

Effects of the pollution by petroleum on the tracheids along the stem of *Podocarpus lambertii* Klotzsch ex Endl., Podocarpaceae

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Received June 21, 2007 – Accepted November 12, 2007 – Distributed May 31, 2009

(With 1 figure)

Abstract

Podocarpus lambertii Klotzsch ex Endl. (Podocarpaceae) is native and a member of the Pinophyta (Gymnosperm) of southern Brazil, locally known as “pinheiro-bravo”. The present work aims to investigate the effects of petroleum on the tracheids dimensions. Wood samples from twenty individuals were studied along the stem, ten being exposed to pollution and ten used as a control set. The wood samples were collected from incisions at three levels: at the ground level, and one and two metres above the ground level. From these samples, sub-samples were selected at the border of the growth layers in the vascular cambium-medulla direction. The methodology followed that traditionally recommended for plant anatomy studies, with analyses done by light microscopy (OLYMPUS – BX41) assisted by the software Image Pro-plus for measurements. Comparison of the individuals exposed to petroleum with the control set, showed that the length, diameter and cell wall width of the tracheids of the former were smaller, a trend which was statistically significant according to the Student’s *t*-test. These traits were observed mainly on the tracheids of the last growth layer, corresponding to the year in which the individuals were exposed to petroleum.

Keywords: phytotoxicity, petroleum, anatomy, wood.

Efeitos da poluição por petróleo nas traqueídes ao longo do caule de *Podocarpus lambertii* Klotzsch ex Endl., Podocarpaceae

Resumo

Podocarpus lambertii Klotzsch ex Endl. (Podocarpaceae), popularmente conhecida por pinheiro-bravo, é uma espécie que pertence ao pequeno grupo de Pinophyta (Gimnospermas) nativas da região Sul do Brasil. O presente estudo teve como objetivo investigar os efeitos da poluição por petróleo nas dimensões das traqueídes que compõem o lenho dessa espécie. Para tanto, amostras do lenho de vinte indivíduos foram coletadas, dez deles expostos à poluição por petróleo e dez usados como controle (coletados em região livre de contaminação). As amostras foram obtidas por meio de incisões paralelas à superfície do solo em três alturas (nível do solo, 1 metro e 2 metros do nível do solo). As subamostras para o estudo da variação estrutural do lenho foram selecionadas no limite das camadas de crescimento no sentido câmbio-medula. A metodologia utilizada para desenvolver o trabalho foi aquela tradicionalmente recomendada para estudos em anatomia vegetal. As mensurações das traqueídes em material macerado, como comprimento, diâmetro e espessura da parede celular, foram feitas pelo software Image Pro-plus em Fotomicroscópio (OLYMPUS – BX41). Nos indivíduos expostos à poluição, o comprimento, diâmetro e espessura da parede das traqueídes foram menores quando comparados aos indivíduos controle e demonstraram diferenças estatisticamente significativas pelo teste *t*-student. Essas tendências foram observadas, principalmente, nas traqueídes da última camada de crescimento, correspondente ao ano em que os indivíduos permaneceram expostos ao petróleo.

Palavras-chave: fitotoxicidade, petróleo, anatomia, lenho.

1. Introduction

The continuous growth of environmental pollution and anthropological disturbances to ecosystems has made the study of abiotic stress responses in plants become increasingly more important in agriculture, forest management, and ecosystem restoration strategies (Alkio et al., 2005).

Crude oil, the raw component of nearly all petroleum products, contains a wide variety of elements combined in various forms (Abb, 1997). Its main components are carbon and hydrogen, which in their combined form are called hydrocarbons. In a broader sense, they are divided into two families: aliphatics (fatty) and aromatics (fragrant). Aliphatics are further divided into three main classes: alkanes, alkenes and cycloalkanes. The alkynes, another type of aliphatic structure, are not commonly found in petroleum hydrocarbons. In the refining process, petroleum products are strongly enriched with hydrocarbons, leaving most crude-based inorganic materials and other types of organic compounds containing sulphur, nitrogen and oxygen in the residual material (Potter and Simmons, 1998).

While reviewing the literature for the present paper, the scarcity of reports on the effects of petroleum pollution in soils as compared to the same type of pollution in aquatic ecosystems was noteworthy. Shifts in the composition of the soil's microbial community, as a response to low-levels of gaseous hydrocarbons from underlying petroleum formations have been investigated as support for further monitoring in cases of accidental spills. Michel et al. (2002; 2005) state that petroleum constitutes a pollutant that can persist in the environment for a long period until the vegetation recovers completely, and its persistence can be explained by the slow biodegradation of hydrocarbons.

According to Green et al. (1996), a variety of reports, patents and scientific papers have addressed the problems of fuel contamination in soils, including chemical characterization and treatment, bioremediation by bacterial metabolism and the impacts on biological organisms in the contaminated soil. The poisoning mechanism and its dose-response are the biggest concern for toxicologists.

