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A BIOMEDICAL APPLICATION OF PIXE - STUDIES OF TRACE ELEMENTS IN HUMAN HAIR, IN CONTROL AND HYPERACTIVE CHILDREN *

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ABSTRACT

Hair samples from hyperactive and control children have been analyzed for their trace elemental contents by PIXE. The main elements examined are S, K, Ca, Fe, Co, Ni, Cu, Zn, Se, Br, Hg and Pb. The significant difference between the elements in control population and in patients suffering from pathological conditions are examined. Investigations of the possible linear and multiple correlations between elements in each population are made. The work indicates that some elements do exhibit variation with pathological state.

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1. INTRODUCTION

Over the last twenty years the importance of trace elements in various biological processes has been underlined. It has been shown that normal growth and health depend on the continuous and sufficient supply of certain elements and the ability to assess levels of these elements is very important. The biological effect of an element depends on its concentration in the organism, and these elements are sometimes present as part per million or less therefore, in sample analysis, factors like contamination, sample size, sample treatment, and the limits of detection are very important. Easily accessible biological samples that validly reflect the status of individuals are needed as well as sensitive and specific technique for assessment of levels. PIXE using hair as the biological sample could be a useful biomedical research tool [1].

2. SUBJECTS AND METHODS

Shreene [2] has defined the hyperactive child as one whose abnormal behaviour is accentuated as he develops. He is prone to cot-rocking and head banging, and his conduct is consistent and troublesome. He is easily excitable and often in tears and seems unable to sit still for more than a minute or two. Hyperactive children have learning difficulties. They seem to require little food and less sleep. Boys are effected more than girls.

In the present work 20 hair samples of hyperactive mentally handicapped children and 20 non-hyperactive mentally handicapped as a control population are kindly selected by the Greaves Hall Hospital, Southport. The subjects were drawn from the same institution, the hair samples were selected so that the two groups under consideration would match with each other as closely as possible. The hyperactive children comprise eight females and twelve males. The average age for the group was 14.3 ± 2.9 years. The control population consisted of children of an average age 11.7 ± 2.8 years, eight males and twelve females. Samples were obtained from corresponding positions on the scalp of the donors. When the hair samples arrived at the laboratory they were removed from their paper envelopes, cut into short segments 1-2 cm in length and stored in labelled plastic screw capped jars.

Unwashed hair samples were digested using teflon bomb [1], the digested hair dopped with Y_{39} (internal standard) was deposited in the form of small drops each of 5 μL volume on a 5 μm kimfal backing. Each target was irradiated for 25 μc with a beam current 10 nA at a proton energy 2.37 MeV. An Al x-ray absorber of 7 μm thickness was inserted between the detector and the irradiated target. Representative spectra of the hyperactive and non-hyperactive groups are shown in Figs. 1 and 2 respectively.

3. RESULTS AND STATISTICAL ANALYSIS

The elemental concentrations obtained from the 10 samples are represented in Tables 1 for the hyperactive and Table 2 for non-hyperactive group. Graphical representation for the mean elemental concentration for the elements measured in the two groups are given in Fig. 3. Tests of significance of differences between hyperactive samples and control group for each mean elemental concentration using the T-test, were performed. Table 3 shows the results of the test. The results of the calculated t, were compared with the tabulated critical values (see Ref. [3]), it is seen that the only significant differences at the p = 0.05 are for K, Ca and Se, for which the hyperactive children show a higher level in K, and lower levels in Ca and Se. K and Ca are also significant at the P = 0.025 but no metal level were significant at the P = .005.

In comparing the individuals in these two groups, there appeared to be no greatly increased or decreased levels of the toxic elements in the hyperactive children examined, but there were a few examples, all in the control group, that did show high levels of Se and Hg. The highest value recorded was 118 ppm for Se, Fig. 4 and the highest Hg was 59.5 ppm, these were not in the same sample though one sample, Fig. 5, did have high levels of Se with 53 ppm and Hg with 30 ppm. It was noticed that these individuals are all females, T-tests between males and females in the control group for Se and Hg showed significance in Se at the P = .025, but not for Hg.

The linear correlation coefficients between concentration of elements, for all possible pairs of elements in the data set for the hyperactive children were calculated and represented in Table 4. Table 5 represents similar calculations for the control group. Each table is divided into two triangles, for ease, in the search for significant values of correlation. The upper triangle gives the correlation coefficients between all pairs of elements, whereas the lower triangle shows only those which are correlated at .05 level. Table 6 represents only the significant correlations at the .05 level with the upper triangle for hyperactive group - and the lower triangle for the control group, for ease, in search for any agreement in the significant values.

