Effects of Simulated Tropospheric Ozone on foliar nutrients levels (Ca^{2+}, Mn^{2+}, Mg^{2+} and K^{+}) of three woody species of high commercial value typical from Campeche, México

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Abstract: - Three months old seedlings of three woody species of high commercial value typical from Campeche, México [*Haematoxylum Campechianum L* (Wood blood tree), *Tabebuia Rosea* (Pink flower tree) and *Swietenia Macrophylla (*Mahogany)] were fumigated during 6 weeks at three different concentrations of simulated tropospheric ozone (at 50, 110 and 250 ppb) using charcoal filtered air within an open-top chamber from june to july in 2009. Visible damages and changes on phtosynthetic pigments levels were identified and nutrients concentrations (Ca^{2+}, Mn^{2+}, Mg^{2+} and K^{+}) were determined. All studied species (Wood blood tree, Pink flower tree and Mahogany) showed sensitiveness to ozone exposure levels, showing decreases in photosynthetic pigments levels as well as changes in nutrients contents. Mahogany and Wood blood tree were the most sensitive species to tropospheric ozone showing greater visible damages .

Key-Words: - Woody species., Foliar damage., Open Top Chambers., Tropospheric ozone., Atmospheric pollution., Campeche, México., Nutrients.

1 Introduction

Tropospheric Ozone is a secondary pollutant produced mainly from photochemical reactions among nitrogen oxides (NO_x), volatile organic compounds (VOC's) and oxygen [1]. Main sources of primary pollutants are anthropogenic sources: NO_x's are generated from motorized vehicles and industrial processes, and VOC's are emitted from vehicles and evaporation of solvents and organic compounds.

Reactions of ozone in environment are very complex and depend on climatic conditions as temperature and solar radiation [2]. Atmospheric pollution is generated in urban and industrial zones, but transport processes in the atmosphere cause increases in polutants concentrations in pristine zones located far away from the locations of the primary sources. For this reason, in a rural zone, the daily emission profiles are different from the typical profiles in urban zones [3]. Background ozone levels in unpolluted air can be anywhere from 20 to 50 ppb [1], though Lefohn et al. [4] 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 [5, 6, 7, 8]. Tropospheric ozone is considered as the most phytotoxic atmospheric pollutant due to its oxidant capacity. It is present in high concentrations in the Northern Hemisphere and cause damages in crops, forests and sensible vegetation [9, 10]; it affects the respiratory system of animals and humans [11]; and is the third most important greenhouse gas, behind the CO_2 and CH_4.

Within the leaf, ozone generates reactive species derived from oxygen (ROS) as such as superoxide radical, hydrogen peroxide and singlete oxygen [12,

13, 14, 15, 16, 17]. If apoplastic detoxificating systems are not enough strong, generated ROS can enter within the mesophill cells and react with the membranes and other cellular components as proteins, causing changes in permeability and fluidity of membranes, damages to enzymes and cause a metabolic and ionic imbalance [18]. This set of damages is called oxidative stress. Oxidative capacity of products derived from ozone will depend on the status of the antioxidant systems of the plant. The most evident effects of ozone on plants are visible damages located on the leaf surface. These damages include chlorosis, white, yellow or reddish spots on the leaves and necrosis [19]. In crops and woody species with a high economical importance, the presence of visible symptoms in leaves can cause a depreciation of the products. Ozone produces a decrease in the net assimilation of CO_2 [10, 20] and induces many processes that explain this decrease in photoshynthesis: stomatal closure, a decrease in CO_2 fixation, a decrease in electron transport efficiency [21], photoinhibition [22], decreases in chlorophyll concentrations [23, 24], reductions in the size of chloroplasts [25, 26] an even their desintegration [27].

Some parameters as chlorophyll content and nutrients concentrations are used to determine if one specie is sensitive or tolerant to a specific air pollutant [28]. Olteanu and colaborators studied the physiological response induced by ozone on gymnosperm species in some insdustrialized areas in Romania and they found that *Pinus sylvestris* showed the greatest sensitivity to ozone, followed by *Pinus nigra* [29]. These species showed a decrease in chlorophyll *a* concentrations and obvious suffering signs (chlorosis, necrosis and defoliation). In studies carried out on Sambucus species (*S. ebulus, S. nigra and S. racemosa*) and *Ginkgo biloba*, total chlorophyll content was determined and a considerable decrease was found [30, 31].

