# Germination and initial growth of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae), in petroleum-contaminated soil and bioremediated soil

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(With 8 figures)

## Abstract

In 2000 there was an oil spill at the Getúlio Vargas Refinery (REPAR) in Paraná. Nearly five years after contamination and the use of bioremediation, a study was carried out to identify the effects of the contaminated soil and the bioremediated soil on the germination and initial growth of *C. xanthocarpa.* The experiment was established with soil from REPAR, with three treatment groups: contaminated soil (C), bioremediated soil (B) and uncontaminated soil (U); with five repetitions of 50 seeds each. There was no significant difference in the percentage of germination and the speed of germination index. The production of total biomass (30 - 60 days) and shoot biomass (60 days) was greater in the bioremediated soil compared to the other treatments. The averages for the root biomass were lower in the contaminated soil than in the bioremediated soil. The shoot length and the total length of the seedling in the contaminated soil and uncontaminated soil were lower than in the bioremediated soil.

*Keywords:* biomass, gabiroba, hydrocarbon contamination, seedling growth.

# Germinação e crescimento inicial de *Campomanesia xanthocarpa* O. Berg. (Myrtaceae), em solo contaminado com petróleo e solo biorremediado

## Resumo

Em 2000, ocorreu um vazamento de petróleo na área da Refinaria Getúlio Vargas (REPAR), no Paraná. Após cerca de cinco anos da contaminação e utilização do processo de biorremediação, pretendeu-se identificar os efeitos do solo contaminado e do solo biorremediado na germinação e no crescimento inicial de *C. xanthocarpa.* O experimento foi montado com solo da REPAR, com três tratamentos - solo contaminado (C), solo biorremediado (B), solo não contaminado (U) - e cinco repetições com 50 sementes cada. Não houve diferença significativa na porcentagem e no índice de velocidade de germinação. A produção de biomassa total (30 - 60 dias) e biomassa da parte aérea (60 dias) foram maiores no tratamento biorremediado em relação aos demais tratamentos. As médias para a biomassa da raiz foram menores no solo contaminado do que no solo biorremediado. O comprimento da parte aérea e o comprimento total da plântula no solo contaminado e no não contaminado foram menores do que no biorremediado.

*Palavras-chave:* biomassa, gabiroba, contaminação por hidrocarbonetos, crescimento de plântula.

## 1. Introduction

In July of 2000 there was an environmental disaster at the Petrobras Getúlio Vargas Refinery (REPAR) in Araucária, PR, spilling four million litres of petroleum. The area has secondary vegetation typical of the Araucaria Pine Forests (*Ombrófila Mista*), in montane and alluvial formations, in addition to floodplain vegetation (*várzeas*) with hydrophyllic plants (especially herbaceous) (Roderjan et al., 2002).

The accident had a substantial effect on the vegetation formations existing at the site, contaminating the soil, the fauna and the Barigui and Iguaçu rivers. Part of this area is being subjected to a bioremediation process aiming to reduce the TPH (Total Petroleum Hydrocarbon) content in the soil to a non-toxic level. Studies are being conducted to understand the influence of petroleum contamination, as well as the bioremediation process on the flora at this site (Bona and Santos, 2003; Maranho et al., 2006, 2009; Inckot et al., 2008).

*Campomanesia xanthocarpa* O. Berg. (Myrtaceae), also known as *gabiroba,* is one of the tree species found in the area, extending from the states of Minas Gerais to Rio Grande do Sul. According to Reitz (1977), it is an heliophytic species, a selective hydrophyte to mesophyte, found quite frequently in Araucaria Pine Forests, especially in the humid soil of the moist forest in isolated patches of trees, and in more open areas of the secondary forests. *C. xanthocarpa* is very important, for not only is it a species common to the REPAR region, but because its fruit is highly appreciated by the fauna.

The impacts caused by accidents with petroleum and its by-products can be evidenced right after a spill; one of the immediate consequences is the death of fauna and vegetation and water contamination. Moreover, the effects from these accidents can remain in the environment for a long period of time, harming the ecosystems (Freedman, 1993).

The potential of the *C. xanthocarpa* species to revegetate areas contaminated with oily residue calls (Mayer et al., 2005) for a better understanding of its germinative potential and growth in the petroleum-affected area. Thus, this work seeks to study the influence of contaminated soil and bioremediated soil on the germination of seeds and initial growth of *C. xanthocarpa*.

## 2. Material and Methods

Ripened fruit was collected from *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) trees in the municipality of Colombo, Paraná. Seeds from approximately 1000 fruit samples were obtained from five trees measuring 10 to 15 m in height, located on the edge of a remaining fragment of subtropical ombrophilous montane forest. Each fruit sample contained an average of nine seeds, amounting to a total of approximately 9000 seeds. To get the seeds, the pulp was removed from the fruit using a strainer under running water. For establishing the experiment, samples were collected from the topsoil layer (at a depth of 0 - 20 cm) in a contaminated area, a bioremediated area and an uncontaminated area, at the REPAR/Petrobrás site in Araucária, PR, where the oil spill took place. Soil samples from the three areas were characterized chemically and physically by the Agronomical Analyses laboratory of the Agricultural Sciences Sector of the Federal University of Paraná (Table 1). The TPH (Total Petroleum Hydrocarbon) analysis of the soil for the three treatments was conducted by LACTEC (Institute of Technology for Development). The soils in the region, in general are red and reddish-yellow latosols, red and reddish-yellow argisols, and cambisols, and all have non-hydromorphic characteristics (EMBRAPA, 1999). The bioremediation was done in situ with the inoculation of autochthonous microorganisms. The process for preparing the soil involved a plow, a rotary hoe, soil correction with limestone and inoculation. These activities were carried out periodically from August, 2000, to the time of soil collection (2005), as needed and according to the concentration of the contaminant in the soil (Carvalho, 2003).