In comparing the calculated correlation coefficients with the tabulated values (see Ref. [4]), it can be seen that there are significant correlations in the hyperactive group at the 0.01 significant level between Ca/Hg, Cu/Pb,

Co/Se, Co/Br, Co/Mg, Co/Pb, Se/Hg, Se/Pb, Br/Hg and Hg/Pb. Also significant at the .05 level, are Ni/Se, Cu/Br and Br/Pb. The highest correlation .796 is between Hg and Pb.

For control group results in Table 5 show at the .01 level, significant correlations between Ca/Cu, Ca/Pb, Co/Ni, Co/Hg, Ni/Hg, and significant at the .05 level, K/Ca, Br/Zn. The highest correlation, .749, is between Ni and Co in this group. For the results shown in Table 6, there is agreement between some of the significant correlations found in the two groups. Specifically the significant correlations for Co/Ni, Co/Hg, and Co/Pb.

4. CONCLUSIONS

From the above results the following conclusions may be drawn:

1. There is a significant difference between the levels of K and Ca in child hyperactive patients and those in controls, with levels in the control being lower in K and higher in Ca than in the hyperactive group.

2. In the group of hyperactive children examined there is no evidence of high levels of the elements Se and Hg. However, in the control group some samples do show very high levels of one or both of these elements.

3. There is a significant difference between the levels of Se in the female to those of the male in this control group.

4. Several elements tended to increase and decrease together in the hair specimens. From Table 6 there are 14 significant correlations for the hyperactive children and 6 significant correlations for the control group at $P < 0.05$. All significant correlations showed positive trends for these two groups. Some significant correlations do support the previous demonstration of elemental interaction in hair analysis. These are between Ca/Pb in the hyperactive and Cu/Pb in the control, are similar to findings reported in [5] in a control group.

Interaction between elements may be important from the point of view of the therapy and/or the prevention of side-effects [5]. Therefore the examination of interactions using linear and multiple correlations, between elements which have been measured simultaneously is of particular value, and further investigations are desirable.

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REFERENCES

[1] N.S. Shakir, Ph.D. Thesis, University of Aston in Birmingham (1984).

[2] C.M. Shreene, "A state of perpetual motion hyperactivity", (World Medicine, 1982).

[3] P.R. Bevington, Data Reduction and Error Analysis for the Physical Sciences, (McGraw-Hill, Inc. USA, 1969).

[4] O.L. Davies and P.L. Goldsmith, Statistical Methods in Research and Production, (Oliver and Boyd, Edinburgh, UK, 1972).

[5] P.J. Barlow, Ph.D. Thesis, University of Leeds (1980).

Table 1

Element levels in ppm for the

hyperactive children group

Sample	Concentration in ppm										
	S	K	Ca	Co	Ni	Cu	Zn	Se	Br	Hg	Pb
A	30182	235	750	2	2	58	225	3	4	3	9
B	45476	116	349	-	2	33	163	6	5	6	5
C	14983	128	229	-	2	32	159	5	8	3	6
D	26324	358	387	5	13	107	157	10	5	6	12
E	23585	311	319	8	8	18	175	17	13	2	9
F	24206	279	485	2	5	58	98	2	-	3	5
G	28126	194	562	3	-	93	119	8	3	5	12
H	26737	595	487	-	3	88	146	10	1	6	9
I	32332	235	538	1	1	114	214	6	9	5	11
J	14135	145	404	6	3	30	140	10	7	14	16
K	30494	89	289	4	2	26	220	8	16	5	9
L	21045	195	322	2	6	20	198	14	3	9	9
M	32439	434	287	1	5	29	177	9	7	4	9
N	14346	60	580	2	4	32	135	7	8	6	11
O	21816	288	255	3	2	35	117	3	12	11	7
P	17261	55	354	4	3	16	161	6	18	15	9
Q	26702	140	486	3	5	17	157	7	12	3	11
R	25340	501	604	3	1	14	188	6	18	-	7
S	26634	90	1245	9	6	35	213	19	27	38	23
T	20050	57	841	3	7	16	180	5	11	3	10
mean±	25111±	225±	489±	3±	4±	44±	167±	8±	9±	7±	10±
σ	7434	153	241	2	3	32	36	4	7	8	4

