Effects of Simulated Tropospheric Ozone on nutrients levels and photosynthetic pigments concentrations of three Mangrove species

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Abstract: - Three months old seedlings of three mangrove species (*Rhizophora mangle, Laguncularia racemosa*, and *Conocarpus erectus*) 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), soluble proteins content and nutrients concentrations (Ca^{2+}, Mn^{2+}, Mg^{2+} and K^{+}) were determined. All mangrove species studied showed sensitiveness to ozone exposure levels, being Red mangrove the most sensitive specie to tropospheric ozone.

Key-Words: - Mangrove., Foliar damage., Open Top Chambers., Tropospheric ozone., Coastal vegetation., Campeche, México., Macronutrients.

1 Introduction

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 [1, 2, 3, 4]. Tropospheric ozone constitutes a phytotoxic risk to crops and natural vegetation [5]. 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 [6]. 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. 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 [7]. 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* [8]. 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 [9]. In studies carried out on *Ginkgo biloba*, chlorophyl contents alter ozone exposure showed the same behaviour [10].

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 [11], nutrient uptake [12], and leaf biochemistry [13]. 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 [14, 15, 16]. 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 [17]. In Lobloly pine seedlings, foliar nutrient contents were not significantly affected by O3 treatment, which indicates that foliar leaching was not exacerbed by elevated [18]. Studies carried out on seedlings of beech, 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 [19, 14, 18].

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 [20, 21, 22, 23, 24]. In Mexico, numerous studies have been carried out on vegetation in the main urban areas and the surroundings of Mexico City [25, 26, 27, 28, 29, 30]. There are 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. Campeche State has the greater percentage of mangrove coverage in Mexico (29.977 %, 196 552 ha). The importance of mangrove as a coastal barrier against hurricanes and the environmental services that it offers is widely known. For all these reasons is important to carry out studies focused to protect this ecosystem. 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 concentrations on three types of mangrove 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. Open-top chambers (3m diameter x 3 m height) were constructed according the scheme descrited by Heagle et al. [31]. OTC was operated from June 22 to July 31 in 2009 during 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 species were exposed for each concentration level (control samples were not exposed). All seedlings were selected under homogeneous conditions of size, foliage and age (three months old). All plants received daily irrigation during the experiment, to keep the soil moisture close to field capacity. 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).

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.

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 and charotenoids content were calculated per foliar mass unit using Lichtenthaler ecuations [32].

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 [33]. 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.

2.3.3 Macronutrients foliar concentrations

Samples were collected and dried in an oven at 80ºC during 24 h. Dried samples were dissolved in nitric acid, perchloric acid and sulfuric acid and a digestion process was caried out in Teflon ® closed flasks (Cole-Parmer) of 100 ml, using as energy source an autoclave equipment. The macronutrients Ca^{2+}, Mn^{2+}, Mg^{2+} and K^{+} were analyzed by direct aspiration with an atomic absorption spectrometer (GBC Avanta). Calibration curves were prepared for each nutrient from 1000 ppm standard [33].

2.4 Severity Scale

2.3.1 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 [34]. Each class shows lower, middle and upper limits expressed as damaged area percentage. Each procesed leaf was classified according to Horsfall-Barratt method [35].

3 Results

3.1.1 Photosynthetic pigments content

Figure 1 (a, b and c) 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 red mangrove, white mangrove and buttonwood mangrove, respectively.

Figure 2 (a, b and c) shows 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 red mangrove, white mangrove and buttonwood mangrove, respectively.

Table 1 shows mean values and percentages of reduction in photosynththetic pigments before and after exposure to ozone different levels.

3.1.2 Soluble protein levels

In Table 2, it can be observed mean values and percentages of reduction in soluble proteins concentrations before and after exposure to ozone different levels. Figure 3 (a, b and c) shows standard deviation, maximum, minimum, and mean soluble proteins levels before and after exposure at 50, 110 and 250 ppb, for red mangrove, white mangrove and buttonwood mangrove, respectively.

