and is also considered as applicable to petroleum-contaminated sites in Venezuela. Promising uses of vetiver are for amelioration of oil-polluted soils, as an “organic pump” or for erosion control to prevent petroleum contaminants from spreading.

In further plant selections for phytoremediation of oil-contaminated soil it is generally advisable to test the species under different oil conditions and diverse agronomic performances. In addition, advanced studies of plant physiology and rhizospheric microbial activity in the presence of PHC are useful in order to get a better knowledge about the species in the system of contaminants, microorganisms and environment.

## 9 ZUSAMMENFASSUNG

Venezuela ist eines der wichtigsten erdölproduzierenden Länder der Welt. Die Ölindustrie bringt durch ihre Bohr- und Förderungsprozesse sowie Raffinerietätigkeiten und unfallbedingte Ölausschüttungen schädigende Umwelteinwirkungen mit sich. Seit dem Erlass strenger Umweltvorschriften, insbesondere in den frühen 1990er Jahren, wurde die Forschung zur Vorsorge und Beseitigung von ölbedingten Verschmutzungen ein fester Bestandteil der venezolanischen Erdölindustrie. Zur Behandlung von ölverschmutzten Flächen werden üblicherweise auf physikalischen und chemischen Methoden basierende Ingenieurstechniken genutzt. Allerdings ist ein wachsendes Interesse an biologisch basierten Methoden sowohl in Venezuela als auch weltweit erkennbar. Phytoremediation stellt eine erfolgsversprechende alternative Technologie dar. Die Methode basiert auf der Nutzung von Pflanzen und ihren assoziierten Mikroorganismen zur Beseitigung, Stabilisierung und Umwandlung von organischen und anorganischen Schadstoffen in Wasser und Boden.

Im Rahmen der Phytoremediationsforschung von Intevop, dem Forschungsinstitut der venezolanischen Ölfirma PDVSA, wurde ein Gewächshausversuch durchgeführt. Vetivergras (*Vetiveria zizanioides* (L.) Nash) aus der Familie der Poaceen wurde als Versuchspflanze gewählt. Die sechs Monate dauernde Studie zielte auf eine Bestimmung der Toleranz von Vetiver bezüglich eines venezolanischen Schweröls im Boden, welches als hoch pflanzenschädigend gilt. Zudem wurde das Stimulierungspotential natürlicher Abbauprozesse von Erdölkohlenwasserstoffen getestet. Die Methode basierte auf vergleichenden Untersuchungen von Pflanzenwuchs und Bodenparametern unter verschiedenen Einflüssen.

Vetiver wurde unter dem Einfluss von Rohöl geschädigt. Trotzdem zeigten die meisten der geschädigten Pflanzen die Fähigkeit zur vegetativen Vermehrung. Zunächst war die Produktion von Ablegern höher im unverschmutzten Boden, aber schließlich wurde sie produktiver unter dem Einfluss der Erdölverschmutzung. Mit einer steigenden Ablegeranzahl im verschmutzten Boden wurde der Unterschied im Durchmesser, verglichen mit Pflanzenproben im unverschmutzten Boden, ebenfalls kleiner und verlor schließlich nach sechs Monaten seine Signifikanz. Trotz deutlich reduzierter Biomasse und Wuchshöhen während allen experimentellen Phasen zeigten die Ableger in Gegenwart von Schadstoffen keine Schädigungen durch giftige Einwirkungen an oberirdischen Pflanzenteilen. Die Wurzelstruktur wurde jedoch unter dem Einfluss von Schadstoffen verändert und die Wurzeloberfläche reduziert. Hinsichtlich unterschiedlicher Düngungen im verschmutzten Boden war anfangs unter mittlerer Düngekonzentration ein erfolgreicherer Wachstum als unter hoher Düngekonzentration festzustellen. Doch im Laufe des Experiments erreichte das Pflanzenwachstum

unter beiden Düngebehandlungen einen ähnlichen Entwicklungsstand. Nach Zusammenfassung der Ergebnisse kann man Vetiver als tolerant gegenüber toxischen Einwirkungen von Rohöl im Boden einstufen. Bezüglich des Öl- und Fettgehalts im Boden wurden deren Abbauraten unter sämtlichen Behandlungsbedingungen als relativ klein bewertet. In der Gegenwart von Vetiver wurde keine signifikante Aktivierung von natürlichen Abbauprozessen festgestellt. Deshalb kann Vetiver als ungeeignet für eine Stimulierung von Rohölabbau im Boden betrachtet werden.