Regarding to changes in nutrients foliar content attributables to Ozone exposures, reported results differ greatly due to a great variability depends on age of the plant (mature trees or seedlings), kind of especie (woody or herbaceous species) and different treatments (at elevated concentrations of CO2, sinergistic effects considering other air polutants, and so on). Ozone may alter tissue nutrient concentrations by affecting nutrient retranslocation [32], nutrient uptake [33], and leaf biochemistry [34]. Some studies have reported that there is no significant statistical change in the concentration of nutrients in wheat seeds when plants are exposed to elevated ozone concentrations [35, 36, 37]. However, wheat plants are affected secondarily by ozone and it is possible that the transport system of nutrients is affected. In snap bean (*Phaseolus vulgaris L)* exposure to ozone decreased the concentrations of calcium, magnesium, iron and manganese in the leaves, but increased potassium, phosphorus and molybdenum concentrations in the pods, being attributable these reductions to increases in ozone concentrations and in the starch content [38].

In Lobloly pine seedlings, foliar nutrient contents were not significantly affected by O3 treatment, which indicates that foliar leaching was not exacerbed by elevated ozone concentrations[39]. Studies carried out on seedlings of red spruce and wheat exposed to high ozone concentrations show that there are not significant changes in nutrients concentrations and that this highly phytotoxic pollutant does not cause significant changes in foliar leaching of nutrients [35, 38]. Thomas and collaborators [39] didn`t find changes in Ca^{2+} in young beech trees (*Fagus sylvatica*) after ozone exposures and mangnesium concentrations were increased whereas potasium concentrations declined after ozone fumigation [40]. Increase in magnesium concentrations was also reported for Scots pine by Utrainen and Holopainen [41]. Changes in the nutrient concentration caused by ozone fumigation have often been observed in different experiments, even though increases as well as decreases were stated for the individual nutrient concentrations [42, 43]. These changes seem to be depend on the soil properties and on the degree of the impact on plant metabolism of ozone fumigation [44]. In Mexico, numerous studies have been carried out on vegetation in the main urban areas and the surroundings of Mexico City [45, 46, 47, 48, 49, 50]. There are not enough reported information about ozone effects on woody tropical species from other regions in Mexico. In Campeche, México; ozone concentrations exceed the Mexican air quality standard due to emissions from the oil and gas industry (a sour gas recompression station and offshore platforms are located in this area). ¾ of the total territory of Campeche State are covered with tropical vegetation including important species such as *Swietenia macrophylla, Haematoxylum Campechianum L* and *Tabebuia Rosea* (located at the surroundings of the Terminos Lagoon and the southeast edge of Campeche State) [52]. The aim of this research was to determine if studied ozone concentrations (50, 110 and 200 ppb) induced visible foliar damages, reductions on photosynthethic pigments and changes

in foliar nutrients of three woody tropical species of high commercial value typical from Campeche, México.

2 Materials and Methods

2.1 Propagation and fumigation

The study site is located within the Botanical Garden of the Autonomous University of Carmen Island (Figure 1). 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 (OTC) has been widely used in studies about the effects of atmospheric pollutants on plants since they were designed by Heagle and collaborators [51]. An OTC (3m diameter x 3 m height) was constructed according the scheme descrited by Heagle.

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 specie. 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) [52]. 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.

Figure 1. Location of the study area.

2.2 Visual assessment and harvest of plants

2.2.1 Harvest of plants

A first sampling was carried out before exposure (SBE) and a second sampling was done after six weeks at the end of the exposure. During the first sampling, one healthy leaf was collected for each individual under study and during the second sampling, leaves with visible damage were selected for each individual considered. 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 it was carried out a diseases and insects control to prevent any damage different from that induced by ozone treatment. A visual assesment of the plants was made once a week. Number of leaves and senescent leaves were counted, and all plants were observed for visible injuries.

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, *total* chlorophyll, chlorophyll *a*, chlorophyll *b* and total charotenoids contents were calculated per foliar mass unit using Lichtenthaler ecuations [52]. Chlorophyll concentrations were expressed as mg g-1 of fresh weight (fw).

