Effects of Simulated Tropospheric Ozone on soluble proteins and photosynthetic pigments levels of four woody species typical from The Mexican Humid Tropic

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Abstract: - Three months old seedlings of four woody tropical species [*Haematoxylum Campechianum L* (Wood blood tree), *Tabebuia Rosea* (Pink flower tree), *Cedrela odorata* (Red Cedar) and *Swietenia Macrophylla (*Mahogany) were fumigated during 6 weeks at three different concentrations of ozone (50, 110 and 250 ppb) using charcoal filtered air within an open-top chamber from june to july in 2009. Visible damages were identified, effects on phtosynthetic pigment levels (chlorophyll *a*, chlorophyll *b*, total chlorophyll and total charotenoids) and soluble proteins content were determined. All studied species (Wood blood tree, Pink flower tree, Red Cedar and Mahogany) showed sensitiveness to ozone exposure levels, showing decreases in photosynthetic pigments levels and soluble proteins contents. Mahogany and Wood blood tree were the most sensitive species to tropospheric ozone showing greater visible damages.

Key-Words: Woody tropical species., Foliar damage., Open Top Chambers., Tropospheric ozone., Coastal vegetation., Mexican Humid Tropic.

1 Introduction

Changes in lad use and air pollutants increased concentrations during the last decades have affected biogeochemical cycling of vegetation. Background ozone levels in unpolluted air can be anywhere from 20 to 50 ppb [1], though Lefohn et al. [2] argued for occasional background levels over 60 ppb resulting from stratospheric input. Polluted regions can have ozone levels peaking as high as 400 ppb [1]. Concentrations of tropospheric ozone in rural areas are higher on averaged compared to urban areas due to a long-range transport, downward mixing of stratospheric air, lack of nocturnal ozone depletion and diurnal cycling of radiation sentence [3, 4, 5, 6]. Tropospheric ozone constitutes a phytotoxic risk to crops and natural vegetation [7]. In Europe and USA criical levels for ozone are currently processed to protect crops, forests and natural vegetation against adverse effects due to high concentrations of ozone [8]. This has resulted in broad research of the response of a significant number of plant species from different countries around the world to ozone levels. When vegetation species are exposed to airborne pollutants, most plants experience physiological changes before exhibiting visible damage to leaves. Ozone enters the intercellular spaces in leaves/needles almost exclusively through the stomata [9], it is then converted into a reactive oxygen species [10]. When high-concentrations of ozone diffuse into plants, the strong oxidizing power injuries the plant tissues, resulting in visible damage, such as white, yellow or reddish spots on the leaves [11]. Some parameters as chlorophyll content, proteins levels and nutrients concentrations are used to determine if one specie is sensitive or tolerant to a specific air pollutant [12]. Olteanu and colaborators made researches regarding the physiological response induced by atmospheric pollutants on gymnosperm species in some insdustrialized areas in Romania and they found that *Pinus sylvestris* showed the greatest sensitivity to air polutants, followed by *Pinus nigra* [13]. These species showed a decrease in chlorophyll a concentrations and obvious suffering signs (chlorosis, necrosis and defoliation). In a research carried out on Sambucus species (*S. ebulus, S. nigra and S. racemosa*), different treatments were applied at 40 ppb and 70 ppb of ozone during 106 days, after 44 days total chlorophyll content was determined and

a considerable decrease was found [14]. In studies carried out on *Ginkgo biloba*, chlorophyl contents alter ozone exposure showed the same behaviour [15].

Riikonen et al [16] found that soluble protein content in leaves of silver birch was slightly decreased during three years of exposure to elevated concentration of ozone. An acceleration of leaf senescence by ozone was reported by Grandjean and Fuhrer [17] who found that ozone exposure resulted in reductions in the contents of chlorophyll and soluble protein and increases in the activity of proteolytic enzymes; typical changes of those which occur in senescing leaves.

