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NUCLEAR AND RELATED ANALYTICAL METHODS APPLIED TO THE DETERMINATION OF CR, NI, CU, ZN, CD AND PB DURING THE STUDIES OF DISTRIBUTION OF THE METALS BETWEEN *FERRALITIC - RED* SOIL AND *SORGO* PLANT.

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ABSTRACT

Several studies about the influence of heavy metals onto the growth of vegetables have been carried out in Cuba by the Ministry of Agriculture with the aim to evaluate the effects resulting of the continuous application of fertilizers and other materials to the crop soils. The analysis of metal contents in soil and vegetable samples is often troublesome due to the low concentration levels to be determined.

During the present work different EDXRF, AAS and ASV methods were applied and compared for the evaluation of Cr, Ni, Cu, Zn, Cd and Pb contents in *ferralitic -red* soil and *sorgo* plant samples. Several Certified Reference Materials (CRM) (inorganic and organic matrixes) were analyzed in order to evaluate the performance of the analytical procedures and the bias and precision of the results.

A study was performed growing *sorgo* plants in several series of pots where different quantities of metals were added to the soil substrate. The observed correlation between the metal contents in soil and plants resulting after such experiment, as well as the influence of different additions of each metal onto the plant growth is also presented.

1. INTRODUCTION

Pollution of crops with heavy metals has been studied in many countries during the last decades [1, 2]. Some studies to determine the influence onto the growth of vegetables resulting of the continuous application of fertilizers and other products (phosphate rocks, zeolites, pesticides, etc.) in the agricultural practices have been carried out in Cuba in the last years [3, 4]. Another major sources of the environmental pollution of soils and crops are the industrial discharges and the fuel engine based transport. The main objective of those researches has been to evaluate some effects of contaminants (such as Cr, Ni, Cu, Zn, As, Cd, Hg, Pb among others) in animals and human beings.

Some studies to determine the concentration of several elements in soils and plants have been performed in the Institute of Soils (Ministry of Agriculture of Cuba) during the last years. However, the determination of heavy metals in soils and plants has been limited due to the low concentration levels of the metal, that require the performance of high sensitive analytical methods.

Several analytical procedures have been developed for the determination of trace elements in soils and plants: Neutron Activation Analysis (NAA) [5, 6], Atomic Absorption Spectropho tometry (AAS) [7, 8, 9, 10], X-Ray Fluorescence Analysis (XRF) [6, 8, 11], and others.

The results of the determination of Cr, Ni, Cu, Zn, Cd and Pb in *ferralitic - red* soil and *sorgo* plant samples, using Energy Dispersive XRF (EDXRF), AAS and Anodic Stripping Voltammetry (ASV) methods were reported in previous papers [8, 10].

The present work describes the results of an study performed growing *sorgo* plants in series of pots where different quantities of metals were added to the soils. The contents of these elements were determined by the above-mentioned methods in *ferralitic - red* soil and *sorgo* plants. The observed correlation between the metal contents in soil and plants resulting after such experiment, as well as the influence of different additions of each metal onto the plant growth is presented.
The performance of the analytical procedures as well as the determination of the bias and precision of the results was evaluated analyzing Certified Reference Materials (CRM) and by an inter-comparison of the results obtained by the different methods.

2. EXPERIMENTAL

Reagents.

- High purity water (distilled and de-ionized, < 0.05 μ S/cm).
- Cr, Ni, Cu, Zn, Cd and Pb standard solutions were used to perform the addition of metals to the soil as specified in table 1.
- Cr, Ni, Cu, Zn, Cd and Pb high purity solutions were used to prepare the calibration curves for the AAS and Anodic Stripping Voltammetry (ASV) analysis.
- Biological and non-biological CRM used in the present work are related in table 2 [12].