Table 2

Element levels in ppm for the

non-hyperactive control group

Sample	Concentration in ppm										
	S	K	Ca	Co	Ni	Cu	Zn	Se	Br	Hg	Pb
CA	22628	54	562	2	2	19	117	45	32	7	11
CB	11833	99	281	10	10	37	94	17	27	7	10
CC	22023	87	422	3	4	20	175	3	9	6	3
CD	20861	191	822	11	14	31	256	13	-	31	17
CE	17254	55	359	-	2	23	149	8	6	10	7
CF	19063	102	423	2	8	37	263	9	4	11	11
CG	18556	62	356	2	3	18	145	5	10	5	3
CH	23323	49	649	5	6	22	198	53	12	30	4
CI	29920	56	1565	2	4	20	137	118	-	9	10
CJ	29508	43	770	2	2	22	167	3	1	5	5
CK	20366	89	732	3	3	22	180	7	9	-	6
CL	18098	107	421	2	4	31	189	3	4	4	3
CM	15673	252	347	11	8	45	178	42	3	60	-
CN	22977	215	895	4	4	62	152	4	7	6	7
CO	32614	263	426	4	3	50	197	-	6	5	4
CP	19972	210	1227	3	6	366	152	9	1	9	9
CQ	20921	134	1510	-	3	227	171	7	7	16	23
CR	16991	117	672	3	3	125	196	7	24	2	3
CS	26045	102	752	1	5	52	149	10	18	5	5
CT	22852	126	855	1	6	185	204	7	19	4	13
mean ±	21898±	121±	702±	4±	5±	71±	173±	18±	10±	12±	8±
1 σ	4576	69	372	3	3	54	41	28	9	14	6

Table 3

Comparison between hyperactive and non hyperactive control groups for elemental levels

Element	mean levels ppm hyperactive group	mean levels ppm non hyperactive group	"t" value	significance 95 %
S	25111 ± 7434	21898 ± 4576	1.65	not sig.
K	225 ± 153	121 ± 69	2.77	significant
Ca	489 ± 241	702 ± 372	2.15	significant
Co	3 ± 2	4 ± 3	0.53	not sig.
Ni	4 ± 3	5 ± 3	0.83	not sig.
Cu	44 ± 32	71 ± 54	1.27	not sig.
Zn	167 ± 36	173 ± 41	0.58	not sig.
Se	8 ± 4	18 ± 28	1.65	not sig.
Br	9 ± 7	10 ± 9	0.23	not sig.
Hg	7 ± 8	12 ± 14	1.19	not sig.
Pb	10 ± 4	8 ± 6	0.97	not sig.

Table 4

Correlation coefficients, r, for element/element levels in the hair of hyperactive children (20 samples)

Upper triangle: all correlation coefficients

Lower triangle: only those which are significantly correlated at the 0.05 level

	S	K	Ca	Co	Ni	Cu	Zn	Se	Br	Hg	Pb
S	-	0.215	0.033	-0.261	-0.105	0.288	0.311	-0.022	-0.12	-0.119	-0.15
K		-	-0.181	-0.227	0.088	0.345	-0.104	0.021	-0.281	-0.32	-0.259
Ca			-	0.331	0.061	0.067	0.32	0.248	0.403	0.530	0.645
Co				-	0.436	-0.21	0.113	0.651	0.593	0.575	0.653
Ni				0.436	-	0.046	-0.014	0.452	-0.009	0.116	0.257
Cu						-	-0.113	-0.099	-0.469	-0.082	0.116
Zn							-	0.324	0.424	0.158	0.26
Se				0.651	0.452			-	0.349	0.557	0.639
Br				0.593		-0.469			-	0.571	0.453
Hg			0.530	0.575				0.557	0.571	-	0.796
Pb			0.645	0.653				0.639	0.453	0.796	-

Table 5

Correlation coefficients for element/element levels in the hair of the non hyperactive cortrol population (20 samples)

Upper triangle: all calculated correlation coefficients
Lower triangle: only those which are significantly correlated at the 0.05 level

	S	K	Ca	Co	Ni	Cu	Zn	Se	Br	Hg	Pb
S	–	-0.006	0.350	-0.240	-0.23	-0.132	-0.057	0.266	-0.164	-0.275	0.005
K		–	0.041	0.466	0.359	0.377	0.263	-0.243	-0.288	0.388	0.016
Ca			–	-0.307	-0.086	0.548	-0.056	0.387	-0.286	-0.078	0.570
Co		0.466		–	0.749	-0.216	0.090	0.091	-0.023	0.653	-0.068
Ni				0.749	–	0.034	0.386	0.05	-0.10	0.505	0.293
Cu			0.548			–	-0.001	-0.214	-0.055	-0.068	0.394
Zn							–	-0.246	-0.414	0.246	0.115
Se								–	-0.041	0.315	0.017
Br									–	-0.29	-0.002
Hg				0.653	0.505					–	-0.034
Pb			0.570								–