Three treatments were established: treatment 1 - petroleum-contaminated soil (C); treatment 2 - bioremediated soil (B) and treatment 3 - uncontaminated soil (U). The experiment was conducted using plastic trays (25 × 36 × 6.5 cm), each treatment had five repetitions of 50 seeds each. The treatments were kept inside a greenhouse with misting every 20 to 30 minutes, the minimum temperature recorded was (17 °C) and the maximum (39 °C).

After the seeds were sown, germination was monitored on a daily basis. During this period, data was obtained for calculating the percentage (%) and *Speed of Germination Index* (SGI). This rate was calculated using the formula suggested by Maguire (1962) (Equation 1):

$$SGI = \frac{G1}{N1} + \frac{G2}{N2} + ... + \frac{Gn}{Nn} \quad (1)$$

Where, G1, G2 and Gn = number of seedlings computed in the first, second and final count;

N1, N2 and N3 = number of days after planting the seeds. The relative frequency of germination was calculated, in order to study the behaviour of the seeds in the germination process over time, using the formula by Labouriau and Agudo (1987) (Equation 2):

$$fi = \frac{ni}{\sum_{i=1}^{k} ni} \quad (2)$$

In which: ni = number of seeds germinated on day i and k = last day of observation. To check the variation of seed germination over time, the synchronization rate

**Table 1.** Physical-chemical attributes of the contaminated soil (C), bioremediated soil (B) and uncontaminated soil (U). (V = base saturation, T = cation exchange capacity, TPH = Total Petroleum Hydrocarbon, pH SMP = hydrogen potential calculated using the Shoemaker, Mac lean and Pratt buffer method).

| | C | B | U |
|---|---|---|---|
| Sand (%) | 25 | 20 | 26 |
| Silt (%) | 25 | 35 | 21 |
| Clay (%) | 50 | 45 | 53 |
| pH $CaCl_2$ | 4.2 | 5.8 | 3.7 |
| $Al^{+3}$ (cmolc.$dm^{-3}$) | 4.1 | 0 | 6 |
| $Al^{+3}$ + $H^{+}$ | 14.10 | 2.40 | 17.60 |
| CaMg | 1.3 | 9.3 | 2.0 |
| T | 17.84 | 21.21 | 18.30 |
| V (%) | 21 | 89 | 4 |
| pH SMP | 4.60 | 7.00 | 4.30 |
| C (g.$dm^{-3}$) | 26.9 | 19.6 | 23.8 |
| N (g.$kg^{-1}$) | 2.2 | 1.7 | 2.4 |
| P (cmolc.$dm^{-3}$) | 1.60 | 1.20 | 3.50 |
| $K^{+}$ (cmolc.$dm^{-3}$) | 0.24 | 0.21 | 0.10 |
| $Ca^{+2}$ (cmolc.$dm^{-3}$) | 2.0 | 16.8 | 0.4 |
| TPH (mg.$kg^{-1}$) | 5809 | 2713 | 243 |

suggested by Labouriau and Agudo (1987) was applied (Equation 3):

$$E = \sum_{i=1}^{k} fi \log 2fi \tag{3}$$

In which: fi = relative frequency of germination; log 2 = base logarithm 2 and k = last day of observation.

To study the growth, ten seedlings were collected per tray, totalling 50 seedlings per treatment, with 30 and 60-days old. These were pressed and dehydrated at 100 °C. The main root length was measured, as well as hypocotyls, epicotyls, and shoot; the total biomass of the seedling and the cotyledonal area of the eophylls. The leaf area was estimated using the Sigma-Scan program Pro Version 5.0, using a digitalized image on a desktop/table scanner linked to the computer. Both the biomass of the eophylls and the leaf area of the 30-day old seedlings were not measured, since at this phase the seedlings from the contaminated treatment still had not developed eophylls. The results obtained were submitted to analysis using the program MSTAT-C®. The data was analysed statistically, following an entirely random outline with three treatments and five repetitions each. The data were submitted to the Bartlett test to check for homogeneity of the variances. After, ANOVA was done to check for statistical differences between the averages tested. In cases where the result of the ANOVA was significant, the Tukey test was used, at a 5% probability level.

## 3. Results and Discussion

Germination - The emergence of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seedlings began on the 11th day after the establishment of the experiment for the three treatments. The germination test was concluded 35 days after planting. A high percentage of germination was found in all three treatments, with 92% in the petroleum-contaminated soil and the bioremediated soil, while the uncontaminated soil was 86%. There was no significant difference between the treatments for the total germination and the SGI (C = 2.97; B = 2.97 and U = 2.56), ($p > 0.05$).