Although the hydrocarbons from oil pollution in the environment are usually diffused to low concentrations, and their biodegradation is the subject of numerous studies (Strickland, 1990; Green et al., 1996; Michel et al., 2002; 2005), information on their toxicity to the plants is still limited. The presence of petroleum in the soil affects its diversity, canopy and productivity. Little is known about the chronic effects of oil pollution (Strickland, 1990). The immediate toxic effect tends to be caused mainly by molecules of low molar mass that are quickly degraded. The chronic toxic effects, however, are due to molecules of high molar mass, generally aromatic, that present lower toxicity, but are persistent, causing a longer lasting effect (Spies et al., 1996). It is well known

that hydrocarbons are harmful to animals and plants (Pothuluri and Cerniglia, 1994; Dorn et al., 1998; Lin and Mendelssohn, 1998; Dorn and Salanitro, 2000). The inhibition of germination and the reduction of plant growth as well as its death are indicators of the toxicity of hydrocarbons (Powell, 1997; Rivera-Cruz et al., 2002; Hernandez-Valencia and Magger, 2003; Rivera-Cruz and Trujillo-Narcía, 2004).

Although oil hydrocarbons are common pollutants, and their biodegradation is the subject of numerous studies, information on their toxicity to the plants in the soil is limited. The reaction of some plants like *Avena sativa* L., *Secale cereale* L. and *Hordeum vulgare* L., after the contamination of ground and water with oil products was studied by Petukhov et al. (2000). These authors discuss the negative effects of oil contamination on seed germination, reduction of the total biomass and the length of the roots. They also suggest that these plants could be used as test organisms in analyzing the toxicity of this pollutant in soil and water.

Maranho et al. (2006a) investigated the effect of petroleum pollution on the leaf structure of *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae), and concluded that leaf anatomy revealed a large variability related to pollution. Thibes-Rodrigues et al. (2006) evaluated the nutritional status of *Sebastiania commersoniana* (Baill.) L. B. Sm. and Downs growing on soil contaminated with petroleum. Contamination resulted in a decrease in K and an increase in both Mg and Fe content in the biomass of this specie. Maranho et al. (2006b) studied the tolerance of *Sebastiania commersoniana* (Baill.) L. B. Sm. and Downs in soil contaminated with petroleum. The results showed that *S. commersoniana* displayed metabolic strategy for tolerance to the anoxic environment, with occurrence of expressive morpho-anatomical modifications to the formation of new roots, increase both in the content of chlorophyll and in the stomata density.

The study of plant behavior in petroleum contaminated soils allows the identification and selection of oil-pollution-indicating species. Thus, the present work aims to investigate the effects of petroleum pollution on the dimensions of tracheids of *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae). This species, locally known as "pinheiro-bravo", is a member of the diverse family of conifers and is a typical Pinophyta (Gymnosperm) from southern Brazil. Besides being a native species, it was selected also because it was observed to survive for one year after a petroleum spill (Funpar, 2001), while other species, typical of the same forest formation (Forest with "Araucária"), died immediately after being exposed to the pollutant.

2. Material and Methods

The material was collected on the ground of Presidente Getúlio Vargas Refinery (REPAR), located in Araucária, Paraná, Brazil (25° 34' 02,5" S and 49° 20' 53,5" W),

where four million liters of oil were spilled during an accident in July of 2000. The region is moderately hilly, and the area of study, being on a ridge, had a deep layer of oil infiltration, which drained towards a plain. The vegetation in the area may be characterized as a secondary succession stage of the Atlantic Rainforest, locally known as Forest with "Araucária". Part of this vegetation, which was directly impacted by the spill, had its stratum integrally dead.

Wood samples of different individuals of *Podocarpus lambertii* Klotzsch ex Endl. were collected one year after they had been exposed to pollution by petroleum. Twenty individuals were evaluated, ten of which had been exposed to the oil pollution and ten were used as a control set from the same region, but in an area not affected by the spill. According to Maranhão et al. (2006c), the wood samples were 20 cm wide. The wood samples were collected from incisions at three levels: at the ground level, and one and two metres above ground level. From these samples, seven sub-samples were selected at the border of the growth layers in the vascular cambium-medulla direction, including the layer formed during the period of contact with the petroleum.

In order to verify the dimensional variation of tracheids along the stem of the *P. lambertii*, wood samples at three different heights in the axial section were studied. The samples were macerated using procedures described by Franklin (1946). The material analysis, as well as the measurements were performed with light microscopy (Olympus – BX-41). Fifty measurements were taken in each layer for each variable analyzed, which included length, diameter and cell wall width. Statistical tests were conducted using the Statsoft Statistica 6.0 software package. Data were analyzed using the Shapiro-Wilk test to verify the normality of the distributions. The statistical significance of the differences was evaluated by the Student's *t*-test, comparing the dimensional variation of tracheids between growth layers along the stem of *Podocarpus lambertii* Klotzsch ex Endl, with $p < 0.05$ being considered significant by the authors.

3. Results

The tracheids dimension of individuals exposed to petroleum was smaller when compared with the control individuals (Figure 1a-c). The variation in length, diameter and cell wall width of the tracheids in individuals exposed to oil and those used in the control set was evaluated (Tables 1 and 2). The length and cell wall width showed a well-defined pattern on the last growth ring in comparison with the other rings on individuals exposed to the oil. In these samples, the length and cell wall width of the tracheids was smaller when compared to the control. These tendencies were mainly observed on the tracheids of the last growth layer, corresponding

to the year in which the individuals had been exposed to the oil (Figure 1d-k).