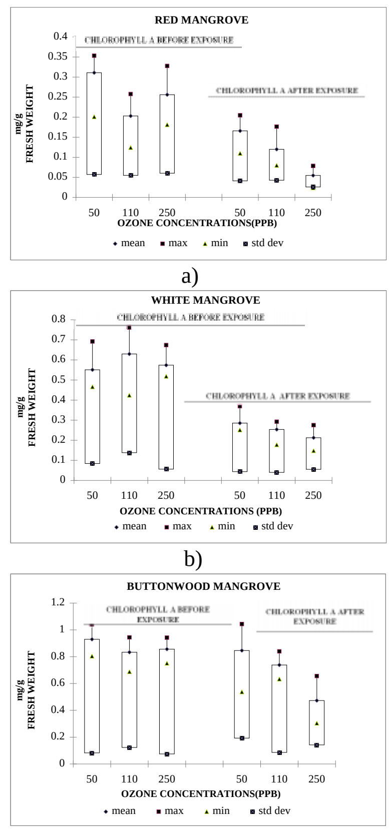

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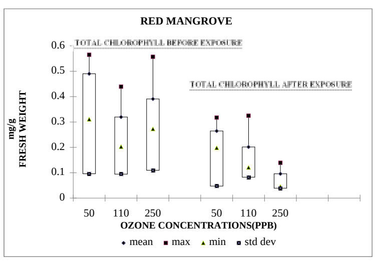
Figure 1. Standard deviation, maximum, minimum, and mean concentrations of chlorophyll a for red mangrove (a), white mangrove (b) and buttonwood mangrove (c) at different levels of ozone exposure.

Table 1. Mean concentrations and percentages of reduction in total chlorophyll and charotenoids before and after exposure to ozone different levels (RM: Red mangrove, WR: White Mangrove, BM: Buttonwood mangrove.

Specie	Total Chlorophyll			Total Charotenoids		
	SBE (mg/g fw)	SAE (mg/g fw)	Reduction (%)	SBE (mg/g fw)	SAE (mg/g fw)	Reduction (%)
RM						
50ppb	0.49	0.26	46.00	1.17	0.56	51.91
110 ppb	0.32	0.20	36.94	1.21	0.38	72.26
250 ppb	0.39	0.09	75.52	1.36	0.09	92.13
WM						
50ppb	1.31	1.52	60.08	0.18	0.15	15.67
110 ppb	1.22	0.46	62.39	0.16	0.13	16.39
250 ppb	1.26	0.40	67.81	0.21	0.12	42.23
BM						
50ppb	1.47	1.27	13.60	0.26	0.25	4.24
110 ppb	1.28	1.11	13.76	0.24	0.14	1.527
250 ppb	1.40	0.81	42.13	0.23	0.14	37.28

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



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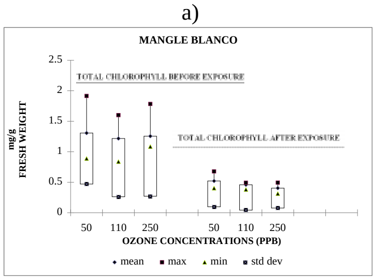

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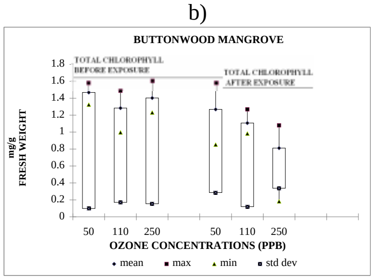

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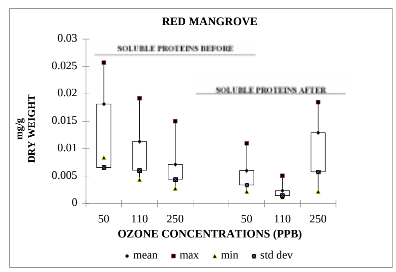
Figure 2. Standard deviation, maximum, minimum, and mean concentrations of total chlorophyll for red mangrove (a), white mangrove (b) and buttonwood mangrove (c) at different levels of ozone exposure.

Table 2. Mean concentrations and percentages of reduction in soluble proteins before and after exposure to ozone different levels (RM: Red mangrove, WR: White Mangrove, BM: Buttonwood mangrove.

Specie	Soluble Proteins		
	SBE (mg/g dw)	SAE (mg/g dw)	Reduction (%)
RM			
50ppb	0.0181	0.005	66.98
110 ppb	0.011	0.002	79.32
250 ppb	0.007	0.013	-
WM			
50ppb	0.012	0.011	6.43
110 ppb	0.006	0.009	-
250 ppb	0.012	0.007	92.85
BM			
50ppb	0.0156	0.004	70
110 ppb	0.013	0.008	34.27
250 ppb	0.0247	0.0105	57.37

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



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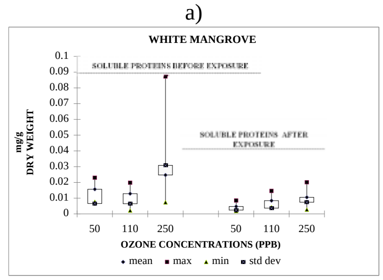

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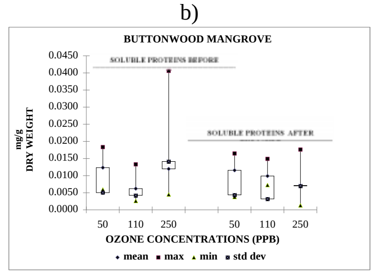

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Figure 3. Standard deviation, maximum, minimum, and mean soluble proteins levels for red mangrove (a), white mangrove (b) and buttonwood mangrove (c) at three different levels of ozone exposure.