Table 1*: Metal additions to soil substrates*

Element	Sample number	Resulting concentration (μg/g)
Ni	1 - 4	100
	5 - 8	200
	9 - 12	400
	13- 16	800
Cd	17 - 20	2
	21 - 24	4
	25 - 28	12
	29 - 32	24
Pb	33 - 36	50
	37 - 40	100
	41 - 44	200
	45 - 48	400

Element	Sample number	Resulting concentration (μg/g)
Zn	49 - 52	200
	53 - 56	400
	57 - 60	800
	61 - 64	1600
Cu	65 - 68	50
	69 - 72	100
	73 - 76	200
	77 - 80	400
Cr	81 -84	200
	85 - 88	400
	89 - 92	800
	93 - 96	1600

Blank samples (without addition): 97 – 104

Table 2. *Certified Reference Materials (CRM)*

Soils and sediments	Biological matrixes
NRCC PACS-1	NIST-SRM-1567 (Wheat Flour)
IAEA SL-1	NIST-SRM-1572 (Citrus Leaves)
IAEA SOIL-7	NIST-SRM-1575 (Pine Needles)
NRCC MESS-1	NIES-CRM-9 (Sargasso)
NIES CRM-02	NRCC-DOLT-1 (Dogfish Liver)
NRCC BCSS-1	NRCC-DORM-1 (Dogfish Muscle)
NRCC MESS-2	NIST-SRM-1566 (Oyster Tissue)
NRCC PACS-2	NRCC-Tort-1 (Lobster Hepatopancreas)
NRCC PACS-1 + NRCC MESS-2 (1 : 4)	
NRCC PACS-1 + NRCC PACS-2 (1 : 1)	

Procedures

I. Sample preparation to study the distribution of the metals between the soil and the vegetable

One kg of the soil taken at 0 - 20 cm. depth layer was homogenized. Then, solutions of Cr, Ni, Cu, Zn, Cd and Pb were added to the soil, which was then homogenized and put in a plant pot. The added concentrations are shown in *Table 1*. Thirty days after adding the metals, 6 *sorgo* plants were seeded in each pot and grown-up for 45 days. After that term, the aerial leafs of the plants were collected and dried at 65^0C until constant weight was obtained.
One hundred four soil samples and eighty-eight vegetable samples were subjected to metal analysis.

II. Analytical methods

II.I. EDXRF analysis

- External standard method using the Compton peak for the correction matrix effects (I/C) [13, 14] was employed. Calibration curves were obtained by measuring pressed pellets (aerial density exceeding 0.6 g/cm^2) of several soil and sediment CRM. The same procedure was followed for the analysis of biological materials, but preparing pellets of higher aerial density (0.9 g/cm^2).

- Emission - Transmission (ET) method [15].
  Thin pellets (~ 0.04 g/cm2) were prepared to determine the sample mass attenuation coefficient for the X-ray fluorescent line of each element. To quantify the concentration of each metal in soil and vegetal samples pellets of ~ 0.6 and ~ 0.9 g/cm^2 respectively were measured. The calculations were carried out using an instrumental sensitivity calibration curve, experimentally determined for K (Ni, Cr and Zn) and L (Pb) fluorescent lines.

The EDXRF spectrometer consisted of a Si(Li) detector (r_ε ~ 190 eV for Mn-K_α), an ^{109} Cd radioisotope annular source (247 MBq) and a modular NIM-BIN spectrometric track. Due to the low activity of the ^{109} Cd source, the measuring time had to be selected of 10000 s. The EDXRF spectra fitting was performed with the AXIL-QXAS package [16], distributed by the International Atomic Energy Agency (IAEA). The SAX program [17] was used for quantification in the case of ET procedure.

II.2. AAS and ASV analysis

- One g of the vegetable or soil samples was dissolved using HF, HClO4 and HNO3 concentrated acids as described in [8, 10]. The final solution volume was 25 mL.
  Calibration curve method was employed for the determinations of the metal concentrations. In some cases the standard addition method was used as an alternative analysis.

A model SP-9 Pye Unicam Spectrophotometer and a PA4 Polarographic Analyzer (Differential Pulse Polarography as operation mode, and Anodic Stripping Voltammetry (ASV) as Program) were used for AAS and ASV determinations respectively.
All calibration curves were elaborated according to Guide ISO 8466 [18], which permits also to evaluate the detection limits (DL) of the methods.

