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DETERMINATION OF HEAVY METALS IN *Eucalyptus grandis*, MANURED WITH BIOSOLID, BY NEUTRONS ACTIVATION ANALYSIS

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ABSTRACT

The biosolid is a mud resulting from the biological treatment of wasted liquids. It is considered as a profitable alternative and important to minimize the environmental impact generated by the sewages thrown in the sanitary lands. The utilization of biosolid in forest cultures, as the *Eucalyptus grandis*, is of great economic and scientific interest, because it promotes not only the use of sewage residues, but also a fertilization prices reduction. The objective of this work was to detect the presence of heavy metals in *Eucalyptus grandis* sample fertilized with different quantities of biosolid. For the experiment, we used the plantation of Estação Experimental de Ciências Florestais of Itatinga, lincked to ESALQ of Universidade de São Paulo – USP. The eucalyptus were planted in March of 1998 and collect with five years old. The used biosolid was produced by ETE of Barueri – SP, classified as kind B. The samples were prepared in Universidade Estadual Paulista of Itapeva. For the determination of heavy metals presence in eucalyptus samples, an analysis technique by neutronic activation (NAA) was used followed by gamma rays spectroscopy. The samples were irradiated in the Nuclear Reactor IEA-R1 of IPEN-SP, followed by the measure of induced gamma rays activity, using a Detector HPGe. The presence, mainly of Br, Mn, Na and K, was detected in all analyzed samples.

INTRODUCTION

In the modern world there is a non controlled production of residues and many are the sources that spread them in the environment in an indiscriminate way. They can contain extreme large quantities of heavy metals and other highly polluting dejects [1].

The final destiny of the sewage mud is one of the most serious environmental problems faced nowadays, once the metals potential toxic presence is undeniable [2]. It is necessary to find solutions, urgently, for the treatment of generated residues and to minimize their production [1]. For this, some alternatives are being used and studied. The options may be: by controlling the wasted residues in sanitary lands that do not hold the excessive quantity of these residues and finally contaminate the surroundings; or by using through recovery of degraded soils;

production of: fuel, light concrete aggregates, ceramic bricks, concrete, ceramic glasses; the use of biosolid as a fertilizer for the improvement of agricultural soils, provided the environmental conditions are respected: kind of soil, culture, etc. [2]

The biosolid is a mud resulting from the biological treatment of wasted industrial and sanitary liquids, considered as a profitable alternative and important to minimize the environmental impact generated by the sewages thrown in the sanitary lands. It is used as fertilizer mainly in forest plantings by the following aspects: it is a non-eatable culture, minimizing the direct contamination risk of men and animals by food digestion; increases the biomass; releases nutrients gradually (organic); elevates the pH; elevates the macro-nutrients concentration; enlarges the trees diameter and height; and reduces the costs by application in large areas, compared with fertilizers [1].

On the other hand, the biosolid presents negative aspects, such as: to provide non-balanced nutrients; to favor the presence of pathogenic organisms; and to make possible the presence of heavy metals. Thus, it can cause the contamination of soil, springs, groundwater and water currents [1,3].

The utilization of biosolid in forests cultures is of great economy and scientific interest, because besides promoting a discard of sewer residues with a useful and valuable objective for the environment, promoting reduction the fertilization prices, since it does not promote the contamination of the cultivated areas.

The use of the biosolid as fertilizer in *grandis* forest cultures of the Eucalyptus has been stidied intensively [4]. Research evaluates the possibility of biosolid be responsible or not for the contamination by heavy metals in the tree itself, damaging its metabolism and consequently the quality of its wood, as well as the possibility of contamination of soil, groundwater, rivers and other live beings [4,5].

Eucalyptus grandis has an excellent productivity potential due to its wood characteristics, such as: lightness, few cracks, white core, resistance to weather, and others. These characteristics allow a great number of utilities for eucalyptus nowadays.

In eucalyptus cultures, the presence of pathogens and heavy metals damage directly important aspects: the metabolism of the tree (reducing the plant growing); the integrity of other living beings (eatable mushrooms may be contaminated by pathogens, for example), and in the utilization of wood, as in the whitening of the cellulose in the manufacture of paper.

In the industrial field, the interference of heavy metals found in the wood whitening process of the cellulose represents a very important economic problem. The whitening is a purification process where the non cellulose compounds are eliminated or transformed the into others that reflect the white light.

The kinds and the concentration of metals present in the wood and in the pulp may damage the whitening process of cellulose, because the metals [6, 7] are absorbed by the pulp through the water of the process, interfere in the performance of hydrogen peracides and peroxides in the whitening of the pulp, promoting its decomposition.

There, it is essential to monitor the concentration limit values of biosolid contamination to avoid the occurrence of dissemination not only in the eucalyptus wood, but in the soil or groundwater, causing economic problems, and to take into consideration mainly the losses for all ecosystems – the preservation of life.