Although no statistical differences were found between the treatments for the variables already described, it was possible to observe from the germination curves, differences in the behaviour of the species between the three treatments. For a better understanding of the germination process of the seeds, it is important to consider not just the final percentage, but also the germination curves, which demonstrated the germinative behaviour over time. It is necessary to examine the germination curves in detail, since two or more seed samples can have precisely the same final percentage of germination, but also have distinct germination curves. Such differences provide important implications when interpreting the results (Santana and Ranal, 2004).

The curves for the bioremediated treatment group and the uncontaminated group are almost parallel, with only a slight delay in the latter in relation to the former. On the other hand, the curve for the contaminated treatment group, is not only delayed compared to the others, it is also more irregular (Figure 1). Although the synchronicity index was not statistically different, in the contaminated soil this variable exhibited the greatest coefficient of variation (C = 15.5; B = 5.0; U = 8.6), evidencing heterogeneity among the repetitions, which could indicate that the contamination has a minor influence on the germinating behaviour of the species. The synchronicity index represents the degree of homogeneity of germination over time, the higher values being representative of a more irregular germination behaviour (C = 3.36; B = 2.91; U = 2.98).

Considering the relative frequencies of germination of *C. xanthocarpa*, it was found that the first peak in germination occurred in the bioremediated treatment on the 15th day, followed by the uncontaminated treatment on the 16th day. For the contaminated soil, there was a delay in relation to the other treatments occurring on the 19th day. The stabilization of the germination curves in the bioremediated and uncontaminated soils was observed after reaching this point. In the contaminated soil, on the other hand, a second peak in germination was observed on the 28th day (Figure 2), demonstrating greater irregularity of germination in this treatment.

The germination of seeds can be inhibited by the toxic effect of petroleum or by unfavorable soil conditions (Merkl et al., 2004). That is why various authors have studied the effects of soil contamination on seed germination, (Adam and Duncan, 1999, 2002; Banks and Shultz, 2005; Agbogidi et al., 2006; Ogbonna et al., 2007), however, there are very few that have looked at tree species.

Adam and Duncan (1999, 2002) investigated the effects of diesel oil on the growth of various plants, including grass, leguminous and cultivated species. At relatively low levels of diesel oil, there was a delay in the speed of germination index and percentage of seed germination and a reduction in plant growth. These results were due to the physical impediment of the oil, which in turn, makes it difficult to transfer water and oxygen present in the soil to the seeds.

Agbogidi et al. (2006) found there was a reduction in the percentage of germination of *Dennettia tripetala* Baker f. seeds with the increased concentration of crude oil. According to these authors, the crude oil present in the soil can retain oxygen, keeping it from being transferred to the seeds. The oxygen stress that occurs in the seeds can affect the embryo's respiratory system and its viability.

Banks and Shultz (2005) studied different plant species subjected to oil pollution, *Lactuca sativa* L., *Panicum miliaceum* L., *Raphanus* L., *Trifolium pratense* L. and *Triticum aestivum* L. The majority of the seedlings tested showed a decrease in the number of germinated seeds with the increase in the oil contamination; *Lactuca sativa*, however, was tolerant of petroleum contamination.

For some of the *C. xanthocarp*a seeds the death of the embryo was observed in the petroleum-contaminated soil treatment, though this was not significant, because it had no influence on the seed germination. This probably occurred as a result of oil penetrating into the interior of the seed or a lack of water and oxygen, which are essential to the development of the embryo (Baker, 1970; Chaîneau et al., 1997).

Kramer and Koslowski (1960) assert that both a lack of water in the soil and an excess of water reduce or inhibit germination, and that the latter would reduce the oxygen supply. The impermeabilization of the seed coating, due to the presence of a contaminant like oil, can also be a factor that interferes with the seed's water absorption.

For a plant that grows in ideal conditions there is contact between the root and soil matrix, allowing the supply of oxygen and water. However, if the roots grow in a petroleum-polluted environment, the soil particles are covered in a hydrophobic layer that reduces water availability. This causes anoxic stress and hydric stress and then the hydrophobic nature of the pollutant also causes chemical stress (Peña-Castro et al., 2006). Therefore, the presence of organic hydrocarbons in the soil in large quantities can block the absorption of water and nutrients by the plants (Baker, 1970; Udo and Fayemi, 1975; Reis, 1996).

In the case of *C. xanthocarpa*, despite the variations in the germinative behaviour evident in the germination curves, they are small in magnitude and were not significant. The high germinating capacity and the high seed vigour make it possible for the species to "resist" the negative effects of the contaminant on germination. In this phase, the plant depends more on the existing reserves in the embryo than on the external environment.

