

Research Articles

Phytotoxicity of Fresh and Weathered Diesel and Gasoline to Willow and Poplar Trees

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Abstract. The toxicity of fresh and weathered gasoline and diesel fuel to willow and poplar trees was studied using a tree transpiration toxicity test. Soils were taken from an abandoned filling station. Concentrations in the samples were measured as the sum of hydrocarbons from C₅ to C₁₀ (gasoline) and C₁₂ to C₂₈ (diesel). Concentrations ranged from 145 to 921 mg/kg gasoline and 143 to 18231 mg/kg diesel. The correlation between log soil concentration and toxicity to willows (*Salix viminalis* x *schwerinii*) was highly significant for the diesel fraction (r²=0.81, n=19) and for the sum of hydrocarbons (r²=0.84, n=19). The EC₅₀ (50% inhibition of transpiration) for the sum of hydrocarbons was determined at 3910 mg/kg (95% C.I., 2900 to 5270 mg/kg) and followed a log-normally distributed sigmoidal curve. The EC₁₀ was 810 mg/kg (95% C.I., 396 to 1660 mg/kg). The results were verified with artificially mixed diesel and gasoline contaminated soils, and two willow and one poplar species (*S. viminalis*, *S. alba* and *Populus nigra*). Fresh diesel at about 1000 mg/kg showed no effect on *S. alba*, although *P. nigra* was more sensitive. 10000 mg/kg seriously affected the transpiration of all species, silver willow (*S. alba*) being the least sensitive. Free phase diesel killed all trees within six weeks. Fresh gasoline at 1000 mg/kg was deadly for all trees, hence was more toxic than weathered gasoline. Survival of poplars and willows planted at the abandoned filling station was compared to the laboratory findings. There was some correlation, but in the field, trees also suffered from other stress factors than fuel pollution.

Keywords: Diesel; gasoline; phytoremediation; poplars; toxicity; soil, pollution; willows

study of Banks et al. (cited in Erickson et al. 2000) investigated the reduction of petroleum hydrocarbons on the Persian Gulf coast, ryegrass (*Lolium*) being the most promising option. Biodegradation of jet fuel (JP-8), from C₆ to C₁₈-hydrocarbons, was studied in the presence of vegetation by Karthikeyan et al. (1999).

Aside from grasses, trees of the family *Salicaceae* (poplar and willow) are often used (Schnoor 1997). These trees grow fast, possess a deep rooting ability and transpire large amounts of water. Some species can stand flooding and possess an air transport system to aerate the root zone (Grosse et al. 1996). About 350 different willow species are known. This genus is distributed world wide except for some islands in South-East Asia and Australia (omnicyber 2001). Willows, like poplars, can easily be interbred to give new hybrids with selected properties, e.g. for generating sustainable energy.

In 1999, an in-situ phytoremediation was started at a former gas filling station in Axerved, Rønnede, Denmark. The gas station had operated from 1956 to 1990. The polluted site, in total 1400 m², is located in an area with sensitivity for the drinking water supply. The soil is polluted with up to 20,000 mg/kg gasoline and diesel compounds to a depth of 3 m. Stones were removed, the area was fertilized with 40 tons of chicken manure and harrowed before, in April 1999, 2500 willow (*Salix schwerinii* x *viminalis*) and 500 poplar (*Populus trichocarpa*) cuttings were planted out. At that time, no quantitative data had been available on the phytotoxicity of diesel and gasoline to willow and poplar. Laboratory experiments were conducted to fill this gap.

1.1 Properties of diesel and gasoline compounds

Typical pollutants in the soil of abandoned gasoline stations are the residues of gasoline and diesel fuels. Gasoline is composed of hydrocarbons, mainly alkanes and cycloalkanes (35 to 65 mass%) and monoaromates (25 to 42 mass%), with a molar weight between 44 g/mol (propane, C₃) and 142 g/mol (decane, C₁₀) (ARAL 2001). During weathering in soil, a shift of composition due to microbial activity, leaching and volatilization is usually observed. Diesel is mainly composed of alkanes (40 to 70 mass%), cycloalkanes (10 to 25 mass%), alkenes (up to 5%) and aromates (10 to 30%). The latter