The sewage treatment stations of must direct the produced mud to the fabrication of biosolid, following the procedures of CETESB (1999) [8], which determines the limit values for the metals concentration in soils, mud and agriculture soils, and thus must also follow the current legislation about the subject, as it is indicated in Table 1, as it follows.

Table 1 – CETESB Procedure - p 4.230 – mud application of biological treatment systems in agriculture areas – criteria for the project and operation (technical manual) – issued in D.O.E. of 04.12.99

PARAMETER	DIRECTIVE 86/278/EEC (1986)		
	Attachment 1A Limit values for metals concentration in the soil (mg/kg)	**Attachment 1B** Limit values for metals concentration in the mud (mg/kg)	**Attachment 1C** Limit values of metals quantities that can be added annually in agriculture soils (kg/ha.year)
Arsenic	-	-	-
Cadmium	1 to 3	20 to 40	0.15
Lead	50 to 300	750 to 1200	15
Cobalto	-	-	-
Copper	50 to 140	1000 to 1750	12
Chromium	(1)	(1)	(1)
Mercurio	1 to 1.5	16 to 25	0.1
Molibdenio	-	-	-
Nickel	30 to 75	300 to 400	3
Selenium	-	-	-
Zinc	150 to 300	2500 to 4000	30

In this work, the method used for the determination of heavy metals presence in the eucalyptus samples was the Neutron Activation Analysis. It is a sensitive technique for the analysis of multi-elements in a qualitative and quantitative way [9,10,11]. This analysis consists of pumping a certain material followed by the measure of radioactivity induced. In general, the irradiation is done with thermal neutrons and the result is measured using a spectrometry of gamma rays emitted by each radioisotope. Once each isotope produced in the activation process has its own characteristics of emission (half-life ($t^{½}$) and energy of particles or gamma radiation emitted), it is possible to determinate quantitatively the concentration through comparison standards. This is an analysis method non destructive that allows, in some cases, to determine the concentrations of 20 to 40 elements, in a single sample.

OBJECTIVE

The objective of this work is to study the application and the application viability of the Neutrons Activation Analysis method in order to verify heavy metals presence in the wood of eucalyptus. Generally, for this analysis physics-chemical methods are applied [10]. To evaluate if the used biosolid results in an increase of the heavy metals level absorbed by the tree. To compare quantitatively samples of eucalyptus fertilized with different aliquot of biosolid (10, 20, 30, 40 $ton.ha^{-1}$) with samples of non fertilized material with biosolid [10].

MATERIALS AND METHODS

To achieve this work, the eucalyptus plantation of the 'Estação Experimental de Ciências Florestais' in Itatinga, linked to 'ESALQ' of 'Universidade de São Paulo – USP' was used. It is a region of 830m of medium altitude, located between the parallels 23°02'01" and 23°02'30" South latitude and the meridians 48°37'30" and 48°38'34" Greenwich West longitude [12].

In this region, the weather is mesothermic humid (Cwa), soil Latossol Red - Yellow type with dense vegetation. The soil is Latossol Red - Yellow with relief softly waved and with texture medium-sandy dystrophic psamitico (sand = 830 g kg-1, silt = 50g Kg-1 and clay = 120 g kg-1, in the layer of 0-20 cm) [12,13].

The eucalyptus were planted in March, 1998, in a system of minimum cultivation in casualized blocks. Nine kinds of treatments were applied to the plantations, where we considered only four, with doses variations of biosolid: the first treatment without addition of biosolid; the second, third, fourth with ten, twenty and forty tons by hectare of biosolid respectively, with addition of mineral potassium.

The biosolid used for the experiment was produced in the 'Estação de Tratamento de Esgoto' of Barueri and classified as kind B (SENEA, 1999; Cetesb, 1999; EPA; [1,14]), what means that the concentration of faeces in the residue is inferior to 2 million $NMPg^{-1}$ of total solids [15]. It has organic material content around 300 $g.kg^{-1}$ and relation C:N of 4.3 humidity around 60% and pH (in water) equal to 12 [16]. Some data of the biosolid used (heavy metals concentration and the presence of patogenics) are presented in the following tables:

Table 2: Polluting heavy metals concentration in biosolid of ETE Barueri – São Paulo. [17]

Polluting Heavy Metal	Concentration in $mg.Kg^{-1}$, dry base					EPA 40 CFR Part 503	CETESB Procedure P 4.230
	1997		1998	1999 (1)		Exceptional Quality	Maximum Limit
	07/03 (2)	18/12	08/12	30/11	05/12 (3)		
Arsenio	5.3	NR	NR	NR	NR	41	75
Cadmium	7.7	7.6	24	8	6	39	85
Lead	152	152	371	180	131	300	840
Copper	619	664	1207	718	492	1500	4300
Mercurio	1.6	0.67	NR	NR	NR	17	57
Molibdenio	5	NR	<24	NR	5	-	75
Nickel	211	268	**595**	358	276	420	420
Selenium	1.4	NR	NR	NR	NR	100	100
Zinc	1850	1800	3810	2330	2198	2800	7500

(1) In 1999 polymers were used and in the other years lime and ferric chloride for the conditioning of biosolid; (2) The analysis in 1997 was carried out in Canada;(3) The analysis in 1999 was carried out in UNESP / Jaboticabal;(NR) – Analysis not carried out; (*) All the othes analysis were carried out in CETESB laboratory - São Paulo [80].

