KACILDA NAOMI KUKI

EMISSÕES DA INDÚSTRIA DE MINÉRIO DE FERRO COMO FATORES DE RISCO ECOLÓGICO EM ESPÉCIES OCORRENTES NA RESTINGA DE ANCHIETA, ESPÍRITO SANTO, BRASIL

> Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de *Doctor Scientiae*.

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À MINHA FAMÍLIA

Dedico

AGRADECIMENTOS

BIOGRAFIA

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SUMÁRIO

RESUMO

KUKI, Kacilda Naomi, D.Sc., Universidade Federal de Viçosa, abril de 2007. **Emissões da indústria de minério de ferro como fatores de risco ecológico em espécies ocorrentes na restinga de Anchieta, Espírito Santo, Brasil**. Orientador: Marco Antônio Oliva Cano. Co-Orientadores: Milene Faria Vieira e José Cambraia.

A vegetação de restinga localizada no litoral do estado do Espírito Santo encontra-se exposta à contaminação por poluentes emitidos pela atividade das indústrias mineradoras de ferro, o minério de ferro em pó e a deposição úmida ácida. Os possíveis efeitos das emissões sobre a vegetação foram avaliados mediante das alterações bioquímicas, fisiológicas e ecológicas, em cinco espécies nativas: *Ipomoea pes caprae, Canavalia rosea, Sophora tomentosa, Guapira pernambucensis* e *Schinus terebinthifolius.* O monitoramento em campo revelou que a proximidade com a fonte emissora dos poluentes leva a um incremento do conteúdo de ferro nos tecidos foliares, o que pode colaborar para o desenvolvimento de toxidez. Os resultados fenológicos indicaram que as fenofases reprodutivas, floração e frutificação, são negativamente afetadas pela exposição à deposição de material sólido particulado de ferro, especialmente em *S. tomentosa*. Em experimentos de germinação e crescimento inicial, observou-se que a exposição à acidez e ao material sólido particulado de ferro afetou diferentemente as espécies estudas. Os resultados revelaram que *G. pernambucensis* e *S. terebinthifolius* apresentaram maior resistência*,* uma vez que a germinabilidade, o índice de tolerância radicular e o ganho de matéria seca total destas espécies permaneceram inalterados em todos os tratamentos, enquanto que *S. tomentosa* foi significativamente afetada*.* A deposição simulada de material sólido particulado de ferro e de névoa ácida sobre a porção aérea de *S. terebinthifolius* e *S. tomentosa* provocou o desenvolvimento de um estresse oxidativo somente em *S. tomentosa.* Nesta espécie, foi observada uma redução da taxa fotossintética líquida, enquanto que o extravasamento de eletrólitos, o conteúdo de ferro e de malondialdeído, nas folhas, aumentaram. Esta situação foi acompanhada por um aumento significativo na atividade de enzimas antioxidantes, superóxido dismutase e catalase. Ao contrário, as plantas de *S. terebinthifolius* foram capazes de impedir o desenvolvimento do estresse oxidativo, em parte por evitarem o acúmulo tóxico de ferro em suas folhas.

Os resultados obtidos permitem concluir que as emissões provenientes das atividades da indústria de minério de ferro podem afetar a vegetação da restinga em seus diferentes níveis de organização e em longo prazo, provocar um declínio na quantidade e diversidade daquelas espécies mais sensíveis.

ABSTRACT

KUKI, Kacilda Naomi, D.Sc., Universidade Federal de Viçosa, April, 2007. **Iron ore industry emissions as ecological risk factors for plant species of Anchieta restinga, Espírito Santo, Brazil**. Adviser: Marco Antônio Oliva Cano. Co-Advisers: Milene Faria Vieira and José Cambraia.

The restinga vegetation of the Espírito Santo state coast is exposed to the pollutants emitted by iron ore industry, the iron ore solid particulate matter and the wet acid deposition. To investigate the possible effects of these pollutants on the vegetation, biochemical, physiological, and ecological processes were evaluated. For that matter five native species were assessed: *Ipomoea pes caprae, Canavalia rosea, Sophora tomentosa, Guapira pernambucensis* and *Schinus terebinthifolius*. Field monitoring revealed that plants located near the source of pollutants had greater iron content in the leaves, which might collaborate to phytotoxicity. Phenological results indicate that the reproductive aspects of these species, flowering and fruiting, can be negatively affected by the deposition of iron ore solid particulate matter, especially in *S. tomentosa*. In the germination and initial growth experiments it was observed that the exposure to acidity and iron ore solid particulate matter affected differently the species studied. The results reveal that G. *pernambucensis* and *S. terebinthifolius* presented a greater resistance, since the germination rate, the root tolerance index and the net gain of dry matter in these species remained unaffected even upon stress treatments, while *S. tomentosa* was significantly affected. The simulated deposition of iron ore solid particulate material and acid mist on the aerial portion of the plants induced an oxidative stress in S. *tomentosa*. In this species the photosynthetic rate was reduced, whereas the electrolytes leakage, the malondialdehyde and iron contents in the leaves increased. This situation was accompanied by a significant increase in the activity of antioxidant enzymes, superoxide dismutase and catalase. Conversely, plants of *S. terebinthifolius* were able to avoid the development of oxidative stress, in part due to its capacity to maintain a normal content of iron in the foliar tissue. These results allow the conclusion that the emissions from the iron ore industry can affect the restinga vegetation in its different organization, levels and as a long term consequence, alter its composition.

1. Introdução Geral

O litoral brasileiro abriga vários resquícios de restinga, um ecossistema que outrora ocupava grandes extensões da costa nacional (Suguio e Tessler, 1984). O termo restinga refere-se ao cordão arenoso de origem quaternária, depositado ao longo das idades geológicas e que abriga uma série de comunidades vegetais, temporal e espacialmente distintas entre si. As diferentes comunidades vegetais representam, em parte, os estádios sucessionais do ecossistema. Assim sendo, as formações arbóreas encontram-se mais afastadas do oceano, enquanto que as comunidades pioneiras e herbáceas são encontradas próximas à zona das marés (Rizzini, 1979).

No Estado do Espírito Santo ocorrem inúmeros fragmentos de restinga. Entretanto, a vegetação original encontra-se fortemente alterada devido à intervenção humana. As porções remanescentes são formadas por cerca de oito comunidades ou formações vegetacionais básicas: halófitas, halófita-psamófita, pós-praia, ''Thicket'' de Myrtaceae, ''Scrub'' de Palmae, ''Scrub'' de *Clusia*, ''Scrub'' de Ericaceae, mata seca, mata periodicamente inundada, mata permanentemente inundada e brejo herbáceo (Pereira, 1990). Toda a cobertura vegetal desempenha papel significante na fixação das dunas arenosas e, consequentemente, na estabilidade do ecossistema (Araújo e Lacerda, 1987).

As formações vegetacionais mais próximas à zona das marés, halófita-psamófita e pós-praia, ocupam área de solo arenoso nutricionalmente deficiente e sujeita à constante ação do aerossol marinho (Andrade, 1977). Comunidades que se desenvolvem em tais circunstâncias são geralmente formadas por número seleto de espécies vegetais, as quais apresentam mecanismos adaptativos capazes de suplantar as condições vigentes do ambiente (Oosting e Billings, 1942; Boyce, 1954). As comunidades halófita-psamófita e pós-praia desempenham papel fundamental na dinâmica sucessional e na preservação da vegetação de restinga, visto serem responsáveis pela fixação das dunas arenosas, evitando a movimentação excessiva delas e colaborando para a estabilização de um solo propenso ao desenvolvimento de espécies vegetais mais exigentes (Pereira, 1990).

O Espírito Santo é um estado em franco desenvolvimento econômico e abriga várias indústrias do setor minerador de ferro em seu litoral. A presença destas indústrias pode contribuir para a alteração da fitofisionomia da vegetação de restinga,

uma vez que suas emissões de dióxido de enxofre (SO_2) e de material sólido particulado (MSP) são potencialmente deletérias (Wong e Tam, 1977; Lopez *et al.*, 2000). Os vegetais, em particular, apresentam elevada sensibilidade e especificidade a uma série de poluentes, exibindo sintomas precoces de injúria (Treshow, 1984). Contudo, a sensibilidade dos vegetais não depende exclusivamente da espécie, sendo que o estádio de desenvolvimento em que se encontram durante o período de exposição aos poluentes interfere nas suas reações ao estresse (Alexeyev, 1995).

O SO_2 pode afetar diretamente a vegetação ao ser absorvido na forma de gás pelas folhas e desencadear alterações metabólicas nas células do mesofilo, ou de forma indireta ao colaborar para a acidificação das deposições úmidas, como a chuva e a neblina (Fenn e Kiefer, 1999). A deposição ácida, por sua vez, contribui para a modificação da composição química do solo, o que pode interferir nos padrões fisiológicos, de crescimento e de desenvolvimento de espécies vegetais susceptíveis (Fan e Wang, 2000). As plantas, em suas diferentes etapas biológicas, podem ser direta e igualmente afetadas pelas deposições ácidas. A germinação do tubo polínico, durante a fecundação, pode ser inibida por diferentes tipos e intensidade de acidez (Paoletti e Bellani, 1990). Similarmente, o poder germinativo das sementes pode ser reduzido, caso o pH do substrato em que se encontram decaia além do limite tolerado pela espécie (Ryan *et al.,* 1975). Plântulas são particularmente sensíveis às deposições ácidas, sendo, por isso, muitas vezes utilizadas em programas de biomonitoramento em áreas afetadas por essa forma de poluente (Koricheva *et al.,* 1997; Momen *et al.*, 2002).

A poeira ou MSP consiste de matéria subdividida em diminutas frações suficientemente pequenas para serem suspensas e carreadas pelo vento sob a forma de aerossol. O MSP fino ($MP_{2.5}$; 0-2.5 μm $\varnothing$), e o MSP grosso (MP_{10}; 2.5-10 μm $\varnothing$) são as frações usualmente responsáveis pelas alterações biológicas em organismos vivos. O MSP afeta as plantas tanto por mecanismos físicos, ao perturbar o balanço de radiação, ao causar abrasão e aquecimento foliar e ao danificar o controle estomático; ou por mecanismos químicos ao provocar a lixiviação de nutrientes das folhas e ao afetar o pH, o estado nutricional e a microflora do solo (Grantz *et al.*, 2003; Prusty *et al.*, 2005). A magnitude do impacto do MSP ao ambiente dependerá das características de suas partículas, da intensidade e freqüência das deposições, bem como da susceptibilidade e do estágio de desenvolvimento dos organismos afetados. Sabe-se, porém, que os efeitos desta forma de poluição podem atingir

patamares suficientes para causar modificações na estrutura das comunidades vegetais expostas (Wong *et al.*, 1978). A natureza química e física do MSP varia de acordo com a fonte emissora. No caso das indústrias de mineração, o MSP está diretamente relacionado com o minério explorado (Farmer, 1993).

Durante os processos de exploração e beneficiamento do minério de ferro (hematita ou pirita) é gerado o minério de ferro em pó, ou material sólido particulado de ferro (MSP_{Fe}), que emitido pelas indústrias, a exemplo do que ocorre no Espírito Santo, representa um aporte adicional deste elemento no ambiente (Wong e Tam, 1977; Uhlig e Junttila, 2001). O ferro é um dos minerais mais abundantes na crosta terrestre, porém a maior parte não se encontra prontamente biodisponível às plantas. O ferro está presente nos solos principalmente em dois estados de oxidação: Fe^{3+} (íon férrico) e Fe^{2+} (íon ferroso). Na presença de oxigênio o Fe^{2+} é rapidamente oxidado a Fe^{3+}, o qual é pouco solúvel em água, precipitando-se na forma de oxihidrato de ferro (FeOH-FeOOH) que é virtualmente inacessível aos organismos vivos (Schmidt, 2003).

As plantas superiores desenvolveram diferentes estratégias para absorção, transporte e armazenamento do Fe. Em dicotiledôneas a estratégia I é a mais comum. Neste caso, o mecanismo de absorção de ferro pelas plantas envolve a redução do pH na rizosfera, pela excreção de prótons (H^+) e ácidos orgânicos. Desta forma, o estado redox da superfície radicular é alterado, permitindo a solubilização do Fe^{3+} e sua posterior redução a Fe^{2+}. Essa reação é catalisada pela redutase férrica, presente nas membranas das células epidérmicas das raízes, e o Fe^{2+} resultante é diretamente absorvido por um transportador específico. Outra forma de absorção de ferro, denominada estratégia II, presente apenas em gramíneas, envolve a liberação de fitosideróforos, que são aminoácidos não-protéicos cuja função é quelar o Fe^{3+}, no meio externo, e carreá-lo para as raízes. Uma vez no córtex, o ferro passa de uma célula a outra através de plasmodesmos, até chegar ao xilema onde é encontrado na forma de citrato férrico (Briat *et al.*, 1995; Briat e Lobreaux, 1997).

Adicionalmente, sob a forma gasosa ou iônica solúvel, e quando em contato com a superfície das folhas, os elementos químicos podem ser absorvidos via epiderme. Esta estratégia de absorção contribui para o acúmulo total de elementos essenciais e não essenciais. Em locais onde há presença significativa de poluentes em suspensão na atmosfera é possível ocorrer o acúmulo tóxico de elementos químicos nos tecidos foliares (Lau e Luk, 2001; Pugh *et al.*, 2002).

O íon ferro, no interior celular, pode formar ligações complexas com o oxigênio, o nitrogênio e o enxofre, estando comumente associado a sistemas enzimáticos. Aliado a essa característica, o ferro apresenta grande potencial redox, o que imprime a esse elemento papel importante na transferência de elétrons em processos metabólicos de oxirredução, como fotossíntese e respiração (Guerinot e Yi, 1994; Suh *et al.*, 2002). Como elemento essencial às plantas, o Fe é requerido em baixíssimas concentrações. Contudo, determinadas condições ambientais podem interferir na sua homeostase. Solos com fortes condições redutoras e baixo pH, fraco poder de redução das raízes, e contínuo fornecimento de ferro ao sistema, a partir de uma fonte externa, podem elevar as concentrações do ferro biodisponível (Vitosh *et al.*, 1994; Connolly e Guerinot, 2002; Azevedo e Chasin, 2003).

Os danos causados pela toxicidade do ferro podem ser diretos, por meio da absorção e acúmulo excessivo do elemento, ou indiretos, quando altos teores de ferro na solução do solo resultam na sua precipitação sobre as raízes, formando uma crosta de óxido férrico, que altera a absorção de outros nutrientes como fósforo, potássio e zinco (Hansel, *et al.*, 2001) Em situações de acúmulo excessivo de ferro nos tecidos, ocorre a potencialização da geração de radicais hidroxil ($OH^\bullet$), através da reação de Fenton. A ação deste radical livre, juntamente com outros intermediários de oxigênio reativos (IORs), pode causar danos irreversíveis às membranas celulares, ocasionando o colapso celular e conseqüente deterioração do tecido, fenômeno denominado de estresse oxidativo (Kampfenkel *et al.*,1995; Gallego *et al.*, 1996; Becana *et al.*, 1998; Bartakova *et al.*, 2001).

As plantas apresentam vários mecanismos enzimáticos e não-enzimáticos para se protegerem contra o estresse oxidativo gerado pelo excesso de ferro (Sinha *et al.*, 1997; Vansuyt *et al.*, 1997). Os sistemas enzimáticos são representados pelas enzimas dismutase do superóxido (SOD), catalases e peroxidases. Os sistemas não enzimáticos consistem de moléculas orgânicas como a glutationa, os carotenóides, o ascorbato, o tocoferol, o ubiquinol, o ácido úrico e lipóico, que podem reagir diretamente com os IORs, neutralizando-os (Mittler, 2002).

As respostas dos vegetais em função da exposição a agentes poluentes são indicativas do grau de severidade imposto pelo estresse. Sob esta concepção, as alterações não visíveis de caráter bioquímico ou fisiológico, os biomarcadores, e as alterações visíveis em indivíduos, os bioindicadores, ou na população da espécie, os

indicadores ecológicos, são empregados como ferramentas de avaliação de risco ambiental (Klumpp *et al.*, 2004).

