

## STUDY OF ENVIRONMENTAL CONTAMINATION IN GROWTH TREE RINGS OF *COPAÍFERA LANGSDORFF* BY SR-TXRF: EVALUATION OF Cr, Ni, Cu, Zn AND Pb

Bruna Fernanda Faria<sup>1</sup>, Silvana Moreira<sup>1</sup> and Ana Elisa S. de Vives<sup>2</sup>

<sup>1</sup> Faculdade de Engenharia Civil Arquitetura e Urbanismo  
Universidade Estadual de Campinas  
Caixa Postal 6021  
13093-852 Campinas, SP  
bffaria@yahoo.com.br

<sup>2</sup> Faculdade de Engenharia, Arquitetura e Urbanismo  
Universidade Metodista de Piracicaba  
Rodovia Santa Bárbara D'Oeste/Iracemápolis, km 01  
13450-000 Santa Bárbara D'Oeste, SP  
aevives@unimep.br

### ABSTRACT

Some arboreal species present annual cycles of growth and sleeping, registered in the log by different anatomical structures - the growth rings. With the objective of verifying the industrial activity in the city of Bauru, SP samples were submitted to a quantitative analysis in order to verify the bio-accumulation of metals. For that Synchrotron Radiation Total Reflection X-Ray Fluorescence was used. Samples of *Copaifera Langsdorfii* (Copaíba) were collected close to Municipal Forest of Bauru, SP located at 200m of distance of a disabled company of energy accumulators (batteries) known by receiving several penalties of CETESB due disagreement with the environmental legislation. Through the quantification of the elements Cr, Ni, Cu, Zn and Pb in the annual growth rings were possible to verify the influence of the battery industry in the local pollution. The temporal variation of Pb showed that after the interdiction of the battery industry the concentrations are close to the reference value, but in the periods previous to the industry interdiction the Pb concentrations were above the reference value. For Cr, 46% of the samples presented superior concentration to the reference value. Zinc presented larger concentration in the period from 1996 to 1998, reaching  $1383 \mu\text{g g}^{-1}$ . For Cu the concentrations were higher than the reference value in almost all periods analyzed, that is, from 1969 to 2004. On the other hand Ni presented great oscillation in its concentration, and the highest values were observed in the period from 1969 to 1971 and from 1999 to 2001, reaching  $87 \mu\text{g g}^{-1}$ .

### 1. INTRODUCTION

With the man and its industrial activity also appeared the environmental pollution. The discovery of the fire and its use were the causes of the first anthropic negative impacts on the environment. However, nowadays, a great amount of different pollutants happens in several

concentrations, being the most important sources of pollutant atmospherics the industries, thermo electrical plants, garbage incinerators, traffic of automobiles, among others [1].

In relation to the industries, emissions originating from productive section can cause the workers' contamination turning it a risk to the human health, besides to damage flora and fauna and to destroy historical monuments and modern constructions.

The ability of several terrestrial plants to absorb and to accumulate metals as calcium, nickel, zinc, manganese, copper, and cobalt turn them very attractive to indicate possible environmental alterations, using them as bioindicator, for example, in the environmental pollution for metals.

The purpose of this work was applied the Synchrotron Radiation Total Reflection X-Ray Fluorescence (SR-TXRF) in order to quantify metals in *Copaifera Langsdorfii* (Copaíba) collected in Bauru, SP, Brazil in a area with historical of contamination by lead and in this way verify the potential use of the specie as bioindicator of environmental pollution.

## 2. EXPERIMENTAL

### 2.1. Instrumentation

The measurements were performed at Synchrotron Light Source Laboratory (LNLS), Campinas (SP). For the excitation a white beam with energy between 4-22 keV, with 2 mm de width and 1 mm de height was employed, under total reflection condition. For X-ray characteristic detection a Ge hyper pure detector with resolution of 150 eV at 5.9 keV was used.