On the other hand, most of the studies have been focused to crops, forest and vegetation from temperate zones and there are not enough studies related to ozone effects on tropical vegetation [18, 19, 20, 21, 22]. In Mexico, numerous studies have been carried out on vegetation in the main urban areas and the surroundings of Mexico City [23, 24, 25, 26, 27, 28]. There is not reported information about ozone effects on tropical vegetation from other regions in Mexico. There are evidences that in Atasta-San Antonio Cárdenas, ozone concentrations exceed the air quality standard for ozone in Mexico. In this region there is a PEMEX sour gas recompression station and several offshore platforms where oil and gas are extracted. Currently, in Mexico it has not been determined a target value to protect vegetation and critical levels for ozone are stablished considering adverse effects only in human beings.

The objetives of this research were to determine if elevated ozone concentrations induced visible foliar damages and if visible injuries were accompanied by reductions on photosynthethic pigments and soluble proteins levels and changes in macronutrients foliar on four tropical tree species exposed to different ozone concentrations using open-top chambers.

2 Materials and Methods

2.1 Propagation and fumigation

The research site is located within the Botanical Garden of the Autonomous University of Carmen Island (Lat. 18º 38' 36''N, Long. 91º 49' 51'' W, elev. 2 m asl) on the southeast edge of Carmen City in Campeche, Mexico. In Figure 1 is showed the location of the site where this research was carried out. Six months old seedlings of Wood blood tree, Pink flower tree and Mahogany were purchased and transplanted into 0.30 m x 0.20 m deep pots (1 seedling/pot). All seedlings were selected under homogeneous conditions of size, foliage and age. All plants received daily irrigation during the experiment, to keep the soil moisture close to field capacity. Open-top chambers (3m diameter x 3 m height) were constructed according the scheme descrited by Heagle et al. [29]. An OTC consists on standardised large cylindrical open-top plastic-covered field chamber where incoming air is charcoal-filtered which inflates a double wall of the lower half of the chamber and moves outward through perforations all around the inner wall, moving under and over the plants inside and then upward and out of the chamber. This up-draft, open-top chamber is the most widely used device for studying Ozone effects on plants in the field. OTC was operated during six weeks from June 22 to July 31 in 2009 at the day-time from 08:00 to 16:00 h. Experiment was conducted at three ozone exposure levels: 50, 110 and 250 ppbv using charcoal filtered air (CF) and exposures were conducted every two days for each species. A total of 24 individuals for each specie were considered, six individuals for each concentration level and six control samples which were not exposed. Ozone was generated; every day from 08:00 to 16:00 h, by an ozone generator (Model 700 API) and dilutions with CF air were performed using two mass flow controllers. Ozone-levels in the OTC were measured by using an ozone analyzer (Advanced Pollution Instrumentation Model 4000). The environment in an open-top chamber can be different than that in open ambient air. Changes can occur in temperature and humidity, for this reason, inside and outside the chamber were taken measures of temperature and relative humidity. In addition, open-air ambient plots were included to determine possible chamber effects.

2.2 Visual assessment and harvest of plants

2.2.1 Harvest of plants

A first sampling was carried out before exposure and a second sampling was done after six weeks at the end of the exposure. Dry weights of the foliar tissue samples were determined by drying the vegetal tissue at 80ºC for 48 h.

2.2.2 Visual assesment

During the experiment visual assesments of the plants were made once a week. Number of leaves and senescent leaves were counted, and all plants were observed for visible injuries.

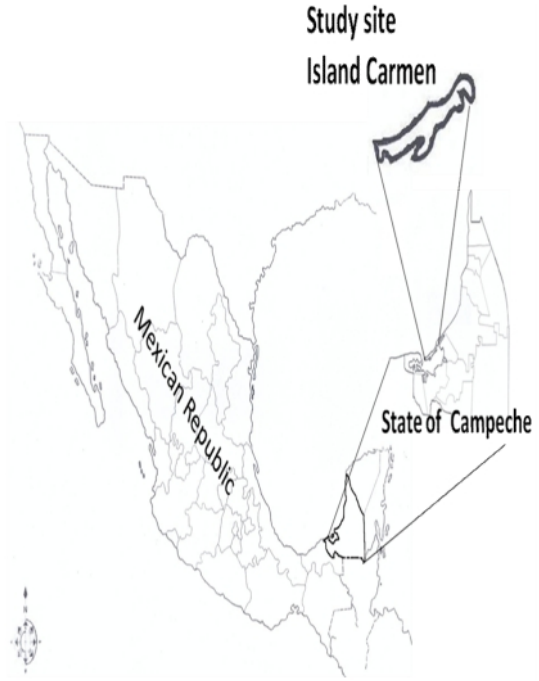


Figure 1. Site location of this research.