Table 6

Significant correlation coefficients at 0.05 level for Element/Element in the hair

upper triangle: for hyperactive children

lower triangle: for non hyperactive children

	S	K	Ca	Co	Ni	Cu	Zn	Se	Br	Hg	Pb
S	–										
K		–									
Ca			–							0.53	0.645
Co		0.466		–	0.436			0.651	0.593	0.575	0.653
Ni				0.749	–			0.452			
Cu			0.548			–			0.469		
Zn							–				
Se								–		0.557	0.639
Br									–	0.571	0.453
Hg				0.653	0.505					–	0.796
Pb			0.571								–

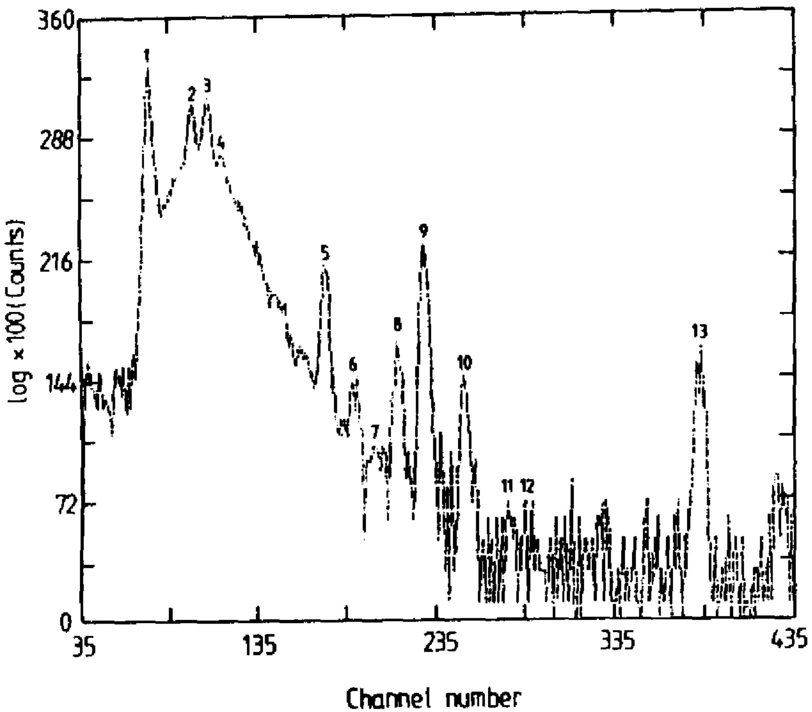

Fig 1 Representative PIXE spectrum for one of the hair sample from the hyperactive group.

1 - S (K α + K β) 8 - Cu (K α)
2 - K (K α) 9 - Zn (K α)
3 - Ca (K α) 10 - Zn (K β)
4 - Ca (K β) 11 - Pb (L α)
5 - Fe (K α) 12 - Se (K α)
6 - Fe (K β) 13 - Y (K α)
7 - Ni (K α)