Individuals in the control set had the lower length tracheids located next to the medulla. In the medulla-cambium direction, these values increased until stabilizing near a maximum (Table 1). The same pattern was observed along the stem. In plants exposed to pollution, a similar behavior was observed in the medulla-cambium direction and the first growth layer (Table 1). Differences in diameter and cell wall width were not observed among the growth rings of individuals used as control, while in plants exposed to pollution there was a marked reduction in these dimensions at the last growth ring, when compared to those observed in the inner rings.

Although several authors state that genetic and environmental effects are difficult to distinguish, the results obtained here indicate a trending the size of tracheids of the individuals exposed to oil pollution, thus suggesting that such alteration is due to environmental causes (Tables 1 and 2).

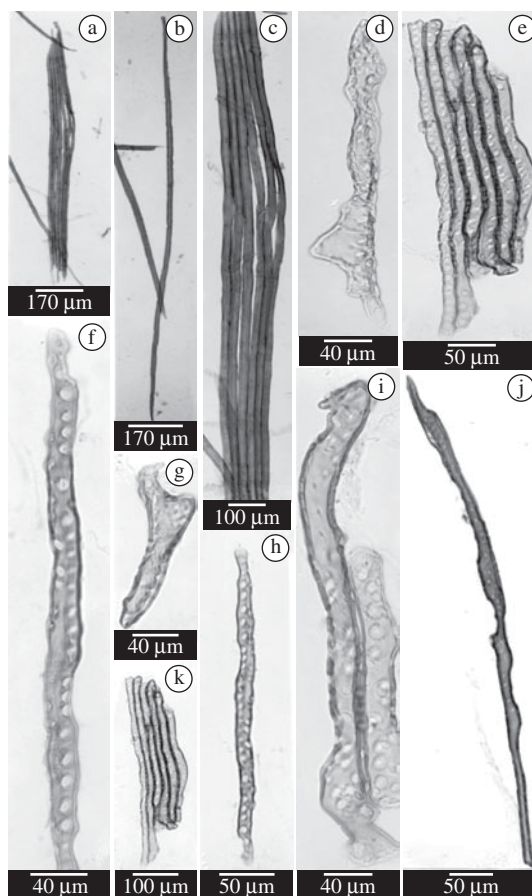


Figure 1. Differences between tracheids of last growth layer of *Podocarpus lambertii* Klotzsch ex Endl.. a-c) Tracheids of control set individuals; d-k) Tracheids of individuals exposed to pollution by petroleum.

Table 1. Dimensional variation of tracheids along the stem of *Podocarpus lambertii* Klotzsch ex Endl. of control set and individuals exposed to pollution.

Dimensions of tracheids (µm) of control set									
Layers	Length			Diameter			Cell wall width		
	0 m	1 m	2 m	0 m	1 m	2 m	0 m	1 m	2 m
1	1903.1 ± 220.8	1981.5 ± 220.6	2031.5 ± 199.4	27.2 ± 5.1	27.1 ± 4.9	25.0 ± 4.3	4.5 ± 1.2	4.9 ± 1.2	5.0 ± 1.2
2	1881.8 ± 221.9	1955.6 ± 147.6	1945.8 ± 187.1	26.1 ± 4.2	26.8 ± 5.0	26.6 ± 5.1	4.6 ± 0.9	4.9 ± 1.1	4.9 ± 1.1
3	1804.8 ± 298.5	1889.3 ± 213.2	1934.5 ± 218.9	26.8 ± 5.0	27.2 ± 4.5	27.8 ± 5.0	4.6 ± 1.1	4.8 ± 1.0	4.8 ± 1.1
4	1859.6 ± 260.4	1946.5 ± 312.0	1776.0 ± 265.3	25.8 ± 4.0	26.1 ± 5.3	24.7 ± 4.8	4.6 ± 1.0	4.7 ± 1.2	4.7 ± 1.1
5	1987.6 ± 236.5	1986.5 ± 201.6	1945.8 ± 187.1	25.3 ± 4.2	28.1 ± 4.6	24.4 ± 4.3	4.4 ± 1.0	4.9 ± 1.1	4.7 ± 1.1
6	1802.3 ± 285.8	1749.0 ± 236.1	1934.5 ± 218.9	27.1 ± 4.6	28.1 ± 4.4	26.1 ± 3.5	4.6 ± 1.1	4.9 ± 1.1	5.0 ± 1.0
7	1690.3 ± 322.8	1896.5 ± 197.1	1776.0 ± 265.3	24.3 ± 4.7	28.2 ± 5.3	23.0 ± 4.0	4.5 ± 1.1	4.9 ± 1.1	4.7 ± 1.2