1 Introduction

Phytoremediation is a technique for cleaning polluted soils by use of vegetation. For organic pollutants, it is most frequently used for petroleum products (US-EPA 2000). Erickson et al. (2000) provided an overview of recent phytoremediation projects of petroleum. One example was the clean-up of an olefin production area (Qui et al. 1997), where a variety of millet *Panicum coloratum* was found to be superior in removing PAH contamination, compared to the other investigated plant species and non-vegetated soil. A

Table 1: Properties of typical gasoline compounds (Rippen 2000)

Property	<i>m</i> -xylene	<i>n</i> -octane	<i>n</i> -hexane	benzene
class	monoaromatic	light alkane	light alkane	monoaromatic
CAS-no.	108-38-3	111-65-9	110-54-3	71-43-2
sum formula	C ₈ H ₁₀	C ₈ H ₁₈	C ₆ H ₁₄	C ₆ H ₆
water solubility (mg/L) 20°C	160	0.72	12.8	1760
sat. vapor pressure (Pa) 20°C	800	1610	16400	10100
Henry's Law constant (m ³ /m ³)	0.22	103	57	0.2
log K _{ow} (m ³ /m ³)	3.18	5.18	4.01	2.17
half life in air (h)	17	44	66	350
EC ₅₀ algal test mg/L **	4.9	>0.74 *	10	310
LC ₅₀ fish mg/L ***	3.8–43	0.38	200	3.2–33

* > water solubility

** mostly green algae *Chlorella vulgaris*, reduction of photosynthesis*** mostly fathead minnow *Pimephales promelas* (96 h)**Table 2:** Properties of diesel compounds (PAH Rippen 2000; alkanes Mackay et al. 1993)

Property	<i>n</i> -dodecane	<i>n</i> -decane	naphthalene	1-methyl-naphthalene
class	heavy alkane	heavy alkane	PAH	PAH
CAS-no.	112-40-3	124-18-5	91-20-3	90-12-0
sum formula	C ₁₂ H ₂₆	C ₁₀ H ₂₂	C ₁₀ H ₈	C ₁₁ H ₁₀
water solubility (mg/L) 20 °C	0.0034-0.0084	<0.001-0.05	25	28
sat. vapour pressure (Pa) 20 °C	15.4–32.5	170–238	7.2	8.3
Henry's Law constant (m ³ /m ³)	30	12–28	0.023	0.011
log K _{ow} (m ³ /m ³)	5.64–7.2	4.82–6.7	3.37	3.87
half life in air (h)	nd	2.4–24	23.8	7.3
EC ₅₀ algal test mg/L	nd	nd	19	>3; <30
LC ₅₀ fish mg/L	nd	nd	2	9

nd: no data

include diaromates (4 to 8%, e.g. naphthalene), and PAH (e.g. alkylated anthracene). The alkanes range mainly from C₁₀ to C₂₅, only a small fraction (<2 mass%) is longer (ARAL 2001).

Physicochemical properties of typical diesel and gasoline constituents are given in Tables 1 and 2. They have in common that they are all volatile (high vapor pressure). All diesel compounds have a small water solubility and a high lipophilicity (log K_{ow}). The aquatic toxicity, if given, is rather high.

1.2 Uptake of alkanes into plants

No experimental data are available on the uptake of alkanes into higher plants. Simulations with a plant uptake model (Trapp et al. 1994) indicate rapid uptake of long-chain (heavy) alkanes into fine roots, but slow translocation to stems and leaves, due to the very low solubility in water and high sorption to roots. Short-chain (light) alkanes are more mobile, but have a high vapor pressure. The model predicts that, after they are taken up into trees and translocated, they are either quickly metabolized or volatilized. Monoaromatics behave similar to light alkanes, PAH more like heavy alkanes.