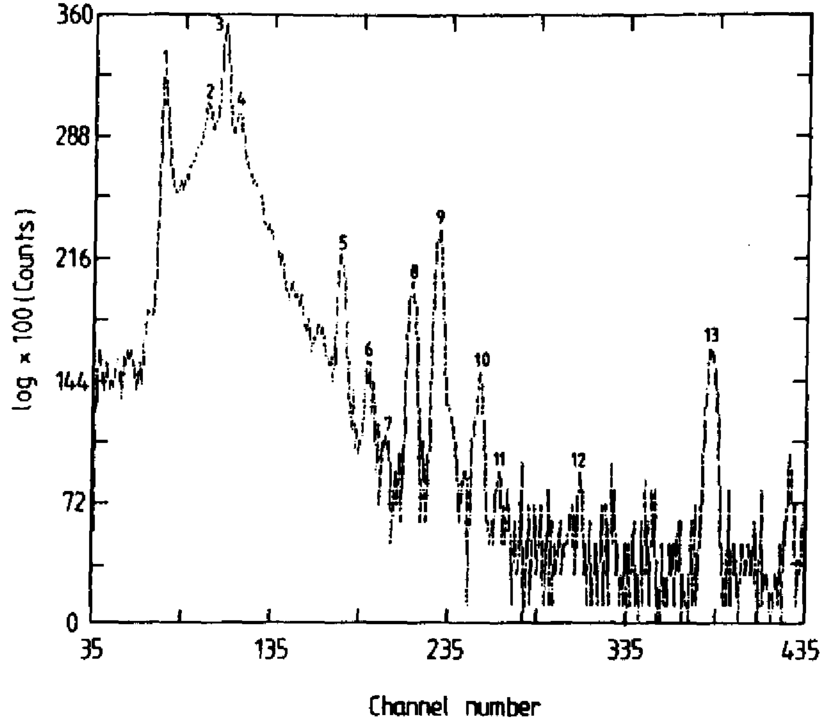

Fig 2 Representative PIXE spectrum for one of the hair sample from the non-hyperactive control group.

1 - S (K α + K β) 8 - Cu (K α)
2 - K (K α) 9 - Zn (K α)
3 - Ca (K α) 10 - Zn (K β)
4 - Ca (K β) 11 - Hg (L α)
5 - Fe (K α) 12 - Br (K α)
6 - Fe (K β) 13 - Y (K α)
7 - Ni (K α)

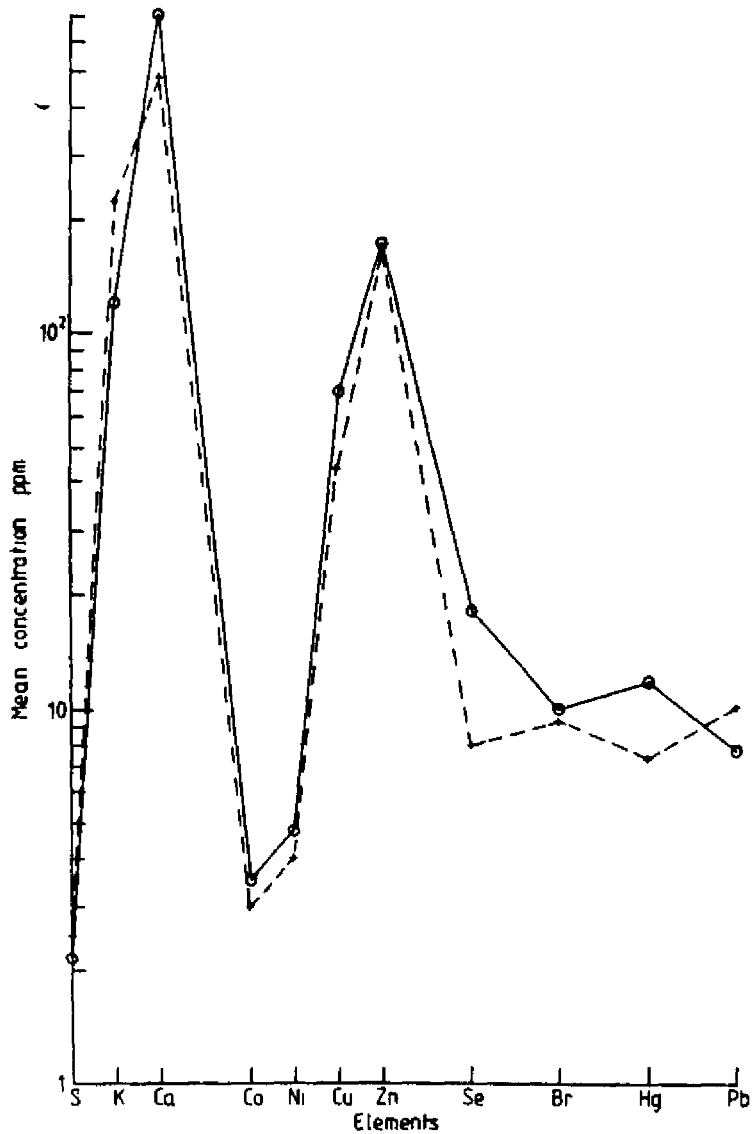

Fig 3 Mean concentrations in ppm for elements measured in hyperactive and non-hyperactive control (+ hyperactive, o control)