2.3.2 Foliar nutrients concentrations

Samples were collected and dried in an oven at 80ºC

during 24 h. Dried samples were grinded and digested with nitric, hydrochloric and sulphuric acid in Teflon ® closed flasks (Cole-Parmer) of 100 ml, using as energy source an autoclave equipment. Subsequently, digested samples were filtered to determine the foliar nutrients: Ca^{2+}, Mn^{2+}, Mg^{2+} and K^{+} by atomic absorption spectrophotometry using direct aspiration (GBC Avanta). Calibration curves were prepared for each nutrient from 1000 ppm standard [53].

3 Results

3.1.1 Photosynthetic pigments content

Table 1 shows mean concentrations and standard deviation of chlorophyll *a* (Chl*a*) and *total* chlorophyll (Chl*t*) before (SBE) and after ozone exposures (SAE) 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. The percentages of reduction in these photosynthetic pigments after ozone exposures are shown in Table 2.

Table 1. Mean concentrations and standard deviation of *total* chlorophyll and chlorophyll *a* before and after ozone exposures.

Specie	Chl*a*		Chl*t*	
	SBE (mg/g fw)	SAE (mg/g fw)	SBE (mg/g fw)	SAE (mg/g fw)
HM				
50ppb	3.679 ± 0.179	1.815 ± 0.097	6.040 ± 0.304	2.923 ± 0.136
110 ppb	2.424 ± 0.285	1.152 ± 0.139	4.222 ± 0.306	2.025 ± 0.101
250 ppb	1.661 ± 0.076	0.837 ± 0.064	2.906± 0.078	1.470 ± 0.045
TR				
50ppb	1.158 ± 0.115	0.560 ± 0.034	2.359 ± 0.408	1.114 ± 0.060
110 ppb	0.857 ± 0.074	0.347 ± 0.058	1.227 ± 0.200	0.700 ± 0.068
250 ppb	0.449 ± 0.033	0.075 ± 0.013	0.710 ± 0.038	0.245 ± 0.051
SM				
50ppb	1.235 ± 0.278	0.721 ± 0.084	2.749 ± 0.420	1.278 ± 0.254
110 ppb	1.629 ± 0.067	0.682 ± 0.178	2.483 ± 0.379	1.103 ± 0.184
250 ppb	1.415 ± 0.436	0.362 ± 0.055	2.074 ± 0.472	0.657 ± 0.054

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure. HM: *Haematoxylum Campechianum L*; TR: *Tabebuia Rosea*; SM: *Swietenia Macrophylla*.

In Table 2, it can be observed the percentages of change for *total* chlorophyll, chlorophyll *a* and *total* charotenoids, before and after ozone exposures 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. Percentages of change in *total* chlorophyll (Chl*t*), chlorophyll *a* (Chl*a*) *and total* charotenoids (Cht*t*) before and after ozone exposures.

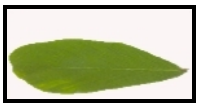
Specie	Chl*t*	Chl*a*	Cht*t*
	Change Percentage %)	Change Percentage(%)	Change Percentage (%)
HM			
50ppb	-51.666	-50.665	-24.210
110 ppb	-52.035	-52.475	-29.196
250 ppb	-49.415	-49.608	-26.509
TR			
50ppb	-52.777	-51.640	-19.917
110 ppb	-42.950	-59.509	-67.608
250 ppb	-67.889	-83.296	-87.234
SM			
50ppb	-53.510	-41.619	-38.437
110 ppb	-55.577	-58.133	-45.940
250 ppb	-68.322	-74.416	-36.946

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure. HM: *Haematoxylum Campechianum L*; TR: *Tabebuia Rosea*; SM: *Swietenia Macrophylla*.

3.1.2 Visual Assessment and Foliar Damage

Figures 2, 3 y 4 show a sample of representative foliar damages founded in individuals for the three tropical woody species studied after six weeks of exposure to different levels of ozone. Damages assessment was carried out considering both visible 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 foliar nutrients). Damages foliar percentage was calculated from 18 digitalized images for each of the studied species showing visible damages. A severity scale was obtained from representative images for each class. Table 3 shows the severity scale and class distribution for each studied specie.

Necrotic lesions



Interveinal chlorosis

Figure 2. Foliar damages observed after ozone exposures in Wood blood tree.