1.3 Toxicity of diesel and gasoline compounds to plants

Toxicity data of BTEX, naphthalene and a few alkanes in solution and soil were measured by Hulzebos et al. (1993) with the lettuce (*Lactuca*) growth test. Alkane fuel constituents (heptane, octane, decane) had a low toxicity (EC₅₀ >1000 mg/kg) for uptake from soil, while naphthalene was more toxic (EC₅₀ = 100 mg/kg). For octane and decane, the aqueous toxicity to lettuce was above water solubility (see Tables 1 and 2). For the compounds heptane, toluene, naphthalene and *o*-xylene, EC₅₀-values between 1.7 and 13 mg/l were found for the uptake from hydroponic solution. The metabolic products of alkane degradation, fatty acids, have a lower toxicity, but higher water solubility. Octadecane acid (stearin acid, C₁₈H₃₆O₂) has an EC₅₀ to green algae (growth rate) between 10 and 50 mg/L (Rippen 2000).

Phytotoxicity of diesel fuel contamination in soil was observed in concentrations of less than 100 mg/kg for *Tradescantia* hybrids (Spiderwort) within 1 week of exposure time (Green et al. 1996). No mutagenic action was found. Most sensitive was the photosynthesis system II.

2 Materials and Methods

2.1 Soil

Soil samples polluted with various amounts of diesel and gasoline were collected from uncased 6" drill holes each at 0.5 m depths. The soil samples were stored in plastic bags in the dark at 4°C.

2.2 Chemical analysis of soil samples

The concentrations of $C_5 - C_{10}$ (gasoline fraction, including monoaromatics) and $C_{12} - C_{28}$ (diesel fraction, including PAH) in the soils were measured with GC-FID. The procedure for the GC-analysis of soil samples is based on the method required by the Danish EPA (Miljøstyrelsen 1998), with few modifications. Pyrex tubes with lids were used for extraction of 15 to 20 g soil with 9 ml of pentane after the addition of 8 ml of pyrophosphate, employing overnight shaking. Before analysis, the extract was diluted five times with pentane, when necessary.

Standard gasoline range organics (GRO) were provided from Sigma-Aldrich, Denmark. The standard contained benzene, toluene, *m*, *p* and *o*-xylene, ethylbenzene, methyl tert-butyl ether, 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene and naphthalene. Standard diesel range organics (DRO) from the same supplier contained long-chain alkanes from $C_{10} - C_{28}$.

Soil samples were also analyzed for volatile organic compounds using a PID-probe (photo ionization detector). The instrument (HNU HW 101; 10.2 eV UV-lamp) was able to detect BTEX, phenol, naphthalene, tetrachloroethylene and trichloroethylene. Calibration was performed with 100 mg/L of isobutylene as a standard. In the field, samples of 100 g each were transferred to diffusion-tight containers and stored at 5°C for a max. of 48 h. PID measurements were performed after equilibration for 4–24 h at 20°C.

2.3 Plant uptake and toxicity tests

The test system was described in Trapp et al. (2000). Photosynthesis and transpiration, and therefore also growth and transpiration, are closely related to each other. The test uses transpiration, growth and water use efficiency as a toxicity parameter. Experiments were carried out at two different locations in the lab. At the first location in the institute garage (during summer and autumn), the temperature was not controlled, but it was at $22 \pm 2^\circ\text{C}$ during the daytime, while the relative humidity was at $65 \pm 5\%$. The second location (autumn and winter) was a fully climatized cellar room with a temperature at $24.5 \pm 0.5^\circ\text{C}$, and relative humidity at $45 \pm 5\%$. Hybrid willow cuttings (*Salix schwerinii x viminalis* var. Björn) were provided by Svalöf-Weibull, Svalöf, Sweden, and later along with all other trees by Aage Bach, Tylstrup, Denmark. The cuttings had a length of approximately 40 cm and were pre-grown for three weeks in a bucket with tap water. Once rooted and with leaves, they were transferred into 500 ml foil-wrapped Erlenmeyer flasks that contained approx. 400 ml of modified ISO 8692 nutrient solution (ISO 1997). The flasks were sealed at the neck and placed