Vetiver gilt allgemein als eine nutzbringende Pflanze im Boden- und Wasserschutz und kann diesbezüglich auch auf ölverschmutzten Flächen eingesetzt werden. Eine erfolgsversprechende Nutzung von Vetiver in der Melioration von ölkontaminierten Böden wäre somit möglich. Vetiverpflanzen als „organische Pumpsysteme“ oder im Erosionsschutz zur Verhinderung von Schadstoffausbreitung könnten weitere erfolgreiche Anwendungen sein.

Allgemein ist bei der Auswahl von Pflanzenarten zur Anwendung in der Phytoremediation von ölverschmutzten Böden eine vergleichende Untersuchung unter dem Einfluss verschiedener Zusammensetzungen und Konzentrationen von Erdölkohlenwasserstoffen sowie diverser landwirtschaftlicher Maßnahmen empfehlenswert. Zusätzlich sind weiterführende Studien über Pflanzenphysiologie und Rhizosphärenprozesse in der Gegenwart von Erdölkohlenwasserstoffen hilfreich für eine Erweiterung der Kenntnisse der jeweiligen Pflanzenart in Wechselwirkung mit Schadstoffen, Mikroorganismen und Umwelt.

## 10 RESUMEN

Venezuela es uno de los países del mundo con mayor producción de petróleo. La explotación del crudo ha causado diversos impactos ambientales especialmente por los procesos de perforación, producción, refinación y los derrames accidentales. Desde el decreto de estrictas leyes ambientales, especialmente en los años noventa del siglo pasado, la investigación, la prevención y la recuperación de áreas contaminadas de crudo han sido una parte integral de la industria petrolera de Venezuela. Para la recuperación de suelos contaminados de petróleo se utilizaban tradicionalmente técnicas químicas y físicas, pero hay también un interés creciente en la utilización de métodos biológicos tanto en Venezuela como en el resto del mundo. Fitorremediación es una de las más eficaces tecnologías alternativas. Este método usa plantas y microorganismos asociados para la eliminación, estabilización y modificación de contaminantes orgánicos e inorgánicos en el agua y el suelo.

Dentro de las actividades de investigación de la fitorremediación en Intevep, el cual es el instituto de tecnología de la compañía venezolana de petróleo (PDVSA), se conducía un experimento a nivel de invernadero. La Poácea vetiver (*Vetiveria zizanioides* (L.) Nash) se seleccionó como planta experimental. El ensayo, que duró seis meses, apuntaba a la determinación de la tolerancia de vetiver a un crudo pesado fitotóxico en el suelo. Además, se estudió el potencial de la planta para estimular la biodegradación de hidrocarburos de petróleo en el suelo. El método se basó en la comparación del crecimiento de las plantas y los parámetros del suelo con diferentes tratamientos.

El crudo pesado dañaba al vetiver; pero, las plantas desarrollaron la capacidad de reproducirse vegetativamente. Primero, la producción de hijos fue mayor en el suelo sin contaminantes y luego, fue más eficiente bajo la influencia del petróleo. Con un creciente número de hijos en el suelo contaminado se disminuía la diferencia en el diámetro de plantas en el suelo contaminado y sin contaminantes hasta que al final del experimento se perdió su importancia. Durante todo el estudio las biomásas y alturas de las plantas que crecieron en el suelo contaminado eran significativamente reducidas. Sin embargo, no hubo señales de efectos tóxicos en las partes aéreas de los hijos de vetiver; pero las estructuras de las raíces sufrieron modificaciones y las superficies de las raíces se redujeron por la presencia de contaminantes en el suelo. En cuanto a los diferentes tratamientos de fertilizantes en el suelo contaminado se observó un mayor crecimiento en el tratamiento del nivel medio que en el tratamiento de fertilizantes del nivel superior. Con el tiempo el crecimiento de las plantas había logrado el mismo nivel de desarrollo en ambos tratamientos de fertilizantes. Resumiendo los resultados se puede evaluar la especie vetiver como una planta tolerante a los efectos tóxicos de crudo pesado en el suelo. En cuanto a los contenidos de aceite y grasa en el suelo se registraron niveles de biodegradación muy bajos en todos los tratamientos de crudo pesado. Tampoco hubo una actividad

biodegradable de los hidrocarburos significativamente excesiva en la presencia de vetiver. Por eso se evaluó el vetiver como una planta inadecuada para estimular la biodegradación del crudo pesado en el suelo.

Por lo general, el vetiver es una planta muy útil en la conservación del suelo y el agua. Con respecto a esto, se descubrió que sería apropiada también para aplicarla en sitios contaminados de petróleo. Por lo tanto, sería posible utilizar eficazmente la especie vetiver en la melioración de los suelos contaminados por el crudo. Es por ello que las plantas de vetiver podrían ser utilizadas como “bombas orgánicas”, o en el control de la erosión para prevenir la distribución de contaminantes.