Pouca informação existe acerca dos efeitos da toxicidade de ferro em espécies vegetais tropicais. A ação conjunta da deposição úmida ácida e do MSP$_{Fe}$, configura uma situação de distúrbio às comunidades vegetacionais, uma vez que o efeito de um pode ser potencializado pela ação do outro. O processo germinativo e o estabelecimento inicial das plântulas são particularmente susceptíveis a esta forma de estresse (Wong *et al.,* 1978). Em indivíduos adultos tal exposição pode afetar os processos metabólicos, resultando numa menor produtividade e vigor, necessários ao crescimento e a ocorrência das fenofases (Thompson *et al.,* 1984).

A presença da indústria do setor minerador traz benefícios sócio-econômicos concretos às regiões onde se instala como é o caso do litoral capixaba. Mas agregado aos benefícios existe risco real de impacto ao ambiente. Apesar das plantas de restinga possuírem mecanismos que lhes permitiram colonizar um ambiente natural de condições extremas, a inserção de novos elementos ao sistema, como o MSP$_{Fe}$ e a deposição ácida, pode desencadear reações que comprometem a dinâmica e a composição florística do ecossistema. Portanto, uma cuidadosa avaliação dos efeitos dos poluentes, resultantes da atividade mineradora de ferro, sobre a vegetação de restinga possibilitará a geração de informações que permitirão auxiliar no biomonitoramento e na avaliação de risco ecológico em áreas afetadas pela atividade, além de fornecer informações necessárias aos programas de conservação e recuperação de áreas nativas.

2. Objetivo Geral

Este estudo teve como objetivo avaliar os efeitos potencialmente deletérios das emissões da indústria de beneficiamento de minério de ferro sobre componentes da vegetação de restinga. Para tanto, foram investigados a ação dos poluentes mais característicos desta atividade industrial, o minério de ferro particulado e a deposição úmida acida, sobre diferentes aspectos bioquímicos, fisiológicos e ecológicos de espécies nativas da restinga: *Ipomoea pes caprae* L. Sweet (Convolvulaceae) e *Canavalia rosea* (Sw.) DC. (Legumiosae), *Sophora tomentosa*

L. (Leguminosae)*, Guapira pernambucensis* (Casar.) Lundell (Nyctaginaceae) e *Schinus terebinthifolius* Raddi (Anarcadiaceae).

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CAPÍTULO I

ASSESSMENT OF ECOLOGICAL RISKS FOR TROPICAL COASTAL VEGETATION INDUCED BY IRON ORE INDUSTRY EMISSIONS

Abstract. In the coastal zone of Espírito Santo state, Brazil, fragments of restinga, a natural and already disturbed ecosystem share its space with an increasing number of iron ore industries. The iron ore dust and the SO_2 originated from the processing activities of the industries can interfere on the vegetation composition of the adjacent ecosystem through variable levels. This study was undertaken in order to evaluate the impact of the industry emissions on restinga vegetation, using physiological and phenological parameters as indicators. Foliar samples of *Ipomoea pes caprae*, *Canavalia rosea*, *Sophora tomentosa* and *Schinus terebinthifolius*, were collected at three increasing distances from an ore industry (1.0, 5.0, and 15 km), and had their dust deposition, chlorophyll and Fe contents assessed. Phenological monitoring, focused on shooting, flowering and fruiting, was also performed throughout a year. The results showed that edafics and mineral constitution of the plants were affected and the chlorophyll content of the four species increased with the proximity to the industry. Phenological data revealed that the reproductive effort, as fruit production, has been affected by the emissions. *S. tomentosa* was the most sensitive species. The integrate survey indicates that the best choice to assess the ecological risks on restinga vegetation due to ore industry activities has to be simultaneously based on different parameters rather than using isolated biochemical or ecological data. Thus it will be possible to evaluate the ecosystem health and provide needed information to restoration programs without a bias interpretation.

Key words: coastal ecosystem, ecological indicator, particulate matter, phenology, restinga.

1. Introduction

It is fairly accepted that atmospheric pollution can trigger environmental associated problems. A stationary source of pollutant, such as ore industries, can generate and emit considerable amount of particulate matter (PM) and sulfur dioxide (SO_2), therefore contributing to increase dry and wet deposition on vicinity ecosystems (Lopes *et al.*, 2000; Grantz *et al.*, 2003).

Chemical and physical injuries are among the effects caused by coarse and fine PM on living organisms. Plants, in particular, are prone to suffer in larger scale, especially due to their inability to escape from stressors (Yunus and Iqbal, 1996). Depend on its size, the PM deposited on the leaf surface can affect its metabolism by blocking the light, obstructing stomata aperture, increasing leaf temperature, and altering pigments and mineral contents of the organ (Paling *et al.*, 2001; Naidoo and Chirkoot, 2004). Vegetation damages imposed by PM can also be enhanced if trace metals are present in its constitution or by simultaneous acid deposition. Once deposited on the soil, the metals may accumulate to phytotoxic levels and in association with low pH modify the substrate composition and nutrient availability to the plants (Farmer, 1993; Uhling and Juntilla, 2001). The combination of these effects can contribute to the development of oxidative stress and alter plant growth and reproduction, bringing changes on the dynamic and composition of ecosystems (Wong *et al*., 1978; Gallego *et al*., 1996; Bartakova *et al*., 2001; Alvarez, *et al*., 2003; Kozlov and Zvereva, 2004). Nonetheless, much of the impacts caused by any atmospheric pollution are time and space related, hence the effects can vary not only with the sensitivity of the organism but also with the season or period of exposure to the pollutants.

Restinga is a tropical coastal ecosystem of quaternary origin found along the Brazilian littoral. The vegetation that comprises this ecosystem has a crucial role on the stability of the sand dunes and the security of its biodiversity (Pereira, 1990). The coastline of Espírito Santo state, Brazil, hosts fragments of restinga, and a growing number of iron ore industries. The industrial activities involve processing, beneficiation, stocking and shiploading of the ore. As a result, SO_2 and iron ore dust, a type of dry PM, are constantly added into the atmosphere, as spontaneous or spills occurrences.

Although coastal vegetation is adapted to marine aerosol, a natural form of airborne particles (McCune, 1991; Barrick, 2003), the addition of PM, as iron dust, can impose a new form of stress to the vegetation. The impact can affect the whole structure of the ecosystem, disrupting its balance, as ecological risks set in. While isolate effects of pollutants on plants indicate the integrity of a particular species, an integrated view of those effects on different species can help evaluate the vegetation integrity (Dale and Beyeler, 2001).

The restinga ecosystem is protected by law and many efforts have being done to ensure its preservation, despite the intense anthropogenic disturbance. However, few studies concerning the impact caused by ore industry activities are available. Most ecological assessments judge the impact only trough physiological or ecological data alone. Thus, the present research aimed to investigate the strain created by the emissions of the iron ore industry on the restinga vegetation, through their influence on phenological data as well as on biochemical traits of growing plants, providing useful information for restoration programs.

2. Materials and Methods

2.1. Study site

Fragments of restinga occur in Anchieta, state of Espírito Santo and according to Köppen´s classification, the climate in this region is Aw, tropical warm and humid with a non pronounced dry season during winter months. The annual precipitation is about 900 mm, the average air humidity 70 % and the average temperature 23.4 ^{o}C.

Located at 0.5 km from the sea shore at 20° 46' 21.0" S and 40° 34' 52.3" W, and surrounded by native flora, there is an iron ore industry complex. The industry plant includes a stockyard, two furnaces and a port terminal for shipment of the iron pellets and sinter, the final products. The main ore exploited is *itabirito*, composed basically of hematite (Fe_2O_3) and quartz (SiO_3), and its beneficiation and handling generate iron ore particles, mainly coarse particulates (Table 1). Also, SO_2 is emitted by the furnaces during the pelletization process which contributes to increase the total particulate matter in suspension (TPM) or dust.

Table 1. Average composition of the iron ore powder after beneficiation

Component	%(dry basis)
Fe	67.1
FeO	0.96
SiO_2	1.10
Al_2O_3	0.30
CaO	0.10
MgO	0.02
Cu	0.006
Na_2O	0.007
P	0.040
S	0.003
Mn	0.033
Moisture	9.60

To study the impact of these pollutants on vegetation, three sites were selected along the coast, based on their proximity to the source and the commonness of the plant species of interest. The distances of the first, second and third site were 1.0, 5.0 and 15.0 km from the industry complex (Figure 1).
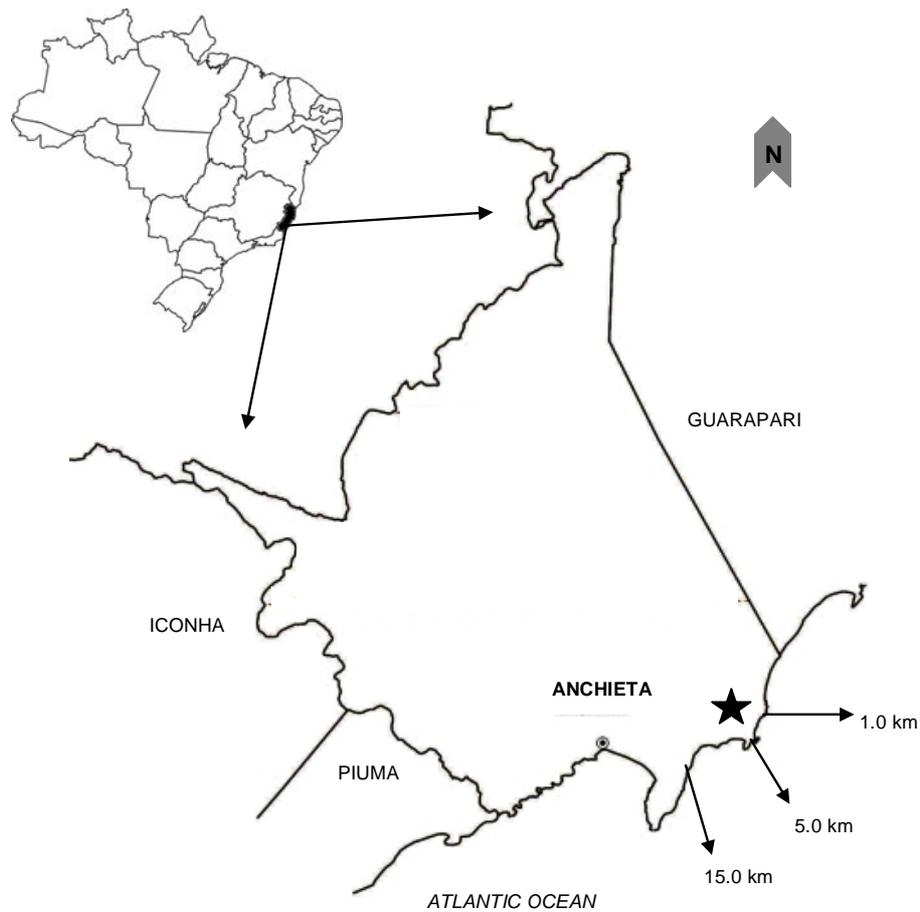

Figure 1. Map of Anchieta municipality, Espírito Santo state-Brazil, with location of the 3 assessed sites (1.0, 5.0 and 15.0 km). The (★) represents the location of the iron ore industry plant.

2.2. Plant material and experiment

Four species were chosen to have their phenological and biochemical traits assessed: *Canavalia rosea* DC, (Leguminosae - Papilionoideae), *Ipomoea pes caprae* L. (Convolvulaceae), *Schinus terebinthifolius* Rardii (Anarcadiaceae) and *Sophora tomentosa* L. (Leguminosae - Papilionoideae). The selection was based on their ecological importance and abundance on the areas of study. *C. rosea* and *I. pes caprae* are both crawling, fast growing herbaceous species. Their growing habit helps contain sand dunes from shifting (Gross, 1993; Patiño *et al.*, 2002). *S. terebinthifolius* and *S. tomentosa* are arbustive species. *S. terebinthifolius* is a species with great ecological plasticity, commonly found in several tropical ecosystems, while the distribution of *S. tomentosa* is restrict to tropical coastal ecosystems (Lenzi and Orth, 2004 b; Nogueira and Arruda, 2006).

On each sample site, 10 individuals per species were selected and marked, according to their health and size homogeneities. Since *S. terebinthifolius* is a dioecious species only female plants, previously selected, were marked. To mark *C. rosea* and *I. pes caprae*, 10 m^2 plastic frames were used to individualize the dense ground cover produced by these crawling species.

The deposition of TPM on the leaf surface and the iron (Fe) and pigments contents in the leaves were analyzed every trimester along the year 2005. The TPM was determined on plants of all three sites, while the Fe and pigments analysis was done in plants of the first and third site.

The TPM deposition was estimated on five leaves, from the third or fourth nodes, each randomly collected from five out of the 10 marked plants. Aided by a soft paint brush, the leaves were individually washed with 20 ml of distilled water in pre-weighed glass flasks. After 48 hours of decantation at room temperature the flasks were transferred and kept into a non ventilated oven at 40°C until the water had been completely evaporated. After cooling, the flasks were re-weighed. The leaf area was measured by a plan meter (Delta MK2 – Delta Devices Ltd, England). The amount of TPM deposit was expressed as mg per mm^2 of leaf area (Prusty *et al.*, 2005).

To estimate the content of foliar pigments three foliar discs (59 mm^2), from the leaves used to determine TPM deposition, were incubated at room temperature in 7 ml of dimethyl sulfoxide (DMSO) in glass vials covered with aluminum foil. The incubation period varied from 48 hours for *I. pes caprae*, *S. terebinthifolius* and *S.*

tomentosa to 72 hours for the thicker foliar discs of *C. rosea.* The absorbance of chlorophyll *a*, chlorophyll *b* were recorded on a spectrophotometer (U - 2000 UV/Vis – Hitachi Ltd, Japan) and their concentration calculated as pigment content per area (μg mm^{-2}), using the equations cited by Wellburn (1994).

For the determination of Fe content, 10 to 15 leaves were randomly collected from three sample plants. These leaves were thoroughly washed in distilled water and dried at 70°C during 72 hour in a ventilated oven. The dry leaves were grounded in an electrical stainless steel mill and 0.5 g samples were digested in HNO_3:$HClO_4$ acid concentrate solution (3:1v/v) at 200°C. Digests were analyzed for Fe by atomic absorption spectrophotometry (GBC Avanta - GBC Scientific Equipment Ltd, Australia) (Kampfenkel *et al.*, 1995).

Phenologycal phases of the four species were monthly monitored during the year of 2005. This monitoring survey was carried out on the marked plants of the first (1.0 km) and third (15.0 km) site. The shooting, flowering and fruiting were recorded per plant, using the score method proposed by Fournier (1974), on a scale of 0-4 further transformed into percentage.

Composed samples of the soil from the 1.0 and 15.0 km sites were analyzed for their physical and chemical composition, by a commercial soil laboratory. Meteorological and emissions data were also collected from microclimate stations located in the industry complex. The amount of SO_2 in the atmosphere was determined by a Tri-gas monitor (TriGas 1/110V - Energética Indústria e Comércio LTDA, Brazil) and the amount of TPM in suspension in the air was determined by a Hi-Vol air sampler (AGVPTS1- Energética Indústria e Comércio LTDA, Brazil).

2.3. Statistical analysis

The data were submitted to analysis of variance (ANOVA) using a statistical program package (SAEG/UFV) and the effects of significant interactions examined in detail. The means of each parameter were further compared by Tukey´s test at *P≤0.05*.

3. Results

3.1. Meteorology, emissions and soil properties

The rainfall precipitation in the area occurred mainly in the first semester of the year when the research was conducted, with its peak at the beginning of the winter season (Figure 2a). The maximum and the minimum average temperatures in the region did not exceed 25°C and 15°C, respectively. The wind direction was predominantly northeast with average high speed of 1.9 m s^{-1}.