3.1.3 Visual Assessment and Foliar Damage
Figure 4 (a, b and c) shows foliar damages founded in individuals for the three mangrove 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 species studied with visible damages. Severity scale was obtained from representative images for each class. In Table 3, the severity scale and class distribution for each species studied are shown.

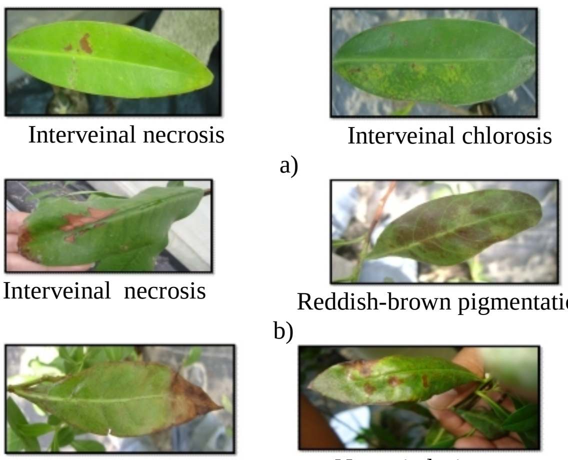


Figure 4. Foliar damages observed after the exposure period in red mangrove (a), white mangrove (b) and buttonwood mangrove (c).

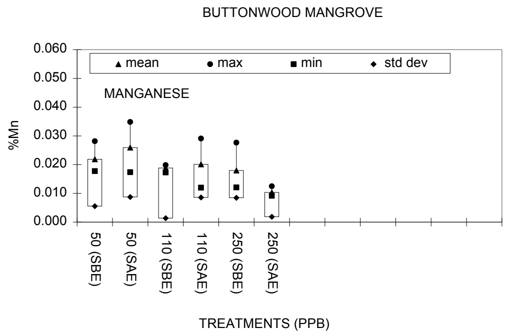
3.1.4 Statistical Analysis

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 $\alpha=0.05$ between samplings (before and after exposure to ozone). Duncan's test was performed using SAS-package, release 6.06 [36] to find significant differences among the three studied species for photosynthetic pigments, soluble proteins levels and nutrients content (at $P= 0.05$). Chlorophyll A, Chlorophyll B and Total chlorophyll showed different behaviour for the three mangrove species. Pheophytinization indexes for Buttonwood and white mangrove did not show significant differences but significant differences were found for Red mangrove. Total charotenoids concentrations did not show significant differences among the three mangrove species. Between Buttonwood and White mangrove did not exist significant differences, however, Red mangrove showed a different behavior.

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

Severity Scale				
Red Mangrove				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	1.79% (110 ppb)	2.51% (250 ppb)	13.03% (250 ppb)	24.13% (250 ppb)
Class Distribution	50%	16.67%	2.22%	11.11%
White Magrove				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	4.39% (110 ppb)	11.09% (110 ppb)	35.99% (250 ppb)	74.97% (250 ppb)
Class Distribution	38.89%	33.33%	22.22%	5.56%
Buttonwood Mangrove				
	Class 1	Class 2	Class 3	Class 4
Damaged Area	3.05% (110 ppb)	15.30% (110 ppb)	39.23% (250 ppb)	50.90% (250 ppb)
Class Distribution	27.78%	22.22%	38.89%	11.11%

3.1.5 Macronutrients foliar concentrations
Figure 5, 6 and 7 shows standard deviation, maximum, minimum, and mean concentrations in percentage of a) Mn^{2+}, b) K^{+}, c) Mg^{2+} and d) Ca^{2+} before and after exposure at three different levels of ozone (50, 110 and 250 ppb) for buttonwood mangrove, white mangrove and red mangrove, rspectively. Tables 4 to 7 show mean values and percentages of reduction in nutrient contents before and after exposure to ozone different levels for Manganese, Potassium, Magnesium and Calcium, respectively.