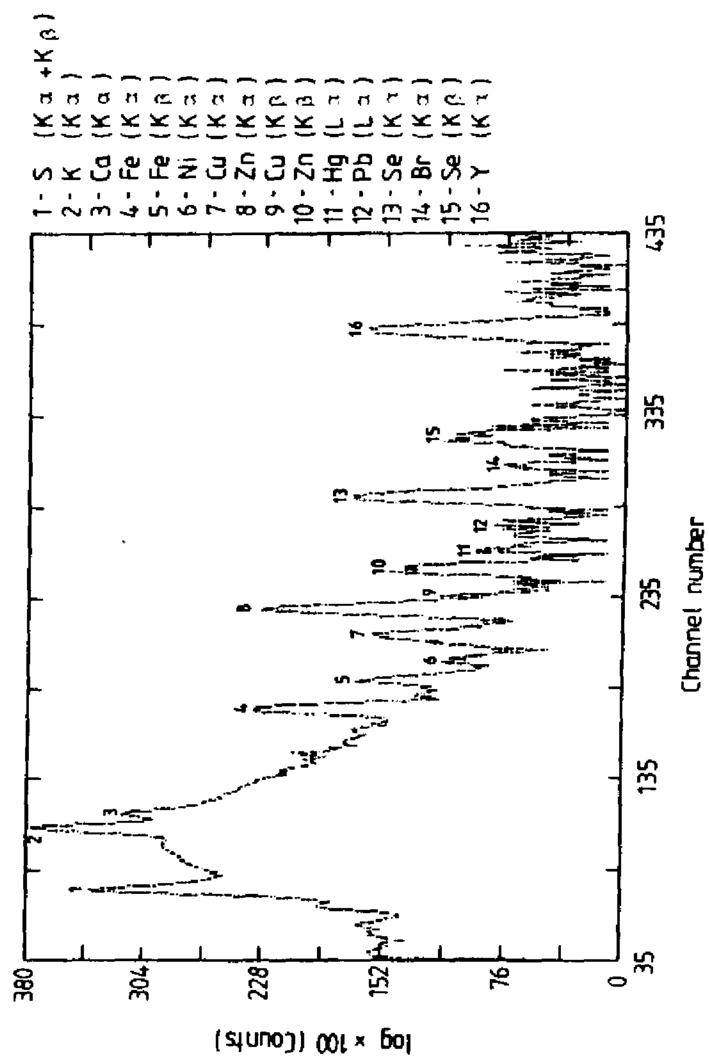

Fig 4 PIXE spectrum for the hair sample C1, showed high Se level.

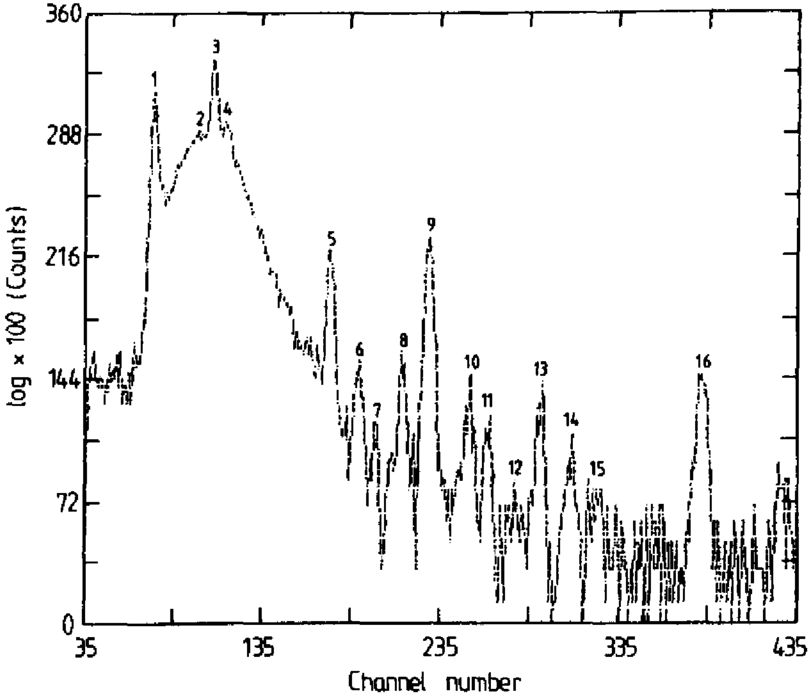

Fig 5 PIXE spectrum for the hair sample CH, showed high level in Se and Hg.

1 - S (K α + K β)	9 - Zn (K α)
2 - K (K α)	10 - Zn (K β)
3 - Ca (K α)	11 - Hg (L α)
4 - Ca (K β)	12 - Pb (L α)
5 - Fe (K α)	13 - Se (K α)
6 - Fe (K β)	14 - Br (K α)
7 - Ni (K α)	15 - Se (K β) + Pb (L β)
8 - Cu (K α)	16 - Y (K α)