Throughout the year the local emission reports showed that the SO$_2$ level in the air was below the primary pattern (80 µg m^{-3}) defined by the Brazilian National Council for the Environment (Resoluções CONAMA, 1992). Higher values of the TPM in suspension were detected in the second semester, probably due to low or absence of precipitation. In three specific months, January, August and October, the TPM in suspension (Figure 2b, c) exceeded the threshold of the primary pattern (80 µg m^{-3}) as established by CONAMA.
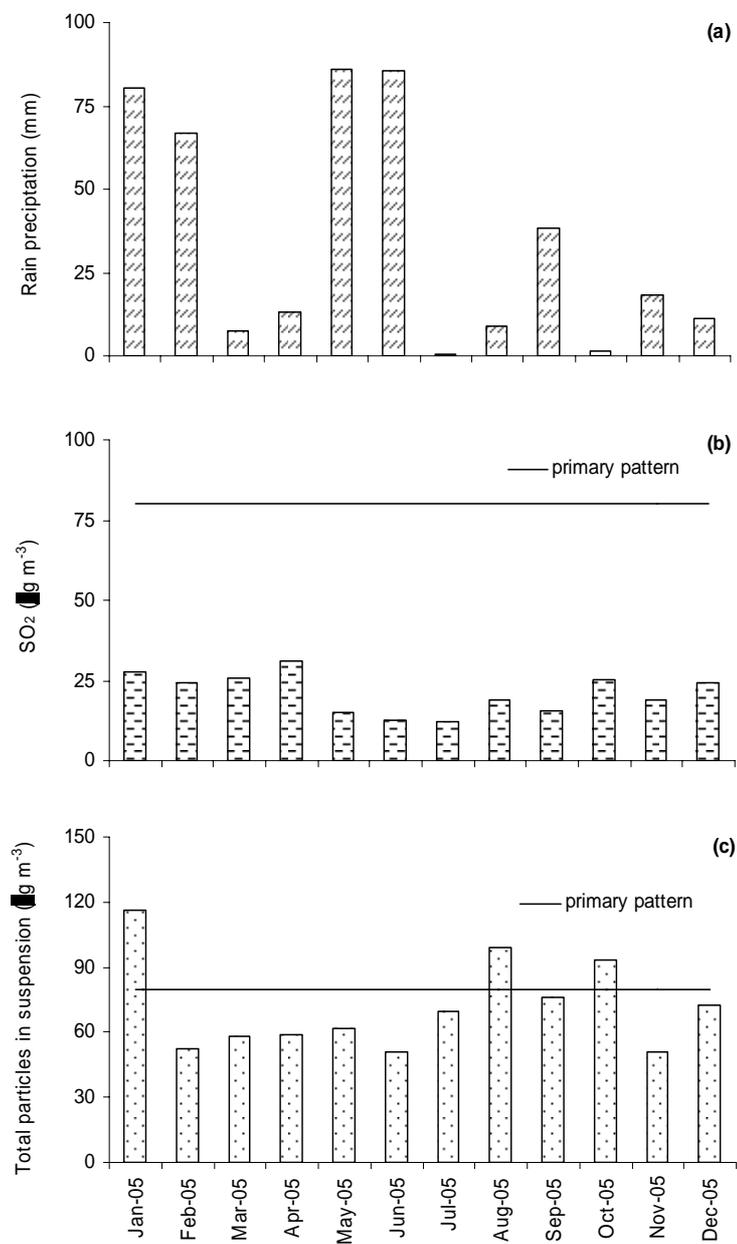
Figure 2. Meteorological (a) and emissions (b, c) data of year 2005 collected from a microclimate station located in the vicinities of the iron ore plant.

The soil from the sites located at 1.0 and 15.0 km from the industry showed different characteristics (Table 2). The 1.0 km site showed lower mineral content and high acidity when compared to the site located at 15.0 km from the source of the emissions. The iron content of the soil samples collected from the 1.0 km site was also higher than the 15.0 km site.

Table 2. Physical and chemical characteristics of the soil composed samples collected from the 1.0 and 15.0 km sites from thre emission source

Component	1.0 km	15.0 km
P (mg dm^{-3})	3	15.5
K (mg dm^{-3})	6	10
Na(mg dm^{-3})	0	0
Ca^{2+} (cmolc dm^{-3})	0.1	1.4
Mg^{2+} (cmolc dm^{-3})	0	0.2
Al^{3+} (cmolc dm^{-3})	0.1	0
Fe (mg dm^{-3})	10.6	8.9
Zn (mg dm^{-3})	0.5	0.6
Mn (mg dm^{-3})	3.6	4.9
Cu (mg dm^{-3})	0.2	0.3
pH in water	4.7	7.3
CEC (cmolc dm^{-3})	0.22	1.63
Organic Matter (dag kg^{-1})	0.27	0.81
Granulometry (dag kg^{-1}) :		
Coarse	93	94
Fine	1	1
Silt	1	2
Clay	5	4
Class	sand	sand

3.2. Plant experiment

3.2.1. Total particulate matter deposition

The deposition of dust or TPM on the leaves of *I. pes caprae, C. rosea, S. tomentosa* and *S. terebinthifolius*, was significantly affected by the interaction between local and time of analyses ($P \leq 0.05$, *F* test).

All four species, markedly *S. tomentosa*, showed high TPM deposition in the second semester of the year during the low rainfall period. September was the month when the highest amount of dust was intercepted for most of the species. This result is consistent with the pattern of TPM in suspension observed in the same period (Figure 2c). The pattern of TPM deposition also varied with distance (Figure 3).
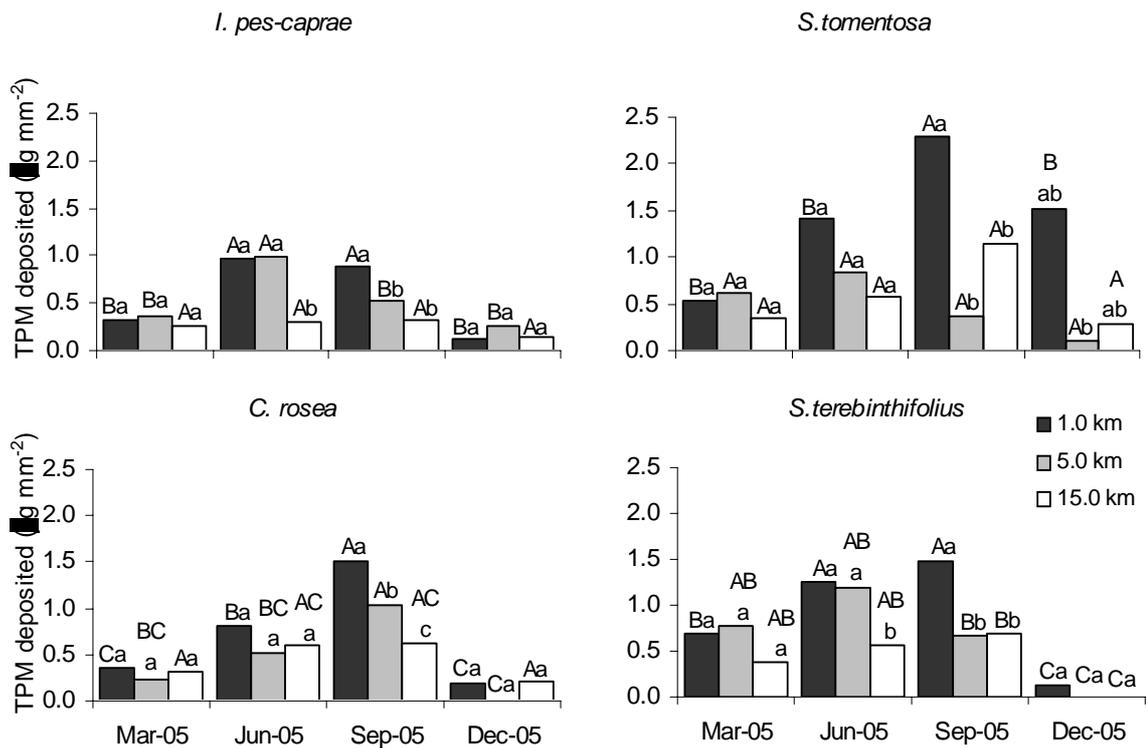
Figure 3. Mean spatial and temporal variation of the Total Particulate Matter (TPM) deposited on the leaf surface of *I. pes caprae, C. rosea, S. tomentosa* and *S. terebinthifolius*. Bars marked with different capital and lower case letters differ from each other on the temporal and spatial basis, respectively. Probability level $P \leq 0.05$ (Tukey´s test).

3.2.2. Total iron content

The total Fe content in the leaves of *I. pes caprae*, *C. rosea*, *S. tomentosa* and *S. terebinthifolius* was significantly affected by the interaction between local and time (P≤0.05, *F* test), revealing that the exposure to the source of emissions might influence the chemical constitution of the plants, either by modifying the soil properties, or by the deposition of iron particulates on the leaf surface.

The accumulation of total Fe by all four studied species, especially in *S. tomentosa*, was greater on plants growing adjacent to the industry. A variation on the total Fe content was also observed along the year, but less pronounced in *S. tomentosa*. For the other three species, higher values of Fe content were generally found in the first semester, the rainy period (Figure 4).
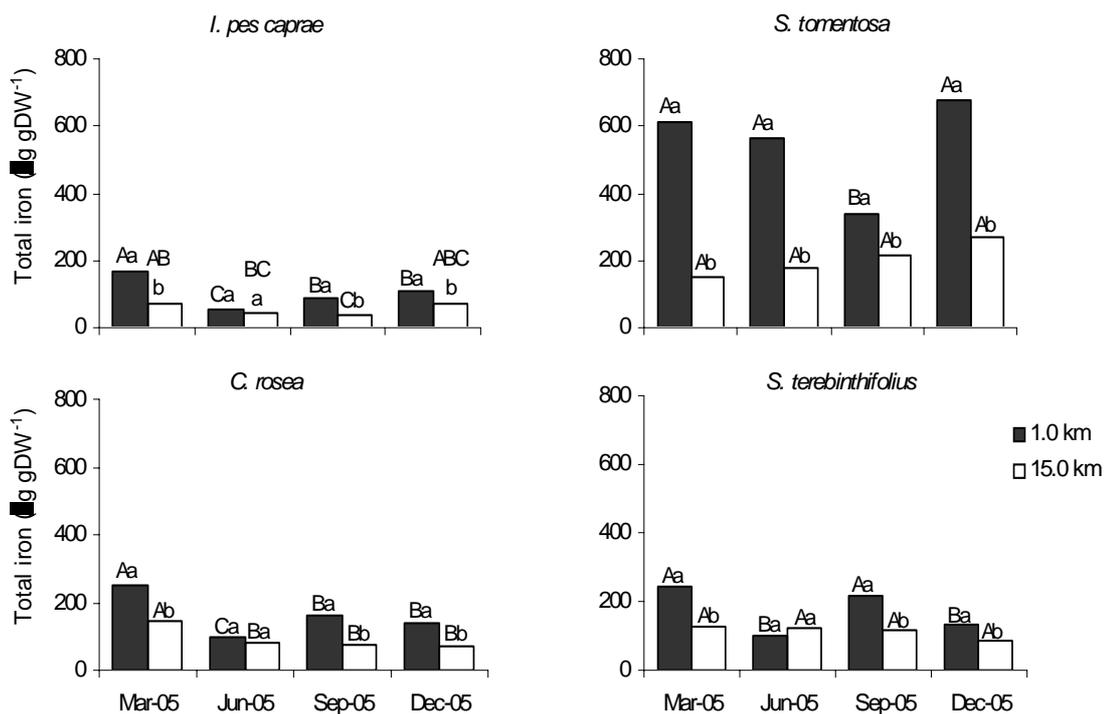
Figure 4. Mean spatial and temporal changes of the total iron content in the leaves of the *I. pes caprae, C. rosea, S. tomentosa* and *S. terebinthifolius*. Bars marked with different capital and lower case letters differ from each other on the temporal and spatial basis, respectively. Probability level *P*≤ 0.05 (Tukey´s test).

3.2.3. Total chlorophyll content

The effect of time and space on chlorophyll content of the leaves of the four species studied was variable. The total chlorophyll content of *I. pes caprae* and *S. tomentosa* was significantly affected by the interaction between time and local ($P \leq 0.05$, *F* test). The total chlorophyll content of *C. rosea* was only affected by the local where the plants were growing ($P \leq 0.05$, *F* test). The total chlorophyll content of *S. terebinthifolius* was influenced both by the local and the time of analyses ($P \leq 0.05$, *F* test).

Total chlorophyll content of *I. pes-caprae*, *C. rosea* and *S. terebinthifolius* was generally higher on the 1.0 km site (Figure 5), where the amount of Fe in the soil (Table 2) and the load of particulate matter on the leaves (Figure 3) were higher than those observed on the 15.0 km site. *S. tomentosa* did not showed significant difference in the chlorophyll content, except on March. The influence of time in chlorophyll content was variable among the studied species along the year. That might be linked to the pattern of new leaves production.
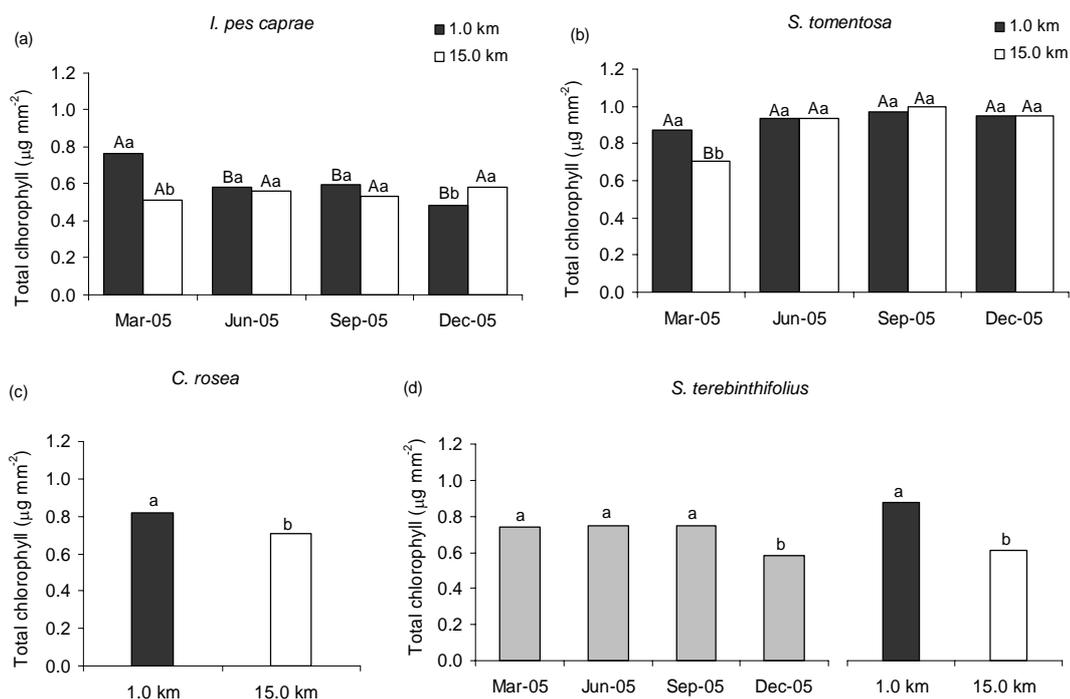
Figure 5. Mean spatial and temporal variation of total chlorophyll content (chl*a*+ chl*b*) in the leaves of *I. pes caprae, C. rosea, S. tomentosa* and *S. terebinthifolius*. Bars marked with different capital and lower case letters differ from each other on the temporal and spatial basis, respectively (a, b). Bars marked with different lower case letter differ from each other (c, d). Probability level $P \leq 0.05$ (Tukey´s test).

3.2.4. Phenology observation

The phenologycal monitoring of *I. pes caprae, C. rosea, S. tomentosa* and *S. terebinthifolius* revealed that all phenophases were, at some degree, affected by the proximity of the emission source (Figures 6 a, b and 6 c, d). Fluctuations in the intensity and pattern of the phenophases between plants at different distances were observed. The production of new leaves on plants growing at the 1.0 km site tended to be less intense than those growing at the 15.0 km site. Most of the sprouting in *I. pes caprae* and *C. rosea* occurred at the first semester, during the rainy period. Both *S. tomentosa* and *S. terebinthifolius* had two peaks of shooting along the year. The flowering phenophase in *I. pes caprae* and *S. tomentosa* was pronouncedly affected in the 1.0 km site while *C. rosea* and *S. terebinthifolius* kept their pattern. Except for

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I. pes caprae, the fruit production was also diminished in those plants, notably in *S. tomentosa* and *S. terebinthifolius.*

Figure 6 a, b. Phenology of *I. pes caprae* and *C. rosea* growing at 1.0 km and 15.0 km far from the iron ore industry plant at Anchieta restinga / ES. The bars represent the standard error (SE) of 10 individuals monitored.