3. RESULTS AND DISCUSSION

3.1. Quality control of the results.

The concentration of Cr, Ni, Cu, Zn, Cd and Pb resulting of the analysis of the CRM by AAS, ASV and EDXRF (ET) are shown in tables 3 and 4. The bias and precision (expressed as confidence interval for $\alpha = 0.05$, and n=4) were, in general, better than 10 % for all analyzed elements. For concentrations close to the detection limits (DL) the values for these parameters were consequently worse (see table 5). In the case of EDXRF (ET) method the bias and the precision values were greater than those obtained by AAS and ASV methods. The latter is caused by the extremely low activity of the employed ^{109}Cd source. The detection limits for ASV were the lowest when compared with the rest of the methods. As will be seen below, the high sensitivity of this method allowed determining ultra trace concentrations of Cd and Pb in the vegetable samples.

***Table 3**. Analysis of Non-Biological Certified Reference Materials.*

Element		*Non-Biological Certified Reference Materials (µg/g)*								
		Soil-7			**Pacs-1**			**SL-1**		
		FRX (ET)	**EAA**	**ASV**	**FRX (ET)**	**EAA**	**ASV**	**FRX (ET)**	**EAA**	**ASV**
Cr	**C. C.**		**60 ± 12**			**113 ± 8**			**104 ± 9**	
	O. C.	ND	61 ± 4	ND	ND	106 ± 6	ND	ND	109 ± 4	ND
	Bias %		2.0			6.20			4.81	
	Precision %		6.86			5.66			3.67	
Ni	**C. C.**		**(26)**			**44.1 ± 2**			**44.9 ± 7.6**	
	O. C.	ND	24.8 ± 1.7	ND	ND	47.3 ± 2.5	ND	ND	42.4±3.8	ND
	Bias %		4.62			7.26			5.56	
	Precision %		6.86			5.29			8.96	
Cu	**C. C.**		**11 ± 2**			**452 ± 16**			**30.0 ± 5.4**	
	O. C.	< LD	12.4 ± 0.7	ND	453 ± 24	464 ± 10	474 ± 28	< LD	30 ± 2	32 ± 1
	Bias %		11.29		0.22	2.65	4.87		1.33	6.66
	Precision %		6.65		5.30	2.16	5.91		6.57	3.13
Zn	**C. C.**		**104 ± 6**			**824± 22**			**223 ± 10.0**	
	O. C.		105 ± 8	ND	831 ± 34	825 ± 36	847 ± 36	229 ± 12	216 ± 15	211 ± 8
	Bias %		0.96		0.90	0.12	2.80	2.70	7.30	5.38
	Precision %		7.61		4.10	4.35	4.25	5.24	6.98	3.79
Cd	**C. C.**		**(1.3)**			**2.4 ± 0.2**			**0.26 ± 0.05**	
	O. C.	ND	ND	1.15 ±0.08	ND	< LD	2.7 ± 0.2	ND	< LD	< LD
	Bias %						12.5			
	Precision %			6.95			7.41			
Pb	**C. C.**		**60 ± 8**			**404 ± 20.2**			**37.7 ± 7.2**	
	O. C.	ND	75 ± 7	ND	378 ± 18	397 ± 8	411 ± 20	ND		40.7 ± 7.2
	Bias %		25.0		6.44	1.73	1.73			7.96
	Precision %		9.33		4.76	2.01	4.87			4.67

C.C Certified Concentration O.C Obtained Concentration
( ) Not certified ND Not determined