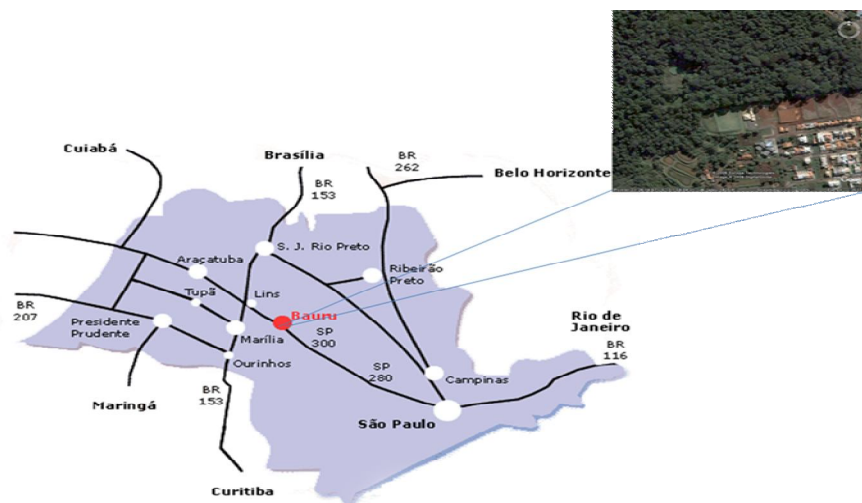
### 2.2. Sampling

Samples of *Copaifera langsdoffii* were collected at Municipal Forest Area located in Bauru city, SP, Brazil (Figure 1). This site is located to 220 m far way a battery industry (Ajax S.A.), known by its atmospheric contamination. Table 1 shows the geographic coordinates of the sampling site and of the Ajax plant.

**Table 1. Geographic coordinates of the sampling site and the Ajax plant.**

	Distance (m)	Latitude	Longitude
<b>Battery plant (AJAX)</b>	-	22°19'38.40''S	49°0'30.15''W
<b>Sampling Site</b>	220	22°19'44.07''S	49°0'25.11''W

Sampling is based on the method classified as non-destructive which consists in the removal of wood cylinders (10 mm diameter) by a Pressler probe introduction in the trees [2], illustrated in the Figure 2.



**Figure 1. Bauru city, São Paulo state and in detail the Municipal Forest Area**



**(a)**



**(b)**

**Figure 2. Pressler probe (a) and sampling in course (b).**

### 2.3. Standard and Sample Preparation

The annual growth three rings were identified and dated in the Forest Research Institute of São Paulo University (Piracicaba, SP). The period sampled correspond to 1966 up to 2004, totalizing 39 rings. Each sample is composed by three rings, totalizing in this way 13 samples. The samples were crushed and 100 mg were submitted to a humid extraction procedure (nitric acid/hydrogen peroxide). The final volume was completed to 10 mL with deionized and demineralized water.

For SR-TXRF analysis, the extracts were prepared adding to 1 mL of each sample, 100  $\mu\text{L}$  of Gallium ( $102.5 \text{ mg L}^{-1}$ ) used as internal standard in order to eliminate the geometry effect, because the thin film formed on the holder sample hasn't regular geometry.

After that, 5  $\mu\text{L}$  of this solution was putted on Perspex support and dried with infrared lamp. All samples and standards were prepared in triplicate and analytical blanks were also analyzed.

## 2.4. System Calibration and Quantitative Analysis by SR-TXRF

The system calibration and the quantitative analysis depend on the determination of the sensitivity of the system for the element of interest. For TXRF analysis isn't necessary to correct the matrix effect, and the fluorescent intensity of the element is directly proportional to its concentration (equation 1).

$$R_i = S_{Ri} \cdot C_i \quad (1)$$

where:

$C_i$  = concentration of the element **i** in the sample ( $\text{mg L}^{-1}$ );

$S_{Ri}$  = relative sensitivity for the element **i** (non-dimensional) and;

$R_i$  = relative counts ( $\text{mg.L}^{-1}$ ).

For elements contained in the standard solutions is possible to obtain a calibration curve,  $S_{Ri} = f(Z_i)$  for K and L series and in this way to determine the relative sensitivity for the elements present in the samples to be analyzed.

## 2.5. Detection Limits

The detection limits (DL) for K and L series were calculated using the equation (2), based on the background intensity of each element present in the characteristic X-ray spectra.

$$DL = 3 \sqrt{\frac{I_{bi}}{T} \frac{C_{Ga}}{I_{Ga} S_{Ri}}} \quad (2)$$

where:  $I_{bi}$  and  $T$  are the background intensity and the measuring time, respectively.

# 3. RESULTS AND DISCUSSION

## 3.1. System Calibration

The standards were measured by 100 s and after the measurements; spectra were adjusted for acquisition of the fluorescent intensities employing the Quantitative X-ray Analysis System (QXAS) software [3].