Figure 6 c, d. Phenology of *S. tomentosa* and *S. terebinthifolius* growing at 1.0 km and 15.0 km far from the iron ore industry plant at Anchieta restinga / ES. The bars represent the standard error (SE) of 10 individuals monitored.
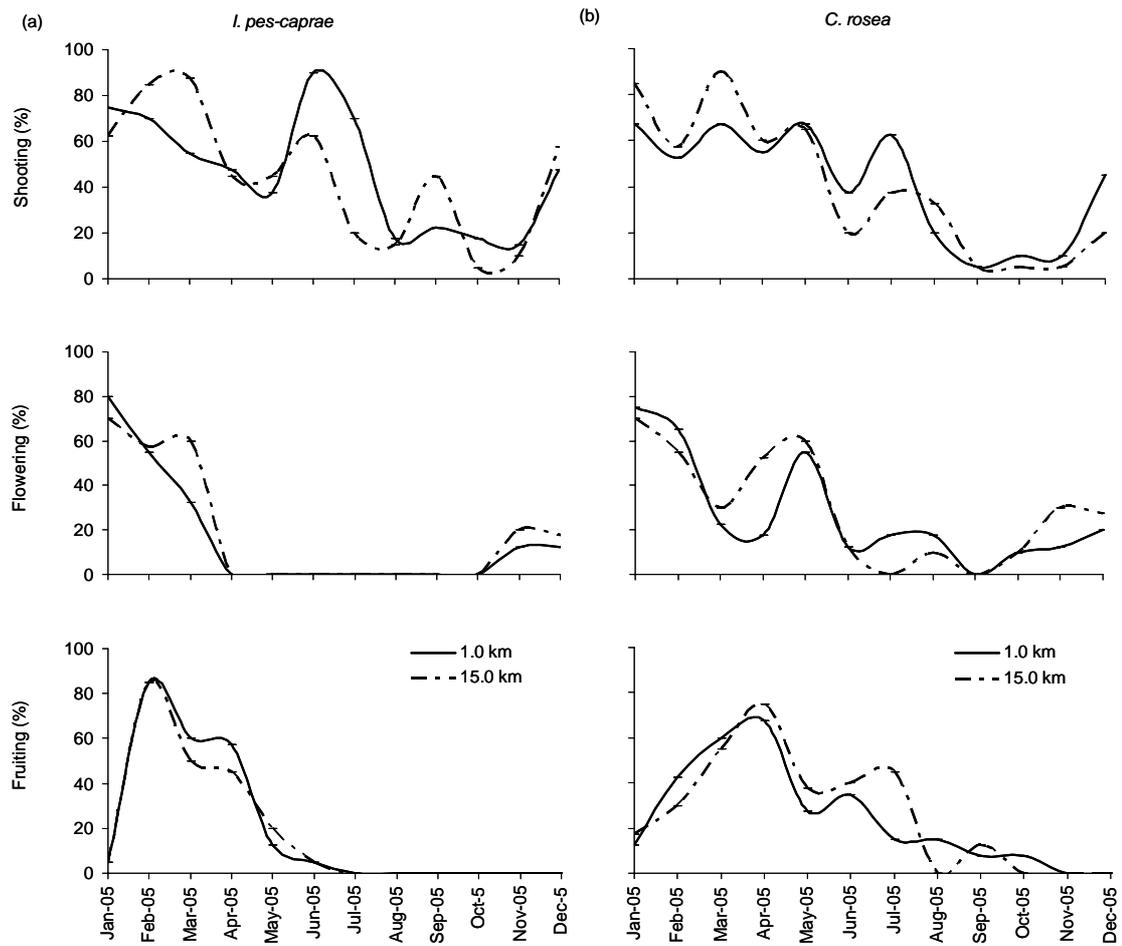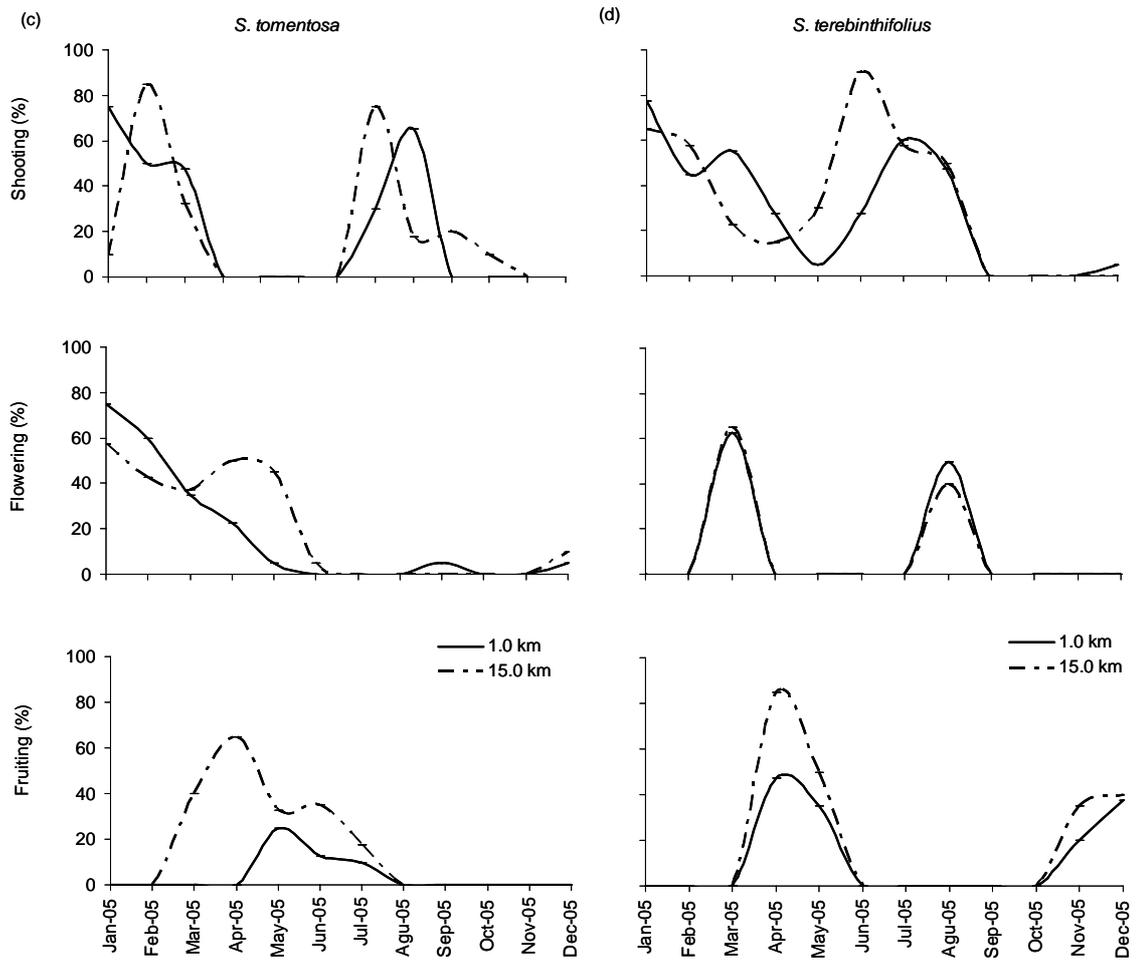
4. Discussion

Acid precipitation, both wet and dry, can alter soil pH and its mineral content. The nature of the pollutants in this study, iron ore particulates associated with the acidification potential of the SO_2 emitted by the industry, might be responsible for the differences found in the soil composition of the studied sites (Table 2). The pronounced dust load on the 1.0 km site indicates that this local is affected on more regular basis than the 15.0 km site (Figure 3). Modifications on soil properties are one of the consequences of intense ecosystems exposure to atmospheric pollution. Such effect has an important role on the chemical composition of the plant tissues (Monni *et al.*, 2001; Pugh *et al.*, 2002).

Regardless of that, in no circumstance the amount of iron found in the soils of 1.0 and 15.0 km sites exceed typical values (Azevedo and Chasin, 2003). However, lower pH of the soil in the 1.0 km site might contribute to enhance solubility of essential micronutrients, including Fe (Engleman and McDiffett 1996; Connolly and Guerinot, 2002; Momen *et al.*, 2002). Greater nutrient availability can interfere in the physiology and nutrient content, and if it does not reach toxic levels, the plant growth and development might be improved. The physical and chemical characteristics of sandy soils, as those of the studied sites, are often limiting factor for plant establishment and survival. Thus few species are able to overcome the harshness of the environment. Any permanent modification on the properties of the soil might interfere on the plant composition of the ecosystem.

As observed, the higher deposition of TPM in all four studied species occurred in the second semester, the less pronounced rainy season. Although in September the rain fall was unusually pronounced, the higher deposition of TPM on all four species could be explained by the timing of sampling, which occurred before the precipitation took place.

During rainy periods most of the particulate matter, either in suspension or deposited on surfaces, is usually washed out. The coarse fraction (2.5-10 μm∅) of the TPM is most affected by this meteorological component while the fine fraction (<2.5 μm∅) often stays on surfaces as a persistent layer. Depending on the leaf surface characteristics, more or less particles can become adhered to it (Grantz *et al.*, 2003). The size of the particulates also dictates much of their deposition fate, thus the coarse fraction often precipitates on areas near the source opposite to the fine

particles, which tend to be carried by the wind to greater distances (Farmer, 1993). The amount of TPM deposited on leaves of *S. tomentosa* was generally higher than in other species. The presence of trichomes (data not measured) on both leaf surfaces of this species might have helped the trapping of particulate matter, in contrast to those glabrous species or with fewer trichomes on their leaf surfaces *(I. pes caprae, C. rosea* and *S. terebinthifolius).*

Physical alterations on plants have been attributed to the dust coarse particles (Hirano *et al.*, 1995). This fraction of PM can abrade or smother the leaves, causing weakness, temperature rising or impairing light to reach the photosynthetic tissues (Naidoo and Chirkoot, 2004). The fine fraction, on the other hand, can affect plants by affecting stomata aperture and if it penetrates within the mesophyll, it may modify its chemical balance (Silva *et al.*, 2006). Depending on the species sensitivity, the outcomes can be either beneficial or detrimental.

In all four studied species, the Fe content was higher in the site near the source of pollutants (Figure 4). In *S. tomentosa* the higher content of total Fe happed during the first semester (rainy season) might be due to greater water availability, elevated temperature and irradiance, which together can increase the plant growth rates and the uptake of mineral nutrients by the roots. Besides, since the TPM deposition in this specie is higher, a previous TPM accumulation could have increased the total Fe content in the leaves. The foliar absorption is also a possible route of nutrients intake and this process can be aided by the combination of constant acid and particulate matter deposition on the leaves (Grantz *et al.*, 2003). Similar conditions are found on the local of assessment in this studied, therefore, increasing the possibility of a higher accumulation of Fe by the leaves of exposed species.

Fe is an essential micronutrient involved in chlorophyll synthesis and many metabolic reactions on plant tissues. Even though Fe content in the leaves was significantly affected by spatial and temporal components, the values found in *I. pes caprae*, *C. rosea* and *S. terebinthifolus* never reached the values considered phytotoxic (> 500 ppm) (Levy *et al.*, 1999). It is possible that these species are able to resist the iron toxicity by avoiding the over-accumulation of the mineral.

In *S. tomentosa* plants growing in the 1.0 km site, however, Fe content clearly exceeded phytotoxic levels indicating that this particular species might not be able to regulate the nutrient uptake. Fe excess in foliar tissue might elicit an oxidative

stress or enhance photo damage leading to a reduction on the photosynthetic capacity (Sinha *et al.*, 1997; Suh *et al.*, 2002).

The plant response to toxicity caused by trace elements depends on the species sensitivity and the environmental conditions (Narayan *et al.*, 1994). Synergic pollution stress, such as acid deposition and overload of particulate matter can collaborate to diminish the development and the reproduction of susceptible species while resistant species will thrive in a disturbed environment (Salemaa *et al.*, 2001; Alvarez *et al.*, 2003).

Near the source of emissions the TPM deposition and the content of chlorophyll in all four studied species were higher (Figure 3 and 5), despite the fact that the presence of heavy dust layers on leaves can negatively affect chlorophyll synthesis (Prusty *et al.*, 2005). Its synthesis and foliar content can also be affected by the age of the leaf and the amount of nutrients available. The content of chlorophylls on leaves are often negatively correlated to atmospheric pollution (Monni *et al.*, 2001; Prusty *et al.*, 2005), but increments can also be observed depending on the type of the pollutants or excess of mineral to which they are exposed (Päivöke and Simola, 2001; Silva *et al.*, 2006).

The higher Fe content in the leaves of all four species in the 1.0 km site clearly shows that Fe was accumulated in larger quantities by the plants of this site. Since Fe is necessary to chlorophyll synthesis (Briat *et al.*, 1995), these plants might be favored by the circumstances. The greater Fe content found in *S. tomentosa* at 1.0 km site might be partially stored as chelates in the apoplastic space (Connolly and Guerinot, 2002; Bartakova *et al.*, 2001). That could prevent the onset of an oxidative stress and avoid chlorophyll degradation and cellular damage.

The observed temporal influence on chlorophyll content (Figure 5) in all four studied species is probably related to the phenological component of the species, which is driven by seasonal variation and by self-regulation of the plants (Van Schaick *et al.*, 1993). During hot rainy periods tropical plants often invest in the outburst production of new leaves, while senescence is synchronized with periods of low rain fall (Van Schaick *et al.*, 1993). In the case of *I. pes caprae*, *C. rosea* and *S. terebinthifolius* leaf production occurred throughout the year, although unevenly (Figures 6 a, b, d). This assures that new and mature leaves are always present, and sampling of leaves can be performed with low variation along the year. *S. tomentosa* invested in the production of new leaves in two different periods of the year. The

lower content chlorophyll in the plants of the 15.0 km site, in March 2005, is probably due to the age of the leaves not yet totally mature at sampling time (Figure 5b). The sampling time almost overlapped summer outburst of leaves (Figure 6c).

The setting of new leaves, flowers and fruits relies on a series of factors, including the ones above analyzed. Organic and mineral nutrition and water availability are major requirements for the investment on new organs and reproductive structures. Under severe pollution exposure plant growth and productivity can be reduced (Momen *et al*., 2002; Brun *et al*., 2003; Chauhan *et al*., 2004). The greater amount of particulate matter deposited on the plants of all studied species growing in the 1.0 km site might decrease their overall production. Iron ore dust can do so by overheating or smothering the leaves (Naidoo and Chikoot, 2004; Silva *et al*., 2006).

Even though the Fe content in the leaves of *I. pes caprae*, *C. rosea* and *S. terebinthifolius* was higher in the 1.0 km, the toxic level was never observed. Therefore this component is probably not associated with trace metal toxicity. However the toxic levels of Fe in *S. tomentosa* leaves could generate, at some point, oxygen reactive intermediates, which might affect the plant productivity (Becana *et al*., 1998).

Since the increase in chlorophyll content of all studied species was favored by the proximity to the source of pollutant, this component was probably not a limiting factor for the observed decreases of the phenophases of the four species assessed.

The fruit setting in the 1.0 km site was the phenological aspect most affected in two out of the four species assessed*, S. tomentosa*, and *S. terebinthifolius.* Since the chlorophyll content and the iron levels in the soil were not limiting factor, the decreased values on flowering and fruiting should be linked to other ecological factor. In *S. tomentosa*, the toxic potential of iron accumulation could additionally interfere in the reproductive success. The formation of seeds and fruits generally depend on pollination and fertilization processes. The viability of pollen grain is an important factor on the successful set of seeds. Some atmospheric pollutants can have a genotoxic effect, causing the plants to produce unviable pollen grain (Iannotti *et al*., 2000). Acidity and particulate matter can also interfere on fertilization processes by weakening the reproductive structures or impairing the germination and growth of pollinic tube (Paolletti and Bellani, 1990; Gottardini *et al*., 2004).