2.3 Chemical determinations

2.3.1 Photosynthetic pigments content

Samples were processed and weighed inmediately after collection. Pigments were extracted using a 80% acetone-20% water solution. Extracts were centrifuged at 1500 rpm during one minute and absorbances were measured in an UV-visible Hach DR201Q spectrophotometer at 663.2, 646.8, 470, 430, and 665 nm. Finally, chlorophyll total, chlorophyll a, chlorophyll b and total charotenoids contents were calculated per foliar mass unit using Lichtenthaler ecuations [30].

2.3.2 Soluble proteins levels

Samples were extracted with 10 ml of a buffer solution of potassium phosphate 0.1 M at pH 7.4. Proteins were precipitated adding 1 ml of trichloroacetic acid (at 10%) to 1 ml of the extract, then stirring and let it stand overnight in refrigeration. The next day, sample was centrifuged from 5 to 10 minutes at 10 000 rpm. The sediment obtained was dissolved into 1 ml of NaOH and let it stand for two hours. 50 ml of the sediment were taken and then 250 µl of distilled water and 1.7 ml of Folin reactive mixture were added and let it stand during 10 minutes [31]. Finally, absorbance was measured at 750 nm in an UV-visible Hach DR201Q spectrophotometer. The calibration curve was prepared from bovine serum, at concentrations in water at 200 µg / ml. The curve was prepared whenever required in the same way as samples.

2.4 Severity Scale

2.4.1 Damaged Area Percentage

Damaged leafs were scanned and processed using Adobe photoshop CS e Image Tool for Windows v. 1.28 (UTHSCSA1995-97). Foliar damaged percentage was used to obtain a severity scale by 2LOG v1.0 program [32]. Each class shows lower, middle and upper limits expressed as damaged area percentage. Each procesed leaf was classified according to Horsfall-Barratt method [33].

3 Results

3.1.1 Photosynthetic pigments content

Mean values and percentages of change in the content of total chlorophyll and total charotenoids before and after exposure to ozone different levels are shown in Table 1. Figure 2 (a, b, c and d) shows standard deviation, maximum, minimum, and mean concentrations of chlorophyll a before and after exposure at three different levels of ozone (50, 110 and 250 ppb) for *Haematoxylum Campechianum L* (Wood blood tree), *Tabebuia Rosea* (Pink flower tree), *Swietenia Macrophylla (*Mahogany) and *Cedrela Odorata* (Red Cedar), respectively.

In Figure 3 (a, b, and c), it can be observed values for standard deviation, maximum, minimum, and mean concentrations of total chlorophyll before and after exposure at three different levels of ozone (50, 110 and 250 ppb) for *Haematoxylum Campechianum L* (Wood blood tree), *Tabebuia Rosea* (Pink flower tree) and *Swietenia Macrophylla (*Mahogany), respectively. Table 2 shows mean values and percentages of change in chlorophyll *b* and chlorophyll *a/b* ratio.

3.1.2 Soluble protein levels

In Table 3, it can be observed mean values and percentages of reduction in soluble proteins concentrations before and after exposure to ozone different levels. Figure 4 (a, b, c and d) shows standard deviation, maximum, minimum, and mean soluble proteins levels before and after exposure at 50, 110 and 250 ppb, for *Haematoxylum Campechianum L* (Wood blood tree), *Tabebuia Rosea* (Pink flower tree), *Swietenia Macrophylla (*Mahogany) and *Cedrela Odorata* (Red Cedar), respectively.