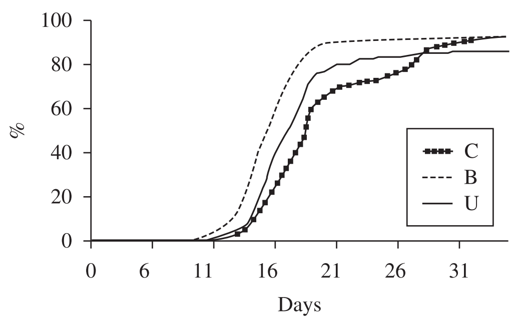


**Figure 1.** Percentage of germination (%) of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seeds, each curve represents the average of five repetitions. (C) - petroleum-contaminated soil; (B) - bioremediated soil; (U) - uncontaminated soil.

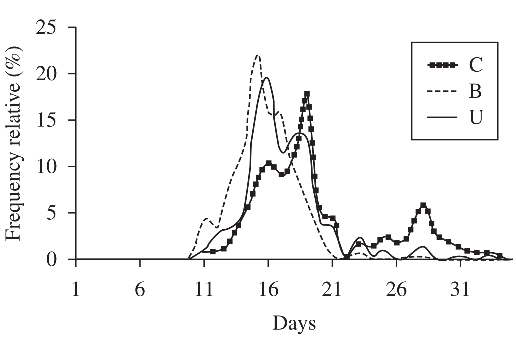


**Figure 2.** Relative frequency of germination of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) in (C) - petroleum-contaminated soil, (B) - bioremediated soil and (U) - uncontaminated soil. Each curve represents the average of five repetitions.

The high germinative capacity of the *C. xanthocarpa* seeds was also reported by Santos (2006). This author found the average rate of various species of Myrtaceae: *Campomanesia guazumifolia* (Cambess.) O. Berg, *C. xanthocarpa*, *Eugenia rostrifolia D*. Legrand, *Myrcianthes pungens* (O. Berg.) D. Legrand and *Psidium cattleyanum* Sabine. Among these species, *C. xanthocarpa* had the highest average speed of germination index, which corroborates the high germination rate indexes observed in the present study. According to Maguire (1962), the speed of germination index is a tool for evaluating seed vigour; the higher the numeric value, the higher the seed vigour will be in the seed samples analysed. Based on the results already described, such as high percentage and speed of germination index, it is possible to affirm that the species studied has high vigour and germinative potential. This fact probably justifies the high percentage of germination in all of the treatments tested.

Biomass and growth - Considering the biomass results, the findings showed that the contamination had no effect on the majority of variables, with the exception of the biomass of the paracotyledons and eophylls in the 60-day seedlings. When compared to the bioremediated treatment and the uncontaminated treatment, the petroleum-contaminated soil exhibited an increase in the biomass of the paracotyledons and a reduction in the biomass of the eophylls (Figure 3a, b).

Alterations in the growth and production of biomass of plants in contaminated soil have been reported by several researchers (Udo and Fayemi, 1975; Malallah et al., 1996; Li et al., 1997; Chupakhina and Maslennikov, 2004; Alkio et al., 2005; Mayer et al., 2005; Merkl et al., 2005; Agbogidi et al., 2006; Peña-Castro et al., 2006; Rezende, 2006; Santos, 2006).

Merkl et al. (2005) observed that *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf. and *Cyperus aggregatus* (Willd.) Endl. presented lower root length and an expressive increase in the root diameter in contaminated soil when compared to the control soil. Chupakhina and Maslennikov (2004) studied plants at roughly 25 days in hydrocarbon contaminated soil; the species that germinated in contaminated soil exhibited a reduction in the biomass and delayed development of the eophylls. In this case, it was found that for *C. xanthocarpa* there was also less biomass in the eophylls at 60 days and a delay in the emission of eophylls in contaminated soil (Figure 3a, b).

With *C. xanthocarpa*, it was found that there is a tendency for the largest values of biomass to occur in the bioremediated treatment. For the root biomass and total biomass of seedlings at 60 days, the bioremediated soil exhibited higher averages than the other treatments (Figure 4a, b). While the shoot biomass of the seedlings at 60 days was larger in the bioremediated treatment only when compared to the contaminated treatment group, not differing statistically from the uncontaminated treatment (Figure 4c). This same tendency is valid for the root biomass of seedlings at 30 days (Figure 4d).

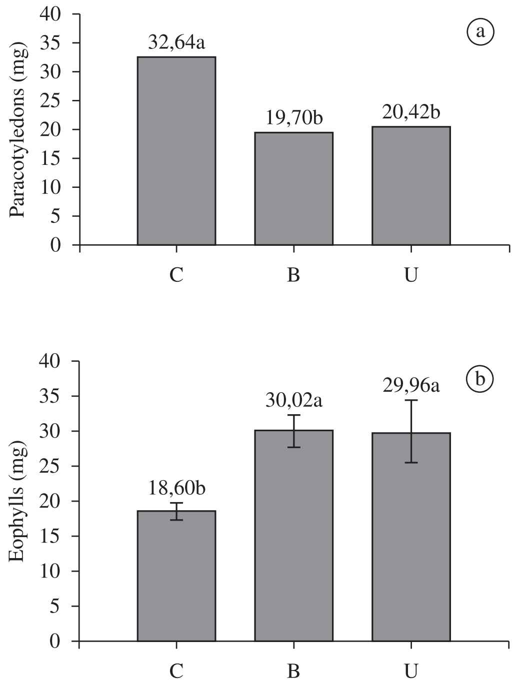


**Figure 3.** a-b) Biomass of the *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seedlings at 60 days, in petroleum-contaminated soil (C), bioremediated soil (B) and uncontaminated soil (U). Each column represents the average of five repetitions, bars indicate the standard deviation. Averages followed by the same letter do not differ statistically ($\alpha = 0.05$).