The presence of pollinators is also relevant to the setting of fruits, if the plant species is dioecius or requires cross-pollination. *S. terebinthifolius, I. pes caprae, C. rosea* and *S. tomentosa* are pollinated by insects, mainly Hymenoptera and Coleoptera (Gross, 1993; Patiño *et al.*, 2002; Lenzi and Orth, 2004a; Nogueira and Arruda, 2006). Atmospheric pollution affects not only organisms but the interaction between them (Kevan, 1999). Pollinator decline due to unfit conditions of the environment, such as those that develop near a source of pollution, can decrease the set of fruits (Kevan, 1999). As a result, natural germplasm resources can be depleted causing changes in the vegetational structure and dynamic of the ecosystem (Brun *et al.*, 2003; Wen *et al.*, 2006).

5. Conclusion

Even though the effects differed among the species, the overall outcome is that the majority of plants growing in the vicinity of the source of emissions are affected. Phenological monitoring, though performed only during one year, evidenced that the ecosystem is under stress. The heavy load of TPM on the plants near the pollution source might be contributing to subtle changes. In a long term perspective it is possible that the iron ore industry emissions will contribute to alter the structure and diversity of the exposed vegetation. To avoid bias, this conclusion was not solely based in one variable studied.

Different ecophysiological parameters and a unified view of the data were used to evaluate the impact caused by the ore industry on restinga vegetation. Adverse effects were pronounced in *S. tomentosa* which accumulated Fe to toxic levels and had its reproductive effort decreased. This species holds the potentiality to be used as an indicator organism. However, to understand the extent of damage on plants, due to dust deposition and over-accumulation of iron in the leaves, further experimentation is required.

6. Acknowledgments

7. References

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CAPÍTULO II

THE EFFECTS OF IRON DUST AND ACIDITY DURING THE EARLY STAGES OF ESTABLISHMENT OF THREE COASTAL PLANT SPECIES

Abstract. The iron ore industries in Brazilian littoral are sources of iron and acid particulates. The impact of these emissions was examined on the early stages of establishment of three plant species: *Guapira pernambucensis*, *Schinus terebinthifolius* and *Sophora tomentosa*, under laboratory and greenhouse conditions. Germination, root tolerance index and initial growth of *G. pernambucensis* and *S. terebinthifolius* were not affected by exposure to different levels of pH and iron dust on the substrate. *S. tomentosa* displayed a deficient germination, and low root tolerance to iron dust and acidity. Initial growth of this species was also affected by increasing doses of iron dust. The iron dust did not increase the iron concentrations to toxic levels in the leaves and stems of the three species. However, toxic values were accumulated in the roots of all treated plants. The ability of the examined species to withstand the effects of iron dust and acidity showed that *G. pernambucensis* and *S. terebinthifolius* would be benefited, opposed to *S. tomentosa* under detrimental field circumstances. This advantage is likely to interfere in the vegetational composition and dynamic of the affected ecosystem.

Key words: atmospheric pollution; ecological risk; iron particulate matter

1. Introduction

As the world demands for metal good increase, the ore industries are compelled to expand their activities which can exacerbate existent ecological problems caused by the sector. Brazil is the world's largest producer of iron ore (UNCTAD, 2006) and the industries constitute a source of geographic and environmental disturbance due to their mining exploitation and the emission of iron dust and sulfur dioxide (SO_2) (Wong and Tam, 1977; Lopes *et al*., 2000; Paling *et al.*, 2001). The iron dust, a form of solid particulate matter, is the major pollutant released by the industry and it is generated along the different stages of the ore handling. Depending on the particle size, wind and landscape features the pollutant can be deposited on the surrounding of the source or carried out. The SO_2 is chiefly emitted when coal is burned in the furnaces during the ore pelletization process, increasing the chances of formation of acid particles (Lopes *et al*., 2000).

The effects of incoming particulate matter on natural ecosystems are linked to its chemical constitution and the severity of the exposure to it. This type of pollutant can directly affect the plant photosynthesis through abrasion, stomata blockage and smothering of the leaves once the particles settle down on the organ surface (Hirano *et al*., 1995; Naidoo and Chirkoot, 2004). Indirect effects may involve chemical and physical modification of the soil properties. Deposition of acid particles can affect the soil pH and contribute to elevate the solubility of heavy metals (Farmer, 1993). In both cases, the accumulation of the particles collaborates to growth reductions and increases the susceptibility of the plants to other stresses. Germination and the early growth stages are the most vulnerable periods of a plant life cycle, thus any alteration of the soil combined with the sensitivity of the species may interfere on the establishment success of key species (Fan and Wang, 2000; Grantz *et al*., 2003). The implication of such impact may reflect on the vegetation dynamic of the ecosystem (Narayan *et al*., 1994; Wen *et al*., 2006).

Particulate matter derived from the crushing and beneficiation of the iron ore is primarily inert. However, when it accumulates in the soil due to heavy deposition or poor drainage and in association with low pH of the substrate, the ore may increase the availability of the iron (Fe) to the plants (Wong *et al*., 1978). Even though Fe is an essential micronutrient, high levels of the element in the soil can lead

to toxicity or nutritional altercations, affecting the proper metabolism of the plant (Connolly and Guerinot, 2002).

On the coastal line of Espírito Santo state in Brazil, the beneficiation and shipload plants of a growing number of iron ore industries are located in areas of natural ecosystem, named restinga (Pereira, 1990). The restinga vegetation is frequently at risk of chronic and acute exposures to iron dust and acid deposition, since constant amount of particles normally escapes during the handling process and spills are inevitable occurrences. This might worsen the already disrupted equilibrium of this coastal system.

This study aimed to verify the possible impact of iron dust and acidity on restinga ecosystem through evaluation of the germination and initial growth of three native species.

2. Materials and Methods

2.1. Plant material

Three native species, *Guapira pernambucensis* (Caesar.) Lundell (Nyctaginaceae), *Schinus terebinthifolius* Rardii (Anarcadiaceae) and *Sophora tomentosa* L. (Leguminosae) were chosen to have their germination and initial growth tested under the influence of iron ore dust and different pH levels. These shrub species constitute the after dune plant community in the restinga ecosystem and help to stabilize the sand dunes (Gross, 1993; Patiño *et al.*, 2002; Lenzi and Orth, 2004; Nogueria and Arruda, 2006).

The ripened fruits of the three species were collected from plants growing on a restinga fragment, Paulo Cesar Vinha State Park (PEPCV), at 30 km north from an iron ore pelletization industry and opposed to the prevalent wind direction, in the Espírito Santo State, Brazil. The seeds were removed, washed in distilled water and soaked in a 5% sodium hypochlorite solution for five minutes and in a 0.1 % Captan solution for one minute to prevent microorganism infestation. After drying at room temperature, the seeds were selected for uniformity in size and used within 15 days.

2.2. Iron dust material

The iron particulate matter used in the experiments was the manufactured powdered ore, the iron fines, composed basically of metallic or elemental iron (Fe^0) and often stockpiled on the open yard at the ore industry plant. The product is mainly coarse (2.5-10 µm $\varnothing$) and larger (> 50 µm $\varnothing$) particles and its composition is described in Table 1.

Table 1. Average composition of the iron particulate matter used in the germination and initial growth experiments.

Component	%(dry basis)
Fe	67.1
FeO	0.96
SiO_2	1.10
Al_2O_3	0.30
CaO	0.10
MgO	0.02
Cu	0.006
Na_2O	0.007
P	0.040
S	0.003
Mn	0.033
Moisture	9.60

2.3. Germination and root tolerance index

The selected seeds were placed in sterile glass Petri dishes (9 cm $\varnothing$) containing germination paper and submitted to treatments with different combination of pH and iron dust levels in a factorial 3 x 2 arrangement with four replicates. The pH values studied were 6.5, 5.0 and 3.0, obtained by adding diluted sulfuric acid solutions. The doses of iron dust used were 0.0 and 0.06 mg mm^{-2}, based on the average amount daily deposited in the vicinity of an iron ore industry (Lopes *et al.*, 2000). The treatment corresponding to the pH 6.5 without iron dust was considered the control. Each treatment was constituted of 100 seeds evenly distributed in four dishes. The Petri dishes were incubated in a germination chamber at constant temperature of 27^oC and with a 16/8 h photoperiod on a randomized block design. The solutions with different pH´s and the iron dust in the dishes were renewed every two days to assure maximum exposure to the intended treatments. The seed was considered germinated when the protruded radicle was 2.0 mm longer. The germinated seeds were daily recorded and the mean cumulative results expressed as percentage.

To determine the tolerance of the roots to iron dust and acidity, 20 pre-germinated seeds with 2.0 mm protruded radicle were evenly distributed in five sterile PVC trays (5 x 20 x 45 cm) containing germination paper with the corresponding treatments of iron dust and sulfuric acid solutions described above.

The trays were covered with polypropylene wrap and kept at a constant temperature of 27°C with a 16/8h photoperiod on a randomized block design, each replicate represented by one tray. When necessary the pH´s solutions were replaced to assure desired pH level. The primary root length was measured at the 1st, 7th and 15th days after the setting of the experiment. The root tolerance index (RTI) was calculated by the equation based on Rout *et al.* (2000):

RTI (%) = (mean root length of treatment/ mean root length of the control) x 100

2.4. Seedling emergence and growth

Under greenhouse condition 480 seeds of the three species were evenly sowed in 24 plastic pots (2.0 L, 9 cm $\varnothing$) filled with soil collected from the Parque Estadual Paulo César Vinha State Park. The soil composition and characteristics were determined by a commercial soil laboratory (Table 2). Treatments consisted of the addition of different amounts of iron dust on the soil surface. The following doses of the particulate were used: 0.0, 3.0, 6.0 and 12.0 g, based on Lopes *et al.* (2000). The experiment was designed in a randomized block arrangement with 6 replicates per treatment, each replicate represented by one pot. The pots were watered with distilled water every two days. The emergence of the seedlings indicated by the expanded cotyledons was recorded at the 7th, 14th, 21st and 28th days after the initial setting of the experiment. The final number of emerged seedlings was expressed as mean percentage.

Table 2. Physical and chemical characteristics of the soil used in the emergence and growth experiment

Soil charcteristics	
P (mg dm^{-3})	8
K (mg dm^{-3})	19
Na (mg dm^{-3})	0
Zn (mg dm^{-3})	0
Fe (mg dm^{-3})	10
Mn (mg dm^{-3})	0
Cu (mg dm^{-3})	0.2
Ca^{2+} (cmolc dm^{-3})	0.06
Mg^{2+} (cmolc dm^{-3})	0.02
Al^{3+} (cmolc dm^{-3})	0
pH	7.5
CEC(cmolc dm^{-3})	0.13
BSI (%)	100
Organic Matter (dag kg^{-1})	0.25
Granulometry (dag kg^{-1}):	
coarse sand	93
fine sand	1
silt	1
clay	5

In order to evaluate the influence of iron dust on the initial growth, on the 35th day, after the initial setting of the experiment, the seedlings were thinned to two plants per pot, at the two to three leaf stage, which comprised the new experimental unit. The number of leaves and height of the seedlings were monthly recorded. Standard Hoagland solution (100 ml) at 1/4 of the strength and pH 6.5 was applied every 15 days per pot. At the end of 180th day, the plants were harvested.

The leaves, stem and roots were separated and washed in distilled water. The roots were additionally rinsed in a 1.0 mM EDTA solution to minimize tissue contamination by external Fe. The total leaf area of each plant was measured by a plan meter (Delta MK2 – Delta Devices Ltd, England). All plant parts were dried at 90°C and the biomass dry weight recorded. Later, 0.5 g samples of the dried organs were subsequently wet digested in nitric-perchloric solution (3:1 v/v) and the

content of total Fe determined by atomic absorption spectrophotometry (GBC Avanta - GBC Scientific Equipment Ltd, Australia) (Kampfenkel *et al.*, 1995).

2.5. Statistical analysis

The data of all experiments were submitted to analysis of variance (ANOVA) using a statistical program package (SAEG/UFV). The means of each parameter examined were further compared by Tukey´s test at *P≤0.05*.

3. Results

3.1. Germination and root tolerance index

Iron dust and acidity exposures did not interfere on the germination of *G. pernambucensis* and *S. terebinthifolius*. However the germination of *S. tomentosa* was significantly ($P \leq 0.05$) reduced by either different pH or iron dust (Figure 1). During the germination period most of *S. tomentosa* seeds under iron dust exposure developed a dark coloration on their coat followed by spoiling.
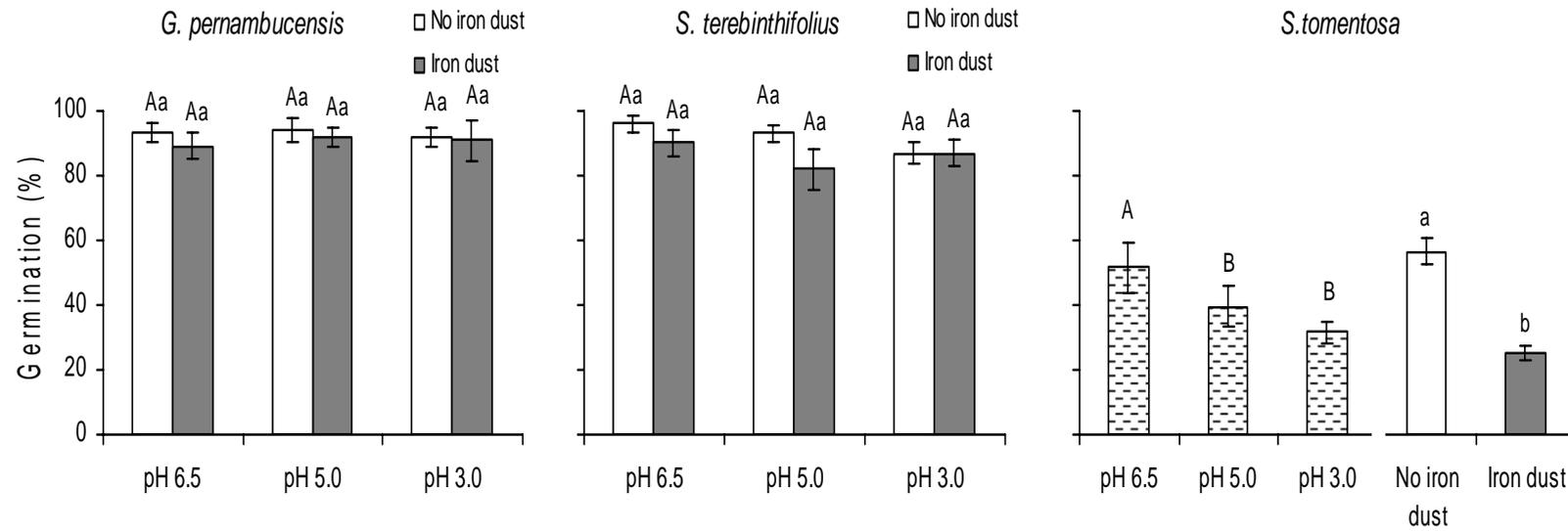
Figure 1. Germination of *G. pernambucensis*, *S. terebinthifolius* and *S. tomentosa* under different pH (6.5, 5.0 and 3.0) and iron dust (0.0 and 0.06 mg mm^{-2}) exposure. Significant differences between pH levels and iron dust doses are indicated by capital and lower case letters, respectively, at *P*≤0.05, according to Tukey´s test. Bars represents mean ± standard error (n = 4).

The RTI in *G. pernambucensis* and *S. terebinthifolius* did not differ (*P≤0.05*) from the control treatment, indicating that these species were tolerant to the imposed treatments. In *S. tomentosa,* however, RTI was negatively affected by acid pH and iron dust (Table 3). In the presence of iron dust the radicles of *S. tomentosa* displayed a stunted growth and their surface were usually covered by a reddish stained coat, probably originated from the deposition of iron oxides or plaques (Figure 2).