Table 1. Mean concentrations and percentages of change in total chlorophyll and charotenoids before and after exposure to ozone different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rose.*, SM: *Swietenia Macrophylla*, CO: *Cedrela Odorata.*

	SBE (mg/g fw)	SAE (mg/g fw)	Change Percentage (%)	SBE (mg/g fw)	SAE (mg/g fw)	Change Percentage (%)
HM						
50ppb	3.679	1.815	-50.666	0.665	0.504	-24.210
110 ppb	2.424	1.152	-52.475	0.572	0.405	-29.196
250 ppb	1.592	0.837	-47.424	0.464	0.341	-26.509
TR						
50ppb	2.359	1.114	-52.777	0.241	0.193	-19.917
110 ppb	1.227	0.700	-42.950	0.460	0.149	-67.608
250 ppb	0.763	0.245	-67.889	0.329	0.042	-87.234
SM						
50ppb	2.749	1.278	-53.510	0.320	0.197	-38.437
110 ppb	2.483	1.103	-55.577	0.542	0.293	-45.940
250 ppb	2.074	0.657	-68.322	0.452	0.285	-36.946
CO						
50ppb	5.134	3.299	-35.742	0.952	0.573	-39.857
110 ppb	4.165	2.596	-37.671	0.609	0.531	-12.932
250 ppb	4.897	2.069	-57.749	0.208	0.239	+14.814

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure. (-) indicates a reduction percentage between samplings. (+) indicates an increase percentage between samplings.

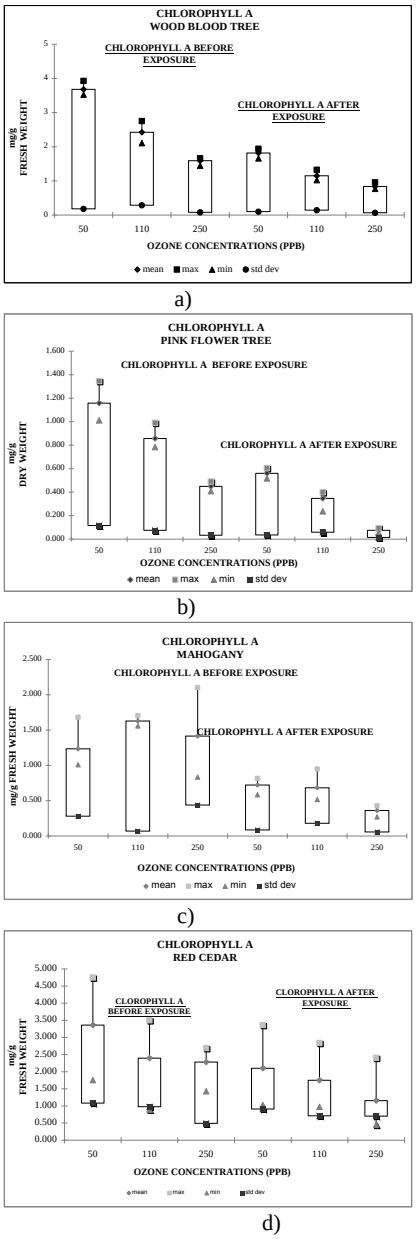
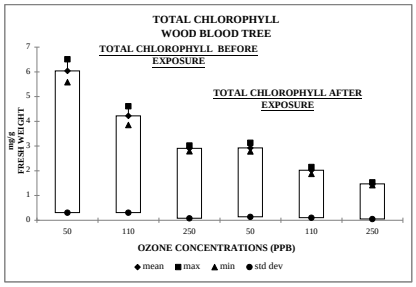
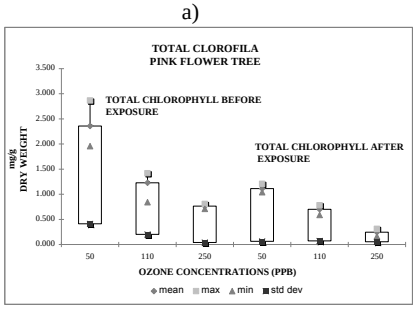


Figure 2. Standard deviation, maximum, minimum, and mean concentrations of chlorophyll a for a) *Haematoxylum Campechianum L* (Wood blood tree), b) *Tabebuia Rosea* (Pink flower tree), c) *Swietenia Macrophylla (*Mahogany) and d) *Cedrela Odorata* (Red Cedar) at three different levels of ozone exposure.