The relative sensitivities were calculated for K (equation 3) and L (equation 4) series.

$$S_{Ri} = \exp(-18.15101 + 1.08479Z - 0.01223Z^2 - 1.25719 \cdot 10^{-4} Z^3) \quad (3)$$

$$R^2 = 0.98292$$

$$S_{Ri} = \exp(-20.54286 + 0.36209 \cdot Z - 0.00109 \cdot Z^2 - 3.2353 \cdot 10^{-5} Z^3) \quad (4)$$

$$R^2 = 0.99502$$

### 3.2. Detection Limits for Wood Samples

After the fittings of the wood sample spectra the detection limits were calculated and they are presented in the Table 2.

**Table 2. Detection Limits for Wood Samples determined by SR-TXRF.**

Element	Atomic Number (Z)	DL ( $\mu\text{g g}^{-1}$ )
Al	13	103.25
Si	14	65.05
S	16	15.61
Cl	17	7.71
K	19	2.10
Ca	20	1.18
Ti	22	0.41
Cr	24	0.18
Mn	25	0.13
Fe	26	0.10
Co	27	0.08
Ni	28	0.10
Cu	29	0.09
Zn	30	0.09
As	33	0.11
Sr	38	0.28
Pb	82	0.27

### 3.3. Methodology Validation

With the purpose to validate the methodology employed a standard reference materials called Trace Elements in Natural Water (SRM 1640) supplied by National Institute of Standards and Technology (NIST). The measured and certified values were presented in the Table 3.

**Table 3. Measured, certified and reference values for Trace Elements in Natural Water (NIST/SRM 1640)**

Element	Measured Value ( $\mu\text{g kg}^{-1}$ )	Certified Value ( $\mu\text{g kg}^{-1}$ )
Ca*	$6.974 \pm 0.03^*$	$7.045 \pm 0.089^{* \#}$
K	$908 \pm 7$	$994 \pm 27^{\#}$
V	$12.96 \pm 2.07$	$12.99 \pm 0.37$
Cr	$39.8 \pm 0.85$	$38.6 \pm 1.6$
Mn	$109.6 \pm 5.63$	$121.5 \pm 1.1$
Co	$17.24 \pm 0.74$	$20.28 \pm 0.31$
Ni	$20.8 \pm 1.5$	$27.4 \pm 0.8^{\#}$
Cu	$75.8 \pm 1.68$	$85.2 \pm 1.2^{\#}$
Zn	$54.8 \pm 0.96$	$53.2 \pm 1.1^{\#}$
Rb	$2.32 \pm 0.88$	$2.00 \pm 0.02^{\#}$
Sr	$112.99 \pm 5.51$	$124.2 \pm 0.7$

\*  $\text{mg kg}^{-1}$

n=15 (fifteen determinations)

<sup>#</sup>reference values

$\alpha=0.05$  (95% confidence level)

San Joaquim Soil supplied by NIST as Standard Reference Material was submitted to the same procedure of extraction used for tree ring samples and the results were showed in the Table 4.

**Table 4. Measured and certified values of the standard reference material San Joaquim Soil (NIST/SRM 2709).**

Element	Measured Value ( $\text{mg kg}^{-1}$ )	Certified Value ( $\text{mg kg}^{-1}$ )
Ca	$17916 \pm 1006$	$18900 \pm 500$
V	$113 \pm 8$	$112 \pm 5$
Cr	$141 \pm 6$	$130 \pm 4$
Mn	$549 \pm 6$	$538 \pm 17$
Fé	$35836 \pm 2000$	$35000 \pm 1100$
Cu	$36.4 \pm 3.0$	$34.6 \pm 0.7$
Zn	$104 \pm 5$	$106 \pm 3$

### 3.4. Quantitative Analysis for Wood Samples

After the calculation of the relative sensitivities, the concentrations of the elements present in the samples were determined and they are showed in the Table 5.