Dimensions of tracheids (µm) of individuals exposed to pollution									
Layers	Length			Diameter			Cell wall width		
	0 m	1 m	2 m	0 m	1 m	2 m	0 m	1 m	2 m
1	1065.4 ± 465.0	1196.8 ± 266.7	1331.8 ± 271.8	24.5 ± 6.2	25.0 ± 5.2	24.3 ± 6.6	2.7 ± 0.8	0.8 ± 2.8	2.7 ± 0.7
2	2046.6 ± 271.7	2061.9 ± 288.9	2034.5 ± 315.7	26.0 ± 5.3	26.9 ± 7.0	26.4 ± 5.2	4.1 ± 0.9	1.1 ± 4.3	4.2 ± 1.0
3	2097.0 ± 315.6	2050.7 ± 235.3	2054.1 ± 269.3	28.2 ± 6.7	26.4 ± 5.0	26.3 ± 5.6	4.6 ± 1.0	1.1 ± 4.4	4.4 ± 1.1
4	2160.4 ± 300.9	2042.1 ± 234.7	2043.5 ± 295.5	26.2 ± 5.6	26.5 ± 5.4	25.6 ± 5.9	4.1 ± 1.0	1.0 ± 4.4	4.3 ± 1.0
5	2051.3 ± 348.5	2055.3 ± 321.0	2074.4 ± 259.9	26.7 ± 5.8	25.4 ± 5.6	26.0 ± 5.7	4.5 ± 1.2	0.9 ± 4.2	4.3 ± 0.9
6	1939.4 ± 351.5	2026.6 ± 395.4	2017.5 ± 381.4	26.4 ± 5.1	25.6 ± 5.6	26.0 ± 5.0	4.2 ± 1.0	0.9 ± 4.3	4.0 ± 0.9
7	1917.6 ± 347.8	1982.1 ± 397.7	1898.7 ± 517.0	25.7 ± 5.5	25.9 ± 5.3	25.7 ± 6.0	4.2 ± 1.0	0.9 ± 4.2	4.1 ± 0.9

* The numbers of Layers indicates the growth layers in the vascular cambium-medulla direction. 0 m, 1 m and 2 m indicates the heights at which wood samples were collected.

Table 2. Results of Student's *t*-test comparing the dimensional variation of tracheids between growth layers along the stem of *Podocarpus lambertii* Klotzsch ex Endl.

Results of Student's <i>t</i> -test between growth layers																		
Layers	Control set									Individuals exposed to pollution								
	Length			Diameter			Cell wall width			Length			Diameter			Cell wall width		
	0 m	1 m	2 m	0 m	1 m	2 m	0 m	1 m	2 m	0 m	1 m	2 m	0 m	1 m	2 m	0 m	1 m	2 m
1/2	0.17	0.19	0.00	0.03	0.74	0.00	0.37	0.65	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1/3	0.00	0.00	0.00	0.38	0.74	0.00	0.24	0.28	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1/4	0.04	0.20	0.00	0.00	0.07	0.57	0.10	0.11	0.01	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00
1/5	0.00	0.72	0.23	0.00	0.13	0.40	0.51	0.41	0.01	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00
1/6	0.00	0.00	0.46	0.67	0.10	0.00	0.33	0.99	0.47	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00
1/7	0.00	0.00	0.00	0.00	0.07	0.00	0.97	0.77	0.00	0.00	0.00	0.00	0.01	0.03	0.01	0.00	0.00	0.00
2/3	0.00	0.00	0.00	0.07	0.40	0.01	0.62	0.51	0.74	0.00	0.41	0.23	0.00	0.69	0.72	0.00	0.47	0.00
2/4	0.10	0.74	0.00	0.44	0.30	0.00	0.68	0.17	0.32	0.00	0.22	0.67	0.46	0.87	0.04	0.29	0.12	0.06
2/5	0.00	0.10	0.00	0.18	0.01	0.00	0.02	0.82	0.20	0.96	0.75	0.03	0.13	0.00	0.40	0.00	0.02	0.13
2/6	0.00	0.00	0.04	0.05	0.00	0.45	0.98	0.49	0.05	0.00	0.01	0.15	0.14	0.01	0.59	0.30	0.98	0.02
2/7	0.00	0.00	0.00	0.00	0.01	0.00	0.15	0.93	0.07	0.00	0.00	0.00	0.48	0.06	0.08	0.03	0.04	0.25
3/4	0.07	0.16	0.49	0.04	0.04	0.00	0.65	0.64	0.30	0.00	0.70	0.35	0.00	0.96	0.08	0.00	0.37	0.31
3/5	0.00	0.00	0.00	0.00	0.04	0.00	0.11	0.74	0.39	0.00	0.99	0.15	0.00	0.00	0.34	0.09	0.00	0.04
3/6	0.93	0.00	0.00	0.99	0.13	0.00	0.83	0.17	0.04	0.00	0.02	0.02	0.00	0.02	0.68	0.00	0.34	0.00
3/7	0.00	0.89	0.00	0.00	0.07	0.00	0.27	0.32	0.09	0.00	0.00	0.00	0.00	0.29	0.05	0.00	0.00	0.00
4/5	0.00	0.34	0.00	0.43	0.00	0.61	0.05	0.23	0.74	0.00	0.44	0.06	0.24	0.03	0.13	0.00	0.00	0.54
4/6	0.00	0.00	0.00	0.02	0.00	0.00	0.52	0.05	0.00	0.00	0.06	0.06	0.48	0.04	0.06	0.37	0.00	0.00
4/7	0.00	0.11	0.00	0.01	0.00	0.00	0.19	0.09	0.68	0.00	0.00	0.00	0.22	0.22	0.51	0.06	0.00	0.00
5/6	0.00	0.00	0.54	0.00	0.69	0.00	0.11	0.31	0.00	0.00	0.01	0.00	0.79	0.72	0.75	0.00	0.00	0.00
5/7	0.00	0.00	0.00	0.07	0.96	0.00	0.55	0.64	0.64	0.00	0.00	0.00	0.00	0.14	0.48	0.00	0.94	0.03
6/7	0.00	0.00	0.00	0.00	0.97	0.00	0.36	0.97	0.00	0.13	0.00	0.00	0.05	0.30	0.61	0.78	0.02	0.32