a)



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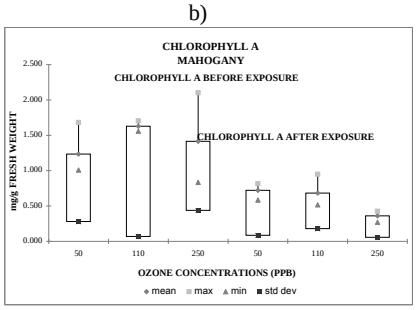

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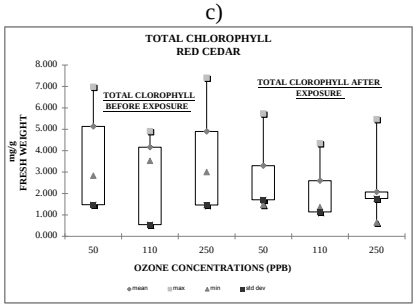


d)

Figure 3. Standard deviation, maximum, minimum, and mean concentrations of total chlorophyll for a) *Haematoxylum Campechianum L* (Wood blood tree), b) *Tabebuia Rosea* (Pink flower tree), c) *Swietenia Macrophylla (*Mahogany) and d) *Cedrela Odorata* (Red Cedar) at different levels of ozone exposure.

Table 2. Mean concentrations and percentages of change in chlorophyll b and chlorophyll a/b ratio before and after exposure to ozone different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rose.*, SM: *Swietenia Macrophylla*, CO: *Cedrela Odorata.*

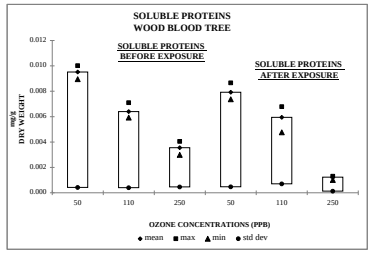
Specie	Chlorophyll *b*			Chlorophyll *a/b* ratio		
	SBE (mg/g fw)	SAE (mg/g fw)	Change Percentage (%)	SBE (mg/g fw)	SAE (mg/g fw)	Change Percentage (%)
HM						
50ppb	2.361	1.108	-53.070	1.558	1.638	+5.134
110 ppb	2.581	1.192	-53.816	0.939	0.996	+2.907
250 ppb	2.009	1.025	-48.979	0.792	0.816	+3.041
TR						
50ppb	1.044	0.554	-46.934	1.109	1.010	-8.863
110 ppb	0.432	0.351	-18.750	1.983	0.988	-50.163
250 ppb	0.314	0.159	-49.363	1.429	0.477	-66.570
SM						
50ppb	1.515	0.697	-53.993	0.815	1.034	+26.84
110 ppb	0.854	0.420	-50.819	1.907	1.623	-14.892
250 ppb	0.659	0.295	-55.235	2.147	1.227	-42.850
CO						
50ppb	1.753	0.886	-49.458	1.862	2.045	+9.830
110 ppb	1.555	0.712	-54.210	1.806	2.303	+27.520
250 ppb	1.943	0.484	-75.090	1.358	2.027	+49.263

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure. (-) indicates a reduction percentage between samplings. (+) indicates an increase percentage between samplings.

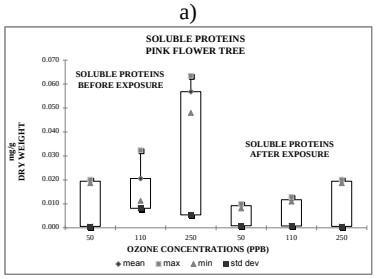
Table 3. Mean concentrations and percentages of reduction in soluble proteins before and after exposure to ozone different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rosea*, SM: *Swietenia Macrophylla* and CO: *Cedrela Odorata*.