**Table 5. Average concentration ( $\mu\text{g g}^{-1}$ ) and variation coefficient (CV) of Cr, Ni, Cu, Zn and Pb in Copaíba wood samples by SR-TXRF.**

Period (years)	Cr	CV	Ni	CV	Cu	CV	Zn	CV	Pb	CV
1968-1966	4.0	7.7	1.5	10.1	20.9	0.9	41.9	1.8	3.2	1.0
1971-1969	94.5	0.7	86.7	0.9	215.3	0.3	514.1	0.4	11.1	5.2
1974-1972	32.9	0.8			341.5	0.4	192.4	0.3	32.4	2.8
1977-1975	222.2	0.7	18.7	5.8	815.3	0.2	147.7	0.4	5.1	4.8
1980-1978	17.8	8.9	10.4	2.2	86.6	0.5	89.0	0.7	3.6	5.4
1983-1981	520.6	1.9	45.1	0.6	36.9	1.0	132.3	0.8	15.8	1.4
1986-1984	432.7	0.7	31.5	0.2	194.8	0.6	193.3	0.7	11.7	4.3
1987-1989	9.9	2.1	7.6	2.6	29.6	0.4	149.6	0.6	8.8	2.9
1990-1992	31.0	6.4	6.5	3.1	58.7	2.3	450.4	0.4	54.9	1.6
1993-1995	22.1	5.1	7.0	3.5	45.3	0.2	265.3	0.6	20.2	6.4
1996-1998	140.3	2.0	13.2	2.6	620.7	0.5	1382.6	0.1	127.9	1.7
1999-2001	889.9	1.4	86.8	2.1	440.6	0.2	275.7	1.2	36.9	8.6
2002-2004	70.6	2.3	8.9	6.9	146.9	0.5	187.5	1.4	10.0	3.0

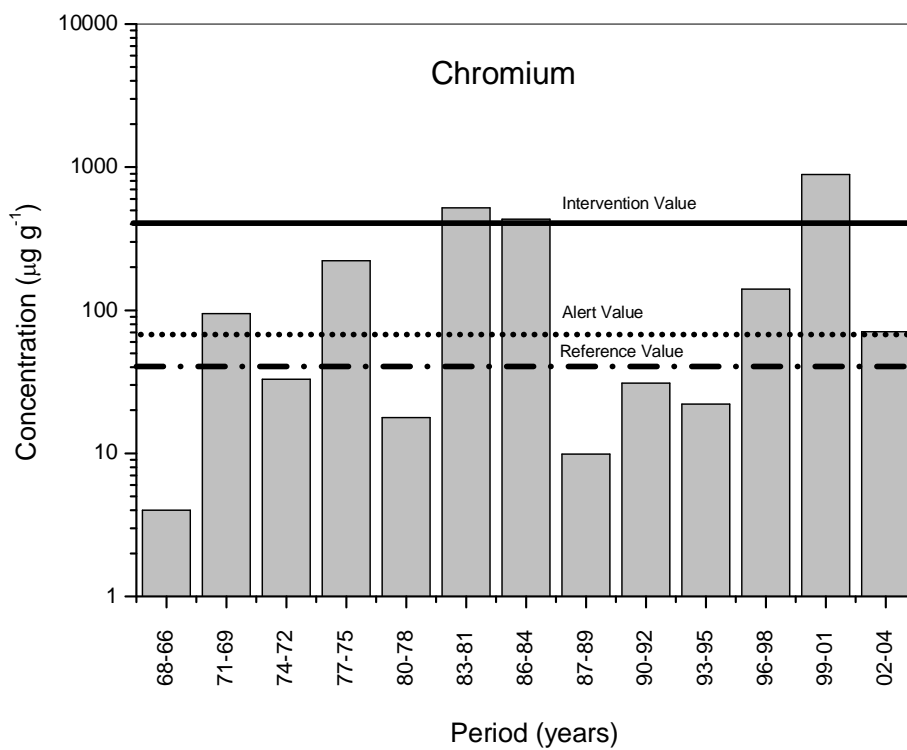
The nutrients necessary to development of the plants can be absorbed of the roots, so it is possible the plants also absorbed the contaminants deposited on its. The permissive values for toxic elements in plants is non existent in the Brazilian legislation Thus, the results were compared to the protection guide values for soil quality, established by the Technology and Environmental Sanitation Company (CETESB), São Paulo state [4].

The guides are: (1) Reference value (R), level quality for clear soil; (2) Alert value (A), indicating a possible variation in the quality soil; (3) Intervention value (I), indication the contamination limit. Above this value exists potential risks, direct or indirect, to the human health, considering exposition scenery agricultural, residential and industrial (Table 6).

