

AN ANNUAL PUBLICATION

VOLUME 13 / JUNE 1984

**HACETTEPE BULLETIN OF
NATURAL SCIENCES AND ENGINEERING**

A BULLETIN PUBLISHED BY
FACULTY OF SCIENCES OF HACETTEPE UNIVERSITY



**HACETTEPE BULLETIN OF
NATURAL SCIENCES AND ENGINEERING**

AN ANNUAL PUBLICATION VOLUME 13 / JUNE 1984

EDITORIAL BOARD:

TIMUR KARAÇAY (CHAIRMAN)
AHMET NOYAN / SONER GÖNEN

SECRETARY

FAHRETTİN SAVCI

A BULLETIN PUBLISHED BY
FACULTY OF SCIENCES OF HACETTEPE UNIVERSITY



SUBSCRIPTION RATES

Annual subscription including postage:

TURKEY : L 250 , FOREIGN : \$ 5.00

Inquiries concerning reprints or subscriptions should be forwarded to :

BACETEPE UNIVERSITESI
FEN FAKULTESI
SEVTEPE , ANKARA , TURKEY

PRINTED AT THE FACULTY PRESS
1984

NACETTEPE BULLETIN OF NATURAL SCIENCES AND ENGINEERING

CONTENTS

A. BIOLOGICAL SCIENCES

1- Kuru, A.

Isolation and Characterization of the Major Reserve Protein Fractions of Soybean (Disappearance of the 2S Protein Fraction During Cold-Precipitation 11S Protein)

7- Zencirci, N.

Etude de la Toxicite et de L'accumulation du Baryum et du Titane Chez Deux Espèces de Gammarus

B. PHYSICAL SCIENCES

19- Adams, F.C-Akyuz, S- Akyuz, T
De Waele, J.K.

Laser Microprobe Mass (Lmass) and Infrared Spectral Analysis of Thorium-Rare Earths Mineral From the Deposits of Eastern Turkey

29- Agustoslu, S- Akyuz, S.

Low Temperature Infrared Spectra of Cd and Ni Diquinoline-Tetracyanonickelate Complexes

33- Eremci, S.

Determination of Exact Curie Temperature by Using Cluster Variation and Constant Coupling Method

41- Öztürk, E.

Proton Spin-Lattice Relaxation Study of Some Hoffmann Type-Complexes of Pyridine and Pyridine Derivatives

45- Severcan, F.

Melittin-Phospholipid Interaction : ESR Studies

C. MATHEMATICAL SCIENCES

53- Bulut, A.

A Note on the Characterization of F-Continuous Functions on Cr Fuzzy Topological Spaces

59- Ibrahimoglu, I.

(C,k)-Summability of the Dirichlet L-Functions

63- Ibrahimoglu, I.

The Value of the Dirichlet L-Functions at Negative Integral Points

69- Khanfar, M.I.

Complex Representations of $PSL(2,7)$

CONTENTS (CONT.)

- 81- Koç, C. A Calculation of the Schur Index
- 87- Koç, C. A Theorem on the Rationality
- 91- Koç, C. Proof of the Serr'e Conjecture
- 95- Koçak, Ş. A Classification Theorem for Principal Bundles With Discrete Group
- 103- Kuruoglu, N- Keles, S. On the Pairs of the Generalized Ruled Surface Correspond to Each Other Under the Exponential Homothetic Motion in E^3
- 111- Kutkut, M. Joint Spectral Sets and the Cartesian Product of Spectral Sets
- 119- Kutkut, M. Some Topological Properties of Certain Classes of Operators
- 129- Sözer, E- Kutsal, A- Sözer, T. Labour Force Status Concepts
- 139- Tok, İ. On the Conditional Variational Principle
- 147- Tok, İ. Sur Le Schema De Bernoulli Conditionnel
- 157- Tutalar, H.İ. On A Construction of Algebraic Function Fields

ISOLATION AND CHARACTERIZATION OF THE THREE MAJOR RESERVE
PROTEIN FRACTIONS OF SOYBEAN.

II. DISAPPEARANCE OF THE 2S PROTEIN FRACTION DURING
COLD-PRECIPITATION OF 11S PROTEIN.

(Soya fasulyesi üç ana depo proteini fraksiyonunun
yalitimı ve belirlenmesi.

II. 11s proteinin sogukta-cökeltimesi sırasında
2s protein fraksiyonunun kaybolması)

Avni KURU

SUMMARY: Cold-soluble and cold-insoluble fractions of the whole protein extract of soybean (*Glycine max L. Merrill cv. Wayne*) seeds were analyzed on a Sepharose-6B column. Disappearance of the 2s fraction protein from the whole protein extract during cold-precipitation strongly supports the occurrence of aggregation of subunits of the fraction proteins of soybean.

INTRODUCTION

Cooling concentrated aqueous extracts of soybean meal causes precipitation of proteins which redissolves on warming (BRIGGS and MANN 1950). GHETIE and BUZILA (1962) introduced the term of plant cryoproteins to designate proteins that precipitate when aqueous extracts of seeds are cooled. The cryoprotein of soybean has been referred to as cold-precipitable protein or cold-insoluble fraction and it consists primarily of the 11S ultracentrifugal component (BRIGGS and WOLF 1957, WOLF and BRIGGS 1958, WOLF and SLY 1967) and cryoprecipitation has been used to purify the 11S component protein by the same workers (WOLF and BRIGGS 1957, WOLF and SLY 1967).

The aim of this work was to investigate the pattern of the major fraction proteins eluted from the Sepharose-6B column after cold-precipitation.

DISAPPEARANCE OF THE 2S PROTEIN FRACTION

MATERIALS AND METHODS

Soybean meal prepared from the dry soybean (*Glycine max* L. Merrill cv. Wayne) seeds was used for protein extraction as described by KURU (1981, 1983). Protein extract in salt-buffer was dialyzed against distilled water for 48 h in a cold room for cold-precipitation of the 11S protein. After dialysis the cold-insoluble proteins were precipitated through centrifugation at 17.000 rpm for 20 min at 4°C in a Beckman SW-25-1 rotor. The precipitate, cold-insoluble protein was dissolved in salt-buffer (32.5 mM K_2HPO_4 , 2.6 mM KH_2PO_4 , pH 7.6, and including 0.4M NaCl, 0.01 M 2-mercaptoethanol, and 10^{-6} M phenylmethylsulphonyl fluoride) and applied to Sepharose-6B column equilibrated with the same buffer. The supernatant, cold - soluble fraction was dialyzed against distilled water and lyophilized. The lyophilized proteins were dissolved in salt-buffer and analyzed by chromatography on Sepharose-6B. In this way the protein components in the cold-insoluble and cold-soluble fraction of the whole extract were compared.

RESULTS AND DISCUSSION

Figure 1 shows the chromatographic separation of the crude 11S protein precipitated in the cold. As seen in this figure, cold-precipitable protein includes a large amount of 15S protein and very little 7S fraction protein, in addition to the main 11S fraction protein. Precipitation of the different fraction proteins in the cold in a varying degree has also been shown by WOLF and SLY (1967), and WOLF et al. (1962).

Figure 2 shows the separation of cold-soluble proteins (supernatant fraction of the cold-precipitated protein) on Sepharose-6B column. This separation shows that a large part of the 11S protein fraction is not precipitated in the cold and that the 15S fraction protein is also present in the supernatant. However, the 2S fraction protein which is not seen in the cold-precipitated fraction, is also not present in the supernatant (cold-soluble) fraction. This observation strongly supports the idea that the 2S fraction protein is readily dissociated into subunits and reassociated with the other fraction proteins to form 15S fraction aggregate protein during manipulation with the protein solution. Actually 2S protein must give a peak higher than 7S protein (HILL and BREIDENBACH 1974, KURU 1981,1983), but as seen in Fig 2, peak corresponding to 2S protein is almost diminished to a negligible degree.