The shoot biomass, the total biomass as well as the biomass of the paracotyledons in 30- day seedlings do not differ statistically among the treatments. This suggests that the effects of contamination, as well as the bioremediation, became more evident after 60 days, a phase in which the seedlings certainly no longer rely on the reserves of the embryo.

The highest values of biomass found in the bioremediated soil can be related to alterations in the physical and chemical characteristics of the soil. This soil was turned over, stimulating greater aeration and, consequently, more favourable conditions for the development of the seedlings. Considering the data in Table 1, it was found that the value of bases saturation (V) is greater in the bioremediated soil (89%), when compared to the uncontaminated treatment group (4%) and the contaminated group (21%). The same occurs with the capacity to retain cations (T). Considering the alterations in the biomass of paracotyledons and eophylls in the 60-day seedlings in contaminated soil, it is possible to observe that bioremediation seems to have minimized the effects of the contaminant, since the values of the treatment matched those of the uncontaminated soil.

Basically, the same general tendency observed in the biomass can also be found in the length of the seedlings. The highest values normally occur in the bioremediated treatment when compared to the others, both for the root length and the total length of the seedlings at 30 and 60 days (Figure 5a, b, c, d). On the other hand, the shoot length did not differ among the treatments. The same factors that affected the biomass may have affected the length of the seedlings, since the aeration of the soil and the increased

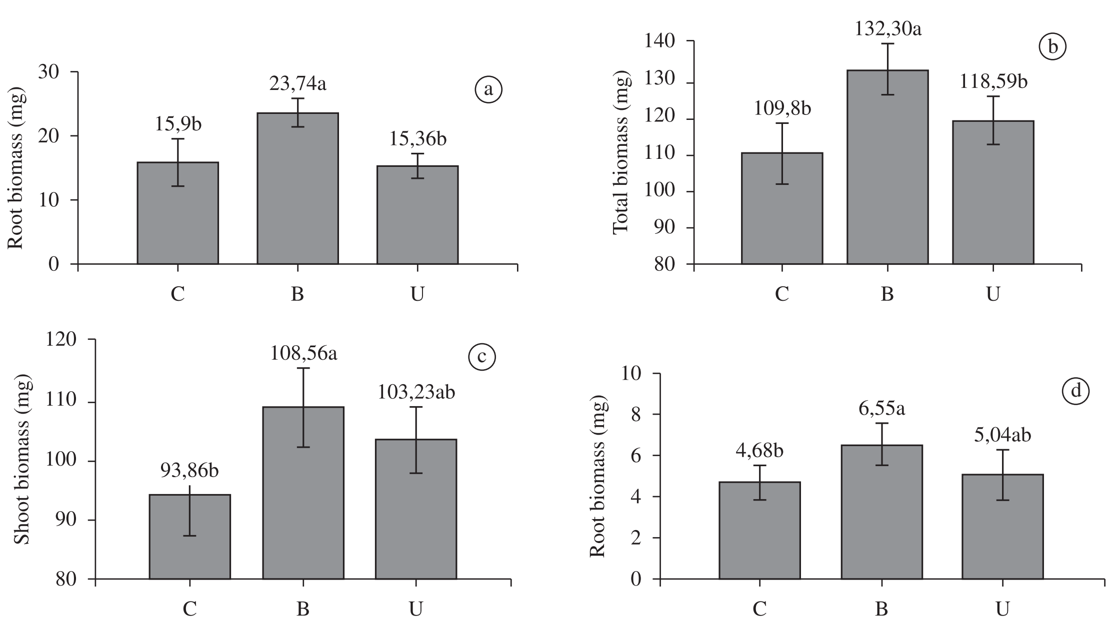


**Figure 4.** Biomass of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seedlings at 60 days (a, b, c) and 30 days (d), in petroleum-contaminated soil (C), bioremediated soil (B) and uncontaminated soil (U). Each column represents the average of five repetitions, bars indicate the standard deviation. Averages followed by the same letter do not differ statistically ($\alpha = 0.05$).