Para la selección de plantas adecuadas en la fitorremediación del suelo contaminado de petróleo generalmente se pudieran recomendar ensayos con diversos tratamientos de crudo y manejos agrícolas. Estudios avanzados de la fisiología de plantas y procesos de la rizósfera en la presencia de hidrocarburos serían muy útiles para ampliar los conocimientos de las especies en la interacción con contaminantes, microorganismos y el ambiente.

## 11 EPILOG

The investigation of remediation methods within the industrial sectors is a very important challenge to reduce risks to health and environment from polluted sites. However, despite these necessary efforts it should not be forgotten that the recuperation of destruction is only a treatment of preceding faults. The true intentions should be focussed on **preventing** environmental impacts. In spite of a growing environmental awareness in many parts of the world during the last few decades, the world-wide devastation of nature has still continued. The importance of an intact environment is still undervalued, and decisions in favor of the environment are usually on the losing side when competing with other interests. Thus, environmental considerations are still far away from the position they should actually take in economy, politics and everybody's life all over the world. Drastic changes in mind at global level may be necessary in order to really satisfy the demands of environmental protection. Especially, powerful industrial sectors, such as the oil industry in Venezuela must act with responsibility in the balancing act between economic enrichment and nature exploitation.

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## 14 GLOSSARY

**Absorption:** The process of one substance actually penetrating into the structure of another substance.

**Adsorption:** Retention of a molecule or molecules on a surface. Adsorption is a physical process which occurs without chemical reaction.

**Aerobic:** In the presence of oxygen.

**Anaerobic:** In the absence of oxygen.

**Bioavailability:** The extent to which a contaminant is available to living things. Lipophilic compounds tend to bind to soil organic matter or soil particles (particularly clay) and are, therefore, typically less available to biota than water-soluble compounds.

**Biodegradation:** The breakdown of organic substances by microorganisms.

**Biomagnification:** Increasing concentrations of a chemical at increasing levels of the food chain due to the consecutive consumption of contaminated, lower trophic-level biota by higher trophic level biota. For example, consumption of grass contaminated with cadmium or methyl mercury will increase concentrations of these contaminants in cattle that feed on the grass.

**Bioremediation:** The use of microorganisms to reclaim soil and water contaminated by hazardous substances.

**Bioventing:** Pumping air into the soil above the water table to provide oxygen to aerobic bacteria.

**Clump:** A cluster of tillers developed originally from a mother plant.

**Consortium:** An interacting group of different microbes that generally result in combined metabolic activities.

**Containment:** In phytoremediation, containment involves using plants to reduce or eliminate the availability of contaminants to other biota. Contaminants are not necessarily degraded when they are contained.

**Culm:** The above-ground part of the stem of a grass.

**Degradation:** The breakdown of a compound into different compounds.

**Enzymes:** Proteins that act as biological catalysts. These chemicals produced by living organisms bring about the digestion (breakdown) of organic molecules into smaller units that can be used by living cell tissues.

**Evapotranspiration:** The combined loss of water from a given area, during a specific time-period, by evaporation from the soil surface and transpiration by plants.

**Ex situ:** Involving excavation or extraction of contaminated soil or water. May involve transport of contaminated material away from the contaminated site, but not necessarily.

**Fibrous root:** Plant roots with many secondary and tertiary branches spreading out from the primary branch.

**Humification:** In phytoremediation, incorporation of the contaminants into soil humus resulting in lower bioavailability.

**Hydrocarbons:** A large class of liquid, solid or gaseous organic compounds, containing only carbon and hydrogen, which are the basis of almost all petroleum products  $\Rightarrow$  petroleum hydrocarbons (PHC).

**Hydrophilic:** Molecules and surfaces that have a strong affinity for water molecules. These molecules tend to be polar in chemical structure.

**Hydrophobic:** Molecules and surfaces that have little to no affinity for water, and typically have more affinity for other hydrophobic substances than for water. These molecules tend to be bipolar or non-polar in chemical structure, such as lipids.

**In situ:** Treatment at the site of contamination without excavation.

**Landfarming:** Spreading contaminated soil thinly over land or a pad with a leachate-collection system.

**Leachate:** Liquids that have moved downward through the soil and that contain substances in solution or suspension.

**Legumes:** A plant whose roots serve as hosts for nitrogen-fixing bacteria, which are in a symbiotic relationship with the plant.

**Lipophilic:** Molecules that are preferentially soluble in lipids or non-polar solvents.

**Metabolites:** The chemical products of changes to a parent molecule, including chemicals participating in metabolism, which is the total of all chemical reactions by which energy is provided for vital processes and new cell substances are assimilated.