DISAPPEARANCE OF 2S PROTEIN FRACTION

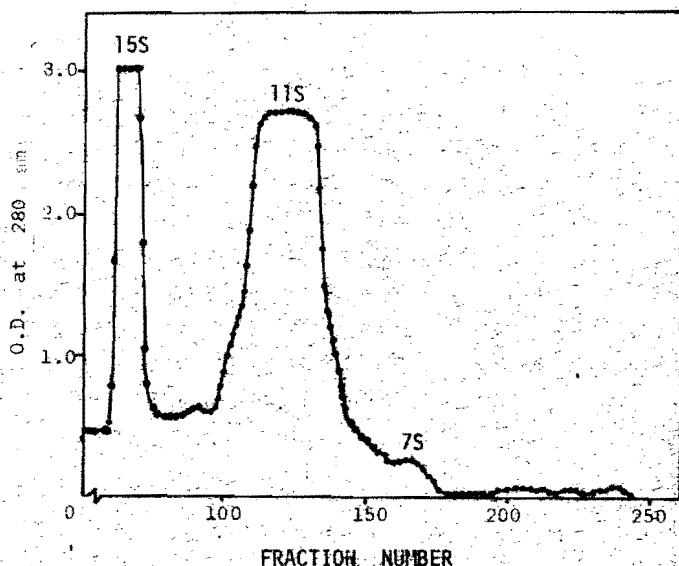


Fig. 1. Elution profile of cold-insoluble proteins from Sepharose-6B column.

The dissociation and association phenomena observed in component proteins of soybean during isolation and purification of the major component protein of soybean is a well known fact (ROBERTS and BRIGGS 1965, KOSHIYAMA 1968). The separation of only three sedimenting classes of proteins with sedimentation coefficients of 2.2S, 7.5S and 11.8S by sucrose density gradient analysis of the soybean storage protein was also demonstrated by HILL and BREIDENBACH (1974). This method of extraction and fractionation requires a minimum of manipulation and results in quantitative recovery of fractions with very little cross contamination (HILL and BREIDENBACH 1974). The appearance of the 15S protein fraction in sedimenting analysis of the protein extract (NAISMITH 1955, WOLF and BRIGGS 1959, WOLF and SLY 1967, ELDRIDGE and WOLF 1967) is probably due to cross contamination and differential denaturation during the employed procedures. This result also shows that more contact with water the soybean protein, has the higher degree of association, dissociation and aggregation phenomena are expected. Because, quick extraction of soybean storage proteins with minimum amount of buffer and separation of protein fractions through Sepharose-6B column chromatography revealed a big peak corresponding to 2S protein in addition to other fraction proteins (7S and 11S) KURU (1981, 1983). Separation of the three major protein components (2.2S, 7.5S and 11.8S) of soybean seeds by direct sucrose density gradient sedimentation (HILL and BREIDENBACH 1974) also support our idea mentioned above. It has been found that large number of globular protein

DISAPPEARANCE OF THE 2S PROTEIN FRACTION

molecules can be associate with themselves to form very large fibrous aggregates by properly varying conditions, e.g., by changing the temperature, concentration of protein or by adding salt (SAKAKIBARA and NOGUCHI 1976). Therefore, it is reasonable to interprete the increase of dissociation and association phenomenon observed in the fraction protein is due to the action of ordered water molecules on the protein surface. WOLF and BRIGGS (1956) reported that the solubility of 11S protein decreases to a minimum in 0.1 M NaCl and increases again in 1 M NaCl. This means that cations exhibit a salting-out effect at low salt concentration and a salting-in effect at higher salt concentration. Therefore, it is also

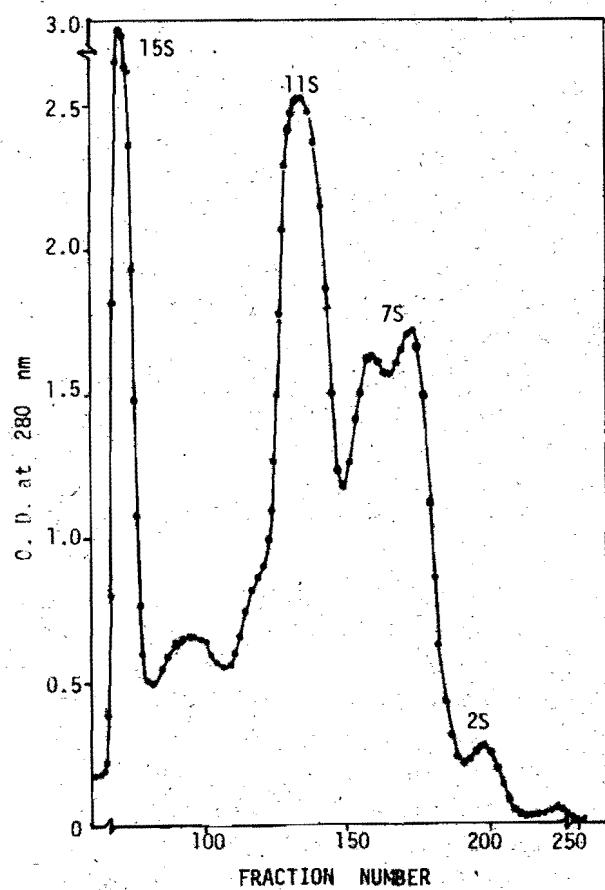


Fig. 2. Elution profile of cold - soluble proteins on Sepharose-6B column.

reasonable to infer that the association phenomenon of protein fractions by contact with water is an instance of ordinary salting-out phenomena.

DISAPPEARANCE OF THE 2S PROTEIN FRACTION

For this reason purification of the protein components from soybean extract by cold-precipitation is not an appropriate procedure. Chromatographic separation of the fraction proteins from the whole protein extract obtained by Method I as described by KURU (1981, 1983) may be advisable for quick separation of soybean storage proteins.

In the experiments designed to prove the disappearance of the 2S protein and the appearance of the 11S protein, it may be useful to mix labelled 2S protein fraction with the other fraction proteins under the condition of cold-precipitation and to observe the radioactivity distribution in fraction proteins, using gel electrophoretic analysis under denaturing conditions.

Acknowledgment. I am greatful to Prof.Dr. J.H. CHERRY of the Department of Horticulture, Purdue University, W.Lafayette, IN, U.S.A. for making available all of the equipment and facilities in his lab during this work. I also express my thanks to IAEA for supporting me financially with the grant of IAEA TMR/7810, Vienna-Austria.

ÖZET

Soya fasulyesi (*Glycine max L.* Merritt cv. Wayne) tohumları total depo proteinini ekstraktının soğukta-eriyen ve soğukta-erimeyen fraksiyonları Sepharose-6B kolonunda analiz edildi. Total protein ekstraktından, soğukta cükelme sırasında 2S fraksiyon proteininin ortadan kaybolması, soya fasulyesi fraksiyon proteinlerinin subunitlerinin agregat oluşturduğunu kuvvetle desteklenmektedir.

REFERENCES

- BRIGGS,D.R., and MANN,R.L. (1950). An electrophoretic analysis of soybean protein. *Cereal Chem.*, 27: 243-257.
- BRIGGS,D.R., and WOLF,W.J. (1957). Studies on the cold-insoluble fraction of the water-extractable soybean protein. I. Polymerization of 11S component through reactions of sulphhydryl groups to form disulfide bonds. *Arch. Biochem. Biophys.*, 72: 127-144.
- ELRIDGE,A.C.; and WOLF,W.J. (1967). Purification of the 11S component of soybean protein. *Cereal Chem.*, 44: 645-652.
- GHETIE,V., and BUZILA,L. (1962). Plant cryoproteins. *Acad. Rep. Populare Romine, Studii Cercetari Biochim.*, 5: 65-72.
- HILL,J.E., and BREIDENBACH,R.W. (1974). Proteins of soybean seeds. I. Isolation