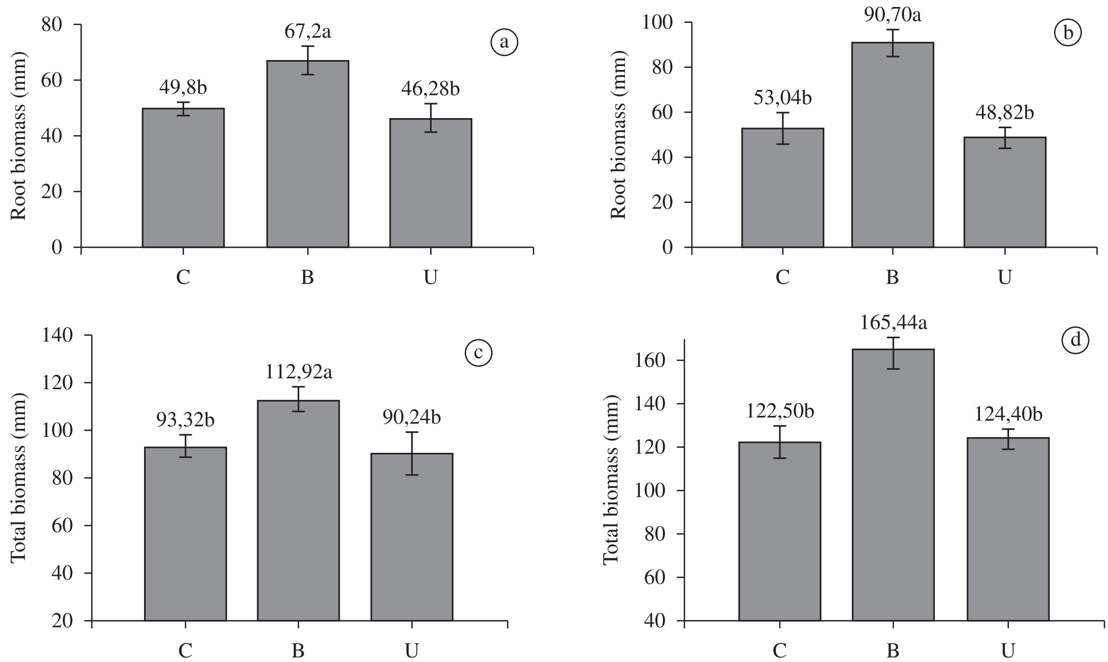


**Figure 5.** Length of the *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seedlings at 30 (a, c) and 60 days (b, d), in petroleum-contaminated soil (C); bioremediated soil (B); and uncontaminated soil (U). Each column represents the average of five repetitions, bars indicate the standard deviation. Averages followed by the same letter do not differ statistically ($\alpha = 0.05$).

availability of nutrients, probably favoured the increase in length.

The leaf area of the seedling paracotyledons at 30 and 60 days exhibited no statistical difference between the treatments. Yet the area of the eophylls (60 days) in the contaminated soil was greater than in the uncontaminated soil (Figure 6).

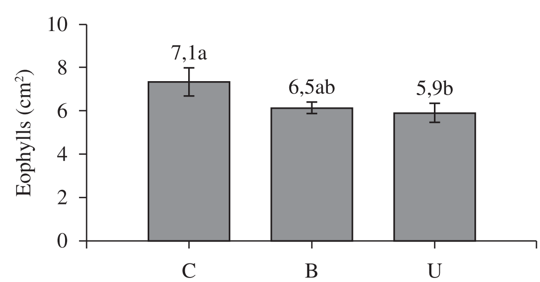


**Figure 6.** Leaf area of the *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seedlings at 60 days, in petroleum-contaminated soil (C), bioremediated soil (B) and uncontaminated soil (U). Each column represents the average of five repetitions, bars indicate the standard deviation. Averages followed by the same letter do not differ statistically ($\alpha = 0.05$).

Ogbonna et al. (2007), studied the effect of bioremediation and crude oil-contamination of soil on the growth of *Abelmoschus esculentus* (L.) Moench. They concluded that when microorganisms and fertilizers are added to the crude oil-contaminated soil, the plants exhibited a greater number of leaves, greater biomass, a higher percentage of germination and a high percentage of degradation of hydrocarbons present in the soil, when compared to the treatments that were not subjected to bioremediation. In the case of the present work, it was also possible to note that the total value of hydrocarbons in the bioremediated soil was reduced, when compared to the contaminated soil (Table 1). As discussed previously, the bioremediation minimized the effect of the contaminant on the biomass of the paracotyledons and eophylls.

Although alterations were not observed in terms of growth (biomass, length and leaf area) in the contaminated soil, morphological alterations were found in various seedlings. V-shaped lesions were observed on the paracotyledons and eophylls (Figure 7a, b, c); necrosis, death of the main root and the formation of new roots (Figure 7d, e, f); wrinkling of the paracotyledons and eophylls (Figure 7g) and even the death of the embryo and some seedlings (Figure 7h). Furthermore, it was found that some of the seedlings in the contaminated soil had an impediment or delay in the expansion of the paracotyledons (Figure 7i), due to the impregnation of a layer of oil and soil particles around the tissues that cover the seed, causing death in some cases. The seedling in the petroleum-contaminated soil exhibited a delay in the emergence of eophylls when compared to the bioremediated soil and the uncontaminated soil (Figure 7j-l).