Interveinal necrosis

Interveinal chlorosis

Figure 3. Foliar damages observed after ozone exposures in Pink flower tree.

Necrotic lesions Interveinal chlorosis

Figure 4. Foliar damages observed after ozone exposures in Mahogany.

Visible damages for the three studied species included interveinal necrosis (death of foliar tissue, which usually turns brown, dark or reddish-brown) and chlorosis (loss of chlorophyll or foliar tissue yellowing) [54, 55]. Some mahogany individuals showed necrotic spots at edges and interveinasl chlorosis.

Table 3. Severity scale and class distribution for each studied specie.

Class Distribution	SEVERITY SCALE	
	Damaged Area (%) LL-UL	Ozone concentration (PPB)
HM		
I	7.55-17.55	50
II	17.55-35.68	110
III	35.68-59.11	250
TR		
I	0-7.55	50
II	7.55-17.55	110
III	17.55-59.11	250
SM		
I	14.95-23.80	50
II	23.80-35.68	110
III	35.68-63.64	250

Note: LL.- Lower level; UL: Upper level. HM: *Haematoxylum Campechianum L*; TR: *Tabebuia Rosea*; SM: *Swietenia Macrophylla*.

3.1.3 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 [56] to find significant differences among the three studied species for photosynthetic pigments and foliar nutrients (at P= 0.05). chlorophyll *a*, chlorophyll *b* and *total* chlorophyll showed different behaviour for the three woody tissue species. The three studied woody species showed a determination coefficient greater than 0.8 in all cases (*total* chlrophyll, chlorophyll *a* and *total* charotenoids), excepting in *Switenia Macrophylla* for *total* charotenoids. 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 decrease.

Table 4. Results of the Duncan test and ANOVA analysis applied to the found levels of foliar nutrients before and after exposure to ozone.

O_3 PPB	Foliar nutrients concentrations							
	% Mn SBE	%Mn SAE	% K SBE	%K SAE	% Mg SBE	%Mg SAE	% Ca SBE	% Ca SAE
HM								
50	0.091	0.236a	3.196	2.664a	0.790	6.103a	34.185	44.27a
110	0.106	0.107a	2.903	3.304a	1.200	5.322a	37.244	53.49a
250	0.064	0.322a	5.473	5.327b	1.127	14.30b	20.026	56.07a
Anova Pr>F		0.082		0.011*		0.025*		0.276
TR								
50	0.021	0.026a	3.362	2.282a	0.312	2.379a	0.750	12.65a
110	0.018	0.005b	4.347	2.142a	1.266	1.405b	0.667	11.40a
250	0.025	0.006b	3.455	0.995b	0.551	2.198a	0.756	8.020a
Anova Pr>F		0.0015*		0.004*		0.0025*		0.072
SM								
50	0.0169	0.004a	1.185	0.998a	0.668	1.218a	12.457	8.711a
110	0.020	0.007a	1.668	0.573a	0.710	1.722a	14.577	14.928b
250	0.013	0.008a	1.343	0.871a	0.795	1.685a	14.795	12.145ab
Anova Pr>F				0.444		0.534		0.014*

Note: *There were found significant differences at 0.05 level. Means with the same letter are not significantly differents according to Duncan Test. **Mn:** Mangenese; **K:** Potasiumm; **Mg:** Magnesium; **Ca:** Calcium; **O_3**: Ozone. **SBE.-** Sampling before exposure. **SAE.-** Sampling after exposure.

In Table 4, it can be observed that for Wood blood tree, K^+ and Mg^{2+} showed significant differences among ozone treatments; for Pink flower tree, Mn^{2+}, K^+ and Mg^{2+}, showed significant differences among treatments; and finally, for Mahogany, there were significant differences among ozone treatments only for Ca^{2+}. Table 5 shows the results of the linear regression analysis applied to the found levels of foliar nutrients after exposure to ozone for the three studied species. Pinkflower tree and Wood blood tree showed a significant linear relation less than 5% at least in two elements ($r \geq 0.9$). The correlation coefficients (r) between changes in all the studied foliar nutrients and ozone treatments for Wood blood tree were positive and it can be concluded with a certainty of 86% to 99% ($0.8657 > r^2 < 0.9999$), that variations in the content of these elements were due to changes in the ozone concentrations. For Pink flower tree, Mn^{2+}, K^+ and Ca^{2+} showed negative correlations, being significant only for K^+ and Ca^{2+}, indicating that these

elements decreased as ozone concentration increased. Mahogany did not show significant correlation between changes in foliar nutrients and ozone concentrations.