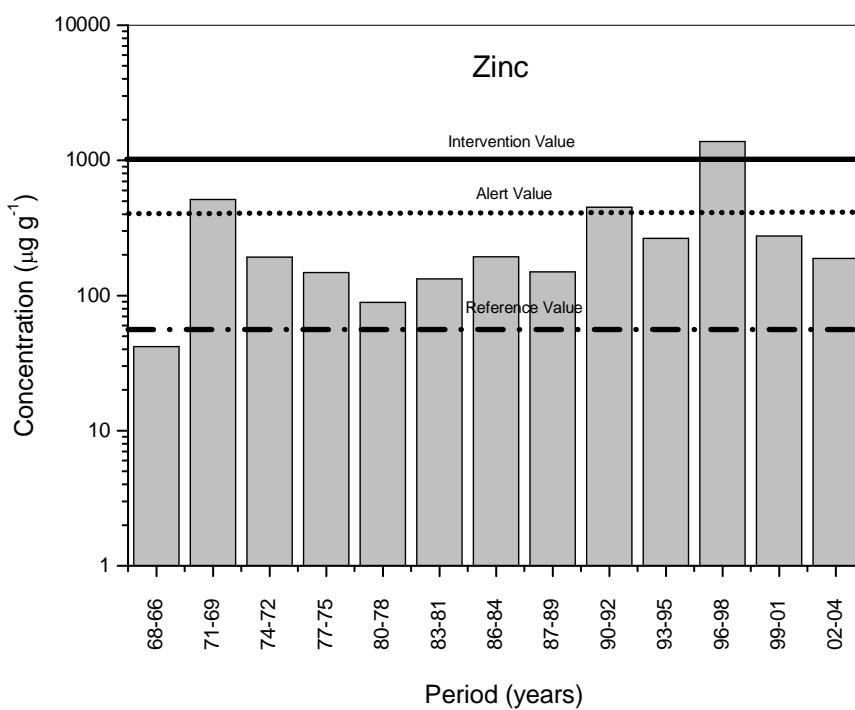
**Table 6. Reference, Alert and Intervention Values for Soils ( $\mu\text{g g}^{-1}$ ).**

Element	Reference (R)	Alert (A)	Intervention (I)		
			Agricultural	Residential	Industrial
Cr	40	75	150	300	400
Ni	13	30	70	100	130
Cu	35	60	200	400	600
Zn	60	300	450	1000	2000
Pb	17	72	180	300	900

Figures 3, 4, 5, 6 and 7 showed the distribution of the elements Cr, Zn, Cu, Ni and Pb by period for the growth three rings of *Copaífera Langsdorffii*. The dash dot line represents the Reference values, short dot line Alert value and Residential Intervention is detached by solid line, all values established by CETESB for soils.



**Figure 3. Distribution of Chromium on Copaiba growth three rings.**

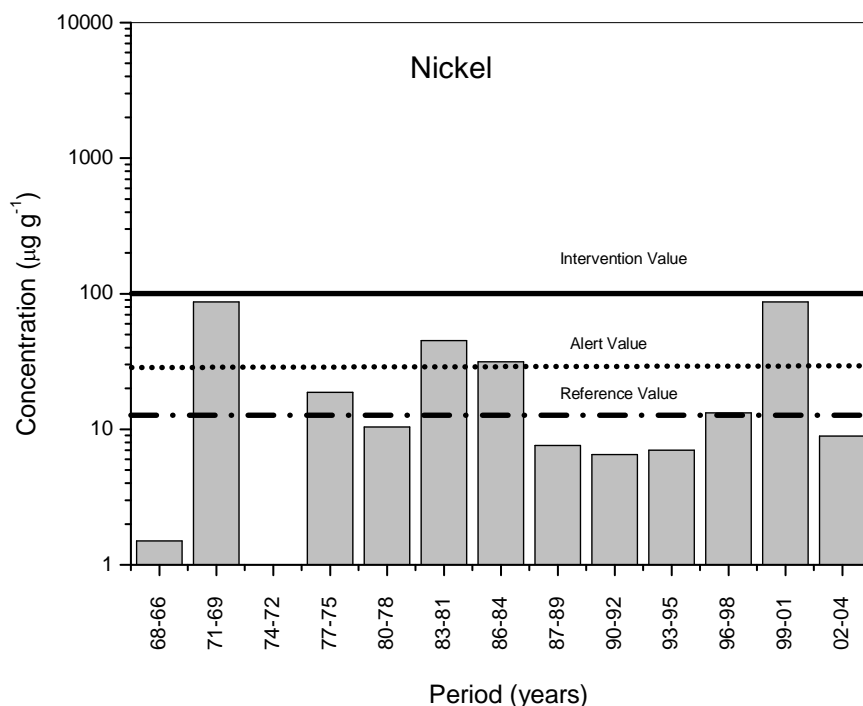


**Figure 4. Distribution of Zinc on Copaiba growth three rings.**



For Cr, as showed in the Figure 3, the periods of 1969-1971, 1975-1977, 1981-1983, 1984-1986, 1996-1998 and 1999- 2001 present concentrations higher than Alert Value adopted by CETESB. In average, 46% of all samples analyzed indicated a possible contamination of the soil for this element [5].