Specie	Soluble Proteins		
	SBE (mg/g dw)	SAE (mg/g dw)	Change Percentage (%)
HM			
50ppb	0.010	0.008	-20.000
110 ppb	0.006	0.006	0
250 ppb	0.004	0.001	-75.000
TR			
50ppb	0.019	0.009	-52.632
110 ppb	0.021	0.012	-42.857
250 ppb	0.057	0.019	-66.667
SM			
50ppb	0.018	0.008	-55.556
110 ppb	0.016	0.007	-56.250
250 ppb	0.012	0.006	-50.000
CO			
50ppb	0.008	0.010	+25.000
110 ppb	0.011	0.011	0
250 ppb	0.010	0.006	-40.000

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure. (-) indicates a reduction percentage between samplings. (+) indicates an increase percentage between samplings.



a)



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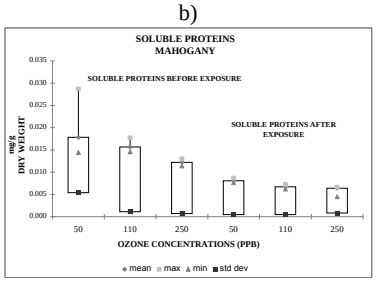

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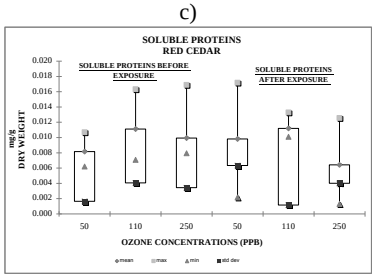

d)

Figure 4. Standard deviation, maximum, minimum, and mean soluble proteins levels for a) *Haematoxylum Campechianum L* (Wood blood tree), b) *Tabebuia Rosea* (Pink flower tree), c) *Swietenia Macrophylla (*Mahogany) and d) *Cedrela Odorata* (Red Cedar) at three different levels of ozone exposure.

3.1.3 Visual Assessment and Foliar Damage

Figure 5 (a, b, c and d) shows foliar damages found in individuals for the four tropical woody species studied after six weeks of exposure to different levels of ozone. Visual assessment was carried out considering both foliar damages (damaged area percentage) and the results obtained from chemicals determinations of biochemical response of plants to Ozone levels (changes in photosynthetic pigments and soluble proteins levels). Damaged foliar percentage was calculated from 18 digitalized images for each specie studied with visible damages.

Severity scale was obtained from representative images for each class. In Table 4, the severity scale and class distribution for each species studied are shown.

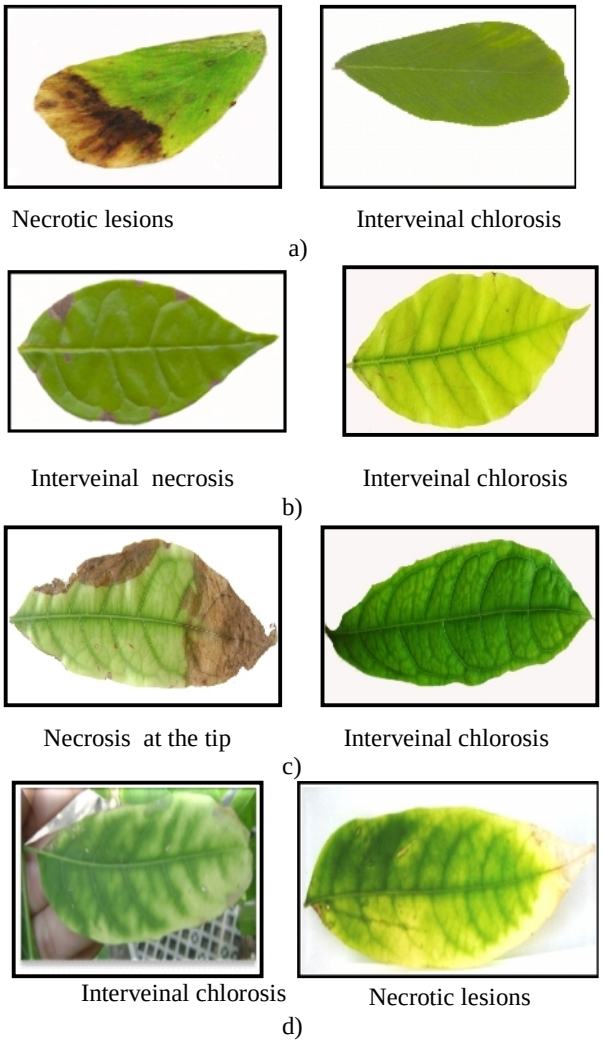

Figure 5. Foliar damages observed after the exposure period in a) *Haematoxylum Campechianum L* (Wood blood tree), b) *Tabebuia Rosea* (Pink flower tree), c) *Swietenia Macrophylla (*Mahogany) and d) *Cedrela Odorata* (Red Cedar) at three different levels of ozone exposure.