DISAPPEARANCE OF THE 2S PROTEIN FRACTION

- and characterization of the major components. *Plant Physiol.*, 53:742-746.
- KOSHIMURA, I. (1968). Factors influencing conformation changes in 7S protein of soybean globulins by ultracentrifugal investigation. *Agric. Biol. Chem.*, 32: 879-887.
- KURU,A. (1981). Soya fasulyesi (*Glycine max L. Merrill* cv. Wayne) depo proteinlerinin saflastirilmalari ve *in vitro* sentezleri. (*Dissertation Thesis, University of Istanbul*)
- KURU,A. (1983). Isolation and characterization of the three major reserve protein fractions of soybean. I. Some external effects on the nature of extracted protein components. *Istanbul Univ. Fen Fak. Mec. Seri B* (in press).
- MAISMIN, W.E.F. (1955). Ultracentrifugal studies on soya bean protein. *Biochem. Biophys. Acta.*, 16: 203-210.
- ROBERTS, R.C., and BRIGGS, D.R. (1965). Isolation and characterization of the 7S component of soybean globulins. *Cereal Chem.*, 42: 71-85.
- SAKAKIBARA, M., and NOGUCHI, H. (1976). Interaction of 11S fraction of soybean protein with calcium ion. *Agric. Biol. Chem.*, 41: 1575-1580.
- WOLF, W.J., BABCOCK, G.E., and SMITH, A.K. (1962). Purification and stability of the 11S components of soybean proteins. *Arch. Biochem. Biophys.*, 99:265-274.
- WOLF, W.J., and BRIGGS, D.R. (1956). Ultracentrifugal investigation of the effect of neutral salts on the extraction of soybean proteins. *Arch. Biochem. Biophys.*, 63: 40-49.
- WOLF, W.J., and BRIGGS, D.R. (1958). Studies on the cold-insoluble fraction of the water-extractable soybean proteins. II. Factor influencing conformation changes in the 11S component. *Arch. Biochem. Biophys.*, 76: 337-393.
- WOLF, W.J., and BRIGGS, D.R. (1959). Purification and characterization of the 11S component of soybean proteins. *Arch. Biochem. Biophys.*, 85: 186-199.
- WOLF, W.J. and SLY, D.A. (1967). Cryoprecipitation of soybean 11S protein. *Cereal Chem.*, 44: 653-668.

DEPARTMENT OF BIOLOGY, UNIVERSITY OF ISTANBUL, TURKEY

ETUDE DE LA TOXICITE ET DE L'ACCUMULATION DU BARYUM ET DU TITANE CHEZ DEUX ESPECES DE GAMMARUS

Dr. Nevin Zencirci

RESUME

Dans ce travail nous avons étudié les effets toxiques de l'accumulation du baryum ($BaCl_2 \cdot 2H_2O$) et du titane ($TiCl_3$) sur *Gammarus pullex* (L.) d'eau douce et *Gammarus locusta* (Crustaces: Amphipodes) marins.

Les expériences ont été faites dans des solutions de métaux d'eau douce et d'eau marine. Les valeurs de DL_{50} (24 h) du baryum sont de 100 ppm pour *G.pullex* et de 75 ppm pour *G.locusta*. Les valeurs de DL_{50} (24 h) du titane sont de 200 ppm pour *G.pullex* et de 150 ppm pour *G.locusta*.

Pour les expériences d'accumulation les concentrations de baryum utilisées sont de 50 ppm, 25 ppm, 10 ppm, 1 ppm et de 0,1 ppm. Les concentrations de titane utilisées sont de 150 ppm, 50 ppm, 25 ppm, 10 ppm, 1 ppm et de 0,1 ppm.

Les valeurs de l'accumulation de métaux ont été déterminées par spectrographie des rayons X.

Les résultats nous ont montrés que les solutions de baryum sont plus toxiques que celles du titane et *G.locusta* est plus sensible que *G.pullex*. Chez deux espèces une très grande accumulation a été rencontrée dans l'estomac, l'hémolymphe et la cuticule. Par contre, une accumulation faible du baryum a été rencontrée dans les muscles et une très faible accumulation de titane dans les yeux.

INTRODUCTION

L'accroissement de la pollution de l'environnement par les métaux toxiques provenant souvent des déchets industriels, a provoqué des problèmes très particuliers dans les mers et dans les eaux douces. L'existence de ces polluants métalliques dans les milieux aquatiques a eu pour conséquence une accumulation de particules dans les organismes qui y vivaient; ces polluants métalliques constituaient un danger, non seulement pour la survie de la faune aquatique, mais aussi pour l'homme.

En partant de cette base, plusieurs chercheurs ont depuis longtemps étudié la question et ont constaté les effets toxiques des solutions de métal sur les organismes vivants. On utilise souvent les différentes espèces de *Gammarus* dans les expériences de pollution parce qu'ils présentent soit des facilités dans leurs élevages, soit une homogénéité pendant les tests faits avec les matières toxiques (Bellan-Santini et Reish, 1977).

D'après les explications ci-dessus faites et par l'étude des effets des ions métalliques sur les deux espèces de *Gammarus* on peut aboutir à des résultats positifs qui pourraient nous expliquer la pollution des eaux. A cette fin, des métaux très toxiques comme le cadmium, le cuivre, le plomb, le zinc et l'étain ont été déjà étudiés (Zencirci 1978-79, 1980a, 1980b).

Dans ce travail on a étudié les effets toxiques de certains métaux tels que le baryum et le titane ayant une très grande usage dans l'industrie et se trouvant en grande quantité dans les eaux douces et dans les eaux des Océans, sur les *Gammarus*.

MATERIEL ET MÉTHODES

Deux espèces de *Gammarus* ont été étudiées dans ce travail. *G. locusta* a été ramassé à Luc-Sur-Mer sur la côte Atlantique en France et intoxiqué pour l'expérience par le baryum ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) et le Titane (TiCl_3) mis en solution dans de l'eau de mer.

G. pulex a été ramassé du Bois de Meudon à Paris et a été intoxiqués par les mêmes métaux mis en solutions dans l'eau douce.

L'application du baryum a été faite en cinq concentrations différentes (50 ppm, 25 ppm, 10 ppm, 1 ppm et 0,1 ppm) sur *G. pulex*, et en seulement deux (25 ppm et 10 ppm) sur *G. locusta*. Pour le titane, l'application a été faite en six concentrations (150 ppm, 50 ppm, 25 ppm, 10 ppm, 1 ppm et 0,1 ppm) sur *G. pulex* et en deux (50 ppm et 25 ppm) sur *G. locusta*.

Afin de pouvoir faire une comparaison avec les résultats obtenus pour les autres métaux (Zencirci 1978-79, 1980a, 1980b) on a utilisée les mêmes concentrations de métal soit 10 ppm, 1 ppm et 0,1 ppm qui constituent une faible dose pour le baryum et le titane.

Pour l'étude à la microsonde, les animaux sont fixés au mélange de Carnoy, inclus à la paraffine et coupés à 7 μ . Les coupés sont collées sur un support de terphane, carbonées, séchées et de paraffinées par le toluène.

L'analyse chimique par spectrographie de rayons X est effectuée à la Microsonde "Cameca MS 46" avec une tension d'accélération de 15 KV, un courant de sonde 40 nA, un diamètre de sonde de 1 μ environ. Le baryum est caractérisé par la raie La_{λ_1} , et le titane est caractérisé par la raie $\text{K}\alpha_{\lambda_1}$. Les valeurs sont exprimées en, chocs par seconde.

RÉSULTATS

Les valeurs de DL_{50} (24 h) du baryum ont été trouvées dans une solution de 100 ppm chez *G. pulex* et de 75 ppm chez *G. locusta*. Pour le titane, les valeurs de DL_{50} (24 h) sont de 200 ppm vis-à-vis de *G. pulex* et de 150 ppm chez *G. locusta*.

Durant les expériences, les taux de mortalité constatés des espèces testées sont indiqués dans le Tableau I.

Tableau I. Les taux de mortalité de *G. pulex* de *G. locusta* dans les solutions de baryum et de titane.

	$\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$	TiCl_3
	50 ppm	25, 10, 1, 0.1 ppm
<i>G. pulex</i>	8 èmej. 820 14 èmej. 840 30 èmej. 840	150 ppm 8 èmej. 820 20 èmej. 840 30 èmej. 840
	25 ppm	50 ppm
<i>G. locusta</i>	8 èmej. 840 14 èmej. 860 30 èmej. 860	10 èmej. 820 17 èmej. 840 30 èmej. 840
		25 ppm 8 èmej. 840 30 èmej. 820

Parmi les sujets expérimentés, on a fixé certains dans le 1^{er}, 4^{ème}, 10^{ème} et 15^{ème} jour de l'expérience pour l'étude à la microsonde. Les valeurs d'accumulation du baryum et du titane dans les organes et les tissus des deux espèces sont indiquées dans le Tableau II.