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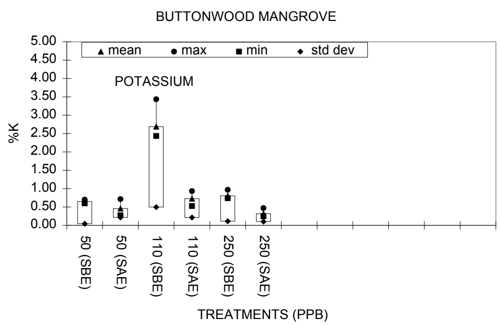

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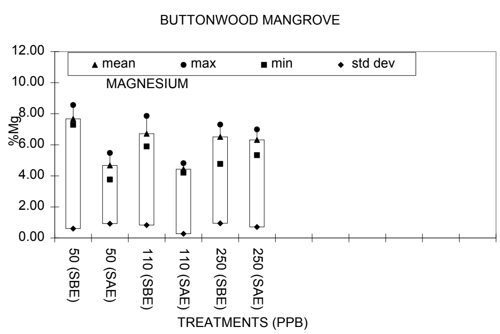

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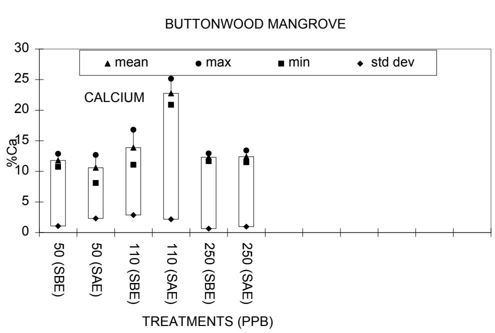
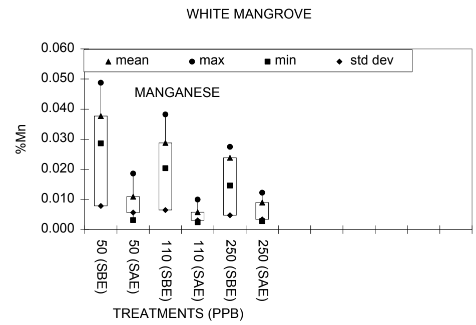


(d)

Figure 5. Standard deviation, maximum, minimum, and mean levels of nutrients for buttonwood mangrove (a) Mn, (b) K, (c),Mg and (d)Ca.



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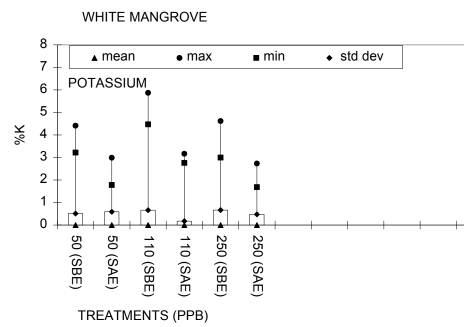

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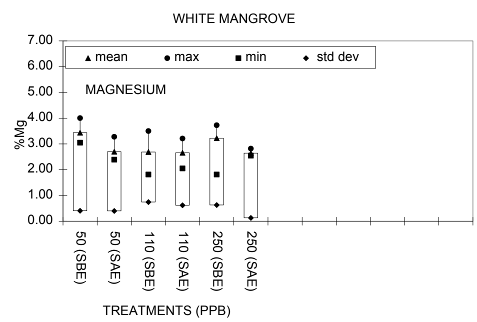

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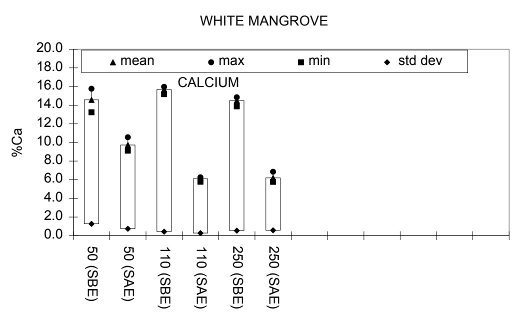

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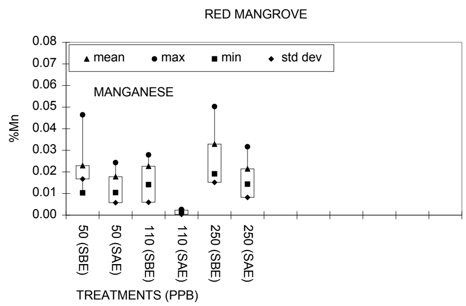
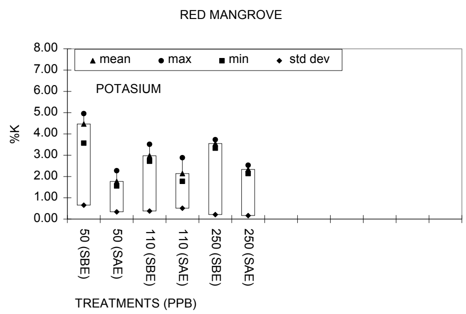
Figure 6. Standard deviation, maximum, minimum, and mean levels of nutrients for white mangrove (a) Mn, (b) K, (c), Mg and (d)Ca.