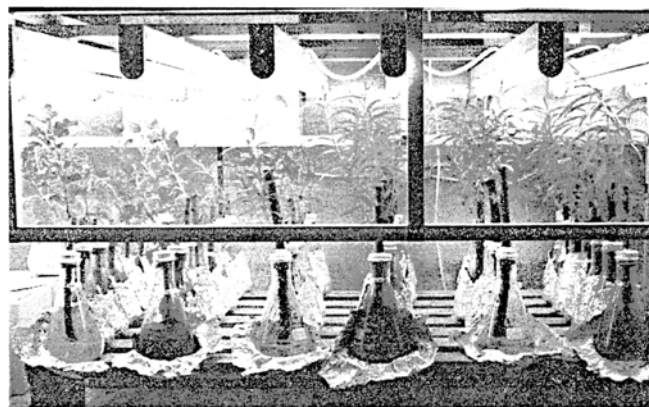


Fig. 1: Toxicity test system with poplar and willow trees in soil and hydroponic solution

under permanent artificial light (a rack of ten fluorescent tubes 36 W/33 with 20 cm distance between each at a height of 65 cm). Five controls contained uncontaminated solution. The weight of the flasks was measured every 24 hours. The weight loss was mainly (approx. 98%) due to transpiration of plants. For every toxicant concentration, four replicates were measured. Toxicity from polluted soils was measured by placing 500 g of polluted soil (unpolluted for the controls) into the Erlenmeyer flasks, and adding 150 ml of nutrient solution. The roots were covered with soil by gently shaking the Erlenmeyer flasks. The experimental apparatus is shown in Fig. 1.

2.4 Toxic endpoint: normalized relative transpiration

To make it possible to compare the toxic effect on cuttings with different initial transpiration (before the toxicant is added), the weight loss was expressed as relative transpiration, by normalizing with respect to the initial transpiration (eliminating the necessity to find cuttings with similar initial transpiration) and with respect to the transpiration of uncontaminated control cuttings (to include the effect of normal growth of the cuttings during the test). The mean normalized, relative transpiration (NRT) is found by;

$$NRT(C,t) = \frac{\frac{1}{n} \cdot \sum_{i=1}^n T_i(C,t) / T_i(C,0)}{\frac{1}{m} \cdot \sum_{j=1}^m T_j(0,t) / T_j(0,0)}$$

where C is the concentration of the compound in solution (mg/l) or soil (mg/kg), t is time period (h, from 0 to 24 h, from 24 h to 48 h, from 48 h to 72 h and so on), T is absolute transpiration (g/h), i is replicate 1,2,...,n and j is control 1,2,...,m.

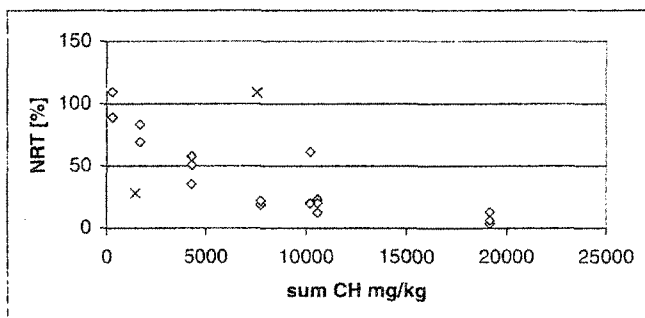
3 Results and Discussion

3.1 Concentrations in Axelved soils

Concentrations of $C_5 - C_{10}$ and $C_{12} - C_{28}$ hydrocarbons are given in Table 3 together with some soil parameters. The differences between the replicates are considerable, expressed by the large standard deviation of the measured concentra-

Table 3: Soil samples and initial chemical concentration (mean of 4 replicates); std denotes standard deviation

Sample ID	depth (m)	Smell	Texture	Sum C ₅ - C ₁₀ (mg/kg)	std	Sum C ₁₂ - C ₂₈ (mg/kg)	std
A22	0.3–0.5	oily	clay	658	271	9 923	4033
A22	0.8–1.0	normal	clay	188	75.2	7 546	5397
A22	1.2–1.5	normal	sandy	921	294	18 231	2081
A22	1.7–2.0	anoxic	sandy	183	42.8	4 100	506
A22	2.5–3.6	anoxic	sandy	224	265	1 448	754
A23	0.8–1.0	oily	loamy	145	n=1	143	n=1
A24	1.0–1.5	oily	nd	165	155	10 032	2043

**Fig. 2:** Initial sum of C₅ - C₂₈ hydrocarbons in soil samples from the abandoned filling station versus the normalized relative transpiration (NRT) of willow cuttings grown in them after 144 h. Symbol x denotes outliers

tions. This indicates an inhomogeneous distribution of the soil pollutants, frequently present in the form of small lumps.