Tableau II. Les valeurs min. et max. d'accumulation du baryum et du titane dans les organes et tissus de *G.pulex* et *G.locusta*.

Organes et Tissus	Baryum		Titane	
	G.pulex	G.locusta	G.pulex	G.locusta
Cuticule	Surf.	0	0	0
	Int.	50-750	85-400	30-600
Epiderme		0	0	0
Estomac		100-1000	190-625	100-3000
	Ant.	0	0	30-280
Mésentéron	Méd.	0	0	0
	Post.	0	0	0
Glande dig.	Ant.	0	0	0
	Post.	0	0	0
Diverticule	Ant.	0	0	0
	Post.	0	0	0
Glandes antén.		0	0	0
Gonades		0	0	0
Muscles		40-250	60-190	0
Hémolymphé		90-3000	400-1000	0
Syst. nerveux		0	0	0
Branchies		0	0	0
Oeil		0	0	Trace

- Les valeurs sont exprimées en chocs par seconde.

Les enregistrements de la raie L^* du baryum et de la raie R^* du titane indiqués dans les Figures 1,2,3 et 4.

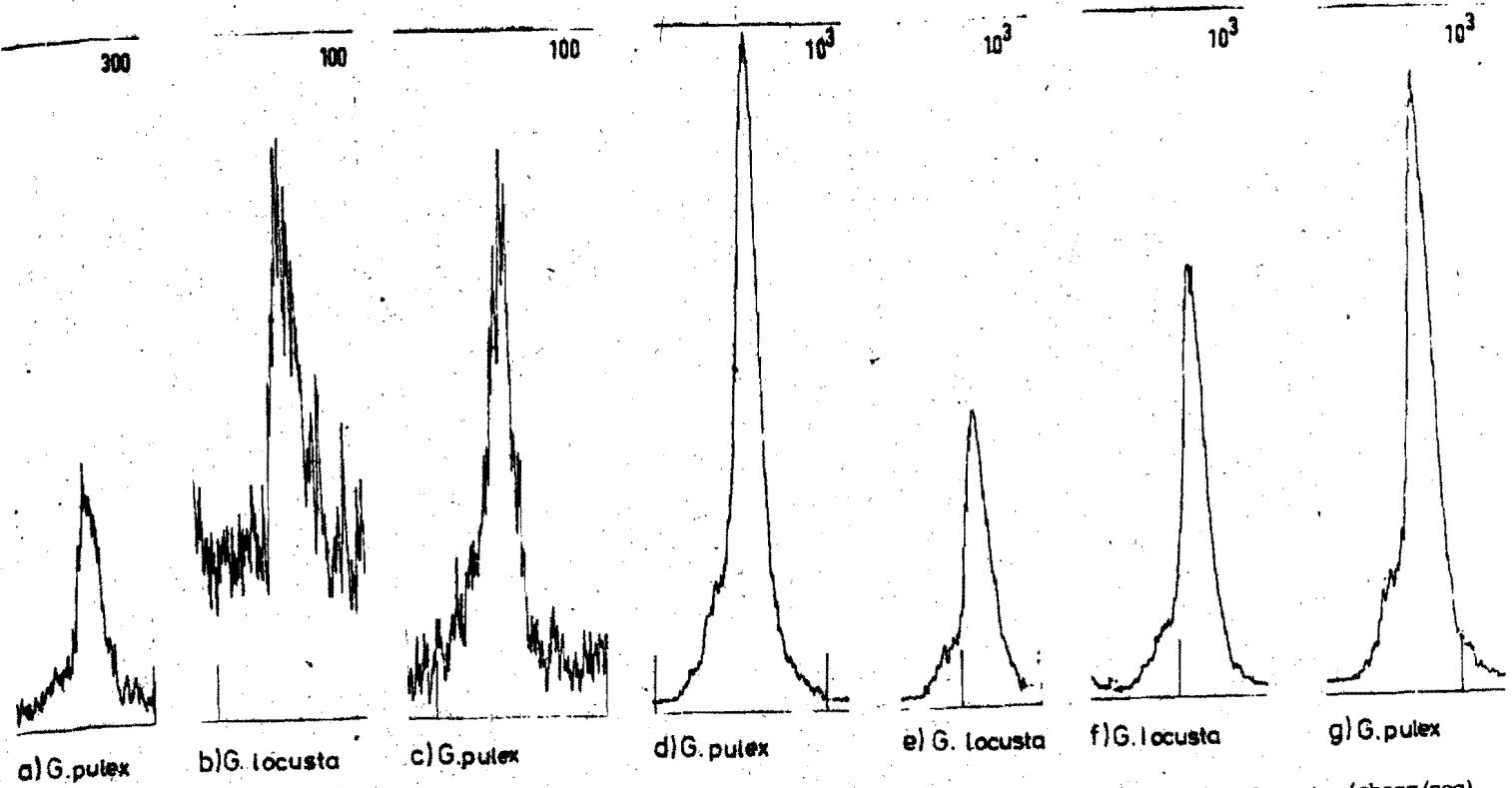


Figure 1. Les enregistrements de la raie L_{α_1} du baryum dans les organes et les tissus des *G. pulex* et *G. locusta* (chocs/sec).

a) Cuticule int., 10 ppm: 1 èr j.	b) Cuticule int., 10 ppm: 1 èr j.
c) Cuticule int., 1 ppm: 1 èr j.	d) L'estomac, 50 ppm: 15 èmej.
e) L'estomac, 25 ppm: 4 èmej.	f) L'estomac, 25 ppm: 10 èmej.
g) L'estomac, 25 ppm: 15 èmej.	

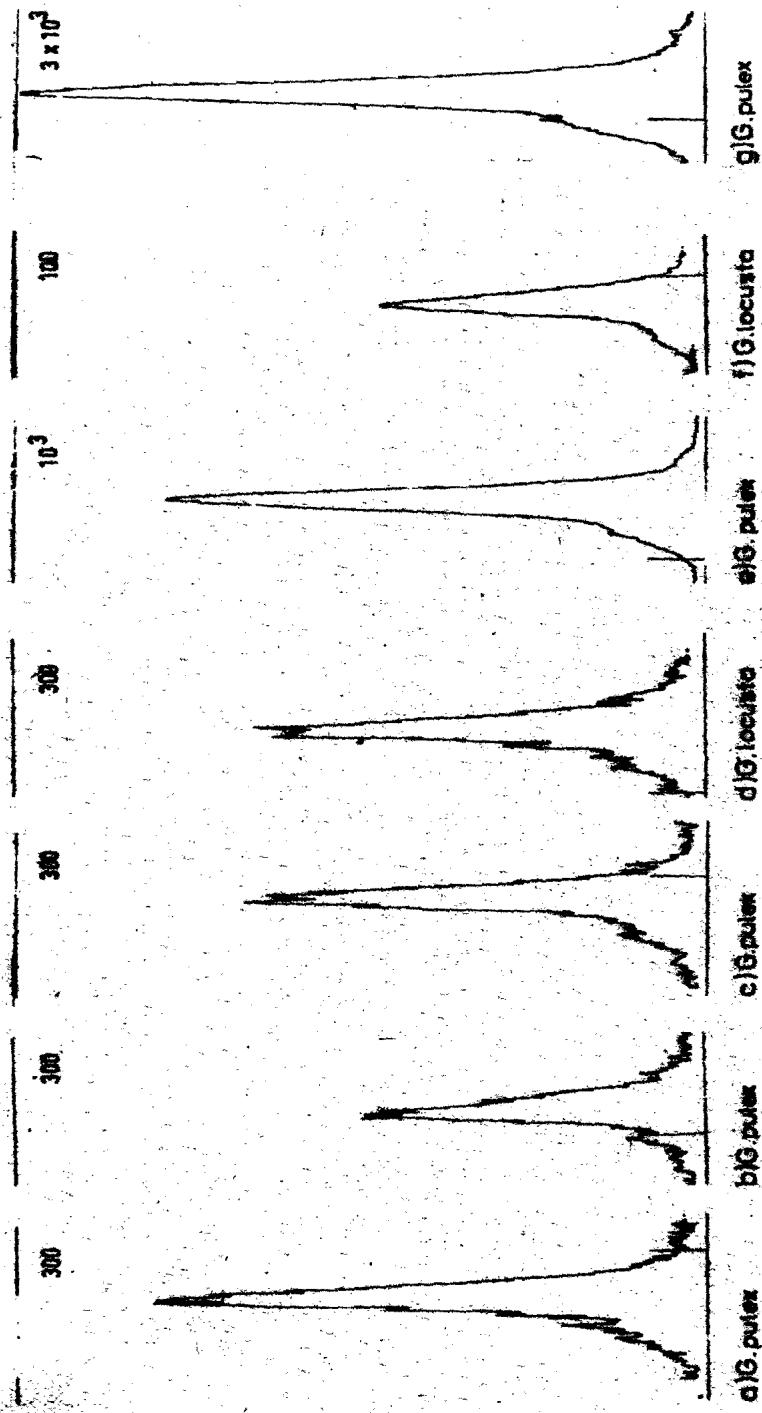


Figure 2. Les enregistrements de la rate Lm_1 du benzyl dans les organes et les tissus des *G. pulix* et *G. lacustris* (chocs/sec).