***Table** 4. Analysis of Biological Certified Reference Materials.*

Element		***Biological Certified Reference Materials (µg/g)***								
		Citrus Leaves			**Oyster Tissue**			**Tort -1**		
		FRX (ET)	**EAA**	**ASV**	**FRX (ET)**	**EAA**	**ASV**	**FRX (ET)**	**EAA**	**ASV**
Cr	**C. C.**		**0.8 ± 0.2**			**0.69 ± 0.27**			**2.4 ± 0.6**	
	O. C.		< LD			< LD			<LD	
Ni	**C. C.**		**0.6 ± 0.2**			**1.03 ± 0.19**			**2.3 ± 0.3**	
	O. C.		< LD			< LD			< LD	
Cu	**C. C**		**16.5 ± 1.0**			**63.0 ± 3.5**			**439 ± 22**	
	O. C.		16.0 ± 0.6	14.9± 0.8		66± 3	61 ± 3		434 ± 12	437 ± 10
	Bias %		3.03	9.70		4.76	3.17		1.14	0.46
	Precision %		3.75	5.37		4.55	2.92		2.77	2.29
Zn	**C. C.**		**29 ± 2**			**852 ± 14**			**177 ± 10**	
	O. C.		31± 2	33 ± 3		874± 30	872 ± 17	171± 15	176 ± 8	
	Bias %		6.89	13.80		2.58	2.35	3.39	0.85	
	Precision %		6.45	9.09		3.43	1.95	8.77	4.73	
Cd	**C. C.**		**0.03 ± 0.01**			**3.5 ± 0.4**			**26.3 ± 2.1**	
	O. C.			< LD			3.1 ± 0.2			26.6±0.7
	Bias %						11.43			1.14
	Precision %						6.45			2.63
Pb	**C. C.**		**13.3 ± 2.4**			**0.48 ± 0.04**			**10.4 ± 1.9**	
	O. C.		15.0 ± 1.0	12.7 ± 0.7		<LD	0.51±0.05			10.9 ± 0.9
	Bias %		12.78	4.51			6.25			4.81
	Precision %		6.66	5.51			9.80			8.26

C.C Certified Concentration O.C Obtained Concentration
( ) Not certified ND Not determined

***Table 5**: Detection Limits (μg/g).*

Samples	***Detection Limits (μg/g)***						
	Method	*Ni*	*Cr*	*Cu*	*Zn*	*Cd*	*Pb*
Biological Material	*VRA*			0.30	0.50	0.023	0.25
	EAA	5.0	7.5	3.5	2.5		3.5
	FRX (ET)	15		20	10		8
Non Biological Material	*VRA*			0.50	0.60	0.10	0.40
	EAA	5.0	7.5	3.5	2.5		3.5
	FRX (I/C)			38	9		15

3.2. Determination of Cr, Ni, Cu, Zn, Cd and Pb contents in the soil and *sorgo* samples. The correlation between the soil-plant metal distribution and the growth of the plants.

The calibration curves obtained for Cu, Zn, and Pb in non-biological CRM by EDXRF (I/C method) are presented in the figure 1. A good correlation coefficient was obtained within the interval of concentration selected. Such curves were used for the determination of these metals in soil samples.

The concentrations determined in the soil and corresponding vegetable samples for experiments with and without metal additions are shown in the tables 6-9. The statistical comparison of the concentration data obtained using the different analytical methods showed that no significant differences between the compared values were observed for all elements. The latter confirms the reliability of the used methods.

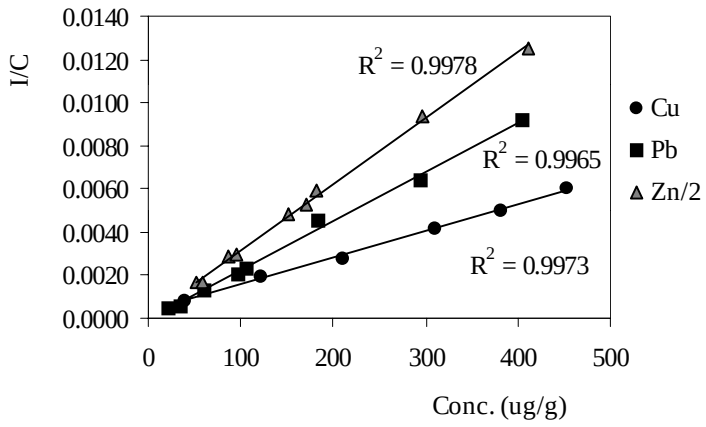


Figure 1. Calibration curves by FRX (I/C method)

***Table 6**: Analysis of soil samples without addition.*

Element	***Obtained Concentration (μg/g)***				
	EDXRF (Std. Add.)	*EDXRF (I/C)*	*EDXRF (ET)*	*AAS*	*ASV*
Cr				232 ± 9	
Ni	296 ± 22			297 ± 10	
Cu		222 ± 14	222 ± 11	200 ± 22	
Zn		212 ± 12	204 ± 11	208 ± 10	220 ± 10
Cd					2.2 ± 0.4
Pb		72 ± 6	74 ± 5	67 ± 3	71 ± 5