3.2 Soil toxicity

In Fig. 2, measured concentrations of hydrocarbons (C₅ - C₂₈) are plotted against the normalized relative transpiration of the willow cuttings 144 h after transplanting into toxic soils. Two obvious outliers (NRT = 109% at 7546 mg/kg and 22% at 1448 mg/kg) were eliminated for the statistical analysis.

In order to statistically verify the correlation between means of transpiration and hydrocarbon concentrations, the square of the Pearson product moment correlation coefficient, r^2 , was calculated (Table 4). None of the correlations between toxic effect after 72 h and soil content was significant at the 5% level (results not shown), but significant correlations ($r^2_{n=19} > 0.456$) could be found for the reduction of transpiration after 144 h.

The correlation with the log of the concentrations was higher in all cases. In the treatment of toxicity data, a log-normal distribution of toxic response is frequently assumed, although a (similar) Weibull dose-response curve might be even more

Table 4: Correlation (r^2) between inhibition of transpiration after 144 h and hydrocarbon content in soil

	C ₅ - C ₁₀	C ₁₂ - C ₂₈	C ₅ - C ₂₈
C	0.39	0.69	0.68
log C	0.40	0.81	0.84

C: correlation with concentration; log C: correlation with log₁₀ of the concentration

appropriate (Christensen and Nyholm 1984). It is no surprise that the amount of C₅ - C₁₀ in soils shows a positive correlation to all toxicity data, because the gasoline (C₅ - C₁₀) and diesel (C₁₀ - C₂₈) pollution of the soils are correlated ($r^2 = 0.67$, $n=8$).

The explained variances (r^2) are up to 84%, indicating that indeed the sum of hydrocarbon pollution of the soil is responsible for the decrease of transpiration of the willow plants. According to the statistical analysis (Table 4), the diesel fraction, which also has the higher concentrations, is more important for the toxic effects of soils from the site.

A sigmoidal curve was fitted to the data obtained, assuming log-normal toxicity data distribution (Andersen 1994), in order to estimate the EC₅₀ of C₅ - C₂₈ residues in soil (Fig. 3). The estimate for the EC₅₀ is 3910 mg/kg, with a 95%-C.I. ranging from 2900 to 5270 mg/kg. This is in agreement with the toxicity of the 4100 mg/kg soil sample (compare Fig. 2). The EC₁₀ is 810 mg/kg (95% C.I. 396 to 1660 mg/kg).

3.3 Verification of the results with artificially contaminated soils

In order to verify the results from the toxicity tests, diesel and gasoline (95 ROZ) were provided from Statoil, Lyngby, and artificially mixed into unpolluted soils taken from the university campus. Additionally, 'free phase' experiments were performed with plants growing in hydroponic solution with a 1–2 cm diesel or gasoline phase on top. In these experiments, *Salix viminalis* (basket willow), *S. alba* (silver willow) and *Populus nigra* (black poplar) were used instead of *S. viminalis x schwerinii* hybrids.

The results for diesel are shown in Figs. 4a and 4b. As previously, 1000 mg/kg diesel decreased the transpiration of *Salix viminalis*, but was not deadly. The plants even recovered. *Salix alba* showed almost no reaction, whereas *Populus nigra* suffered most. With 10000 mg/kg diesel, all three tree species showed a marked response. *Salix viminalis* and *Populus nigra* showed a comparable effect, whereas *Salix alba* was again less sensitive. In free phase diesel, all trees died within weeks (data not shown). It should be noted, however, that some of the *S. alba* recovered partly after a few weeks and made even new leaves, before they dried out finally.