- a) Muscles, 50 ppm: 15 émej.
- b) Muscles, 50 ppm: 1 èr j.
- c) Muscles, 25 ppm: 15 émej.
- d) Muscles, 25 ppm: 15 émej.
- e) Hépatolymphe, 50 ppm: 1 èr j.
- f) Hépatolymphe, 10 ppm: 1 èr j.
- g) Hépatolymphe, 50 ppm: 15 émej.
- h) Hépatolymphe, 50 ppm: 15 émej.

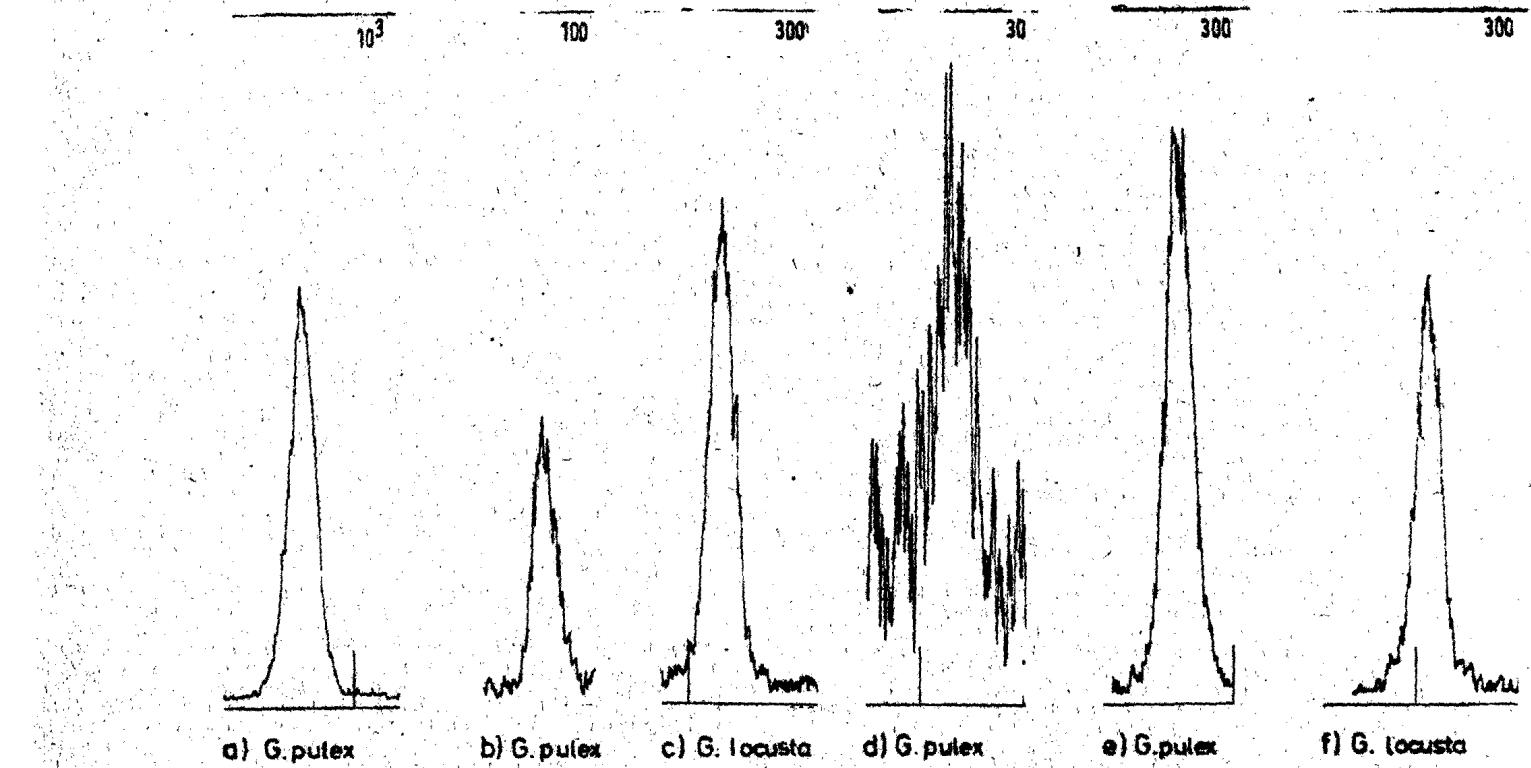


Figure 3. Les enregistrements de la raie K_{α_1} du titane dans les organes et les tissus des *G. pulex* et *G. locusta* (chocs/sec).

- | | |
|--------------------------------------|-------------------------------------|
| a) Cuticule int., 50 ppm: 10 èmej. | b) Cuticule int., 1 ppm: 1 èr j. |
| c) Cuticule int., 50 ppm: 1 èr j. | d) Cuticule int., 0,1 ppm: 1 èr j. |
| e) Mésentéron ant., 50 ppm: 10 èmej. | f) Mésentéron ant., 50 ppm: 1 èr j. |

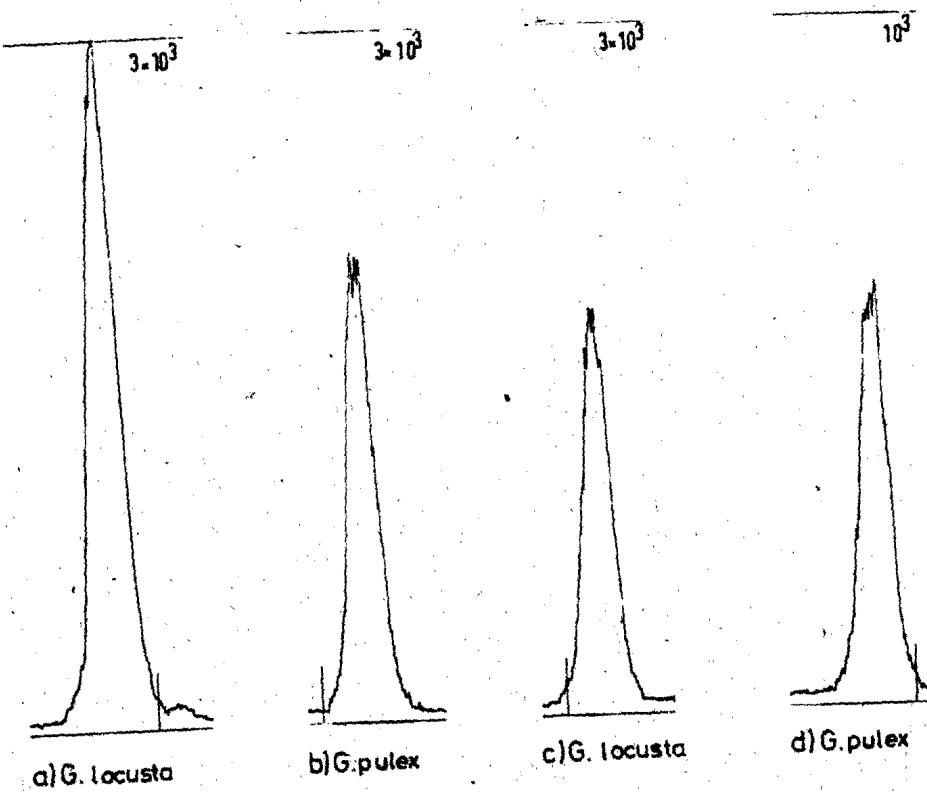


Figure 4. Les enregistrements de la raie $K\alpha_1$ du titane dans les organes et les tissus des *G. pulex* et *G. locusta* (chocks/sec).

a) Estomac, 50 ppm: 10 èmej.
 b) Estomac, 25 ppm: 10 èmej.
 c) Estomac, 50 ppm: 1 èr j.
 d) Estomac, 50 ppm: 1 èr j.