* The numbers of Layers indicates the growth layers in the vascular cambium-medulla direction. 0 m, 1 m and 2 m indicates the heights at which wood samples were collected.

4. Discussion

Fukazawa and Ohtani (1982) in the wood of *Tilia japonica* (Miq.) Simonk. (Tiliaceae), showed that at a height of 5.30 m along the stem, the length of the vascular elements increased in the medulla-cambium direction, and, after reaching a maximum value, it stabilized. At other heights, the variation was very small. On the other hand, Silva (1992) noticed an increase in the vascular elements near the medulla of *Andira parvifolia* Ducke (Leguminosae Papilionoideae). Iqbal and Ghouse (1983) evaluated the average length of the vessel elements in *Prosopis spicigera* L. (Leguminosae Mimosoideae), detecting initial growth from the cambium towards the medulla, and a gradual decline, with some fluctuations, next to the medulla. Urbinati et al. (2003) observed in the *Terminalia ivorensis* A. Chev. wood the same variation pattern detected in the present study.

Thus, in the tracheids on the last growth layer, corresponding to the year when the individuals were exposed to the oil and when the hydric stress was most severe – not due to lack of water in the environment, but due to the presence of oil in the roots, thus compromising the flow of water, and causing increased xylemic tensions – the pattern observed was the presence of shorter tracheids, which may be the plant's attempt to maintain the hydric transport.

Despite the fact that the studies referred to in this and the following paragraphs looked at the wood structure of Magnoliophyta, they all present aspects that should be those considered in the present study. According to Urbinati et al. (2003), the different studies conducted with vascular elements reveal that each species, given its genetic characteristics and environmental conditions in which it developed, presents a different anatomical standard. Trugilho et al. (1996) state that the variations in chemical composition, physical and anatomical aspects of the wood are great among species, but may also occur within the same species, due to age, genetic and, mainly, environmental factors.

Concerning the diameter of the stem, the same trend detected in the radial direction of the individuals in the control set of the present study was observed by Giroud (1977); Fukazawa and Ohtani (1982); Bosman et al. (1994); Helinska (1995); Urbinati et al. (2003). The distinction between young and adult wood can, in some species, be impossible to define based on the diameter of the vascular elements. Butterfield et al. (1993) did not observe variation in the diameter of the vessel elements in *Hieronyma alchorneoides* Allemão (Euphorbiaceae) and *Vochysia guatemalensis* Donn. Sm. (Vochysiaceae). While analyzing the longitudinal variation in *Shorea leprosula* Miq. and *S. pauciflora* King. (Dipterocarpaceae), Bosman et al. (1994) observed that the average diameter in the vascular elements also followed a low consistency standard. However, several trees showed an increase in diameter from the base towards the apex, while two of the trees in the study only pre-

sented a reduction in the diameter of the stem at a height of approximately 10 m.

According to Oever and Baas (1981), the most significant trend found while analyzing the influence of the hydric availability on the wood is a decrease of the size of vascular elements, mainly its diameter, in regions with more droughts. In order to understand the relationship between the organization of the conducting system and environmental factors such as soil pollution by petroleum, two concepts were proposed: “efficiency” and “security”. The conducting system is conditioned by the hydric availability of the environment; therefore, it allows for the best possible conductivity (efficiency), without risk of embolism (security). The water and minerals go up, establishing a pressure gradient from the base to the apex of the plant, as long as the liquids do not suffer volume expansion. If the pressure becomes excessively low, the water may vaporize, forming bubbles, which continue to grow due to diffusion of other gases like N₂, O₂ and CO₂, which are dissolved in water.

Once bubbles are formed, the xylematic pressure raises until it almost reaches equilibrium with the atmospheric pressure, stalling the hydraulic transport. Vascular elements of larger diameter have a hydraulic conductivity, which is higher than that of smaller diameters, i.e., are more efficient in transporting water. This means that, for the same flow rate, a larger diameter will cause less pressure drop than a smaller one. However, if bubbles do form in the larger diameter vascular elements, they will be of greater size, and much harder to dissolve, than in elements of smaller diameter. Thus, there is a small probability of reestablishing the water column. The opposite is true for the elements of smaller diameter (Zimmermann and Brown, 1974; Zimmermann, 1983).