The result for 1000 mg/kg gasoline is shown in Fig. 5. Fresh gasoline in this concentration is fatal for the investigated trees (*S. alba* and *S. viminalis*). At 10,000 mg/kg, the lethal effect occurred faster. Most likely, gasoline is more bio-

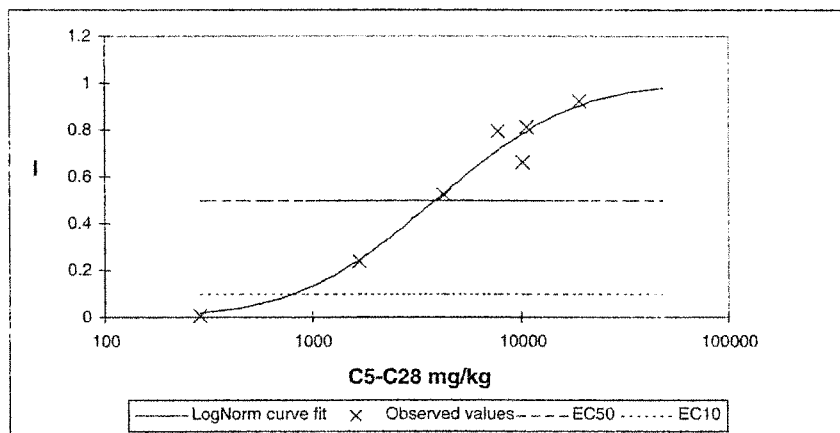


Fig. 3: Log-normal curve fit to soil toxicity data. I is inhibition of normalized, relative transpiration. Crosses indicate the mean of 2 or 3 replicates

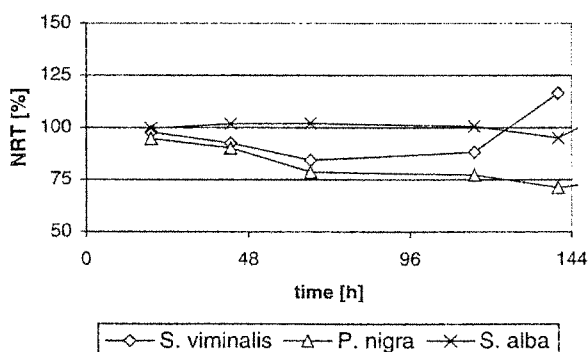


Fig. 4a: Effect of 1000 mg/kg diesel on transpiration of tree species; artificially contaminated soil samples

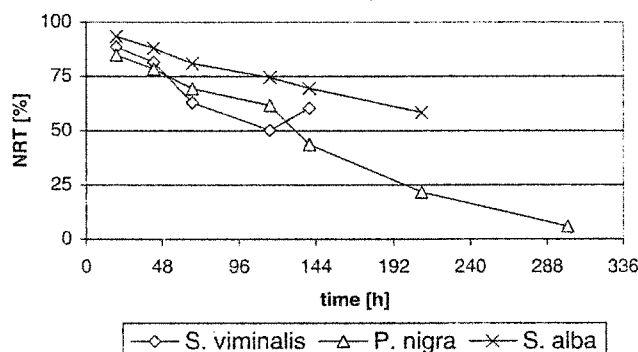


Fig. 4b: Effect of 10000 mg/kg diesel on transpiration of tree species; artificially contaminated soil samples

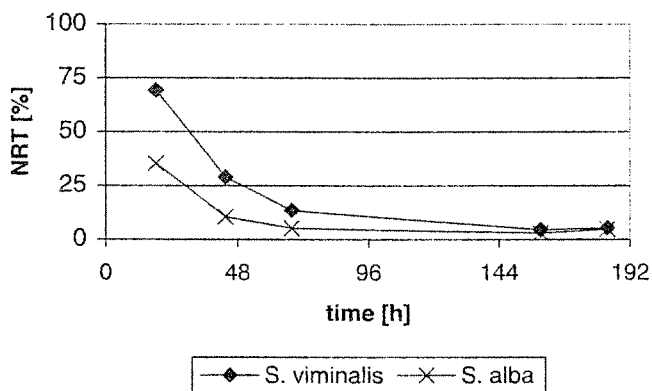


Fig. 5: Effect of 1000 mg/kg gasoline (95 ROZ) on transpiration of trees; artificially contaminated soil samples

available, taken up faster into trees and is more mobile inside the trees than diesel. One soil sample from the site (A22, depth 1.2 to 1.5 m) contained 921 mg/kg gasoline range hydrocarbons. The trees growing in this soil finally died too, but more slowly. This indicates that weathered gasoline is less toxic than fresh gasoline.