Peña-Castro et al. (2006), studied the noxiousness of petroleum on *Cynodon dactylon* L. Pers. plants observing chlorosis and a reduction in the number of leaves and root growth in all of the oil concentrations tested. Alkio et al. (2005) observed necrotic lesions on the leaves of *Arabidopsis thaliana* (L.) Heynh. when subjected to contamination by

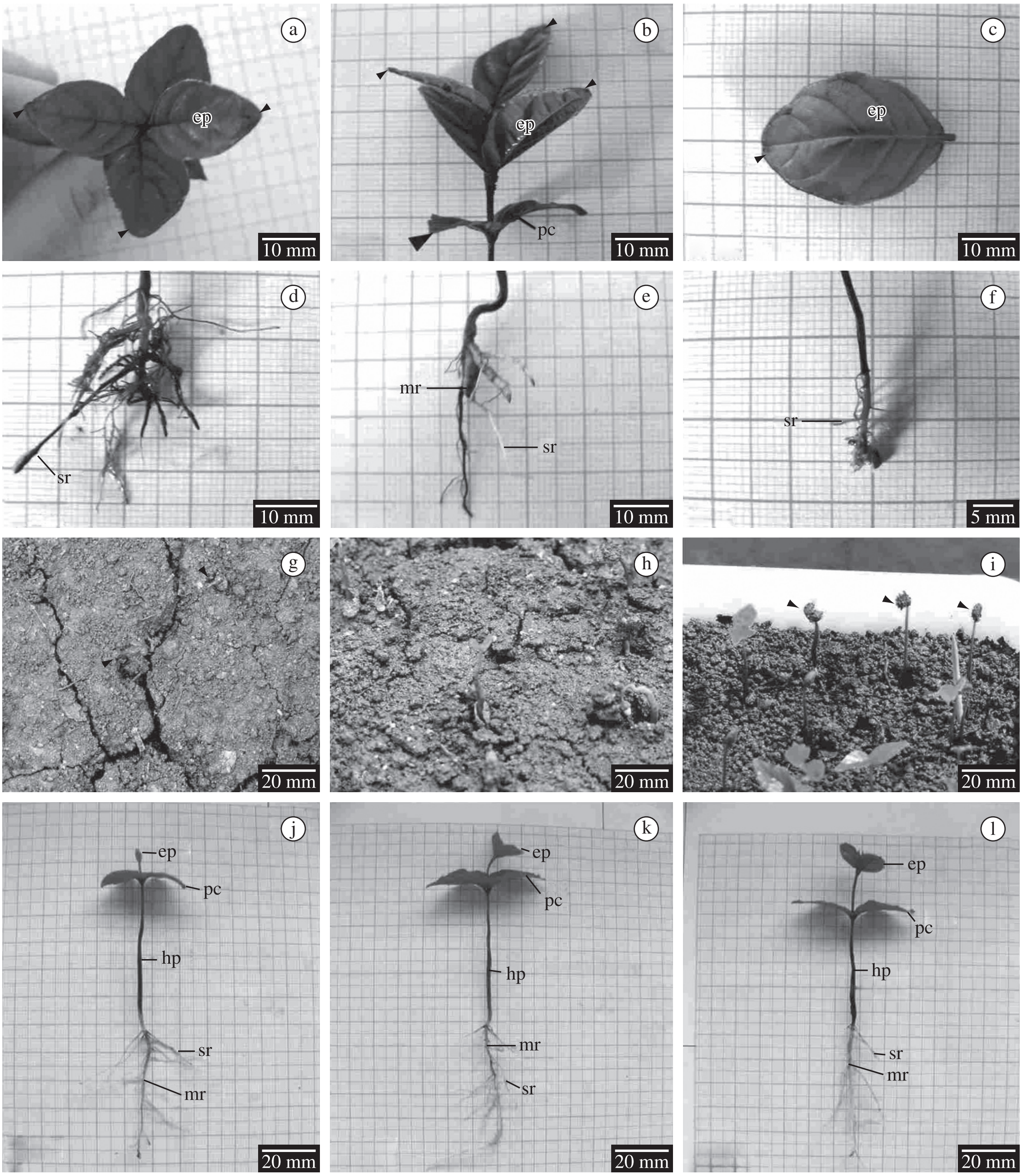


**Figure 7.** Morphology of the *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seedlings growing in petroleum-contaminated soil. a-c) lesions on the paracotyledons and eophylls (arrow); d-f) necrosis of the apex of the main root and formation of secondary roots; g) wrinkle of the paracotyledons and eophylls; h) compacted structure of the petroleum-contaminated soil and mortality of some seedlings (arrow); i) layer impregnated with oil and soil particles in the seed coats (arrow); j-l) seedlings after 30 days in petroleum-contaminated soil; j) bioremediated soil; k) and uncontaminated soil; and l) (ep): eophyll; (pc): paracotyledon; (hp): hypocotyl; (mr): main root and (sr): secondary root.

phenanthrene, in addition to a reduction of radicle growth and the number of leaves.

Agbogidi et al. (2006) reported a reduction in the percentage of seed germination, a reduction in growth and the number of *Dennettia tripetala* (Baker) f. leaves in increased concentrations of crude oil. According to the authors these responses can be attributed to alterations in the soil structure, creating conditions of hydric stress. This fact was also found by Li et al. (1997), the author reported a significant reduction in the growth of grass species as a result of hydrocarbon contamination of the soil, where the soil had a low water content.

Hydrocarbon contamination of the soil can often have various effects, such as preventing the absorption and maintenance of water in the soil. Oil can act as a physical barrier obstructing both the penetration of water and the evaporation of gases into the atmosphere. The plants subjected to oil contamination can exhibit stress due to a hydric deficit and hypoxia (Li et al., 1997).