DISCUSSION ET CONCLUSIONS

Les valeurs très élevées de DL_{50} (24 h) nous ont montré que le baryum et le titane n'avaient pas une très grande toxicité. Nos résultats justifient ceux de Bowen (1966).

Les résultats de DL_{50} obtenus et les taux mortalité constatés au Tableau I, nous ont montré que le baryum était plus毒ique que le titane. En même temps *G. locusta* était plus sensibles que *G. pulex*. Dans nos expériences faites avec les autres métaux toxiques (Zencirci, 1978-79, 1980a, 1980b) on a constaté également que *G. locusta* était plus sensibles que *G. pulex*.

Au terme de notre travail, une grande accumulation de baryum a été constatée dans la cuticule intérieure, l'estomac et l'hémolymphe des deux espèces et aussi une faible accumulation dans les muscles. Pour le titane, une accumulation importante a été observée dans la cuticule, l'estomac des deux espèces, une légère accumulation dans le mésentéron antérieur et une trace d'accumulation dans les yeux (Tableau II).

Une accumulation de baryum a été déjà signalée dans l'intestin et l'hémocyte de *Cerastoderme edule* (*Pelecypoda*) par Martoja et coll (1977); dans l'épitel de l'intestin et dans le tube de malpigi de *Formica polyctena* (*Insecta*) par Jeantet et coll (1974). D'autre part Martoja et coll (1977) ont trouvé une accumulation de titane dans l'hémocyte et l'intestin de *Cerastoderma edule*. Nous n'avons pas eu l'occasion de comparer nos résultats n'ayant pas pu trouver une étude qui a été déjà faite sur l'accumulation de baryum et de titane notamment chez les Crustacés. Mais comme on pourrait constater en examinant plusieurs études que l'accumulation de baryum et de titane se situe dans les différentes parties du système digestif.

Mabillot (1955) a été déjà indiqué que certaines parties du système digestif du *G. pulex* présentent nettement des activités absorbantes, par contre certaines parties du même système ne présentent aucune activité absorbante. Les plus grandes valeurs d'accumulation ont été rencontrées dans les parties qui présentent un caractère absorbant. Il est bien naturel qu'il ya une accumulation de métal une fois que celui-ci soit assimilé. Comme Hryniwiecka-Szyfter (1973) l'a déjà indiqué certaines cellules de l'hepatopanréas des Crustacés ont la possibilité d'accumuler certains métaux.

D'autre part, du point de vue de l'accumulation de métaux on a rencontré une accumulation négligeable de titane dans les yeux tandis qu'il n'y a aucune d'accumulation dans les yeux des sujets traités avec les autres métaux.

Si on étudie le Tableau II, on constatera que les valeurs d'accumulation des métaux sont plus élevées dans les organes et les tissus de *G. pulex* que *G. locusta*. Les résultats nous justifient l'hypothèse d'Amiard (1976) exprimant la toxicité

provocée par les métaux est plus importante chez les espèces d'eau douce que les espèces marines.

Nos expériences nous ont montré que le baryum et le titane n'étaient pas très toxiques pour les deux espèces par contre les valeurs d'accumulation sont très élevées. D'après Nassogne (1970) les animaux aquatiques sont généralement capables de régulariser partiellement leurs contenus ion majeurs. En ce qui concerne les éléments non régularisés, il peut y avoir une accumulation dans toutes les organes, jusqu'à l'apparition des phénomènes de toxicité.

Donc, un métal très toxique peut déclencher assez vite un phénomène de toxicité qui peut amener la mort des sujets avec une très petite quantité d'accumulation. Par contre avec un métal qui n'est pas toxique le phénomène de toxicité commencerait à partir des valeurs d'accumulation relativement élevée.

ÖZET

Bu çalışmada baryum ($BaCl_2 \cdot 2H_2O$) ve titanın ($TiCl_3$) *G.pulex* ve *G.locusta* taze suyu zehirli etki ve birikimleri incelenmiştir.

Deneyde metallerin deniz suyu ve tatlı sudaki çözeltileri kullanılmıştır. LD_{50} (24 saat) değerleri; *G.pulex* için - 100 ppm Ba, 200 ppm Ti; *G.locusta* için - 75 ppm Ba, 150 ppm Ti olarak bulunmuştur.

Birikim denemeleri için; baryum 50, 25, 10, 1, 0.1 ppm'lik dozlarda, titan ise 150, 50, 25, 10, 1, 0.1 ppm'lik dozlarda uygulanmıştır. Metal birikim değerleri X ışınları spektrofotometresi ile tespit edilmiştir.

Deney sonuçları her iki tür için de baryumun titandan daha toksik olduğunu ve *G.locusta*'nın *G.pulex*'den daha hassas olduğunu göstermiştir.

Her iki türün de midde, hemolorf ve iç kütüklinde yüksek bir baryum ve titan birikimi, kaslarında pek yüksek olmayan baryum birikimi, gözlerinde ise çok az bir titan birikimi bulunmaktadır.

BIBLIOGRAPHIE

AMIARD,J.C. Etude expérimentale de la toxicité aigüe de sels de cobalt, d'antimoine, de strontium et d'argent chez quelques Crustacés et leurs larves et chez quelques téléostéens. Rev. Intern. Océan. Méd., 43: 79-93, 1976.

BELLAN-SANTINI, D., et REISH,D.J. Utilisation de Crustacés péricarides marins (Isopodes et Amphipodes) dans les études de toxicologie. Rev. Intern. Océan. Méd., 48: 103-105, 1977.

BOWEN, H.J.M. Trace elements in biochemistry, Academic Press, London, 1966.

HRYNIE WIECKA-SZYFTER, Z. Ultrastructure of organelles accumulating copper and iron in hepatopancreas of Crustacea. Annals Med. Sect. Pol. Acad. Sci., 18(1): 113-15, 1973.

JEANTET, A-Y., MARTOJA, R., et TRUCHET, M. Rôle des sphérocristaux de l'épithelium intestinal dans la résistance d'un Insecta aux pollutions minérales : données expérimentales obtenues par utilisation de la microsonde électronique et du micro-analyseur par émission ionique secondaire, C.R. Acad. Sc. Paris, 278: 1441-44, 1974.

MABILLOT, S. Contribution à l'étude histophysiologique de l'appareil digestif de *Gammarus pulex* (L.) Arch. Zool. Exp. Gen., 92: 20-38, 1955.

MARTOJA, M., L'HONORE, J., et ELKAIM, B. Accumulations de métaux et de particules minérales dans les tissus mous de la coque comestible, *Cerastoderma edule* (Mollusque: Eulamellibranchia) relation avec le milieu. C.R. Acad. Sc. Paris, 284: 1453-56, 1977.

NASSOGNE, A. Le rôle des Invertébrés marins dans le cumul et le transport des radionucléides. Rev. Intern. Océan. Méd., 20: 79-99, 1970.

ZENCIRCI, N. L'étude de l'accumulation et de la toxicité du cuivre et du zinc sur les *Gammarus pulex* (L.) et *Gammarus locusta* (L.). Hacettepe Bul. Nat.Sci. Eng., 7-8: 115-123, 1978-79.

ZENCIRCI, N. Kadmiyum ve Kroman *Gammarus pulex* (L.) ve *Gammarus locusta* (L.) (Crustacea: Amphipoda) Üzerindeki Zehirli Etki ve Birikimlerinin İncelenmesi. Çevre Haberleri, 5: 53-56, 1980 a.