Table 5. Results of the Linear Regression Analysis applied to the found levels of foliar nutrients after exposure to ozone.

	Mn^{2+}	K^{+}	Mg^{2+}	Ca^{2+}
HM				
r	0.5941	0.9979	0.9304	0.8573
r^2	0.3530	0.9958	0.8657	0.7350
TR				
r	-0.6815	-0.9805	0.0512	-0.9989
r^2	0.4644	0.9615	0.0026	0.9978
SM				
r	0.8105	-0.0678	0.6846	0.3494
r^2	0.6570	0.0046	0.4686	0.1221

Note: Mn^{2+}: Mangenese; **K^{+}:** Potasiumm; **Mg^{2+}:** Magnesium; **Ca^{2+}:** Calcium. **HM:** *Haematoxylum Campechianum L*; **TR:** *Tabebuia Rosea*; **SM:** *Swietenia Macrophylla*.

3.1.4 Foliar nutrients concentrations

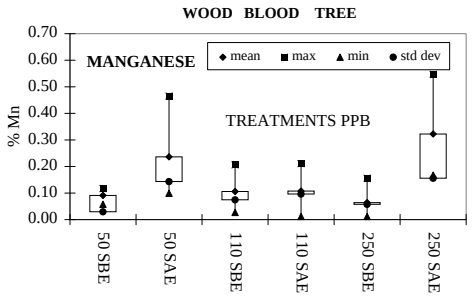
The chemical composition of the substrate used for propagation and cultivation of the studied plants is showed in Table 6.

Table 6. Chemical composition of the substrate used during the experiment for propagation and cultivation of the studied plants.

Element	Mean Concentration
P	0.63 PPM
K	180 PPM
Ca	4061 PPM
Mg	449 PPM
N	0.5 %
Na	330 PPM

Note: P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; N: Nitrogen; **Na**: Sodium.

Figures 5 to 7 show standard deviation, maximum, minimum, and mean concentrations of a) Mn^{2+}, b) K^{+}, c) Mg^{2+} and d) Ca^{2+} before and after exposure at three different levels of ozone for *Haematoxylum Campechianum L*, *Tabebuia Rosea* and *Swietenia Macrophylla*, respectively. Tables 7 to 10 show mean values and percentages of change in foliar nutrients content after exposure to ozone at different levels.



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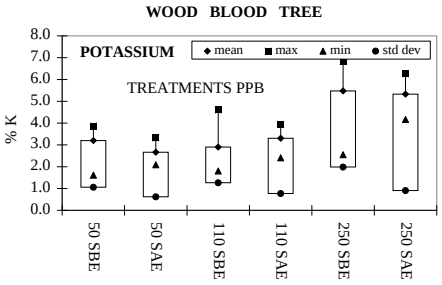

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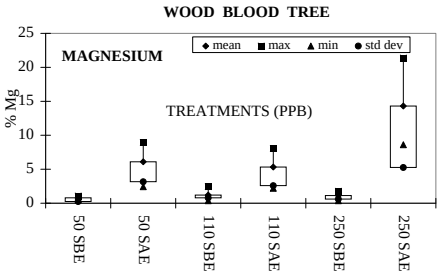

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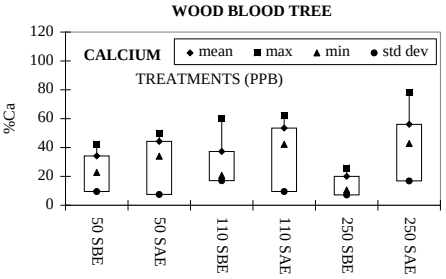
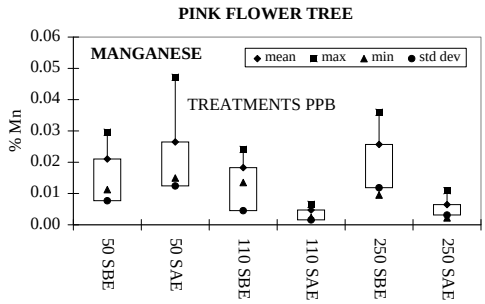


(d)

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

Figure 5. Standard deviation, maximum, minimum, and mean levels of nutrients for *Haematoxylum Campechianum L* (Wood blood tree) (a) Mn^{2+}, (b) K^{+}, (c) Mg^{2+} and (d) Ca^{2+}.