***Table 7**: Analysis of soil samples with addition.*

Element	*Sample No*	*Obtained Concentration (µg/g)*			
		AAS	*ASV*	*EDXRF (I/C)*	*EDXRF (ET)*
Cr	*81 -84*	289 ± 22			
	85 - 88	383 ± 16			
	89 - 92	689 ± 40			
	93 - 96	1278 ± 218			
Ni	*1 - 4*	410 ± 26			
	5 - 8	555 ± 5			
	9 - 12	772 ± 22			
	13 - 16	1132 ± 44			
Cu	*65 - 68*			274 ± 30	283 ± 22
	** 65*	* 248 ± 8	* 236 ± 10	* 299	* 300
	69 - 72			317 ± 32	324 ± 37
	** 69*	* 292 ± 11	* 282 ± 11	* 346	* 353
	73 - 76			444 ± 37	438 ± 42
	** 73*	* 403 ± 13	* 413 ± 15	* 435	* 433
	77 - 80			615 ± 29	582 ± 35
	** 77*	* 576 ± 25	* 626 ± 29	* 608	* 572
Zn	*49 - 52*			447 ± 54	426 ± 62
	** 49*	*`419 ± 13	* 389 ± 15	* 411	* 384
	53 - 56			649 ± 39	618 ± 48
	** 53*	* 633 ± 18	* 613 ± 20	* 652	* 592
	57 - 60			1075 ± 38	1024 ± 37
	** 57*	* 1108 ± 27	* 1028 ± 26	* 1065	* 998
	61 - 64			1900 ± 71	1816 ± 43
	** 61*	* 1800 ± 34	*1858 ± 42	* 1899	* 1810
Cd	*17 - 20*		3.8 ± 0.6		
	21 - 24		6.9 ± 0.7		
	25 - 28		12.6 ± 0.4		
	29 - 32		25 ± 1		
Pb	*33 - 36*			105 ± 17	108 ± 16
	** 33*	* 97 ± 5	* 90 ± 3	* 99	* 98
	37 - 40			136 ± 39	134 ± 21
	** 37*	* 116 ± 8	* 110 ± 5	* 112	* 117
	41 - 44			225 ± 16	202 ± 15
	** 41*	* 256 ± 13	* 248 ± 10	* 240	* 215
	45 - 48			350 ± 32	313 ± 24
	** 45*	* 365 ± 17	* 357 ± 13	* 336	* 299

* Controlled sample by different methods.

Table 8: *Analysis of vegetable samples grown on red-ferralitic soil without addition.*

Element	Obtained Concentration (μg/g)		
	AAS	**ASV**	**FRX (ET)**
Cr	< 7.5		
Ni	16 ± 3		< 15
Cu	8 ± 1	9 ± 1	< 20
Zn	23 ± 2		
Cd		0.58 ± 0.05	
Pb		1.04 ± 0.08	

Table 9*: Analysis of vegetable samples grown on red-ferralitic soil with addition*

Element	***Sample Number***	***Obtained Concentration (μg/g)***		
		AAS	*ASV*	*FRX (ET)*
Ni	*1 - 4*	20 ± 6		-
	5 - 8	19 ± 5		-
	9 - 12	24 ± 5		-
	13 - 16	21 ± 5		-
Cu	*65 - 68*	11 ± 3	10 ± 2	
	69 - 72	10 ± 2	10 ± 2	
	73 - 76	9 ± 1	9 ± 1	
	77 - 80	13 ± 2	14 ± 3	
Zn	*49 – 52*	90 ± 14		94 ± 21
	53 - 56	172 ± 22		178 ± 26
	57 - 60	503 ± 39		561 ± 29
	61 - 64	851 ± 66		878 ± 97
Cd	*17 - 20*		2.1 ± 0.2	
	21 - 24		3.4 ± 0.2	
	25 - 28		5.0 ± 0.4	
	29 - 32		8 ± 1	
Pb	*33 - 36*		0.87 ± 0.05	
	37 - 40		1.3 ± 0.3	
	41 - 44		1.7 ± 0.2	
	45 - 48		2.4 ± 0.2	