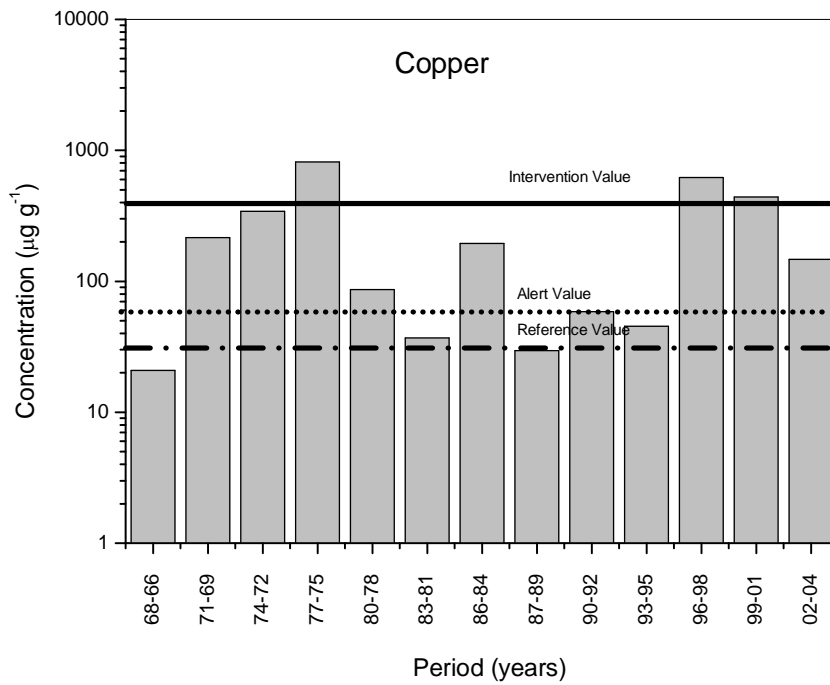
By the Figure 4, just in the period of 1966-1968 the Zn concentration was lower than Reference Value, surpassing the Alert Value in the periods of 1969-1971, 1990- 1993 and 1996-1998.



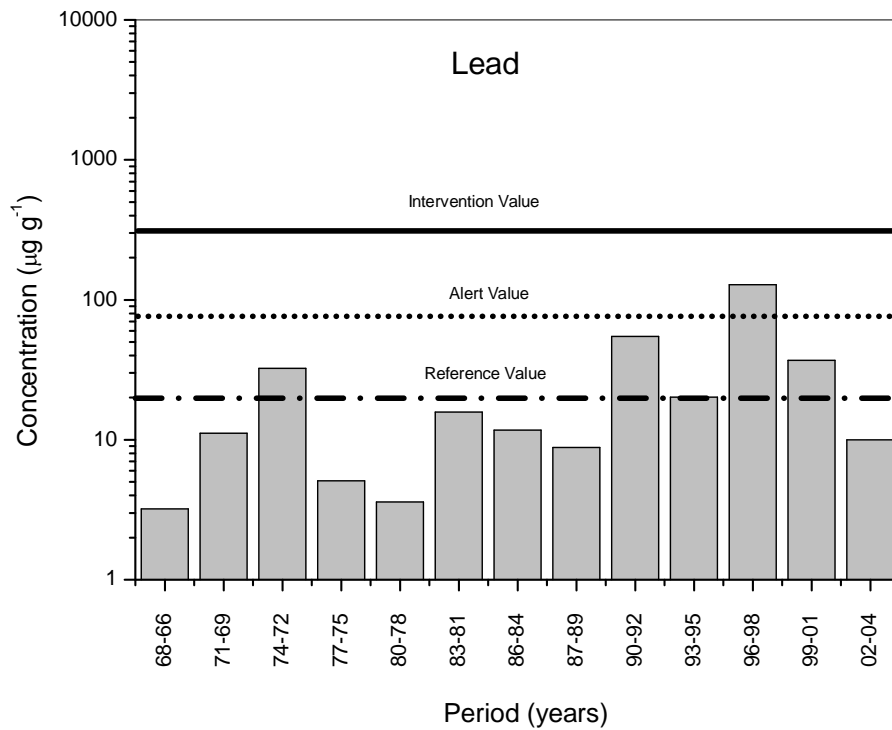
**Figure 5. Distribution of Nickel on Copaiba growth three rings.**