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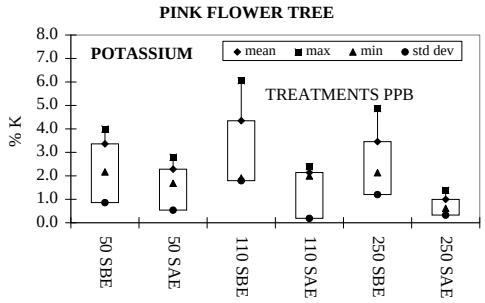

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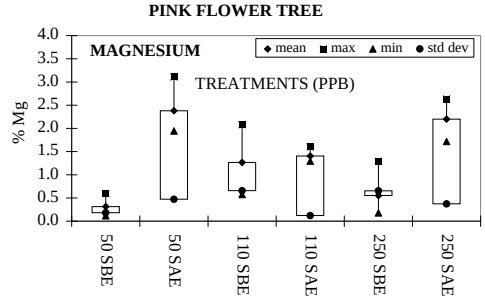

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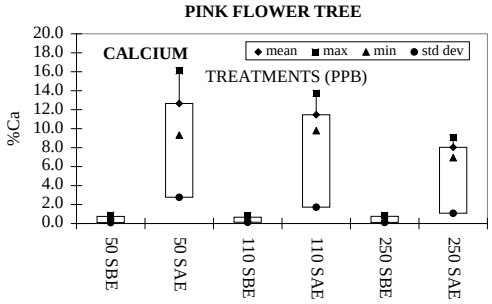

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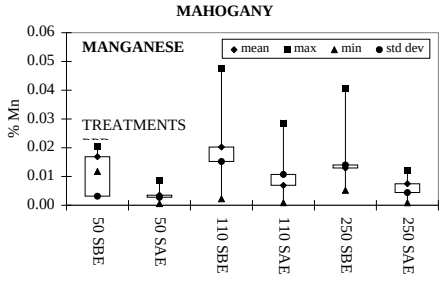
Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

Figure 6. Standard deviation, maximum, minimum, and mean levels of nutrients for *Tabebuia Rosea* (Pink flower tree) (a) Mn^{2+}, (b) K^{+}, (c) Mg^{2+} and (d) Ca^{2+}.



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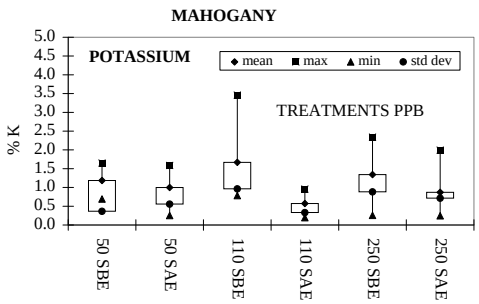

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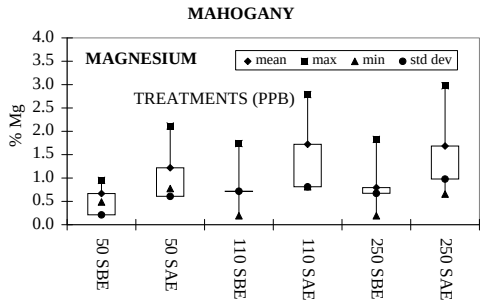
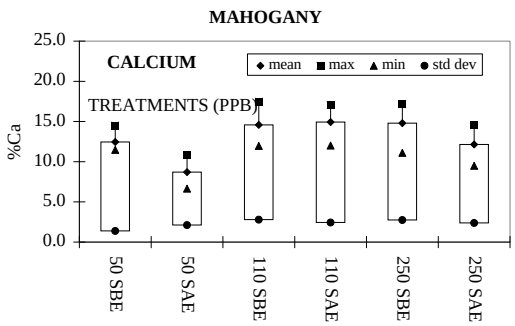


(c)



(d)

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

Figure 7. Standard deviation, maximum, minimum, and mean levels of nutrients for *Swietenia Macrophylla* (Mahogany) (a) Mn^{2+}, (b) K^{+}, (c) Mg^{2+} and (d) Ca^{2+}.