The variations of the dried vegetable mass, and the Ni, Cu, Zn, Cd and Pb concentrations in the plants as a function of the metal content in the soils are presented in the figures 2-6. A remarkable effect was obtained in the cases of additions of Zn and Cr both for the metal soil-plant distribution and for the growth of the plant (see pictures 1-2). In particular, for the latter, the lowest added concentration just provoked the death of the plant. As can be observed, in the case of addition of Ni, no relevant changes were observed when increasing the metal concentration in the soil. A slight increase of the Pb content in the *sorgo* did not affect the mass of the dried vegetable sample.

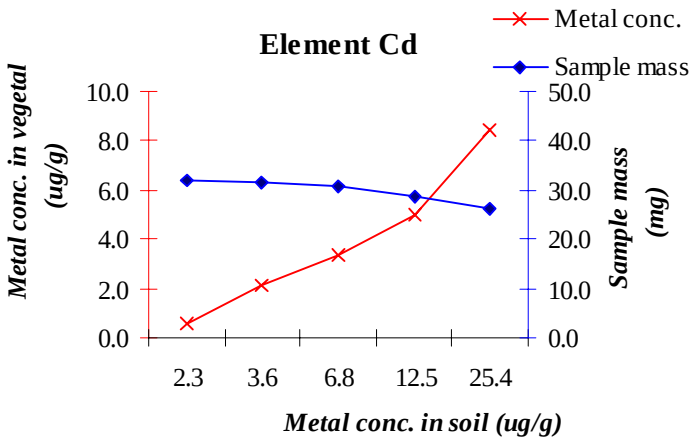


Figure 2. Correlation between Cd soil-plant distribution and the growth of the plants

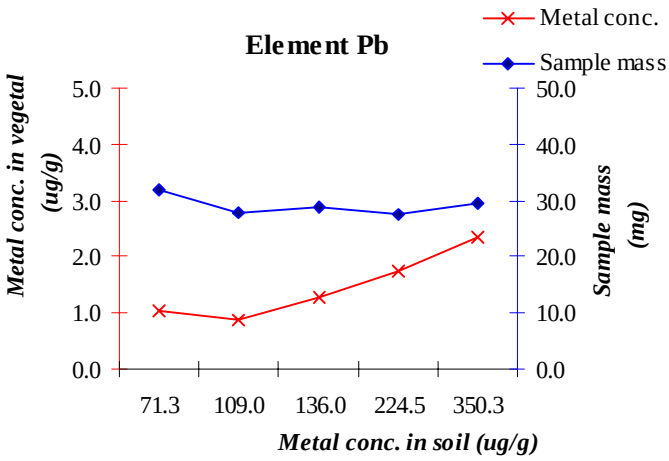


Figure 3. Correlation between Pb soil-plant distribution and the growth of the plants

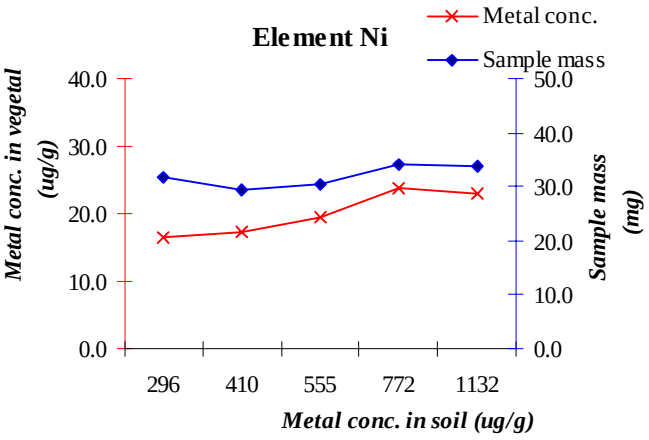


Figure 4. Correlation between Ni soil-plant distribution and the growth of the plants