Table 3. Root tolerance index (RTI) of *G. pernambucensis*, *S. terebinthifolius* and *S. tomentosa* treated with different pH and iron dust.

Treatment	*G. pernambucensis*	*S. terebinthifolius*	*S. tomentosa*
	mean (%)*		
pH 6.5	100^{Aa}	100^{Aa}	100^{Aa}
pH 6.5+Iron dust	91.24±3.65^{Aa}	94.61±4.47^{Aa}	78.56±4.74^{Ab}
pH 5.0	98.96±6.30^{Aa}	86.62±5.70^{Aa}	35.00±1.66^{Ba}
pH 5.0+Iron dust	94.17±3.99^{Aa}	96.19±5.43^{Aa}	18.9±1.13^{Bb}
pH 3.0	92.79±7.75^{Aa}	86.63±5.69^{Aa}	15.88±1.17^{Ca}
pH 3.0+ Iron dust	92.77±8.89^{Aa}	94.50±4.75^{Aa}	15.43±1.41^{Ba}

*Significant differences between pH levels and iron dust treatments are indicated within the columns by capital and lower case letters, respectively, at *P≤0.05* according to Tukey´s test. Mean ± standard error (n = 5).
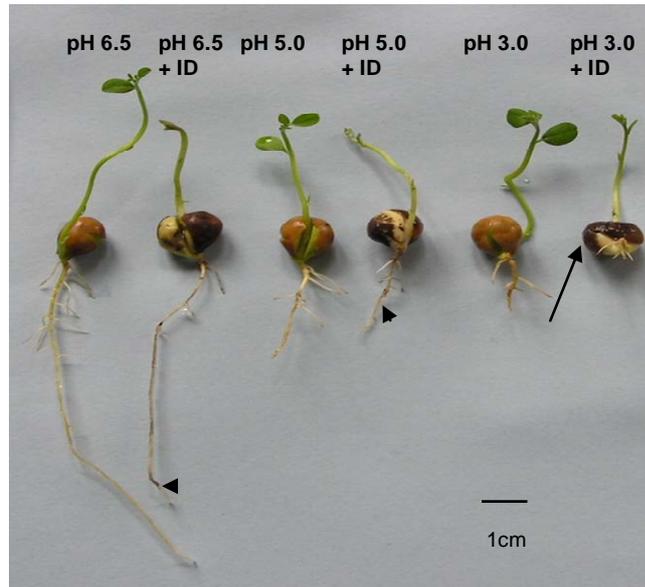
Figure 2. *S. tomentosa* seedlings under different pH and iron dust (ID) treatments. Iron plaque and dark seed coat areas pointed out by arrowheads and arrows, respectively.

3.2. Seedling emergence and growth

Seedling emergence of *G. pernambucensis* and *S. terebinthifolius* was not affected ($P \leq 0.05$) by the different doses of iron dust applied in this experiment (Table 4). Meanwhile, the emergence of *S. tomentosa* was consistently reduced by iron dust, even within the lowest dose applied (3.0 g). The iron dust treatments did not interfere in the growth parameters of *G. pernambucensis* and *S. terebinthifolius.* However, stem height, leaf area and nodule dry weight of *S. tomentosa* were significantly reduced when the seedlings were exposed to iron dust treatments. Roots of *S. tomentosa* were also stained with a reddish coat, which could not be completely removed after the cleansing process.

The Fe content in the organs of *G. pernambucensis* and *S. terebinthifolius* varied according to the treatments. Higher Fe content ($P \leq 0.05$) was found in the leaves and stems of these two species at doses 6.0 and 12.0g of iron dust (Figure 3). In *S. tomentosa* Fe content increased in the stem but decreased in the leaves with iron treatments. In the roots, however, the Fe content was higher in all the species studied under iron dust exposure.

Table 4. Effects of iron dust in the emergence and initial growth parameters on cultivated plants of *G. pernambucensis*, *S. terebinthifolius* and *S. tomentosa.*

Species	Parameters[*]	Treatment			
		0.0 g	3.0 g	6.0 g	12.0 g
G. pernambucensis					
	Emerged seedling (%)	95±3.1^A	96.7±1.6^A	93.3±2.7^A	92.5±3.5^A
	Stem height (cm)	15.5±1.3^A	15.4±1.1^A	14.0±1.5^A	15.7±0.7^A
	Leaf number	17±1.2^A	21.4±1.2^A	20.3±2.4^A	18.5±1.5^A
	Leaf area (cm^2)	127.3±4.7^A	141.2±13.4^A	133.2±11.9^A	142.9±6.6^A
	Total dry weight (g)	9.9±0.7^A	9.5±0.8^A	9.3±0.8^A	11.1±0.3^A
S. terebinthifolius					
	Emerged seedling (%)	88.3±4.5^A	80.8±3.2^A	87.5±2.8^A	82.5±3.3^A
	Stem height (cm)	11.8±0.8^A	12.9±0.6^A	13.4±0.9^A	13.7±1.0^A
	Leaf number	14.7±1.1^A	13.5±0.9^A	16.2±1.1^A	13.6±0.4^A
	Leaf area (cm^2)	77.5±8.2^A	85.5±2.5^A	84.3±6.6^A	97.7±6.6^A
	Total dry weight (g)	3.4±0.1^A	3.3±0.4^A	3.1±0.3^A	3.3±0.2^A
S. tomentosa					
	Emerged seedling (%)	95±3.1^A	75±2.7^B	54.1±3.9^C	50.8±4.3^C
	Stem height (cm)	18.3±1.9^A	13.7±0.9^{AB}	11.0±0.1^B	10.3±0.5^B
	Leaf number	12.5±0.5^A	12.7±0.6^A	13.5±0.3^A	13.3±0.4^A
	Leaf area (cm^2)	269.9±22.3^A	164.2±31.0^B	135.7±9.0^B	123.0±8.8^B
	Nodules dry weight (g)	0.5±0.1^A	0.5±0.3^{AB}	0.3±0.1^{AB}	0.3±0.0^B
	Total dry weight (g)	4.0±0.9^A	5.3±0.3^A	3.71±0.2^A	3.2±0.1^A

[*]Mean values ± standard error (n=6). Different letters between columns indicate significant difference at $P \leq 0.05$, according to Tukey´s test.
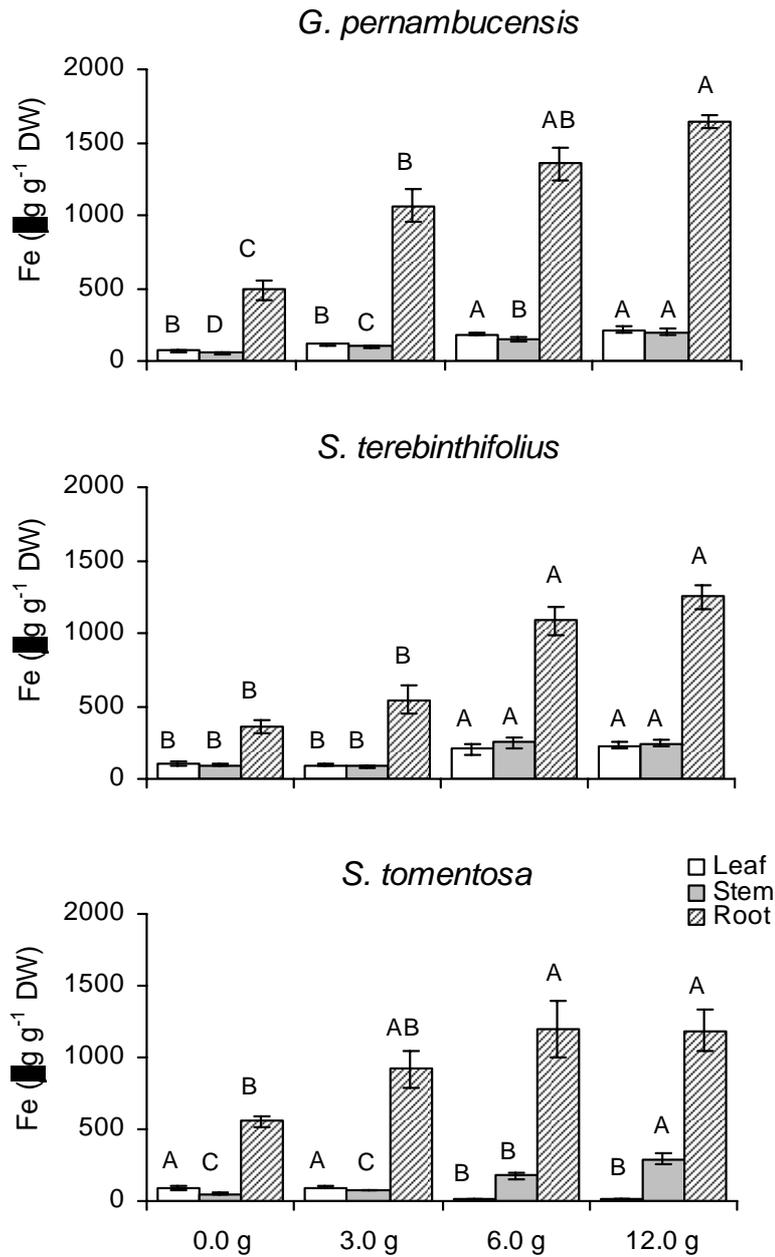
Figure 3. Iron content in leaves, stem and roots of cultivated plants of *G. pernambucensis*, *S. terebinthifolius* and *S. tomentosa*. Different letter in the same organ indicates a significant difference between treatments, according to Tukey´s test (*P*≤ 0.05). Bars represent mean ± standard error (n = 6).

4. Discussion

The pH and the iron dust treatments only affected the germination of *S. tomentosa*, despite adequate rehydration of the seeds in all treatments. Acid condition may alter seed physiology, therefore affecting the germination process (Fan and Wang, 2000). Conditioned by the species sensitiveness, low pH can cause damage to metabolic processes, including the glycolysis, by disrupting pH gradient balance within the cells. A limited supply of energy would interfere in the radicle and hypocotyl growth, decreasing the germination capability (McColl and Johson, 1983). Thus, the reduced germination and low RTI of *S. tomentosa* under pH 5.0 and 3.0 might be related to respiratory alterations in the embryo, which apparently occurred even in a weaker acid condition (pH 5.0).

The browning of the *S. tomentosa* seed coat in the treatments with iron dust is probably due to phenol oxidation. Phenols are commonly present in many parts of the seeds, including the coat and embryo, and are primarily related to defense against herbivory, pathogen infestation and regulation of seed germination (Muscolo *et al*., 2004; Rashid *et al*., 2005). Tissue browning can be the result of increased polyphenol oxidase activity due to excessive Fe^{2+} uptake. Also, increased Fe supply can reduce respiration capacity due to enzyme inactivation by interaction with sulphydryl groups, as observed in *Phragmites australis* (Fürtig *et al*., 1999). Additionally, certain environmental conditions, such as the presence of elemental Fe^{0}, can cause oxidation of phenol to quinines (Rush, *et al*., 1995). Hydroquinone, a reduced quinone, has cytotoxic potential that can diminish cell division rate and growth (El-Bargbathi and Asoyri, 2007). Hence it is believed that the oxidizing characteristic of the iron dust might have accelerated the oxidation of seed coat phenols and/or contributed to the accumulation of internal Fe in the seeds of *S. tomentosa*, which might have affected the germination process.

The presence of a reddish stain on the root surface of *S. tomentosa* may indicate the formation of mineral precipitates. Iron ores are mainly in (hydr-)oxides forms and usually unavailable for plants. After beneficiation, metallic or elemental Fe^{0} is obtained. In the presence of water and dissolved oxygen, elemental Fe^{0} is reverted to its ferrous state Fe^{2+}, which is readily absorbed by roots (Azevedo and Chasin, 2003; Schmidt, 2003; Pereira and Freire, 2005). Nevertheless, Fe^{2+} in the surrounding rizosphere may react with oxygen and precipitate, forming mineral

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plaques of iron oxides (Fe_2O_3) as observed on roots of some wetland plant species (Tanaka, *et al*. 1966). Fe^{2+} oxidation can also be mediated by enzymes such as peroxidases or catalases (Ye *et al*., 1998; Hansel, *et al*., 2001). Depending on the extension of the plaques formed on the roots, they can either diminish the Fe^{2+} uptake or interfere in the absorption of other minerals through physical barrier (St. Cyr. and Campbell, 1996). In the first case, the plaque could act as a protective barrier against metal accumulation and further oxidative stress development (Sinha *et al*., 1997). However, in the second case, the growth and development of the roots can be negatively affected due to inadequate nutrition (Yamauchi, 1989; Ye *et al*., 1998). The low RTI of *S. tomentosa* might be in part linked to the plaque formation phenomena and the cytotoxic effect of phenol oxidation.

The reduced emergence and the improper growth of *S. tomentosa* indicate that during the initial stages of seedling establishment, this species was being affected by iron dust in the soil. High levels of iron oxides in the soil can lead to iron overlays on the roots which, as similar to root plaques, affect the rizosphere interactions (Hansel *et al*., 2001).The reddish coat on the roots of *S. tomentosa* exposed to iron dust treatments shows iron oxides presence on the organ surface and may be an explanation for the poor nodulation and growth observed in this species. Indeed high levels of Fe applied to leguminous species interfered in the growth of aerial organs by affecting the nodulation and survival of the symbiotic *Rhizobium trifolli* (Lie and Brotonegoro, 1969; Whelan and Alexander, 1985).

Vegetation contamination by metals, including Fe, has been observed in areas of iron ore tailings (Wong and Tam, 1977). However, the mean Fe content in the leaves and stems *G. pernambucensis*, *S. tomentosa* and *S. terebinthifolius* did not reach toxic values, whereas in the roots the levels exceeded the values of 500 ppm cited by Pugh *et al*. (2002). Despite that, *G. pernambucensis* and *S. terebinthifolius* did not show any growth reduction or injury symptoms caused by Fe excess, suggesting that these species were resistant to the presence of iron dust in the soil. Regardless the fact that Fe content in the leaves of *S. tomentosa* decreased along the treatments, the content in the roots increased and affected the seedlings growth. In many wetland plant species, such as *Thypha latifolia, Rhizophora mangle* and *Aviccenia schaueriana*, formation of mineral plaques on the root help to prevent toxic accumulation of heavy metal (Ye *et al*., 1998; Machado *et al*., 2005). Since iron plaque were observed on the roots of *S. tomentosa,* the organ high Fe content

could be due to the ineffectiveness of the cleansing process, which could masked the true values.

5. Conclusion

The results obtained in the present study indicate that under field conditions these native species might be diversely affected by the particulates emission of the iron ore industry. While *G. pernambucensis* and *S. terebinthifolius* show resilience, *S. tomentosa* might be in disadvantage under acute exposure to particulate matter, iron or acid, once its initial establishment would be under duress. As a consequence this competitive imbalance, at long term, can affect the restinga vegetational composition. The physiological implications of this environmental stress upon vegetation are still poorly understood and a refined investigation should be taken in account. In this way the knowledge of the extended impact of iron ore emissions upon restinga ecology would be accurate.

6. Acknowledgments

7. References

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CAPITULO III

EFFECTS OF SIMULATED DEPOSITION OF ACID MIST AND IRON ORE PARTICULATE MATTER ON THE PHOTOSYNTHESIS AND OXIDATIVE STRESS GENERATION IN *Schinus terebinthifolius* Rardii **AND** *Sophora tomentosa* L**.**

Abstract. Particulate matters are natural occurrence in the environment. Some industrial sectors as the ore mining and beneficiation industries are emission sources of ore particles and sulfur dioxide. These elements contribute to increase the total particulate matter in suspension in the atmosphere. The effects of the particulate matters originated by the iron ore industries in the vegetation of restinga, a Brazilian coastal ecosystem, were investigated thorough physiological and biochemical parameters. Two species, *Schinus terebinthifolius* and *Sophora tomentosa,* were exposed to simulated deposition of acid mist and iron ore particulate matter on their leaves. The treatments were not capable of inducing an oxidative stress in *S. terebinthifolius*. However its chlorophyll content, maximum quantum efficiency of photosystem II and the electron transport rate were positively affected, while the iron content was unaltered. A greater sensitiveness to the treatments was observed in plants of *S. tomentosa*. The plants exposed to acid mist and iron solid particulate matter presented significant changes in the physiological and the biochemical responses, such as photosynthesis, parameters of chlorophyll *a* fluorescence, antioxidant enzyme activity, membrane permeability, malondialdehyde content and iron content. While *S. terebinthifolius* avoided the stress, *S. tomentosa* used antioxidant enzyme system to partially neutralize the oxidative stress. The results suggested that restinga species use different strategies to overcome the stressful conditions created by the ore industry emissions.