3.4 Survival in the field

The tree cuttings (mainly high-yielding hybrids *Salix viminalis x schwerinii*) were planted in April 1999. During the first summer, some of the cuttings died, inside and outside the plume as well. In October 1999, willows growing on the

site of the highest contamination showed clear signs of toxicity, similar to symptoms found in the lab (hanging and yellowing of leaves, drying out, some dead). On the other hand, trees on the less contaminated area were growing excellently, indicating that soil and climate support the growth of willows. Voids were replanted in February 2000 using cuttings of surviving willows from the site.

In the year 2000, all trees survived. Summer 2000 was colder and with more rain. After the second growing season, in August 2000, the growth of all trees was measured. Some were >4 m tall, but the majority of the willow hybrids were between 2 and 3 m. On the west side (left in Fig. 6), the growth of trees was inhibited in the center of the plume. From the street (right side) to the plume, some rows of trees failed to grow. This is probably an impact from truck driving for soil sampling. On the east side (right in Fig. 6), there does not seem to be a correlation between measured PID soil concentration and growth of trees. Most likely, growth inhibition was due to a combination of drought (the Danish summer in 1999 was hot), over-fertilization (there had been excessive application of manure before planting), non-optimal planting date (end of April) and toxicity of soils. Another reason for the weak correlation to soil toxicity may be that pollutants reside mostly at a depth (>60 cm) which is only sparsely rooted by young willows. Therefore, impact on growth may be small.

The frequency of fungi, herbivorous insects and other diseases was evaluated in September 2000, but there was no

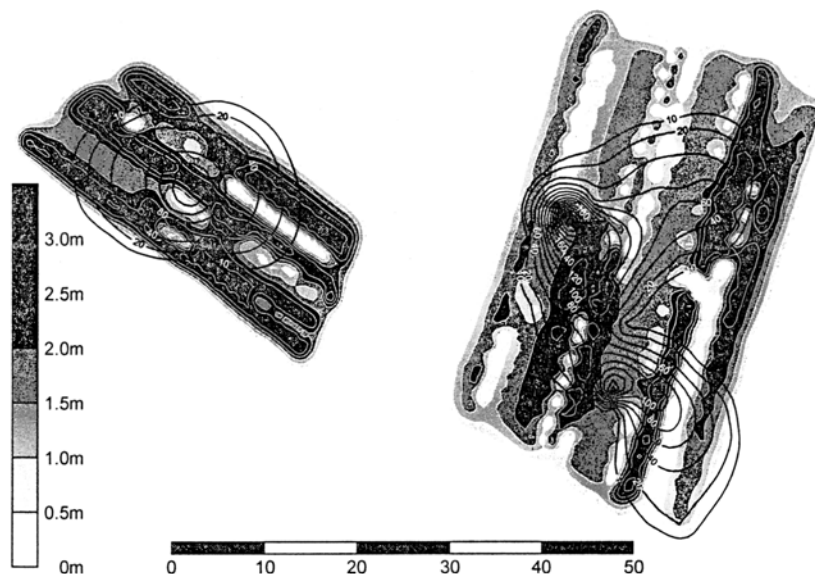


Fig. 6: Growth of trees on the abandoned filling station. Isolines indicate hydrocarbons in soil gas phase, i.e. the smoothed result of the PID analysis (maximum from any depth)

obvious difference between trees growing in different soil pollution concentrations.

4 Conclusions

Phytotoxicity of diesel and gasoline for willow and poplar trees has been tested. Concentrations of about 4000 mg/kg hydrocarbons reduced transpiration to 50%. Outdoors, trees survived higher concentrations, probably because pollutants reside in depths below 60 cm, the main rooting depth. It is concluded that at high soil concentrations, e.g. >5000 mg/kg hydrocarbons, willows cannot be used for phytoremediation due to toxic effects. Therefore, 'hot spots' of pollution should be treated with other methods, such as chemical or biological oxidation, whereas medium to low hydrocarbon levels can be treated with willows.

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