The differences found in the diameters of tracheids can be explained when compared with the growth layers, considering what Larcher (1995; 2000) presented on “the way of the water in the plant”. The greatest velocity of the perspiration flow depends on the anatomical structure of the vessels. In cases with increased tension in the vessels, there is difficulty in water absorption where the water column cohesion can quickly be breached. Once the water column is breached and the pressure gradient is disrupted in the vascular system, air enters the xylem, causing embolism and locally interrupting the perspiration flow. Thus, wood whose xylem presents high hydraulic conductivity is more susceptible to embolism when compared with conducting elements with small lumen diameter.

Flörsheim and Tomazello-Filho (1996); and Flörsheim et al. (1999) did not observe any definitive trends in the cell wall width of the *Myracrodruon urundeuva* Allemão vessels. Klock et al. (2002) observed that the width of cell walls in tracheids of *Pinus taeda* L. is homogeneous throughout all growth layers. Larcher (2000) verified that alterations can occur in the wood of conifers under different types of stress, such as reductions in the width and density of the cell wall. According to Foelkel et al. (1975)

and Larson et al. (2001), the cell wall width increases radially in the trunk, being more pronounced in tracheids belonging to delayed wood.

According to Xu and Johnson (1995), Hester and Mendelssohn (2000), Pezeshki et al. (2000), the contamination with petroleum affects the development of plants due to different physical effects. These authors mention the oil film that covers the roots, modifying water absorption and nutrients, as the main physical effect. Bona and Santos (2003) state that oil diminishes the soil capacity for retaining water, thus interfering with plant growth.

The study reported herein reveals the alterations in the dimensions of tracheids of *P. lambertii* due to exposure to soil contaminated with oil. A clear reduction in the length, diameter and cell wall width of the tracheids was observed. Summarizing, a screening experiment was conducted in order to determine the dimensional variations of tracheids along the stem of *P. lambertii*, which was exposed to an oil spill. Individuals from an adjacent unpolluted site were used as a control set. It is suggested that the variation on the tracheids of *P. lambertii* wood might be used as biosensors or biomarkers for pollution by petroleum. According to Falla et al. (2000), Temmerman et al. (2004), the passive biosensors are plants that already are gifts in the study place. Their use frequently is related to all the area that is being searched, however, when these results are associated with studies about other aspects of the plant in similar conditions, according to Maranho et al. (2006a) it becomes possible to estimate the toxic effects of petroleum on *Podocarpus lambertii* Klotzsch ex Endl.

Acknowledgements — The authors express their thanks to the Universidade Positivo (UP) and Universidade Federal do Paraná (Departamento de Engenharia Florestal) Curitiba, Paraná, Brazil.

References

- ABB, 1997. *ABB's Environmental Management Report*. Stockholm: ABB. 88 p.
- ALKIO, M., TABUCHI, TM., WANG, X. and COLÓN-CARMONA, A., 2005. Stress responses to polycyclic aromatic hydrocarbons in *Arabidopsis* include growth inhibition and hypersensitive response-like symptoms. *Journal of Experimental Botany*, vol. 56, no. 421, p. 2983-2994.
- BONA, C. and SANTOS, OG., 2003. *Adaptações fisiológicas de espécies vegetais crescendo em solo contaminado com petróleo*. Curitiba: FUNPAR; UFPR. Relatório Técnico. 52 p.