Key words: acidity; biomarkers; foliar deposition; iron ore; particulate matter.

1. Introduction

In the littoral of Espírito Santo state, Brazil, a sand coast ecosystem named restinga shares its space with an increasing number of iron ore industries. These industries are generators of dust, iron ore solid particulate matter (SPM_{Fe}), and sulfur dioxide (SO_2), which in contact with water droplets suspended in the atmosphere can generate acid mist (Lopes *et al*., 2000; Grantz *et al*., 2003). Both solid and liquid particulates are components of atmospheric aerosols. Aerosols formed in polluted areas act as efficient mechanisms of dry and wet deposition of some chemicals on vegetation, synergistically, contaminating the ecosystem (Lovett and Kinsman, 1990). In response to the stress, plants can suffer from foliar injuries to modifications in their physiological and biochemical processes that might lead to vegetation decline (Yunus and Iqbal, 1996). Nevertheless, the physiological and biochemical responses to pollution can serve as early biomarkers for stress, providing needed information for ecological risk assessment programs.

Acid wet deposition may primarily affect leaf metabolism through leaching of nutrients, alterations in the cellular pH, enzyme activities, and membrane integrity (Velikova *et al*., 1999). Solid particulate matter may affect plant growth and development by smothering leaf surface, which causes abrasion, temperature elevation, reduction of photosynthetically active radiation and stomatal blockage (Hirano *et al*., 1995; Grantz *et al*., 2003). Also ionic forms of chemical elements in the particulates can enter the mesophyll through the stomatal aperture or fissures in the cuticle (Watmough *et al*., 1999; Lau and Luk, 200), leading to accumulation of essential and non essential elements in the foliar tissue (Lau and Luk, 2001).

Mineral iron in terrestrial ecosystem is found mainly as ores, goethite, hematite and pyrite. Despite the abundant presence of Fe in the Earth crust, it occurs predominantly in the soil in the ferric state (Fe^{3+}), which has low solubility in water and easily precipitates as (hydr)-oxides. Under reductive and low pH conditions, Fe occurs predominantly in ferrous state (Fe^{2+}), a more soluble form that is readily absorbed by living tissues (Fagaria *et al*., 1990).

After beneficiation the iron ore is converted into a metallic or zero-valent Fe (Fe^0). In the presence of water metallic Fe can be oxidized to Fe^{2+} which, depending on the surrounding conductions of pH and oxygen availability, might precipitates as Fe (hydr)-oxides (Pereira and Freire, 2005).

Due to its redox properties, Fe participates in a variety of metabolic processes in which electron transference is required (Hell and Stephan, 2003). However, high Fe content in living tissues can have a phytotoxic effect impairing physiological processes, such as photosynthesis, or predisposing the organism to other stresses (Connolly and Guerinot, 2002; Suh *et al.*, 2002). As a source of Fe, the SPM_{Fe} may also have toxic implications when it combines with wet acid deposition such as rain or mist.

The toxic effects of trace metal in plant tissue are dependent of both, the amount accumulated in it and the sensitivity of the exposed species. High concentrations of free Fe^{2+} in the cell can elicit an increase of the Fenton reaction and catalyze the generation of the reactive oxygen intermediate (ROI), the hydroxyl radical ($\cdot OH$), hence inducing oxidative stress (Becana *et al.*, 1998). Also, under environmental stress, an inadequate flow of electrons can occur in living membranes, such as observed in chloroplast, originating other forms of ROIs. These oxidative intermediates can target the membrane itself, disrupting it. As a consequence, an excess of energy sets in which must be dissipated through heat or fluorescence emissions (Sinha *et al.*, 1997; Padinha *et al.*, 2000; Estevez *et al.*, 2001).

Plants can overcome the effects of oxidative stress through enzymatic and non-enzymatic systems. Superoxide dismutase (SOD, EC 1.15.1.9)), catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC 1.11.1.6) are enzymes commonly involved in the detoxification of ROIs under natural or oxidative stress conditions, while other organic molecules, such as carotenoids, phenols and glutathione act as substrates to neutralize the oxygen reactive intermediates (Mittler, 2002).

This work evaluated the potential effects of simulated deposition of acid mist and SPM_{Fe} on the photosynthesis and oxidative stress in two restinga species, *Sophora tomentosa* L. (Leguminosae) and *Schinus terebinthifolius* Rardii. (Anarcadiaceae), using physiological and biochemical parameters.

2. Materials and Methods

2.1. Experimental design

One-year-old plants, average height of 40 cm, of *S. tomentosa* and *S. terebinthifolius* grown in commercial soil in 5.0 L plastic pots, under green house conditions, were exposed to combined treatment of simulated mist and SPM_{Fe} deposition, in a factorial of 2 x 2. The treatments were: neutral mist (control), neutral mist plus SPM_{Fe}, acid mist and acid mist plus SPM_{Fe}.

The neutral mist consisted of distilled water (pH 6.7±0.2) and the acid mist was a diluted sulfuric acid solution at pH 3.0. The mists were applied to the plants in an adapted mist simulation chamber (Heck *et al*., 1978). The sprinkler nozzles in the chamber delivered fine droplets at a flow rate of 83 ml min^{-1}, under a pressure of 4.0 kg cm^{-2}. The plants were exposed to the mists for 15 minutes under light regime (1000 μmoles m^{-2}s^{-1}). The SPM_{Fe} consisted of metallic iron powder manufactured by an iron ore industry (Table 1), composed by large (> 10 μm $\varnothing$), coarse (2.5-10 μm $\varnothing$) and fine (< 2.5 μm $\varnothing$) particles. Under natural light conditions, the SPM_{Fe} was applied to the plants in an adapted dust simulation chamber (Hirano *et al*., 1995), which delivered an average of 2.5 mg cm^{-2} of particulate. During the exposure, the soil surface was covered with plastic sheets to avoid contamination with the particulates.

The mists and the SPM_{Fe} were applied on the plants every other day for 30 days. The experiments were designed in randomized block arrangement with four replicates per treatments per species.

Table 1. Iron ore particulate matter (SPM_{Fe}) average composition

Parameters	dry basis(%)
Fe	67.17
FeO	0.96
SiO	1.1
Al_2O_3	0.3
CaO	0.1
MgO	0.2
P	0.04
S	0.003
Cu	0.006
Na_2O	0.007
K_2O	0.005
Mn	0.033
Moisture	9.6

2.2. Physiological and biochemical analysis

After 30 days of treatment, different parameters of the plants were evaluated. The gas exchange and chlorophyll fluorescence analysis were performed respectively by an infra red gas analyzer (Li-6400, Li-Cor Inc, EUA) and by a modulated fluorometer (MiniPAM, Heinz Waltz, GmbH) on leaves of the 3rd or 4th node, previously cleaned with distilled water and a soft brush, until particulates were no longer visible. The photosynthetic parameters evaluated were net photosynthesis (A), stomatal conductance (g_s) and internal leaf CO_2 to ambient CO_2 ratio (Ci/Ca). Measurements were done at a temperature of 25°C and irradiance of 1000 μmoles m^{-2}s^{-1}. The fluorescence parameters evaluated were electron transfer rate (ETR) and maximum quantum efficiency of photosystem II (Fv/Fm), after leaf adaptation to darkness for 30 minutes.

For biochemical analysis, the leaves were washed in distilled water and rinsed with a 1.0 mM EDTA solution to minimize contamination by external Fe. leaf discs (5.0 mm ∅) were collected and used fresh or stored frozen in ultra-freezer at -80°C,

and used within 7 days for enzyme assays. The remained foliar material was dried in oven at 75°C, until constant weight, and the dry material used for Fe content determination.

Total chlorophyll content (chlorophyll *a* + chlorophyll *b*) in the fresh leaf discs was determined using dimethyl sulfoxide (DMSO) as extractor, according to Wellburn (1994). Total phenolic content was determined using 0.5 g of fresh foliar discs grounded in methanol, according to Saltveit (2004).

The integrity of membranes was evaluated by membrane permeability and malondialdehyde content (MDA), a by-product of lipid peroxidation. Membrane permeability (MP) was estimated through electrolyte leakage of fresh foliar discs and expressed as relative permeability (%) (Tarhanen *et al.*, 1999). MDA content was determined by thiobarbituric acid (TBA) reactive substance using trichloroacetic acid (TCA) to homogenize 0.2 g of fresh foliar discs (Heather and Packer, 1968).

The total Fe content in the leaves was determine by atomic absorption spectrophotometry (GBC Avanta - GBC Scientific Equipment Ltd, Australia), using an aliquot of 0.5 g of dry weight material, wet digested in a nitric-perchloric acid solution (3:1) at 200°C (Kampfenkel *et al.*, 1995).

For the enzymes assay, 0.2 g of fresh foliar discs was grounded in a mortar with liquid nitrogen and 2.0 ml of homogenizer buffer, in ice bath. The homogenizer buffer consisted of 0.3 M potassium phosphate buffer (pH 6.8), 1.0 mM phenylmethylsulfonyl fluoride in ethanol and 1% polyvinylpyrrolidone (w:v). The homogenate was centrifuged at 15.000 x *g*, at 4°C for 15 minutes. Aliquots of the supernatant were used for the enzymatic assays of SOD (Del Logo *et al.*, 1993), CAT (Havir and McHale 1987) and POX (Kar and Mishra, 1976). SOD activity was determined by the formation of blue formazine, resulted from the photoreduction of the p-nitro blue tetrazolium (NBT), detected at 560 nm and expressed as units of SOD necessary to inhibit 50% of NBT photoreduction at 50% (Beauchamp and Fridovich, 1971). CAT activity was determined by the decay of absorbance at 240 nm, using the 36 M^{-1} cm^{-1} molar extinction coefficient for calculation and expressed as μmoles min^{-1} g FW^{-1} (Anderson *et al.*, 1995). POX activity was determined by the increment of purpurogallin formation detected at 420 nm, using the 2.47 mM^{-1} cm^{-1} molar extinction coefficient for calculation and expressed as μmoles min^{-1} g^{-1} FW of pupurogallin (Chance e Maehley, 1955).

For characteristics description of the foliar surface, mature leaves were examined under binocular scope (Motic SMZ 143, USA).

2.3. Statistical analysis

The physiological and biochemical data were submitted to analysis of variance (ANOVA) using a statistical program package (SAEG/UFV). The means of each parameter examined were further compared by Tukey´s test at *P≤0.05*.

3. Results

The effects of the mists and SPM_{Fe} varied among the parameters assessed in the two species. *S. terebinthifolius* had increased values of ETR, Fv/Fm ratio and total chlorophyll content induced by SPM_{Fe} (Table 2, 4). However mists treatments had no effect on this species.

On the other hand, *S. tomentosa* was affected by mist, SPM_{Fe} or by the interaction of both factors (Table 3). To better understand the effects of the factors, the study of the interactions proceeded in all parameters assessed in this particular species (Table 5).

In *S. tomentosa* the acid mist significantly decreased the net uptake of CO_2 while increased the MP, but these effects appeared to be suppressed by the SPM_{Fe}. The Fe content in the leaves was enhanced when the plants were exposed to SMP_{Fe}, and the MDA content increased when the plants were exposed to both SPM_{Fe} and acid mist (Table 5). There was no significant effect of the interaction of the factors in the ETR, however the main effect of SPM_{Fe} was significant (Table 3), increasing ETR mean values (No SPM_{Fe} = 131.49 and $SPMFe$ = 161.16 at $P \leq 0.05$, *F* test).

S. tomentosa showed high SOD activity after acid mist treatment, while CAT activity was increased by both acid mist and SPM_{Fe} treatment (Table 5).

Table 2. Summary of the analyses of variance for physiological and biochemical parameters of *S. terebinthifolius* exposed to simulated mists and SPM$_{Fe}$ deposition

Source of variation	df	A	g_s	Ci/Ca	ETR	Fv/Fm	Chl	Phenol	Fe	MP	MDA	SOD	CAT	POX
Mist	1	1.29	$0.13e^{-02}$	$0.83e^{-04}$	113.95	$0.27e^{-03}$	$0.61e^{-02}$	$0.33e^{-01}$	754.87	20.43	1736.9	1724.26	77.30	$0.50e^{-01}$
SPM$_{Fe}$	1	0.28	$0.77e^{-05}$	$0.19e^{-02}$	1995.85*	$0.19e^{-01}$*	$0.65e^{-01}$*	$0.16e^{-01}$	36052.52	0.43	4.34	1414.44	1756.70	3.88
Mist x SPM$_{Fe}$	1	0.63	$0.16e^{-02}$	$0.12e^{-02}$	77.88	$0.72e^{-04}$	$0.51e^{-02}$	$0.29e^{-01}$	46.58	$0.31e^{-01}$	963.05	92.23	261.50	0.12
Error	9	0.81	$0.69e^{-03}$	$0.57e^{-02}$	188.71	$0.52e^{-03}$	$0.60e^{-02}$	$0.74e^{-02}$	7642.63	8.52	1638.37	2439.90	1630.88	1.96

* Significant ($P \leq 0.05$) by F test.

A: net photosynthesis, g_s: stomata conductance, Ci/Ca: intern and ambient CO_2 ratio, ETR: electron transfer rate, Fv/Fm: maximum quantum efficiency of PSII, Chl: total chlorophylls, Phenol: total phenols, Fe: leaf Fe content, MP: membrane permeability, MDA: malonaldehyde content, SOD: superoxide dismutase activity, CAT: catalase activity, POX: peroxidase activity.

Table 3. Summary of the analyses of variance for physiological and biochemical parameters of *S. tomentosa* exposed to simulated mists and SPM$_{Fe}$ deposition

Source of variation	df	A	g_s	Ci/Ca	ETR	Fv/Fm	Chl	Phenol	Fe	MP	MDA	SOD	CAT	POX
												Mean square		
Mist	1	1.52*	$0.22e^{-03}$	$0.74e^{-02}$	339.48	$0.11e^{-04}$	$0.19e^{-02}$	0.48e-03	172.26	24.16	192.44	38570.65*	2874.35*	$0.71e^{-01}$
SPM$_{Fe}$	1	0.30	$0.33e^{-03}$	$0.23e^{-04}$	3525.39*	$0.79e^{-03}$	$0.11e^{-01}$	0.18e-01	255912*	5.37	4619.40*	28.75	2235.99	$0.77e^{-01}$
Mist x SPM$_{Fe}$	1	2.44*	$0.79e^{-03}$	$0.24e^{-03}$	2.97	$0.52e^{-04}$	$0.14e^{-02}$	0.16e-01	$0.56e^{-02}$	48.25*	7929.79*	4131.71	3175.93*	0.43
Error	9	0.24	$0.61e^{-03}$	$0.51e^{-02}$	620.66	$0.45e^{-03}$	$0.13e^{-01}$	0.44e-02	13253.65	5.16	701.81	3361.19	516.61	0.52

* Significant ($P \leq 0.05$) by *F* test.

A: net photosynthesis, g_s: stomata conductance, Ci/Ca: intern and ambient CO_2 ratio, ETR: electron transfer rate, Fv/Fm: maximum quantum efficiency of PSII, Chl: total chlorophylls, Phenol: total phenols, Fe: Fe content, MP: membrane permeability, MDA: malondialdehyde content, SOD: superoxide dismutase activity, CAT: catalase activity, POX: peroxidase activity.