(a)



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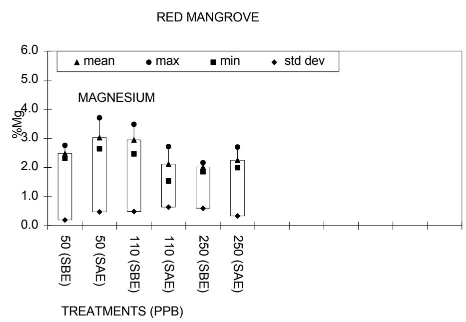

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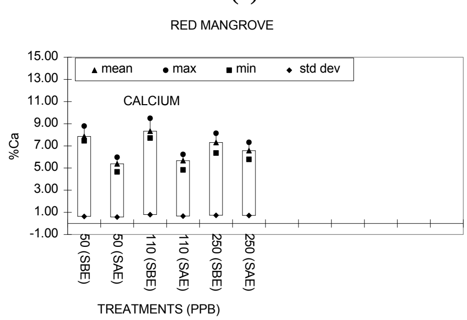

(d)

Figure 7. Standard deviation, maximum, minimum, and mean levels of nutrients for Red mangrove (a) Mn, (b) K, (c) Mg and (d)Ca.

Table 4. Mean concentrations of Manganese and percentages of reduction before and after exposure to ozone at different levels (WR: White Mangrove , BM: Buttonwood mangrove, RM: Red mangrove).

	Manganese Percentage (%Mn)		
Specie	**SBE (mg/g fw)**	**SAE (mg/g fw)**	**Reduction %**
WM			
50 PPB	0.03771813	0.01092947	71.0232893
110 PPB	0.028801	0.00579129	79.892058
250 PPB	0.0238385	0.00899494	62.2671745
BM			
50 PPB	0.02186	0.025925	18.598
110 PPB	0.018825	0.020108	6.820
250 PPB	0.017977	0.010359	-42.378
RM			
50 PPB	0.02291967	0.0177628	22.4997657
110 PPB	0.02261991	0.00229344	89.8609521
250 PPB	0.03285099	0.02142213	34.7900042

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

Table 5. Mean concentrations of Potassium and percentages of reduction before and after exposure to ozone at different levels (WR: White Mangrove , BM: Buttonwood mangrove, RM: Red mangrove) .

	Potassium Percentage (%K)		
Specie	**SBE (mg/g fw)**	**SAE (mg/g fw)**	**Reduction %**
WM			
50 PPB	3.94638412	2.30600089	41.567
110 PPB	5.45634739	2.96130713	45.727
250 PPB	3.74047542	2.32578282	37.821
BM			
50 PPB	0.64803485	0.37421943	42.253
110 PPB	2.68700401	0.72648889	72.963
250 PPB	0.80379697	0.32032297	60.149
RM			
50 PPB	4.46672797	1.76772462	60.425
110 PPB	2.97235608	2.1369338	28.106
250 PPB	3.55033408	2.33066631	34.354

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

Table 6. Mean concentrations of Magnesium and percentages of reduction before and after exposure to ozone different levels (WR: White Mangrove , BM: Buttonwood mangrove, RM: Red mangrove) .

	Magnesium Percentage (%Mg)		
Specie	SBE (mg/g fw)	SAE (mg/g fw)	Reduction %
WM			
50 PPB	3.43575637	2.69586285	21.535
110 PPB	2.68802368	2.65501625	1.228
250 PPB	3.22352837	2.6438481	17.983
BM			
50 PPB	7.66594476	4.66768508	39.111
110 PPB	6.7127938	4.43108191	33.990
250 PPB	6.50937012	6.31146387	3.040
RM			
50 PPB	2.47676278	3.0276468	22.2420987
110 PPB	2.9503726	2.11797763	-28.2132153
250 PPB	2.0189183	2.24927749	11.4100305

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

Table 7. Mean concentrations of Calcium and percentages of reduction before and after exposure to ozone at different levels (WR: White Mangrove, BM: Buttonwood mangrove, RM: Red mangrove).