According to Larcher (2000), in oxygen-deficient soil conditions the growth of the plant roots is interrupted, the apexes of the radicle system die and the adventitious roots develop. Pezeshki, DeLauneb and Jugsujindab, A. (2001), also affirmed that one of the main physical effects of petroleum contamination is the film of oil that covers the roots and changes the flow of water and nutrients to the plants.

Various seedlings in the contaminated soil exhibited death of the main root and the formation of new roots. Gogosz (2008), analysing the anatomy of the seedlings in the same experiment, detected deformations in the radicle apex, necrosis and the presence of a layer enveloping the root, which may be mucilage, in response to the stress, or the contaminant itself (Figure 8a, b).

The morphological responses of the *C. xanthocarpa* seedlings to the contaminant seem to be linked to the disruption in the absorption of water, which in turn, interferes with the absorption of the nutrients necessary for the development of seedlings. In addition to this, there is also the possibility of a decrease in the availability of oxygen for the radicle system.

Oxygen deprivation can increase the ethylene content in plants since it is produced in greater quantities when subjected to conditions of stress. Plants that liberate ethylene in excess can exhibit symptoms like leaf abscission, senescence and necrosis (Taiz and Zeiger, 2004). This fact could explain the necrotic lesions observed in the *C. xanthocarpa* seedlings.

The morphological changes observed in the *C. xanthocarpa* seedlings in the contaminated soil, probably affected its growth. However, the fact that substantial changes in growth were not perceived does not necessarily mean the absence of influence of the contaminant. In order to confirm this it would be necessary to observe the development of the plants for a longer period of time.

## 4. Conclusion

Contamination by petroleum did not change the percentage of germination of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) seeds. On the other hand, it had a negative influence on the development of the seedlings, causing a reduction in the production of biomass and in its growth. Bioremediation of the soil mitigated the effect of the contaminant, resulting in an increase in the biomass and the length of the seedlings.

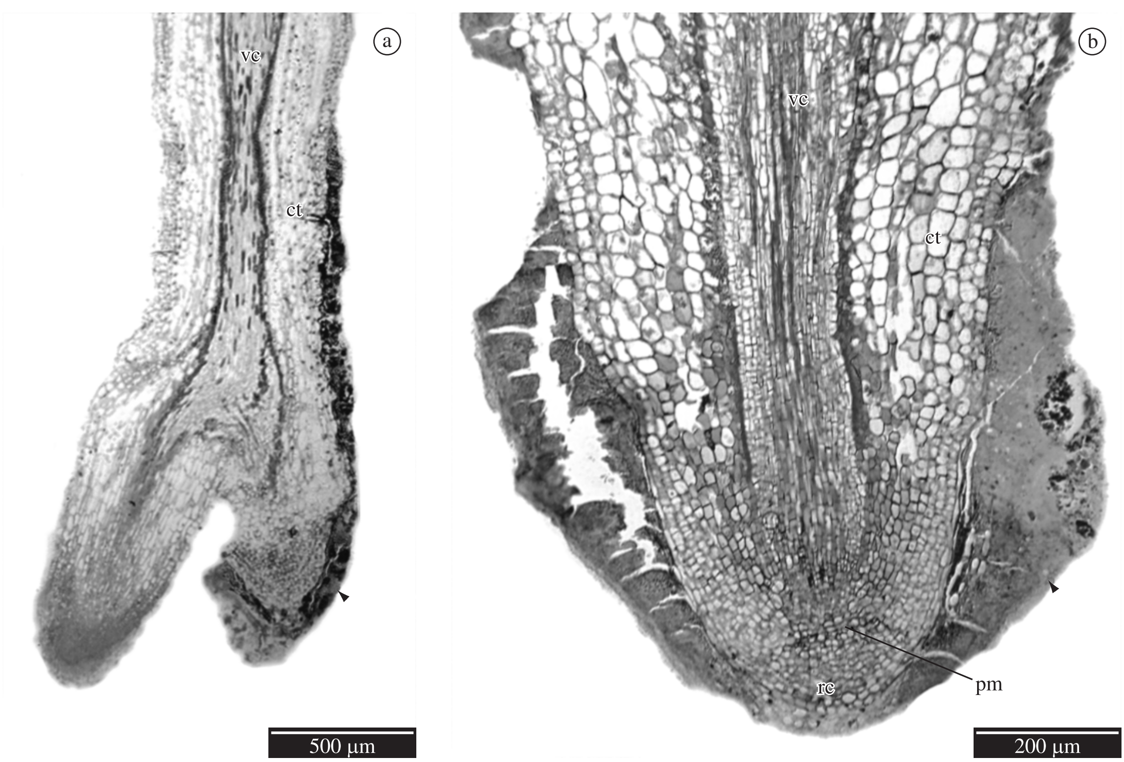


**Figure 8.** Longitudinal sections of the root apex of *Campomanesia xanthocarpa* O. Berg. (Myrtaceae) in petroleum contaminated soil (a, b). ct: cortical tissue; vc: vascular cylinder; rc: root cap; pm: promeristem; arrow: layer of mucilage.

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