ZENCIRCI, N. Contribution à l'étude de l'accumulation et de la toxicité de l'étain et du plomb chez des Crustaces Gammarides Hydrobiologia, 69, 1-2: 179-186, 1980 b.

DEPARTMENT OF BIOLOGY, HACETTEPE UNIVERSITY, ANKARA, TURKEY

LASER MICROPROBE MASS (LAMMA) AND INFRARED SPECTRAL ANALYSES OF THE
THORIUM-RARE EARTHS MINERAL FROM THE DEPOSITS OF EASTERN TURKEY

Freddy C.Adams[†], Sevim Akyüz*, Tanıl Akyüz** and Johan K.De Waele[†]

ABSTRACT

The Ardiçlı (Sofular-Malatya) Th-REE deposit is analyzed. Quantitative chemical analysis and quantitative laser microprobe mass and infrared spectral analysis are performed. The analyses have shown that the mineral is mainly britholite and contains bastnasite and thorite. An appreciable amount of thorium and rare earths (48.7 % as oxides) are present in the mineral.

INTRODUCTION

The Ardiçlı (Sofular-Malatya) Th-REE deposit is located approximately mid-way between the towns of Sivas and Malatya in eastern Turkey. The mineralization occurs as veins and is related to alkali syenite intrusion of the Uppermost Cretaceous age [6]. The mineral contains appreciable amounts of Th and rare earths. Thus it would be interesting to analyse this mineral comparatively by several different techniques. This paper describes quantitative (x-ray fluorescence, optical emission and other methods of analytical chemistry) and qualitative (laser microprobe mass and IR spectral) analyses of this mineral.

EXPERIMENTAL

Ardiçlı Th-REE deposit was obtained from Ardiçlı (Sofular-Malatya). Quantitative analyses of the mineral were carried out on a GE SPG-5 XRF spectrometer, using the Jarrell Ash optical emission spectrograph, and using conventional methods of analytical methodology.

Laser microprobe mass analyses were performed by a LAMMA-500 instrument (Leybold-Heraeus, GmbH, Köln, FRG). This technique makes use of a high-intensity laser pulse to vaporize and ionize a small

amount of the solid sample. The elemental and molecular ions produced are then mass analyzed in a time-of-flight mass spectrometer. A detailed description of the instrument can be found elsewhere [2].

IR spectra of mulls and disks were recorded on a Perkin-Elmer 621 spectrometer which was calibrated using polystyrene film and water bands.

RESULTS AND DISCUSSION

The chemical analysis of the Ardiçli mineral is given in Table 1. The sample contains 48.7 % rare earths and 4.7 % ThO_2 . X-ray diffraction analysis of the sample indicated that it contained bastnasite ($\text{Ce}, (\text{CO}_3)(\text{OH}, \text{F})$) in addition to britholite [1,6]. The XRD diagram taken from the original mineral was poor, probably due to a partial disruption of the crystal structure towards the metamict state by radioactivity within the crystal. A considerable sharpening of the powder lines was obtained by heating the sample for 1 hour at 900°C under a nitrogen atmosphere; britholite bands were then observed clearly [3,6]. We observed the vibrational bands of the CO_3^{2-} group in the IR spectrum of the Ardiçli mineral and chemical analysis of the sample yielded 6.85 percent CO_2 . These findings unequivocally confirm the presence of bastnasite in the sample. The analysis shows 4.7 percent ThO_2 , presumably owing to Thorite [$\text{Th}(\text{SiO}_4)$].

LAMMA analysis

Figure 1(a-f) illustrates typical examples of positive mass spectra, when six consecutive laser shots of around $0.35 \mu\text{J}$ energy are directed at the same location of a single particle of Ardiçli mineral. Thus we could obtain depth profile information by LAMMA.

At the first shot, the molecular ion peaks LaO^+ and CeO^+ at $m/e = 155$ and 156 respectively are the only features of the mass spectrum (Fig. 1-a). These peaks increase significantly in intensity with the subsequent laser shots. At the second shot, the Co^+ peak appears at $m/e = 59$. After the third and fourth shots Ca^+ , CaOH^+ , Y^+ , YO^+ , La^+ , Ce^+ , NdO^+ , Th^+ and ThO^+ peaks are observed. After the fifth and sixth laser shots, the contribution of Li^+ , Na^+ , Al^+ , Bi^+ and K^+ coming from the inner layers of the sample are noticed.

All the elements detected by LAMMA except Co, Bi and Li are also detected by chemical analysis (see Table 1). The lack of detection of Co, Bi and Li by chemical analysis may be caused by a heterogeneous distribution of these elements among the individual particles of the mineral, which results in a low total bulk concentration and may explain the failure of their detection.

TABLE 1. Chemical composition of the Ardiçli Th-REE deposit

component	weight percent	component	weight percent	component	weight percent
<u>XRF</u>					
CeO ₂	26.0	Sm ₂ O ₃	1.2	*CO ₂	6.85
SiO ₂	16.1	Gd ₂ O ₃	1.2	**H ₂ O	1.7
CaO	12.2	Dy ₂ O ₃	0.8	+F ⁻	1.0
La ₂ O ₃	12.0	Pr ₆ O ₁₁	0.5	⁵ U ₂ O ₈	0.08
Nd ₂ O ₃	6.2	SrO	0.4	#S	0.08
ThO ₂	4.7	Eu ₂ O ₃	0.3		
Fe ₂ O ₃	2.2	ZrO ₂	0.3		
Y ₂ O ₃	2.1	Er ₂ O ₃	0.2		
P ₂ O ₅	2.0	Yb ₂ O ₃	0.2		
Na ₂ O	0.5	MgO	0.2		
K ₂ O	0.1	Tm ₂ O ₃	0.1		
Al ₂ O ₃	0.1	BaO	0.04		
T ₁ O ₂	0.05	Pb	0.02		
MnO	0.07	Cu	0.0004		
<u>OES</u>					
<u>Others</u>					

XRF : X-ray fluorescence, OES : Optical emission spectrometry,

Others : * measured by absorption on KOH and weighing the mass difference method.

** (crystal water) measured by absorption on magnesium perchlorate and weighing the mass difference method.

+ measured by distillation-colorimetric method.

⁵ measured by fluorimetric method.

measured by iodimetric titration method (using Leco Instr.).