Table 4. Severity scale and class distribution for each specie studied.

Severity Scale				
Haematoxylum Campechianum L (Wood blood tree)				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	14.47% (110 ppb)	25.39% (110 ppb)	38.22% (250 ppb)	-
Class Distribution	15%	76%	5%	-
Tabebuia Rosea (Pink flower tree)				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	1.33% (110 ppb)	31.9% (250 ppb)	44.8% (250 ppb)	
Class Distribution	15%	37%	44%	-
Swietenia Macrophylla (Mahogany)				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	21.49% (50 ppb)	35.41% (110 ppb)	40.72% (250 ppb)	49% (250 ppb)
Class Distribution	5%	10%	76%	5%
Cedrela Odorata (Red Cedar)				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	10.536% (110 ppb)	19.760% (50 ppb)	41.058% (250 ppb)	-
Class Distribution	47.22%	33.33%	19.44%	-

3.1.4 Statistical Analysis

Average values for all chemical determinations were calculated and used in a one way ANOVA to find significant differences in concentration levels before and after exposure to ozone. All determinations showed significant differences at α=0.05 between samplings (before and after exposure to ozone). Duncan's test was performed using SAS-package, release 6.06 [34] to find significant differences among the four studied species for photosynthetic pigments, and soluble proteins levels (at P= 0.05). Chlorophyll A, Chlorophyll B and Total chlorophyll showed different behaviour for the four woody tissue species.

According Table 5, the four studied woody species show a determination coefficient greater than 0.8 in all cases (total chlrophyll, chlorophyll a, total charotenoids and soluble proteins content), excepting in Switenia Macrophylla (Mahogany) and Cedrela Odorata (Red cedar) in for total charotenoids and soluble proteins, respectively. We can conclude with a certainty greater than 80% that the changes showed in the studied individuals are due to Ozone concentrations and that as ozone concentrations increase most of the photosynthetic pigments and the soluble proteins content decrease.

Table 5. Lineal regression analysis for Total chlorophyll, Chlorophyll a, Total charotenoids and Soluble Proteins for the four studied species exposed to ozone.

	TC	**Ca**	**TCh**	**SP**
***Swietenia Macrophylla* (Mahogany)**				
r	-0.9998	-0.9752	0.6790	-0.9768
r^2	0.9996	0.9511	0.4610	0.9541
***Cedrela Odorata* (Red Cedar)**				
r	-0.9525	-0.9971	-0.9838	-0.8364
r^2	0.9072	0.9943	0.9679	0.6996
***Tabebuia Rosea* (Pink flower tree)**				
r	-0.9800	-0.9876	-0.9999	0.9983
r^2	0.9604	0.9754	0.9999	0.9968
***Haematoxylum Campechianum L* (Wood blood tree)**				
r	-0.9350	-0.9091	-0.9405	-0.9999
r^2	0.8743	0.8265	0.8847	0.9999

Where: TC: Total Chlorophyll; Ca: Chlorophyl a; TCh: Total Charotenoids; SP: Soluble Prtoteins.
(r) indicates a significant lineal relation ($P<0.05$) and (r^2) is the determination coefficient.

4 Discussion

Very often comparison between different experiments is extremely difficult, due to differences in exposure system, ozone exposure, growth conditions, provenance, etc. Another aspect, when comparing species, is which parameter is considered: visible injury, growth or physiological performance, biochemical traits, etc. Because of these reasons it is not surprising that particular species are sometimes classified as very responsive and in other cases not as

sensitive ⇨35, 36, 37⇨. So, caution is needed and therefore the general statement that fast-growing species are more sensitive than slower-growing ones, may be of more use. In the present experiment we exposed four tropical tree species, to exactly the same experimental conditions. All studied species were exposed to ozone concentrations representative for our region, grown and treated in the same type of chambers, soil and growth conditions. Comparing the contents of chlorophyll *a* before and after exposures to ozone, it was found that Wood blood tree, Pink flower tree and Mahogany individuals showed decreases in this photosynthetic pigment.