Calcium Percentage (%Ca)			
Specie	SBE (mg/g fw)	SAE (mg/g fw)	Reduction %
WM			
50 PPB	14.575652	9.72887087	33.253
110 PPB	15.6733304	6.09981669	61.082
250 PPB	14.4804212	6.20034	57.181
BM			
50 PPB	11.7918018	10.5857491	-10.2278913
110 PPB	13.8817641	22.7531729	63.906927
250 PPB	12.3159242	12.4027829	0.70525589
RM			
50 PPB	7.85590127	5.38487764	31.4543621
110 PPB	8.3269599	5.66438943	31.9753007
250 PPB	7.30434552	6.57071329	10.0437779

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

4 Conclusion

According to visible damages, all the mangrove species studied showed damages when they were exposed to different levels of ozone, being buttonwood mangrove the specie that showed visible damages more severe. Red mangrove showed some typical symptoms after the exposure like chlorosis and some leaves showed necrosis in some zones. However Buttonwood mangrove showed more visual damage than red mangrove. White mangrove showed areas with metalic sheen in some leaves as well as necrosis in most of its leaves. Severity Class distribution showed that Buttonwood mangrove was the especie with the highest percentage of individuals who fell into class 3 and 4 at the higher exposure concentrations (110 and 250ppb) with a value of 50%, and White mangrove showed a class distribution percentage of 27.778%.

During the experiment it was observed that when O_3 concentration increased, the three mangrove species showed greater degree of damage. Changes observed in chlorophyll *a* are related to visual damages in the three studied species. Chlorophyll loss was greater at higher ozone concentrations (250 ppb). There were changes in chlorophyll b only for Red and White mangroves. A lower ratio chlorophyll *a*/chlorophyll *b* may be an indicative of damages in leaves induced by photo-oxidation. Some authors [37, 38] 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 [39]. All mangrove species showed a decrease in charotenoids content when ozone concentration increased. Regarding to total chlorophyll/charotenoids it is difficult to stablish if ozone levels produced direct photo-oxidative damages on chlorophyll content of leaves. Pheophytinization indexes (absorbance ratio 435/415) for Red mangrove showed an increase when ozone concentrations increased. A greater pheophytinization index is related to a high degree of air pollution [40]. Buttonwood mangrove did not show significant differences in this index.

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].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. Red mangrove was the most sensitive specie to the studied levels of ozone, followed in an importance order by Buttonwood mangrove and White mangrove.

Most of the macronutrients showed a decrease in their concentrations after ozone exposure. White mangrove showed decreases in Calcium, Potassium, Manganese and Magnesium concentrations after ozone exposure, however, there was not a clear pattern between nutrients concentrations and ozone concentrations. For Buttonwood mangrove, Manganese and Calcium showed increases in concentration after ozone exposure and Magnesium and Potassium showed decreases in concentration after ozone exposure. This mangrove specie did not show a pattern between ozone levels and nutrients concentrations. For Red mangrove, after six weeks of controlled exposures to ozone, Manganese, Potassium and Calcium concentrations showed decreases in concentration. On the other hand, Magnesium showed for this specie an increase in concentration after ozone exposure

Changes exhibited in nutrients concentrations were not due to alterations in soil nutrient concentrations, since ozone treatments had no influence on nutrient concentration. 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 [12]. It is possible that these decreases in nutrients concentration found in this study after ozone exposures are due to the tissue of mangrove seedlings is not 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 macronutrients due to high ozone concentrations

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.

References:

[1] Franzaring, J., Tonneijck, A.E.G., Kooijman, A.W.N., Dueck, T.H. Growth responses to ozone in plant species from wetlands. *Environmental and Experimental Botany*, Vol.44, 2000, pp. 39-48 .

[2] Fuhrer, J., Achermann, B. (Eds.). Critical levels for ozone-Level II. *Environmental Documentation 115,* Swiss Agency for the Environment, Forest and Landscape (SAE FL), 1999, pp. 333.

[3] Wolff, G.T., Lioy, P.J., Wright, G.D, Meyers, R.E and Cederwall,R.T. An investigation of longrange transport of ozone across the midwest and eastern U.S. *Atmospheric Environment*, Vol 11, 1977, pp. 797-802.

[4] Meagher, J.F., Lee, N.T., Valente, R.J., and Parkhurst, W.J. Rural ozone in the southeastern United States. *Atmospheric Environment*, Vol.21, 1987, pp. 605-615.

[5] Kelly, W.A., Wolff, G.T. and Ferman, M.A. Sources and sinks of ozone in rural areas. *Atmospheric Environment*, Vol.18, 1984, pp. 1251-1266.

[6] Krupa, V. And Manning, W.J. Atmospheric ozone: formation and effects on vegetation. *Environmental Pollution*, Vol.50, 1988, pp.101-137.

[7] Liu, Y., and Ding, H. Variation in air pollution tolerance index of plants near a steel factory: Implications for landscape-plant species selection for industrial areas. *WSEAS Transactions on Environment and Development.* Vol 4, No. 1, 2008, pp 24-32.