Table 7. Mean concentrations of Manganese and percentages of change before and after exposure to ozone at different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rosea*, and SM: *Swietenia Macrophylla*.

	Manganese Percentage (%Mn)		
Specie	**SBE**	**SAE**	**Percentage Change %**
HM			
50 PPB	0.0911	0.2362	+159.28
110 PPB	0.1057	0.107	+1.61
250 PPB	0.640	0.3223	-49.64
TR			
50 PPB	0.021	0.0265	+26.19
110 PPB	0.018	0.0047	-73.89
250 PPB	0.025	0.0064	-74.40
SM			
50 PPB	0.0169	0.0035	-79.29
110 PPB	0.0202	0.0069	-65.84
250 PPB	0.0129	0.0075	-41.86

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

Table 8. Mean concentrations of Potassium and percentages of reduction before and after exposure to ozone at different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rosea*, and SM: *Swietenia Macrophylla*.

	Potassium Percentage (%Mn)		
Specie	**SBE (mg/g fw)**	**SAE (mg/g fw)**	**Percentage Change %**
HM			
50 PPB	3.196	2.664	-16.67
110 PPB	2.903	3.304	+13.81
250 PPB	5.473	5.327	-2.67
TR			
50 PPB	3.362	2.282	-32.12
110 PPB	4.347	2.142	-50.72
250 PPB	3.455	0.995	-71.20
SM			
50 PPB	1.185	0.998	-15.73
110 PPB	1.668	0.573	-65.67
250 PPB	1.343	0.813	-39.45

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

Table 9. Mean concentrations of Magnesium and percentages of reduction before and after exposure to ozone different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rosea* and SM: *Swietenia Macrophylla*.

	Magnesium Percentage (%Mn)		
Specie	**SBE (mg/g fw)**	**SAE (mg/g fw)**	**Percentage Change %**
HM			
50 PPB	0.7905	6.1027	+672.01
110 PPB	1.2007	5.3219	+343.23
250 PPB	1.1272	14.306	+1169.16
TR			
50 PPB	0.312	2.379	+662.50
110 PPB	1.266	1.405	+10.98
250 PPB	0.551	2.198	+298.91
SM			
50 PPB	0.6675	1.2177	+82.43
110 PPB	0.7095	1.7224	+142.76
250 PPB	0.7952	1.6853	+111.93

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

Table 10. Mean concentrations of Calcium and percentages of reduction before and after exposure to ozone at different levels (HM: *Haematoxylum Campechianum L.*, TR: *Tabebuia Rosea* and SM: *Swietenia Macrophylla*.

	Calcium Percentage (%Mn)		
Specie	**SBE (mg/g fw)**	**SAE (mg/g fw)**	**Percentage Change %**
HM			
50 PPB	34.185	44.275	+29.52
110 PPB	37.244	53.490	+43.62
250 PPB	20.026	56.074	+180.01
TR			
50 PPB	0.750	12.65	+1586.67
110 PPB	0.667	11.40	+1609.15
250 PPB	0.756	8.020	+960.85
SM			
50 PPB	12.457	8.711	-30.07
110 PPB	14.577	14.928	+2.41
250 PPB	14.795	12.145	-17.91

Note: SBE.- Sampling before exposure. SAE.- Sampling after exposure.

4 Discussion

According to the chemical composition of the substrate used in this study (Table 6), it can be observed that it shows a high content of calcium and organic matter, however, is deficient in nitrogen, phosphorus and potassium. For this reason, calcium concentrations for the three studied species were high due to the alcaline nature of the soil where they were cultivated. The response to ozone exposures in Pink flower tree individuals it was showed as an increase in Mg^{2+} levels. This increase is not toxic but it can cause a deficiency in Ca^{2+} and K^{+} levels [58]. After ozone exposures, Pink flower tree individuals only showed decreases in K^{+} levels whereas Ca^{2+} levels increased. This increase in Ca^{2+} levels can cause a low captation of Mn^{2+} [57, 58], and it can be inferred that chlorotic mottled in leaves is possibly induced by low Mn^{2+} concentrations (which is essential for chlorophyll synthesis). Necrotic lessions may be associated with a decrease in K^{+} [59]. A similar behaviour was found in a study carried out by Elvira and collaborators [60] in *Pinus halepensis M* seedlings exposed to high ozone concentrations. They found that Ca^{2+} levels increased as the age of the leaves and ozone concentrations increased.