The same behaviour was showed in the content of soluble proteins. Red cedar individuals did not showed a clear pattern comparing the samplings before and after ozone exposures. This specie showed changes in proteins soluble, total charotenoids and total chlorophyll contents, but these changes did not show a clear pattern regarding ozone concentrations.

5 Conclusion

According to visible damages, all the studied species showed damages when they were exposed to different levels of ozone, being Mahogany and Pink flower tree the species that showed visible damages more severe. Mahogany, Pink flower tree and Wood blood tree showed some typical symptoms after the exposure like chlorosis and some leaves showed necrosis at the tip. However Mahogany and Pink flower tree showed more visual damage than Wood blood tree. Some Red cedar individuals showed necrotic lesions but there was not a correlation among visual damages and changes in photosynthetic pigments and soluble proteins content. Severity Class distribution showed that Mahogany was the especie with the highest percentage of individuals who fell into class 3 and 4 at the higher exposure concentrations (250 ppb) with values of damaged area between 40 and 49%, where class 3 showed the highest class distribution percentage (76%).

During the experiment it was observed that when O_3 concentration increased, all the studied species showed greater degree of damage. Changes observed in chlorophyll *a* are related to visual damages in the four studied species. Total Chlorophyll losses were greater at higher ozone concentrations (250 ppb). These reduction percentages between samplings (before and after exposure) were greater in Pink flower tree and Mahogany. Wood blood tree, Mahogany and Red Cedar individuals showed important decreases in the content of chlorophyll *b* when they were exposed to ozone at 250 ppb. A decrease in the chlorophyll *a*/ *b* ratio may be an indicative of damages in leaves induced by photo-oxidation (necrosis). Pink flower tree and Mahogany individuals showed a decrease in this ratio when they were exposed to ozone at 110 ppb and 250 ppb, it may indicates that there was photo-oxidation of the pigments. Some authors [38, 39] have found a decrease of this ratio due to effects of ozone.

The most sensible pigment is *B*-carotene, so a degradation of this pigment could be indicative of a photo-oxidative action [40]. All studied species showed a decrease in charotenoids content when ozone concentration increased, but these reduction percentages between samplings (before and after exposure) were more evident in Pink flower tree and Mahogany. Regarding to total chlorophyll/charotenoids it is difficult to stablish if ozone levels produced direct photo-oxidative damages on chlorophyll content of leaves.

All the studied species had the same behaviour regarding to the soluble proteins content, so that there was a decrease in soluble proteins as ozone concentrations were increased. Many air pollutants may induce changes in proteic patterns of the plants such as *Picea abies* (being the proteic alteration a way of adaptation to the produced stress due to ozone) [41, 42]. In a first experiment reported in our last paper [43], other tropical species (three mangrove species) were exposed at the same ozone levels than this study. Mangrove species showed visible damages and decreases in photosynthetic pigments levels and soluble protein content, finding that Red Mangrove plants were the most sensitive to the studied ozone levels.

These preliminary results let us to infer that visible damages and observed changes in soluble proteins and photosynthetic pigments were related to ozone levels used during the controlled exposure, being these more evident at higher ozone concentrations (110 ppb and 250 ppb). Mahogany (*Swietenia Macrophylla*) and Pink flower tree (*Tabebuia Roseae*) were the most sensitive species to the studied levels of ozone, followed in order of importance by Wood blood tree (*Haematoxylum Campechianum L.*) and Red Cedar (*Cedrela Odorata*). Red cedar did not show a clear relation among visible damages and changes in the content of photosynthetic pigments and soluble proteins levels.

In conclusion, it is necessary to carry out a long-term exposure to obtain definitive conclusions about these species and their sensitivity to ozone levels. The biochemical response for other variables that can be affected by trophospheric ozone should be assessed.

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