**Microorganisms:** Includes bacteria, algae, fungi and viruses.

**Mineralization:** The ultimate degradation and recycling of an organic molecule into inorganic materials, such as carbon dioxide and water. In phytoremediation, the mineralization or metabolism of contaminants within plant tissue is also referred to as **phytodegradation**.

**Natural attenuation:** A reclamation approach that relies on natural processes to remediate sites with no human intervention. The natural processes include physical / chemical mechanisms such as dilution, dispersion and adsorption of the contaminant. Biological processes, such as the unassisted growth of plants and microbial communities that break down contaminants, may be involved as well.

**Nutrients:** Elements or compounds essential as raw materials for organism growth and development. Nitrogen, phosphorus, potassium and numerous other mineral elements are essential plant nutrients.

**Octanol-water partition coefficient (log  $K_{ow}$ ):** A measure of a chemical's affinity for water versus lipids or fats. A higher  $K_{ow}$  indicates a greater affinity for lipids than water.

**Organic matter:** In soil, the organic fraction.

**Parts per million (ppm):** A measure of proportion by weight which is equivalent to one unit weight of solute (dissolved substance) per million unit weights of the solution (mg/kg). One liter of water weighs one million milligrams, and one ppm is equal to one milligram per liter (mg/L) for water analysis.

**Organic pump:** Uptake of large quantities of water by plant roots and translocation into the atmosphere to reduce a flow of water. Used to keep contaminated groundwater from reaching a body of water or to keep surface water from seeping into a capped landfill and forming leachate.

**Petroleum:** A naturally occurring mixture composed predominantly of hydrocarbons in the gaseous, liquid or solid phase.

**Phytoaccumulation:** See **phytoextraction**.

**Phytodegradation:** In phytoremediation, plants take up the contaminant and metabolize it to environmentally benign material.

**Phytoextraction:** In phytoremediation, absorption of the contaminant into the plant tissue. Also referred to as **phytoaccumulation**.

**Phytoremediation:** Use of plants to remediate contaminated soil, sediments, surface water or groundwater.

**Phytostabilization:** Use of soil amendments and plants to reduce bioavailability and offsite migration of contaminants.

**Phytotoxicity:** Toxicity in plants.

**Phytovolatilization:** In phytoremediation, refers to the movement of a contaminant out of the soil, into, through and out of a plant, and then into the atmosphere. Also referred to as **transfer** of the contaminants.

**Rhizodegradation:** In phytoremediation, plant roots, their associated microorganisms and excreted products destroy the contaminant in the root zone.

**Rhizofiltration:** Uptake of contaminants by the roots of plants immersed in water. When the roots are saturated with contaminants, they are harvested.

**Rhizosphere:** The surface of plant roots and the region of soil directly surrounding the roots where microbial populations are affected by the presence of the roots.

**Rhizosphere effect:** The direct effect of plant roots and their exudates on microorganisms, including the fact that microbial populations are usually larger within the rhizosphere than in the root-free soil.

**Root exudates:** The compounds that come out of the plant roots and go into the rhizosphere.

**Soil structure:** The combination or arrangement of primary soil particles into secondary units or peds, with secondary units being classified on the basis of size, shape and grade.

**Soil texture:** The relative proportions of sand, silt and clay in soil.

**Tiller:** A shoot growing from the base of the stem of a plant

**TOG:** Total oil and grease.

**Toxic substances:** Chemical elements and compounds that have toxic (poisonous) properties when exposure by ingestion, inhalation or absorption into the organism occurs. There is a large variation in the degree of toxicity among toxic substances and in the exposure levels that induce toxicity.

**TPH:** Total petroleum hydrocarbons.

**Transfer:** In phytoremediation, see **phytovolatilization**.

**Transformation:** Change or modification.

**Translocation:** Movement to a different place. Cellular transport through the plant vascular system (xylem) from roots to other plant tissues: roots ⇔ shoots ⇔ branches ⇔ leaves.

**Transpiration:** The loss of water vapor from plants primarily through pores (stomata) in the leaves.

**Volatilization:** Transfer of a chemical into the atmosphere as a gas or vapor.

**Weathering:** In phytoremediation, the selective reduction of easily-degradable contaminants in soil due to natural processes. In general, the action of external factors such as rain, frost, sun or wind on the Earth's surface.

(CHOMCHALOW, 2000; CUNNINGHAM *et al.*, 1996; FRICK *et al.*, 1999; ITRC, 1999; PETROLEUM COMMUNICATION FOUNDATION, 2003)

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Münster, 07.05.2003

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