In relation to Ni, five samples present concentrations higher than Reference Value and four higher than Alert Value as showed in the Figure 5.

The concentrations for Cu were lower than Reference Value just in two periods 1966-1968 and 1987-1989 (Figure 6).



**Figure 6. Distribution of Copper on Copaiba growth three rings.**



**Figure 7. Distribution of Lead on Copaiba growth three rings.**

For Pb, the periods of 1972-1974, 1990-1992 and 1996-1998 showed concentrations higher to the Reference Value, and the period of 1996-1998 concentration higher than Alert Value (Figure 7).

The detection limits for the elements Cr, Ni, Cu, Zn and Pb were 0.18; 0.10; 0.09; 0.09 and 0.27  $\mu\text{g g}^{-1}$ , respectively.

Levels higher than 30  $\mu\text{g g}^{-1}$  of Pb are considered excessive or toxic and 43  $\mu\text{g g}^{-1}$  is the threshold value indicating death of trees while 10  $\mu\text{g g}^{-1}$  or less is the normal concentration in plants [6]. Pb concentrations founded in this study varied from 3.2 and 127.9  $\mu\text{g g}^{-1}$ . The threshold values were observed between 1990-1992 (54.9  $\mu\text{g g}^{-1}$ ) and 1996-1998 (127.9  $\mu\text{g g}^{-1}$ ). Toxic levels occurred between 1972-1974 (32.4  $\mu\text{g g}^{-1}$ ) and 1999-2001 (36.9  $\mu\text{g g}^{-1}$ ).

Vives et al., (2006) analyzed samples of *Caesalpinia peltophoroides* ("Sibipiruna") collected in Piracicaba city, São Paulo State, which presents high level of environmental contamination in water, soil and air, due to industrial activities, vehicles combustion, sugar-cane leaves burning in the harvesting, etc. Synchrotron Radiation Total Reflection X-ray Fluorescence technique (SR-TXRF) was employed to identify and quantify the elements and metals of nutritional and toxicological importance in the wood samples. In several samples, P, K, Ca, Ti, Fe, Sr, Ba and Pb were quantified. The K/Ca, K/P and Pb/Ca ratios were found to decrease towards the bark. Threshold value for Pb was observed in 1971-1973 (46.5  $\mu\text{g g}^{-1}$ ) while toxic levels were observed in 1974-1976 (32.5  $\mu\text{g g}^{-1}$ ), 1977-1979 (31.5  $\mu\text{g g}^{-1}$ ) and 1983-1985 (30.7  $\mu\text{g g}^{-1}$ ) [7].

Synchrotron radiation analysis was used to investigate the metal content of tree rings collected from paper birch, *Betula papyrifera* Marsh, on transects downwind from two metal smelters (nickel and copper). Concentrations of both total and exchangeable copper and nickel in the soil increased with proximity to the respective smelter, this pattern was reflected only in the nickel content of rings near the nickel smelter; copper content did not vary with distance from either smelter [8].

#### 4. CONCLUSION

Observing the temporal variation for Pb, can be verify that after the interdiction of the battery industry in 2002 it concentration is very close to the Reference Value, but in the previous periods the Pb values were 127.9  $\mu\text{g g}^{-1}$  (1996-1998) and 36.9  $\mu\text{g g}^{-1}$  (1999-2001) indicating the possible use of *Copaifera langsdoffii* specie as bioindicator of the environmental pollution.

Lead pollution is caused by industrial emissions, and on a large scale is caused by vehicular emissions from motors using lead as additive in gasoline. In the Brazilian legislation the addition of Pb as additive in gasoline was prohibited from 1990. The Ajax plant was closed in 2001, and as can be observed Pb concentration in the period of 2002-2004 decrease until the level considered as normal for the plants (10  $\mu\text{g g}^{-1}$ ).

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