[8] Olteanu, Z., Zamfirache, M., and Ivanescu, L. Researches regarding the physiological response induced by atmospheric pollutants on Gymnosperm species in the industrialized areas of Romania. *WSEAS Proceedings of the Int. Conf. On Waste Management, Water Pollution, Air Pollution, Indoor Climate.* Arcachon, France, October 14-16, 2007. pp 223-227.

[9] Cano, I., Cerveró, J., and Sanz, M. J. Ozone effects on three Sambucus species. Environ. Monit . Assess. Vol. 128, 2007. pp 83–91.

[10] He, X.Y., Fu, S.L., Cheng, W., Zhao, T.H., Xu, S., and Tuba, Z. Changes in effects of ozone exposure on growth, photosynthesis, and respiration of Ginkgo biloba in Shenyang urban area. Photosynthetica, Vol. 45, No. 4, 2007. pp 555-561.

[11] Wright, L.M., Lockaby, B.G., Meldahl, R., Thorton, F., and Chappelka, A.H. The influence of acid precipitation and ozone on nitrogen nutrition of young Loblolly pine. *Water, Air and Soil Pollut.*, Vol. 54, 1991. pp 135-142.

[12] Miller, H.G. Dynamics of nutrient cycling in plantation ecosystems. In *Nutrition of Plantation Forest*, ed. G.D. Bowen and E.K.S. Nambiar. Academic Press, Inc. New York, 1984. pp 53-78.

[13] Stow, T.K., Allen, H.L., and Kress, L.W. Ozone impacts on seasonal foliage dynamics of young Loblolly pine. *For. Sci.* Vol 38, 1992. pp 102-119.

[14] Fangmeier, A., Gruters, U., Hogy, P., Vermehren, B., and Jager, H.J. Effects of elevated CO2 nitrogen supply and tropospheric ozone on spring wheat ii. Nutrients (N, P,K, S, Ca, Mg, Fe, Mn, Zn). *Environmental Pollution*, Vol. 96, No. 1, 1997, pp 43-59.

[15] Psaroudaki, A., and Stavrakakis, G. An overview of the impact of tropospheric ozone on the nutritional value of edible plants used in the Mediterranean diet. *WSEAS Proceedings of the 3rd. IASME/WSEAS Int. Conf. On Energy, Environment, Ecosystems and Sustainable Development.* Agios Nikolaos, Greece, July 24-26, 2007. pp 42-50.

[16] Psaroudaki, A. An extensive survey of the impact of tropospheric ozone on the biochemical properties of Edible Plants. *WSEAS Transactions on Environment and Development,* Vol. 3, No. 6, 2007. pp 99-110.

[17] Tingey, D.T., Rodecap, K.D., Lee, E.H., Moser, T.J., and Hogsett, W.E. Ozone alters the concentration of nutrients in bean tissue. *Angew. Bot*, Vol. 60, 1986. pp 481-493.

[18] Edwards, G.S., Sherman, R.E. and Kelly, J.M. Red spruce and Loblolly pine nutritional responses to acidic precipitation and Ozone. *Environmental Pollution,* Vol. 89, 1995. pp 9-15.

[19] Thomas, V.F.D., Braun, S., Fluckiger, W. Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, growth, and nutrient concentrations of young beech trees (Fagus sylvatica). Environmental Pollution, Vol. 143, 2006. pp 341-354.

[20] Skelly, J.M., Savage, J.E., Bauer, M.L. and Alvarado, D. Observations of ozone-induced foliar injury on black cherry (*Prunus Serotina, Var. Capuli*) within the Desierto de Los Leones National Park, Mexico City. *Environmental Pollution,*Vol.95, No.2, 1997, pp. 155-158.

[21] Bauer, L.I. de., and Chávez, A.J. Evaluación de genotipos de capulín (*Prunus serotina)* a oxidantes ambientales. Avances de investigación, C.P. 1991, pp. 141-142.

[22] Bauer, L.I. de., and Krupa, S.V. The Valley of Mexico: Summary of observational Studies on its air quality and effects on vegetation. *Environmental Pollution,* Vol. 65, 1990, pp. 109-118.

[23] Bauer, L.I. de., Hernández, T.T. and Manning, W.J. Ozone causes needle injury and tree decline in Pinus hartwegii at high altitudes in the mountains around Mexico City. *J. Air Pollut. Cont. Assoc.,* Vol.8, 1985, pp. 838.

[24] Hernández, T.T., y Bauer, L.I. de. Evolución del daño por gases oxidantes en *Pinus hartwegii* y *P. Montezumae* Var. *Lindley* en El Ajusco, D.F. *Agrociencia*, Vol.56, 1984, pp. 183-194.