Table 3: Microbiology and parasotology of biosolid of ETE Barueri – São Paulo. [80]

Year	Pathogens Concentrations					
	Fecal Material NMP/g	Salmonella sp NMP/4g	Clostridium Perfringens NMP/g	Bacterial F – Specific UFP/g	Parasitological Viable Eggs /4g	Protozoans Cystitis NMP/g
1997 (Average of 5 samples)	5.4	Absent	$16.6x10^3$	Absent	1.4	NR
1998 (Average of 2 samples)	Absent	Absent	$1.2x10^3$	Absent	Absent	Absent
1999 (Average of 2 samples)	475.000	36.5	NR	Absent	Absent	Absent

The biosolid applications were determined as it follows - first application: 6 months after planting, aplication of KCl, 35 $Kg.ha^{-1}$ of K_2O, in half moon around the trees; second application: 58 $Kg.ha^{-1}$ of K_2O, with the same procedure; third application: 12 and 18 months after planting, aplication in 40 cm long strips, in the center of the parallels.

In each treatment, we analysed 2 trees. The eucalyptus were collected with 5 years old. The samples were prepared with several cuts procedures and in the sequence grinding and sieving, until we obtained eucalyptus wood dust, dried in greenhouse, weighed and placed in ependorf tubes. The procedures were carried out at the Universidade Estadual Paulista of Itapeva.

For the determination of heavy metals presence in eucalyptus samples, we used the Neutron Activation Analysis followed by Gamma Ray Spectroscopy, a sensetive analytical technique for the analysis of multi-elements in a qualitative and quantitative way. The samples were irradiated with neutrons flux of Nuclear Reactor IEA-R1 of Instituto de Estudos Energéticos Nucleares – IPEN – São Paulo followed by the measure of induced gamma rays actitivy, using an HPGe detector.

Trees with good fenotipic profile were selected: straight trunk, good aspect of health and very uniform top, aiming to select trees that represented the answers of the treatments applied [12].

The samples were prepared folowing the methods published by the respective association of valid technical procedures: Associação Brasileira de Normas Técnicas (ABNT, 1984) [12].

The eucalyptus wood dust was dried in greenhouse, weighed, added in ependorfs and identified in Laboratório da Universidade Cruzeiro do Sul – UNICSUL, on 10/16/2006.

Calculation of Metals Concentration:

For the calculation of the quantification of metals found in the analyzed samples, we used the Formula of the Fraction of the Isotope [10,18,19,20,21,22]:

$$F = \frac{C_{OBS.} M.\lambda}{N.\sigma.\varepsilon.m.f.\Phi f_{\gamma}.(1-e^{-\lambda t_i}).e^{-\lambda t_e}.(1-e^{-\lambda t_c}).F_c} \quad (1)$$

Where: Cobs = observed counting; M = element atomic mass;
l = decay constant;
e = measures system eficiency; N = 6.02 10^{23} (Avogadro Number);
s = shock section, in cm^2; m = sample mass, in grammes; f = fraction of target isotope; F = Fraction of isotope in the sample, when the sample is a compound; fg = fraction of gamma decay; ti= irradiation time; te= waiting time between the end of irradiation and the beginning of counting; tc= counting time; Fc = correction factor.

RESULTS

Fig 1 were showed the gamma spectrum and in Table 4 were presented the elements identified in 4 samples (quantities variation of biosolid).