After ozone exposures, Wood blood tree individuals showed an increase in Mg^{2+} levels. According to studies carried out by Rojas [57], this nutrient in excess is not toxic but it can induce a deficiency in Ca^{2+} and K^{+}. Managenese levels were increased after ozone exposures in Wood blood tree individuals. Mn^{2+} in excess may be toxic for the plant [61, 62], so observed visible damages (necrotic lessions) in the individuals of this specie after ozone exposures can be related to high concentrations of this nutrient.

After ozone exposures, Mahogany individuals showed an increase in Mg^{2+} levels, however, it was not found a clear relation between this increase and the pattern of Ca^{2+} concentrations. It can be inferred that produced damages as interveinal chlorosis and necrosis may be induced by a decrease in these nutrients. Edwards and collaborators [62] found that *Pinus taeda L* seedlings exposed to different concentrations of ozone showed high concentrations of Mg^{2+} in foliage and roots. Simmons and Kelly [63] studied this plant at different ozone concentrations , and they did not found effects of ozone in K^{+}, Ca^{2+} and Mg^{2+} levels at any component of the plant neither observed visible damages attributable to ozone. However, they found a decrease in total biomass at higher ozone concentrations. These disagreements in the results may be due to the different exposure periods used in each study. 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. So, caution is needed and therefore the general statement that fast-growing species are more sensitive than slower-growing ones, can may be of more use. In the present experiment we exposed three tropical tree species, to exactly the same experimental conditions. All studied species were exposed to ozone concentrations representative for the studied region, grown and treated in the same type of chambers, soil and growth conditions.

5 Conclusion

According to the obtained results it can be concluded that all the studied species showed visible damages when they were exposed to different levels of ozone, being Wood blood tree and Pink flower tree the species that showed visible damages more severe and a better trend in the changes in foliar nutrients concentrations. These species were the most affected due to ozone concentrations showing determination coefficients for all photosynthetic pigments regarding to ozone concentrations with certainties higher than 90% and 80%, for Pink flower tree and Wood blood tree, respectively. Changes in foliar nutrients concentrations showed a better correlation with ozone concentrations for these two species. Pinkflower tree showed a decrease in K+ and Ca^{2+}, whereas, Wood blood tree showed an increase in K^{+} and Mg^{2+} with significant differences among treatments for both species. Mahogany showed not significant differences and it did not show a clear pattern among treatments, therefore, it can not be inferred with certainty that found damages were induced by ozone concentrations used in this study and it is suggested to carry out long term chronic studies.

Ozone has been shown to increase nutrient concentration in woody tissues and in older and larger trees, due to this kind of plants have a greater capacity for foliar nutrient retention and recycling since foliar production and loss are more balanced than in a seedling [33]. It is possible that decreases found in K^{+} and Ca^{2+} for pinkflower treee after ozone exposures are due to the tissue of the seedlings of this specie is not enough woody at this early age and it does not have the capacity of retention of nutrients. It is

necessary to carried out a whole study that considers other additional nutrients as N, P and to carried out other treatments considering an excess of CO_2 and its influence on nutrients content to obtain reliable results about the changes suffered by nutrients due to high ozone concentrations. Increases in Mg^{2+} found in this study were in agreement with those found in bean tissue by Tingey and collaborators [38] and decreases in K+ were in good agreement with those reported by Edwards [39]and Thomas [40] in *fagus sylvatica* seedlings. In conclusion, it is necessary to carry out a long-term exposure to obtain definitive conclusions about these species and the biochemical response for other variables should be assessed. In addition, it should be noted that there are difficulties in interpreting the results of the exposures using open top chambers (OTC) due to space limitations, it means that only small plants (seedlings) can be used and exposition time only corresponds to a little fraction of the plant`s life [64]. It can be suggested to carry out field studies to observe if these species follow the same behaviour in open field than in OTC.

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