- BOSMAN, MTM., KORT, I., VAN GENDEREN, MK. and BAAS, P., 1994. Radial variation in wood properties of naturally and plantation grown light red meranti (*Shorea* sp., Dipterocarpaceae). *IAWA Journal*, vol. 15, no. 2, p. 111-120.
- BUTTERFIELD, RP., CROOK, RP., ADANS, R. and MORRIS, R., 1993. Radial variation in wood specific gravity, fibre length and vessel area for two Central American hardwoods: *Hyeronima alchorneoides* and *Vochysia guatemalensis*: natural and plantation - grown trees. *IAWA Journal*, vol. 14, p. 153-161.
- DORN, PH. and SALANITRO, J., 2000. Temporal ecology assessment of oil contaminated soils before and after bioremediation. *Chemosphere*, vol. 40, no. 4, p. 419-426.
- DORN, PH., VIPON, T., SALANITRO, J. and WISNIEWSKY, H., 1998. Assessment of the acute toxicity of crude oils using earthworms, microtox and plants. *Chemosphere*, vol. 37, no. 5, p. 845-860.
- FALLA, J., LAVAL-GILLY, P., HENRYON, M., MORLOT, D. and FERARD, JF., 2000. Biological air quality monitoring: a review. *Environmental Monitoring and Assessment*, vol. 64, no. 3, p. 627-644.
- FLÖRSHEIM, SMB. and TOMAZELLO-FILHO, M., 1996. Variação das dimensões celulares no sentido medula-casca e base-topo de árvores de aroeira *Myracrodruon urundeuva* F. F. and M.F. Allemão (Anacardiaceae). *Revista do Instituto Florestal*, vol. 8, no. 2, p. 153-165.
- FLÖRSHEIM, SMB., TOMAZELLO-FILHO, M. and SPEGIORIN, L., 1999. Variação das dimensões no sentido medula-casca e base-topo de árvores de aroeira *Myracrodruon urundeuva* F.F. and A.F. Allemão (Anacardiaceae). *Revista do Instituto Florestal*, vol. 11, no. 2, p. 159-171.
- FOELKEL, CEB., FERREIRA, M., NEHRING, JH. and ROLIM, MB., 1975. Variabilidade no sentido radial de madeira de *Pinus elliotii*. *Instituto de pesquisa e Ciências Florestais*, no. 10, p. 1-11.
- FRANKLIN, GL., 1946. A rapid method of softening wood for microtome sectioning. *Tropical Woods*, vol. 88, p. 35-36.
- FUKAZAWA, K. and OHTANI, J., 1982. Within a tree variation of wood element size in *Tilia japonica*. *IAWA Bulletin*, vol. 3, p. 201-205.
- Fundação da Universidade Federal do Paraná para o Desenvolvimento da Ciência e Tecnologia - FUNPAR, 2001. *Diagnóstico e monitoramento da cobertura vegetal da área de influência direta e indireta do vazamento de óleo da Refinaria Presidente Getúlio Vargas*: REPAR, Araucária/PR. Curitiba: Universidade Federal do Paraná - UFPR. 76p. Fase I – Diagnóstico. Relatório Final.
- GIROUD, B., 1977. Statistical analysis of wood structure variation as related to distance from the pith in *Entandrophragma utile* (Meliaceae). *IAWA Bulletin*, vol. 4, p. 71-75.
- GREEN, BT., WIBER, CT., WOODRUFF, JL., MILLER, EW., POAGE, VL., CHILDRESS, DM., FEULNER, JA., PROSCH, SA., RUNKEL, JA., WANDERSHEID, RL., WIERMA, MD., YANG, X., CHOE, HT. and MERCURIO, SD., 1996. Phytotoxicity observed in *Tradescantia* correlates with diesel fuel contamination in soil. *Environmental and Experimental Botany*, vol. 36, no. 3, p. 313-321.
- HELINSKA, RL., 1995. Variation of vessel lumen diameter in radial direction as an indication of the juvenile wood growth in oak (*Quercus petraea* Liebl.). *Forestry Abstracts*, vol. 56, p. 610-617.
- HERNANDEZ-VALENCIA, I. and MAGGER, D., 2003. Uso de *Panicum maximum* y *Brachiaria brizantha* para fitorremediar suelos contaminados con un crudo de petróleo liviano. *Bioagro*, vol. 15, no. 3, p. 149-155.
- HESTER, MW. and MENDELSSOHN, IA., 2000. Long-term of a Louisiana brackish marsh plant community from oil - spill impact: vegetation response and mitigating effects of marsh surface elevation. *Marine Environmental Research*, vol. 49, p. 233-254.