Table 4: Spectrums of identified elements

Full-energy peak (keV)	Energy (keV) / Element
SAMPLE T1-1	
A – 554.34	554.35 / Br-81
B – 618.28	619.1 / Br-82
C – 846.75	846.76 / Mn-56
D - 1368.55	1368.60 / Na-23
E - 1460.75	1460.83 / K-40
F - 1524.70	1524.58 / K-41
G - 1811.20	1810.72 / Mn-56
SAMPLE T4-8	
A - 554.34	554.35 / Br-81
B - 698.36	698.37 / Br-81
C - 846.75	846.76 / Mn-56
D - 1368.55	1368.60 / Na-23
E - 1460.75	1460.83 / K-40
F - 1524.70	1524.58 / K-41
G - 1811.20	1810.72 / Mn-56
SAMPLE T8-9	
A - 846.75	846.76 / Mn-56
B - 1368.55	1368.60 / Na-23
C - 1524.70	1524.58 / K-41
D - 1642.40	1642.69 / Cl-38
E - 1729.60	1729.58 / Bi-214
F - 1811.20	1810.72 / Mn-56
G - 2112.60	2113.05 / Mn-56
SAMPLE T9-19	
A - 554.34	554.35 / Br-81
B - 776.50	776.52 / Br-81
C - 847.03	846.76 / Mn-56
D - 1369.50	1368.60 / Na-23
E - 1524.70	1524.58 / K-41
F - 1729.60	1729.58 / Bi-214
G - 1811.20	1810.72 / Mn-56
H - 2112.60	2113.05 / Mn-56

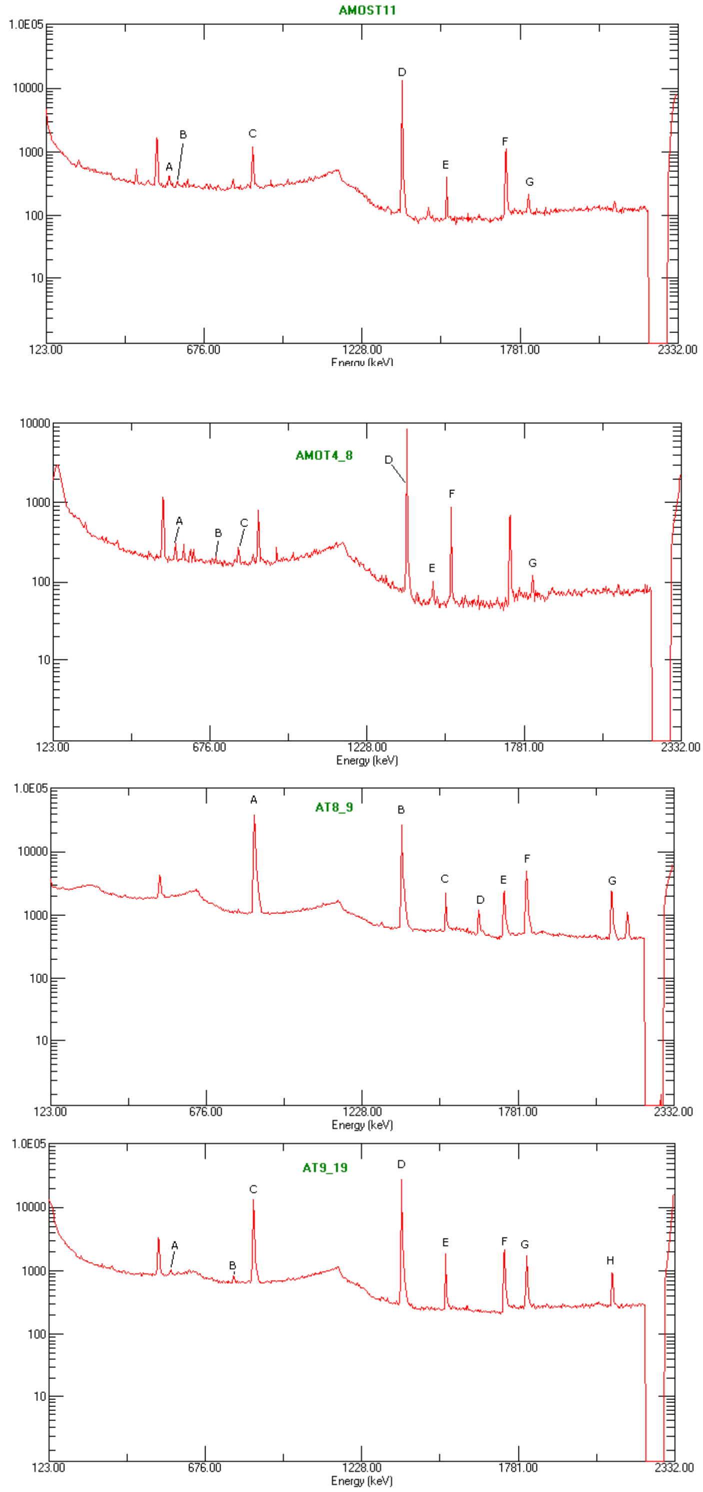

Fig.1- Gamma spectrum of the analised samples: a) sample T1-1; b) sample T4-8; c) sample T8-9; d) sample T9-19

CONCLUSION

Neutron Activation Analysis technique (NAA) showed to be promising in the *Eucalyptus grandis* samples analysis, allowing to verify the presence of phytoavailable heavy metals in the ground.

The following elements were identified: Br, Mn, In and K in all the analyzed samples with the four types of treatments used

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