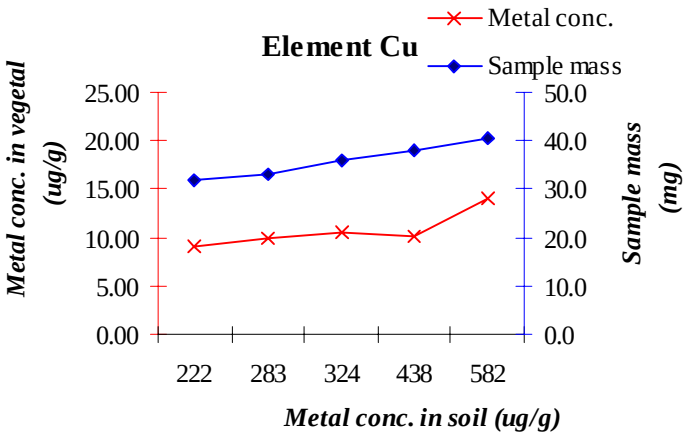


Figure 5. Correlation between Cu soil-plant distribution and the growth of the plants

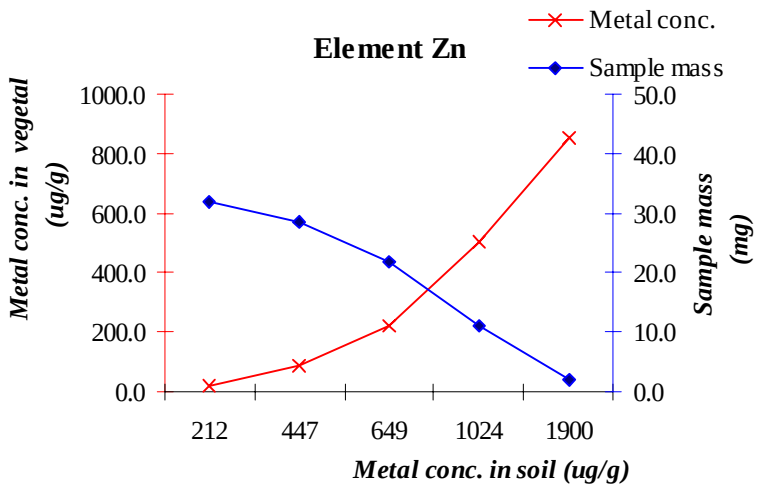
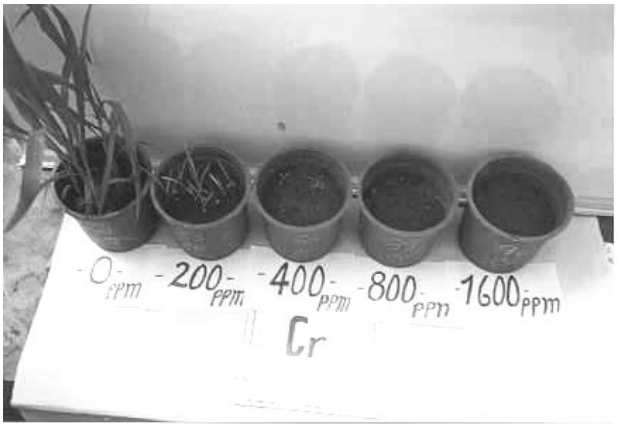


Figure 6. Correlation between Zn soil-plant distribution and the growth of the plants

Picture 1. Experiment of Zn



Picture 2. Experiment of Cr

For copper additions, an opposite effect was observed. A slight increase of metal concentration in the plant as well as of dried sample mass was obtained.

It is necessary to argue that the results reported herein are only part of a larger research studying the influence of different heavy metal contents onto the growth of *sorgo*, which id used in animal feeding, in the most prevalent Cuban soils. A final evaluation resulting of these studies will be presented in the future.

CONCLUSIONS AND RECOMENDATIONS

1. EDXRF, AAS and ASV methods are reliable for the determination of the Cr, Ni, Cu, Zn, Cd and Pb contents in *ferralitic-red* soil and in *sorgo* plant samples.
2. The addition of Cr and Zn to *ferralitic-red* soils in amounts ensuring the reported final concentration levels leads to an unfavorable *sorgo* growth.
3. Similar studies should be carried out in the most frequent types of Cuban soils.

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