[25] Miller, P.R., Bauer, L.I. de., Quevedo, A.N., and T. T. Hernández . Comparison of ozone exposure characteristics in forested regions near Mexico City and Los Angeles. *Atmospheric Environment*, Vol.28, 1994, pp. 141-148.

[26] Furlan, C.M., Moraes, R.M., Bulboras, P., Salatino, A., and M.J. Sanz. Psidium guajava 'Paluma' (the guava plant) as a new bio-indicator of ozone in the tropics. *Environmental Pollution*, Vol.147, 2007, pp. 691-695.

[27] Emberson, L.D., Ashmore, M.R., Murray, F., Kuylenstierna, J.C.I., Perey, K.E., Izuta, T., Zheng, Y., Shimizu, H., Sheu, B.H., Liu, C.P., Agrawal, M., Wahid, A., Abdel-Latif, N.M., Van Tienhoven, M., Bauer, L.I. de., and Domingos, M. Impacts of air polutants on vegetation in developing countries. *Water, Air and Soil Pollution,* Vol.130, 2001, pp.107-118.

[28] Moraes, R.M., Klumpp, A., Furlan, C.M., Klumpp, G., Domingos, M., Rinaldi, M.C.S., and Modesto, I. Tropical fruit trees as bioindicators of indsutrial air pollution in southeast Brazil. *Environmental International,* Vol.28, 2002, pp. 367-374.

[29] Pandey, J., and Pandey, U.C. Evaluation of iar pollution phytotoxicity in a seasonally dry tropical urban environment. *Environmental Monitoring and Assessment,* Vol.33, 1994, pp.195-213.

[30] Singh, J., Agrawal, M., and Narayan, D. Relative susceptiblity of two species of tropical fruit trees to thermal power plant emission. *Tropical Ecology,* Vol. 35, 1994, pp.83-96.

[31] Heagle, A.S., Body, D.E., Heck, W.W. An open-top field chamber to assess the impact of air pollution on plants. *Journal of Environmental Quality,* Vol. 2, pp. 365-36*8.*

[32] Barrantes-Díaz, O. Efectos del dióxido de azufre y del ozono en P*inus halepensis* en cámaras abiertas (open top chambers). Tesis Doctoral. Universidad Autónoma de Barcelona, 2000.

[33] Alcántar, G y Sandoval, M. Manual de análisis químico de tejido vegetal (Guía de muestreo, preparación, análisis e interpretación). *Revista Chapingo,* Publicación Especial. Vol.11, No.10, 1999, pp. 72-73.

[34] Osada-Velazquez, H.K., and Mora-Aguilera, G. 2LOG. Version 1.0 .Programa para desarrollar escalas de severidad por el método de Horsfall-Barrat. Colegio de Postgraduados. Instituto de Fitosanidad. Montecillo, México, 1997.

[35] Horsfall, J.G. and Barrat, R.W. An improved grading system for measuring plant disease. *Phytopathology*, Vol. 35, 1945, pp. 655.

[36] SAS Institute Inc. SAS Package, release 6.06. North Caroline, USA.

[37] Lucas P., Leena, R., and Mehlhorn, H. Needle chlorosis in sitka spruce following a three year exposure to low concentrations of ozone: changes

in mineral content, pigmentation and ascorbic acid. *New Phytologist*, Vol. 124, No.2, 1993.

[38] Takemoto B., Bytnerowicz, A., Dawson, P., Morrison, C., and Temple, P. Effects of ozone on Pinus ponderosa seedlings: Comparison of responses n the first and second growing seasons of exposure. *Canadian Journal of Forest Research*, . Vol.27, No.1, 1997.

[39] Senser M., Kloos, M., and Lütz, C. Influence of soil substrate and ozone plus acid mist on the pigment content and composition of needles from young Norway Spruce trees. *Environmental Pollution*, Vol. 64, 1990.

[40] Anze R., Franken, M., Zaballa, M., Pinto, M., Zeballos, G., Cuadros, M., Canseco, A., De la Rocha, A., Estellano, V., and Del Granad, S. Bioindicadores en la detección de la contaminación atmosférica en Bolivia. *Revista virtual REDESMA.* Red de desarrollo sostenible y medio ambiente. Vol.1, No.1, 2007.

[41] Karenlampi, S., Airaksinen, K., Miettinene, A., Kokko, N., Holopainen, J., Karelampi, L. and Kalajainene, R. . Pathogenesis related proteins in ozone-exposed Norway Spruce. *New Phytologist*. Vol.126, No.1, 1994.

[42] McCool, P., Musselman, R., Younglove, T and Teso, R. Response of kidney bean to sequential ozone exposures. *Environmental and Experimental Botany.* Vol.28, No.4, 1988.