Table 4. Effects of simulated SPM_{Fe} deposition on physiological and biochemical parameters of *S. terebinthifolius*

Parameters	No SPM_{Fe}	SPM_{Fe}
A (μmol_{CO_2} m^{-2} s^{-1})	9.27 [a]	9.54 [a]
g_s (mol m^{-2} s^{-1})	0.08 [a]	0.08 [a]
Ci/Ca	0.71 [a]	0.73 [a]
ETR	66.9[b]	89.23[a]
Fv/Fm	0.69[b]	0.76[a]
Chl (μg mm^{-2})	0.49[b]	0.62[a]
Phenol (ABS g^{-1} FW)	0.76 [a]	0.82 [a]
Fe(μg g^{-1} DW)	383.5[a]	478.44[a]
MP (%)	19.62 [a]	19.29 [a]
MDA (nmol g^{-1} FW)	226.55 [a]	225.50 [a]
SOD (units min^{-1} g^{-1} FW)	142.06 [a]	160.86 [a]
CAT (mmoles min^{-1} g^{-1} FW)	20.63 [a]	41.58[a]
POX (μmoles min^{-1} g^{-1} FW)	0.53 [a]	1.513 [a]

Different letters between columns indicate significant difference, according to *F* test ($P \leq 0.05$).

Table 5. Effects of the interaction between simulated mist and SPM_{Fe} deposition on physiological and biochemical parameters of *S. tomentosa*

Parameter	Neutral mist		Acid mist	
	No SMP_{Fe}	SPM_{Fe}	No SMP_{Fe}	SPM_{Fe}
A (μmol$_{CO2}$ m^{-2} s^{-1})	6.06^{Aa}	5.55^{Aa}	4.66^{Bb}	5.72^{Aa}
g_s (mol m^{-2} s^{-1})	0.07^{Aa}	0.06^{Aa}	0.06^{Aa}	0.08^{Aa}
Ci/Ca	0.54^{Aa}	0.55^{Aa}	0.59^{Aa}	0.58^{Aa}
ETR	126.45^{Aa}	157^{Aa}	136.53^{Aa}	165.35^{Aa}
Fv/Fm	0.80^{Aa}	0.81^{Aa}	0.80^{Aa}	0.81^{Aa}
Chl (μg mm^{-2})	0.83^{Aa}	0.87^{Aa}	0.79^{Aa}	0.87^{Aa}
Phenol (ABS g^{-1} FW)	0.66^{Aa}	0.80^{Aa}	0.72^{Aa}	0.72^{Aa}
Fe(μg g^{-1} DW)	568.75^{Ab}	1368.58^{Aa}	562.15^{Ab}	1362.0^{5Aa}
MP (%)	16.74^{Ba}	19.05^{Aa}	22.67^{Aa}	18.04^{Ab}
MDA (nmol g^{-1} FW)	161.56^{Bb}	240.07^{Aa}	213.02^{Aa}	202.48^{Aa}
SOD (units min^{-1} g^{-1} FW)	135.41^{Ba}	170.23^{Aa}	265.74^{Aa}	236.28^{Aa}
CAT (mmoles min^{-1} g^{-1} FW)	19.21^{Bb}	71.03^{Aa}	74.19^{Aa}	69.66^{Aa}
POX (μmoles min^{-1} g^{-1} FW)	3.59^{Aa}	4.05^{Aa}	4.049^{Aa}	3.86^{Aa}

Different capital letters indicate significant difference between mists treatments and different small letters indicate significant difference between SPM_{Fe} treatments by Tukey´s test ($P \leq 0.05$)

The visible characteristics of the leaves are shown on Table 6. *S. tomentosa* presented trichomes on both surface of the leaf while *S. terebenthifolius* presented glabrous leaf. The leaves of both species are hypostomatic. No markedly chlorosis or necrosis were observed on the leaves of the treated plants.

Table 6. Leaf characteristics of the assessed species

Characteristic	*S. terebinthifolius*	*S. tomentosa*
Morphology	odd pinnate	odd pinnate
Shape	elliptic	ovate
Trichome	glabrous	pubescent
Stomata	abaxial	abaxial

4. Discussion

Different chemical components, including particulate matters, in the atmosphere of polluted areas may be absorbed by leaves and contribute to the accumulation of toxic and non toxic elements (Alexeyev, 1995). Plants species that are able to resist the negative impact of atmospheric pollutants do so through either tolerance or avoidance strategies.

The responses of *S. terebinthifolius* to the treatments indicate that this species is resistant to acid mist and SPM_{Fe} deposition. On the contrary, the SPM_{Fe} seemed to positively affect some physiological parameters. Improvement on ETR, Fv/Fm and chlorophyll content in the leaves of *S. terebinthifolius* exposed to SPM_{Fe} may be due to Fe increment in the foliar tissue. Although not significant, the Fe content in the leaves of the plants treated with SPM_{Fe} was higher than in those not exposed to it (Table 4). In these plants the Fv/Fm was closer to the standard values for healthy plants (0.832 ± 0.004) proposed by Björkamn and Demming (1987). Because Fe is required for the synthesis of chlorophyll and present in many electron transport complexes, i.e. cytochrome b_6/f and ferredoxin molecule (Chereskin and Castelfranco, 1982; Hell and Stephan, 2003), the Fe content in *S. terebinthifolius* in the SPM_{Fe} treatments might have improved the plant physiological responses. Suh *et al*. (2002) reported that under higher availability of Fe, the content of cytochrome b_6/f increased, generating a greater ETR and predisposing the leaves of pea plants to photooxidative damage. However, no effects on photosynthetic parameters and antioxidant enzymes activity were observed in *S. terebinthifolius,* which indicates that both acid mist and SPM_{Fe} treatments were not sufficient to provoke an oxidative response on the exposed plants. Therefore it is possible that *S. terebinthifolius* overcomes the stresses caused by acid and SPM_{Fe} depositions under the field conditions.

In *S. tomentosa* the photosynthesis was only reduced when plants were exposed to acid mist alone. The mitigating effect of SPM_{Fe} on photosynthesis reduction in *S. tomentosa*, caused by acid mist, might be due to physical barrier created by the particulate deposit on the leaves. As observed in many plant species, photosynthesis is sensitive to SO_2, as a gaseous or acid wet contaminant (Saxe, 1991). Although acid deposition can affect photosynthesis on the basis of chlorophyll content (Fan and Wang, 2000), the *S. tomenta* total chlorophyll values did not differ

within the treatments. In *Pinus densiflora* exposed to acid deposition, the reduction on photosynthesis was linked to the increase on the rate of degradation of chlorophyll to pheophytin, despite the increment in total chlorophyll content (Shan, 1996). Since the g_s and the Ci/Ca in *S. tomentosa* were not affected by the imposed treatments, it is possible that the decay on CO_2 gain is linked to other effect than stomatal restriction. Acid deposition on leaves can alter pH balance within the plant tissue, causing a malfunction of metabolic processes. Particularly, the development of low pH in the cytoplasm can alter proton-ionic balance and enzymes activity, including those related to CO_2 fixation (Neufeld *et al.*, 1985). As a consequence, the generation of NADP can be limited, increasing the opportunity of superoxide radical (O^-_2) production, through Mehler reaction, on the reductant side of photosystem I (PSI) (Arora *et al.*, 2002).

According to the values cited by Pugh *et al.* (2002), *S. tomentosa* presented toxic levels of Fe (>500 ppm) when exposed to SPM_{Fe}. This indicates that a foliar uptake was effective. However, the accumulation was independent from the pH of the applied mist, which suggests that the reduction of of SPM_{Fe} into Fe^{2+} was not favored by the acid mist. Because the oxidation of metallic Fe into Fe^{2+} is facilitated by water (Pereira and Freire, 2005) both mist treatments, neutral and acid, could have equally contributed to increase the Fe availability for the foliar uptake. Also, the presence of trichomes on both sides of the leaves may have aided retain the iron particles, even though leaves were cleaned prior to the metal determination. Histochemical analysis on leaves of *Cordia verbenacea* growing in the vicinity of an iron ore industry showed that nanoparticles of Fe were present in the mesophyll, collenchyma and endodermis of the foliar blade presumably due to the iron dust deposition on the leaves (Silva *et al.*, 2006). Since Fe excess increases the content of molecules involved in the electron transfer in the thylakoids (Suh *et al.*, 2002) the higher content of Fe in the leaves o *S. tomentosa*, if available, might have contributed to enhance the ETR response in the plants treated with SPM_{Fe} (Table 5).

Pollutants can generate oxidative stress in plants (Koricheva *et al.*, 1997; Sinha *et al.*, 1997; Vansuyt *et al.*, 1997; Yu *et al.*, 2002). The occurrence of oxidative stress in *S. tomentosa* treated with acid mist and SMP_{Fe} is suggested by increased MP and MDA values found in these plants. Low pH conditions and the phytotoxic accumulation of metals, such as Cu and Fe in living tissues can elicit the production of ROIs (Kim and Jung, 1993; Estevez *et al.*, 2001). ROIs can cause membrane

damage through lipid peroxidation and favor the leakage of cellular electrolytes (Fürtig *et al*., 1999; Edreva, 2005). The higher content of MDA found in the plants of *S. tomentosa* exposed to acid mist and SPM_{Fe} deposition indicates that both pollutants induced lipid peroxidation by either enhancing the production of ROIs or the direct prooxidant effect of Fe on lipid molecules (Becana *et al*., 1998).

The onset of an oxidative stress in *S. tomentosa* is reinforced by the increased activity of SOD and CAT. Increased SOD and CAT activity in plant tissue due to acid deposition and heavy metal accumulation is reported by Koricheva *et al*. (1997). However in *S. tometosa* the SOD activity was only affected by the acid mist. Similarly, the exposure of *Cucumis sativa* to simulated acid deposition increased the activity of SOD and the MDA content while reduced the photosynthetic rate (Yu *et al*., 2002). On the other hand, CAT activity in *S. tomentosa* was affected by both acid mist and SPM_{Fe}. An increased activity of SOD and CAT under Fe excess treatments was reported in *Nicotina plumbaginifolia* (Kampfenkel *et al*., 1995) though the SOD activity had been higher only after a longer period of exposure. Under environmental stress conditions, the antioxidant enzyme system detoxifies the oxygen reactive intermediates in order to keep them at a basal level. However, if the severity of the condition increases, the system may not be able to minimize the oxidative effect, which may compromise the plant vigor and survival.

5. Conclusion

The results in this study suggested that the two restinga species apparently use different survival strategies to overcome the stress of wet and dry deposition originated by the iron ore industry. *S. terebinthifolius* resisted the stress by avoiding the direct effects of acid and SMP_{Fe} deposition on its leaves and the build up of indirect effects caused by Fe phytotoxic accumulation or oxidative stress development. Nevertheless, *S. tomentosa* was affected by acid deposition and the accumulation of Fe in the leaves, which led to an oxidative stress. The use of multi-responses is more suitable to detected oxidative stress rather than the use isolated biomarkers. Therefore the conjunction of physiological and biochemical parameters of *S. tomentosa* evaluated in this study may be used as biomarkers to indicate stress development in plants exposed to iron ore industry emissions under field conditions.

6. Acknowledgments

7. References

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CONCLUSÃO FINAL

Neste estudo evidenciou-se que o impacto das emissões da indústria mineradora de ferro, e suas interações, sob as espécies de restinga não é circunstancial. Os efeitos do material particulado de ferro e de deposição ácida úmida puderam ser detectados em diferentes aspectos biológicos das plantas.

O esforço reprodutivo, a implantação, e o recrutamento de novos indivíduos foram aspectos ecológicos alterados pela exposição ao material particulado de ferro e deposição ácida úmida, efeito este especialmente visível na espécie *S. tomentosa.* Os resultados bioquímicos e fisiológicos, como os observados em *S. tomentosa*, colaboram para a comprovação de que os poluentes em questão podem causar danos aos níveis mais primordiais de organização biológica. Em ultima análise, a somatória destas conseqüências pode colaborar para a aceleração do declínio da composição vegetal da restinga.

Como biomarcadores os processos fisiológicos e metabólicos podem auxiliar em programas de monitoramento e avaliação de risco ecológico. Espécies de ocorrência natural em ecossistemas acometidos pela contaminação por poluentes, podem ser utilizadas como indicadores passivos ou ecológicos de estresse. Em estudos de impacto ambiental, o uso destes organismos é capaz de fornecer informações acuradas acerca do grau de integridade do ecossistema, visto serem eles próprios componentes do sistema. Portanto, o emprego dos resultados e procedimentos descritos nesta investigação auxiliaria na mitigação do impacto das emissões do setor minerador, através de sua aplicação em programas de monitoramento e preservação de ecossistemas em áreas afetadas pela presença de indústrias.

Capítulo 1

Table 1. Summary of the ANOVA of the total particulate matter ($\mu g\ mm^{-2}$) deposited on the leaves of the four studied species growing at three different sites (1.0, 5.0 and 15.0 km) and sampled at the end of each trimester of the year 2005.

Source of Variation	df	Mean square			
		I. pes caprae	*C. rosea*	*S. tomentosa*	*S. terebinthifolius*
Replicates	4	$0.154e^{-01\ NS}$	$0.297e^{-01\ NS}$	$0.792^{\ NS}$	$0.171^{\ NS}$
Local	2	0.580^{*}	0.585^{*}	8.471^{*}	1.145^{*}
Time	3	0.967^{*}	2.256^{*}	3.359^{*}	2.909^{*}
Local x Time	6	0.206^{*}	0.307^{*}	1.958^{*}	0.290^{*}
Error	44	$0.378e^{-01}$	$0.720e^{-01}$	0.648	$0.755e^{-01}$

* Significant at $P \leq 0.05$ by F test; NS not significant

Table 2. Summary of the ANOVA of the total iron content ($\mu g\ gDW^{-1}$) in the leaves of the four studied species growing at two different sites (1.0 and 15.0 km) and sampled at the end of each trimester of the year 2005

Source of Variation	df	Mean square			
		I.pes caprae	*C. rosea*	*S. tomentosa*	*S. terebinthifolius*
Replicates	2	$1.8^{\ NS}$	$111.4^{\ NS}$	$1456.0^{\ NS}$	3090.1^{*}
Local	1	12889.9^{*}	28690.3^{*}	721101.3^{*}	21510.0^{*}
Time	3	5695.4^{*}	13792.9^{*}	37925.4^{*}	9006.3^{*}
Local x Time	3	1911.5^{*}	2278.5^{*}	33657.8^{*}	5660.4^{*}
Error	14	101.2	160.2	2923.1	629.7

* Significant at $P \leq 0.05$ by F test; NS not significant

Table 3. Summary of the ANOVA of the total chlorophyll content (μg mm^{-2}) in the leaves of the four studied species growing in two different sites (1.0 and 15.0 km) and sampled at the end of each trimester of the year 2005

Source of Variation	df	Mean square			
		I. pes caprae	*C. rosea*	*S. tomentosa*	*S. terebinthifolius*
Replicates	4	$0.523e^{-02 \, NS}$	$0.366e^{-02 \, NS}$	$0.757e^{-02 \, NS}$	$0.239e^{-02 \, NS}$
Local	1	$3.733e^{-01 \, *}$	0.127^{*}	$0.120e^{-01 \, *}$	0.620^{*}
Time	3	$0.201e^{-01 \, *}$	$0.208e^{-01 \, NS}$	$0.736e^{-01 \, *}$	$0.698e^{-01 \, *}$
Local x Time	3	$0.528e^{-01*}$	$0.214e^{-01 \, NS}$	$0.190e^{-01 \, *}$	$0.475e^{-02 \, NS}$
Error	28	$0.533e^{-02}$	$0.906e^{-02}$	$0.537e^{-02}$	$0.104e^{-01}$

* Significant at $P \leq 0.05$ by *F* test; NS not significant

Capítulo 2

Table 1. Summary of ANOVA of the mean germination of *G.pernambucensis, S. terebinthifolius* and *S. tomentosa* exposed to different levels of pH and iron dust.

Source of Variation	df	Mean square		
		G.pernambucensis	*S. terebinthifolius*	*S. tomentosa*
Replicates	3	3.26^{NS}	7.15^{NS}	1.15^{NS}
Iron dust	1	2.04^{NS}	12.04^{NS}	376.04^{*}
pH	2	0.54^{NS}	5.54^{NS}	50.66^{*}
Iron dust x pH	2	0.29^{NS}	3.79^{NS}	5.16^{NS}
Error	15	4.09	3.38	3.78

* Significant at $P \leq 0.05$ by *F* test; NS not significant