- IQBAL, M. and GHOSH, AKM., 1983. An analytical study on cell size variation in some arid zone trees of India: *Acacia nilotica* and *Prosopis spicigera*. *IAWA Bulletin*, vol. 4, no. 1, p. 46-52.
- KLOCK, U., BOLZON DE MUÑIZ, GI., NISGOSKI, S. and BITTENCOURT, E., 2002. Características dos traqueóides da madeira juvenil de *Pinus maximinoi* H. E. Moore e de *Pinus taeda* L. In *Annals of the Congresso Iberoamericano de investigación en celulosa y papel*, Outubro 10-12, 2002. Campinas: [s.n.]. p. 1-17.
- LARCHER, W., 1995. *Physiological plant ecology*. New York: Springer-Verlag. 505p.
- LARCHER, W., 2000. *Ecofisiologia Vegetal*. São Carlos: Rima. 531p.
- LARSON, PR., KRETSCHMANN, DE., CLARK III, A. and ISEBRANDS, JG., 2001. *Formation and properties of juvenile wood in Southern Pines: a synopsis*. Madison: Forest Products laboratory. 42p. General Technical Report, FPL-GTR-129, USDA.
- LIN, Q. and MENDENSSOHN, IA., 1998. The combined effects of phytoremediation and bioestimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. *Ecology Engineering*, vol. 10, no. 3, p. 263-274.
- MARANHO, LT., GALVÃO, F., MUÑIZ, GIB., KUNIYOSHI, YS. and PREUSSLER, KH., 2006a. Size variation of tracheids in the stem of *Podocarpus lambertii* Klotzsch ex Endl., Podocarpaceae. *Acta Botanica Brasílica*, vol. 20, no. 3, p. 633-640.
- MARANHO, LT., AZEVEDO, JAM., PREUSSLER, KH. and CARVALHO FILHO, MAS., 2006b. Tolerância e potencial de fitorremediação de *Sebastiania commersoniana* (Baill.) L. B. Sm. and Downs, Euphorbiaceae, em solos contaminados por petróleo. In *The Second International Meeting on Environmental Biotechnology and Engineering*, Setembro 26-29. Cidade do México: CINVESTAV-IPN. p. 1-7.
- MARANHO, LT., GALVÃO, F., PREUSSLER, KH., MUÑIZ, GIB. and KUNIYOSHI, YS., 2006c. Effects of oil pollution on leaf structure of *Podocarpus lambertii* Klotzsch ex Endl., Podocarpaceae. *Acta Botanica Brasílica*, vol. 20, no. 3, p. 615- 624.
- MICHEL, J., HENRY, JR., CB. and THUMM, S., 2002. Shoreline assessment and environmental impacts from the M/T Westchester oil spill in the Mississippi River. *Spill Science & Technology Bulletin*, vol. 7, no. 3-4, p. 155-161.
- MICHEL, J., TREVOR, G., WALDRON, J., BLOCKSISIDGE, CT., ETKIN, DS. and URBAN, R., 2005. Potentially polluting wrecks in marine waters. In *Annals of the 2005 International Oil Spill Conference*, Maio 16. Miami: Oil Spill Response. p. 1-84.
- OEVER, LP. and BAAS, MZ., 1981. Comparative wood anatomy of *Symplocos* and latitude and altitude of provenance. *IAWA Bulletin*, vol. 2, p. 3-24.
- PETUKHOV, VN., FOMCHENKOV, VM., CHUGUNOV, VA. and KHOLODENKO, VP., 2000. Plant biotests of soil and water, polluted with petroleum and petroleum products. *Applied and Environmental Microbiology*, vol. 36, no. 6, p. 652-5.
- PEZESHKI, SR., HESTER, MW., LIN, Q. and NYMAN, JA., 2000. The effects of oil spill and clean up on dominant US ulf coast marsh macrophytes: a review. *Environmental Pollution*, vol. 108, no. 2, p. 129-139.
- POTHULURI, VJ. and CERNIGLIA, EC., 1994. Microbial metabolism polycyclic aromatic hydrocarbons. In CHAUDRY, RG. (Ed.). *Biological Degradation and Biorremediation of Toxic Chemicals*. Portland: Dioscorides. p. 92-123.
- POTTER, TL. and SIMMONS, KE., 1998. *Composition of Petroleum Mixtures*. Massachusetts: Amherst Scientific Publishers. 91p.
- POWELL, R., 1997. The use of plants as "field" biomonitors. In WANG, W., GORSUCH, J. and HUGHES, J. (Eds.). *Plants for Environmental Studies*. Lewis: Boca Raton. p. 47-61
- RIVERA-CRUZ, MC. and TRUJILLO-NARCÍA, A., 2004. Estudio de toxicidad vegetal em suelos com petróleos nuevo e intemperizado. *Interciencia*, vol. 29, no. 7, p. 369-376.
- RIVERA-CRUZ, MC., FERRERA-CERRATO, R., VOLKE, HV., RODRÍGUEZ, VR. and FERNÁNDEZ, LL., 2002. Probioclon microbiana em perfis de suelos afectados por hidrocarburos del petróleo en el estado de Tabasco. *Agrociencia*, vol. 36, no. 2, p. 149-160.
- SILVA, CA., 1992. Variação dimensional dos elementos xilemáticos em duas espécies madeiras da Amazônia. *Acta Amazonica*, vol. 22, no. 2, p. 261-274.
- SPIES, RB., RICE, SD., WOLFE, DA. and WRIGHT, BA., 1996. The effects of the Exxon Valdez oil spill on the Alaskan coastal environment. In RICE, SD., SPIES, RB., WOLFE, DA. and WRIGHT, BA. (Eds.). *Proceedings of the Exxon Valdez Oil Spill Symposium*. Alaska: American Fish Society Symposium. vol. 18. p. 1-16.
- STRICKLAND, RM., 1990. The Pacific Northwest coast: fossil fuel frontier. *Environment Journal*, vol. 6, no. 4, p. 25-77.
- TEMMERMAN, L., BELL, JNB., GARREC, JP., KLUMPP, A., KRAUSE, GHM. and TONNEIJCK, AEG., 2004. Biomonitoring of air pollutants with plants - considerations for the future. In KLUMPP, A., ANSEL, W. and KLUMPP, G. (Eds.). *Urban air pollution, bioindication and environmental awareness*. Göttingen: Cuvillier Verlag. p. 337-373.
- THIBES-RODRIGUES, T., WISNIEWSKI, C., BONA, C., DEDECEK, RA. and SANTOS, GO., 2006. Caracterização nutricional de branquilha (*Sebastiania commersoniana* (Baillon) Smith and Downs - Euphorbiaceae), cultivado em solo contaminado por petróleo. *Floresta*, vol. 36, no. 3, p. 349-358.
- TRUGILHO, PF., LIMA, JT. and MENDES, LM., 1996. Influência da idade nas características Físico-químicas e anatômicas da madeira de *Eucalyptus saligna*. *Revista Cerne*, vol. 2, no. 2, p. 94-11.
- URBINATI, CV., AZEVEDO, AA., SILVA, EAM. and LISBOA, PLB., 2003. Variação estrutural quantitativa no lenho de *Terminalia ivorensis* A. Chev., Combretaceae. *Acta Botanica Brasílica*, vol. 17, no. 3, p. 1-25.
- XU, JG. and JOHNSON, RL., 1995. Root growth, microbial activity and phosphatase activity in oil-contaminated, remediate and uncontaminated soils planted to barley and field pea. *Plant and Soil*, vol. 173, no. 11, p. 3-10.
- ZIMMERMANN, MH., 1983. *Xylem structure and the ascent of sap*. Berlin: Springer Verlag. 143p.
- ZIMMERMANN, MH. and BROWN, CL., 1974. *Trees structure and function*. New York: Springer Verlag. 336p.