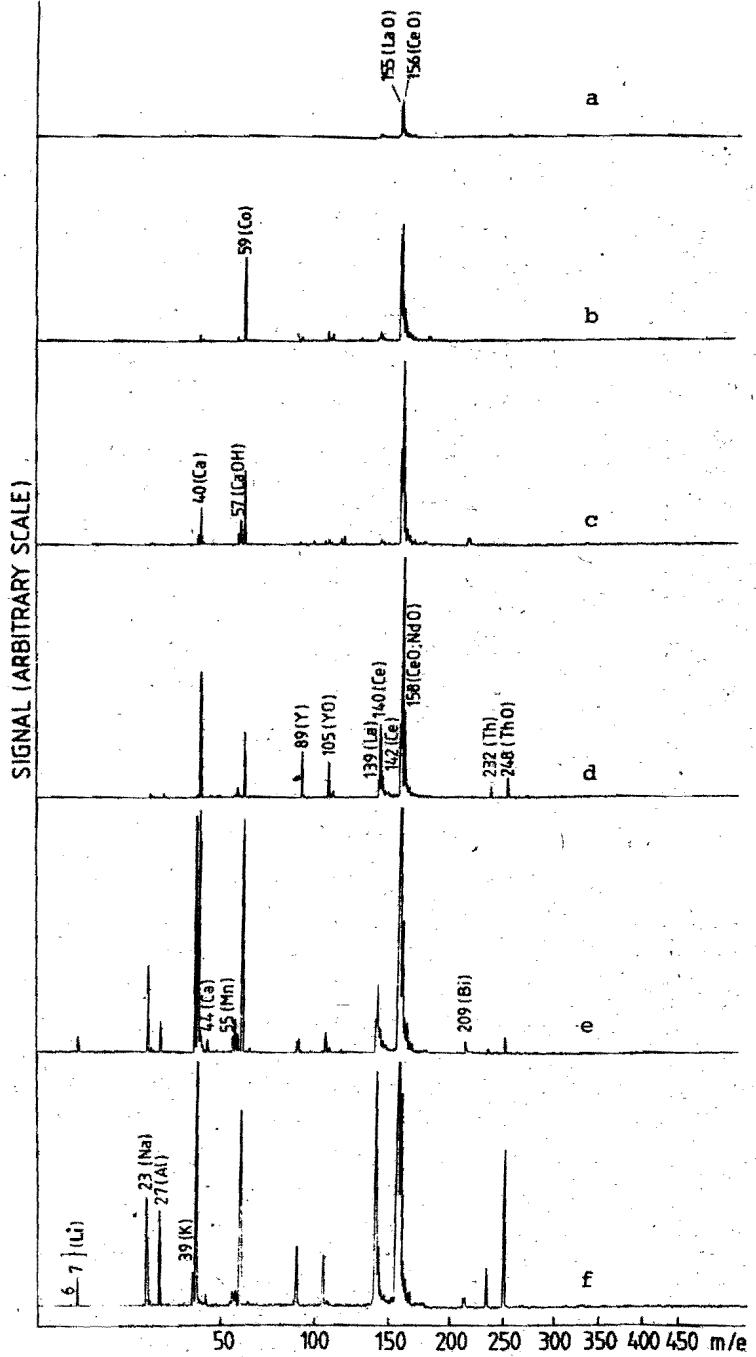


Figure 1. (a-f) Positive mass spectra of Ardiçlı mineral when six consecutive laser shots are directed at the same location of the sample.

Fi

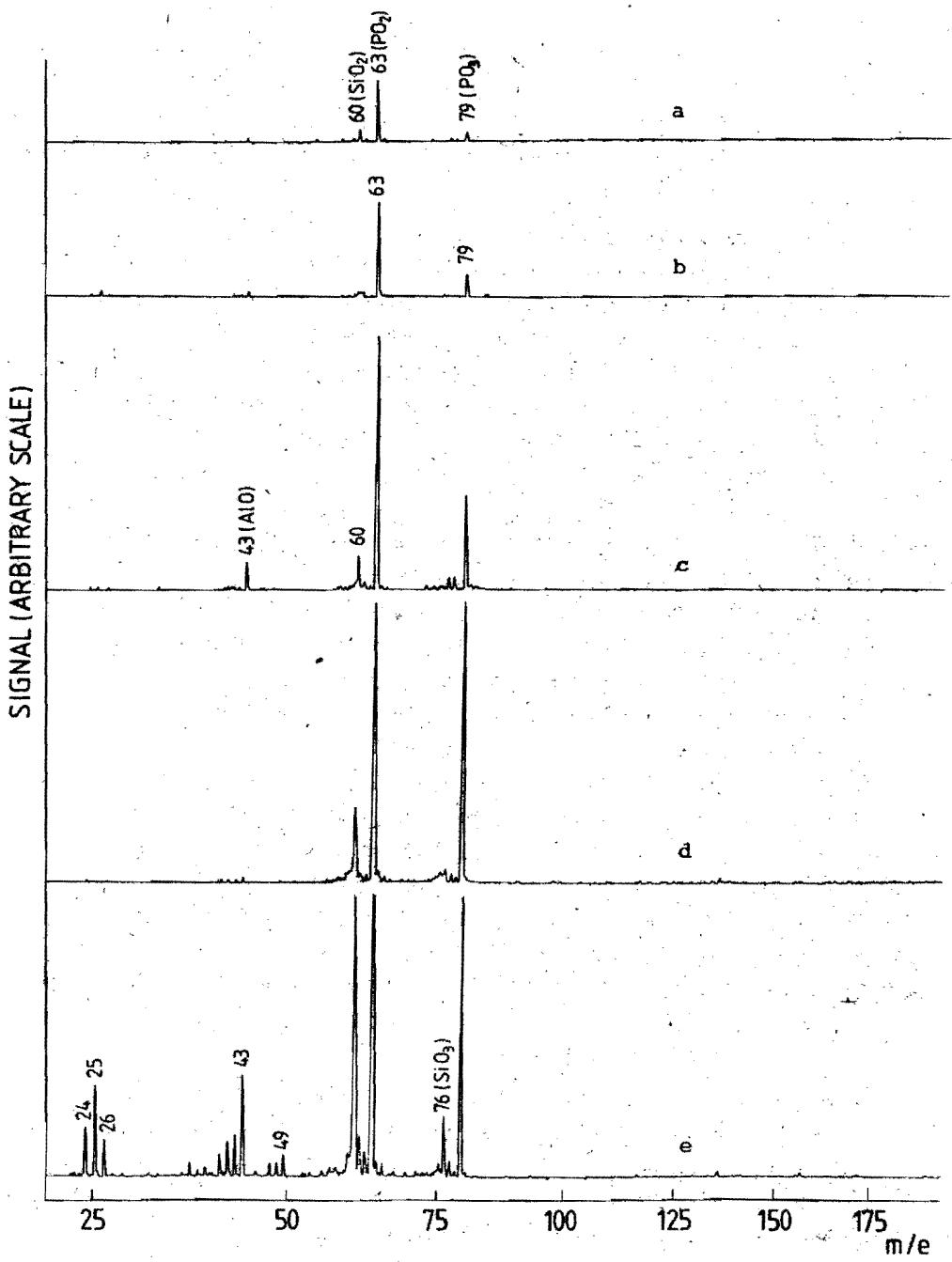


Figure 2. (a-e) Negative mass spectra of Ardiçlı mineral when five consecutive laser shots are directed at the same location of the sample.

Figure 2(a-e) gives negative mass spectra recorded under similar conditions to positive mass spectra. In this case five consecutive laser shots (ca. 0.35 μ J) are directed at the same location of the mineral to obtain depth profile information. On the surface of the particles PO_2^- , SiO_2^- and PO_3^- ions were observed. At greater depths the contributions of AlO^- and SiO_3^- peaks are obtained.

IR spectrum

The IR spectrum of the Ardiçlı mineral is given in Fig.3, and the vibrational wavenumbers are tabulated in Table 2. In the IR spectrum of the mineral there is a medium intense absorption of molecular water at ca. 3400cm^{-1} and a weak absorption at 1620cm^{-1} . The absorption in the region of $1450-1400\text{cm}^{-1}$ is characteristic for carbonates [5], in this case owing to the Bastnasite $[\text{Ce},(\text{CO}_3)(\text{OH},\text{F})]$ content of the mineral. The sharp band at 870cm^{-1} is assigned to deformation vibration of the CO_3^{2-} group $|\gamma(\text{CO}_3)|$.

Silicates show strong absorption in the region of $1000-1100\text{cm}^{-1}$ and $400-500\text{cm}^{-1}$ [4,5]. The strong band observed at 1025cm^{-1} is assigned to asymmetric stretching vibrations of the Si-O bond in SiO_4 tetrahedrons. The IR spectrum of the mineral shows no absorption in the range $600-800\text{cm}^{-1}$, which indicates the absence of, condensed silicon oxygen tetrahedra, Si-O-Si bonds [4].

Rare-earth-oxide bands are observed in the region of $600-300\text{cm}^{-1}$ which overlap with the O-Si-O bending vibration [4]. The La-O stretching vibration is observed as a strong band at 643cm^{-1} whereas, $\nu(\text{Ce}-\text{O})$ is observed as a broad band at ca. 400cm^{-1} in the IR spectrum of pure La_2O_3 and CeO_2 , respectively. These two rare earths are the major elements in the sample studied. We observed a weak band at 602cm^{-1} and a number of bands between 573 and 434cm^{-1} . We tentatively assigned the band of 602cm^{-1} to La-O stretching vibration and other bands between $573-434\text{cm}^{-1}$ to $\delta(\text{SiO}_4)$ and rare-earth-oxide vibrations.

ÖZET

Ardiçlı (Sofular-Malatya) yöresinden alınan Th-REE minerali, niçel olarak kimyasal yöntemlerle ve nitel olarak laser mikroprob kütle ve kırmızı-altı spektroskopileri ile analizlendi. Analiz sonuçlarına göre mineral çoğunlukla britolittir, bastnasit ve thoriti de içermektedir. Mineralde dikkate değer miktarda thoryum ve nadir toprak elementleri oksitleri (%48.7) bulunmaktadır.

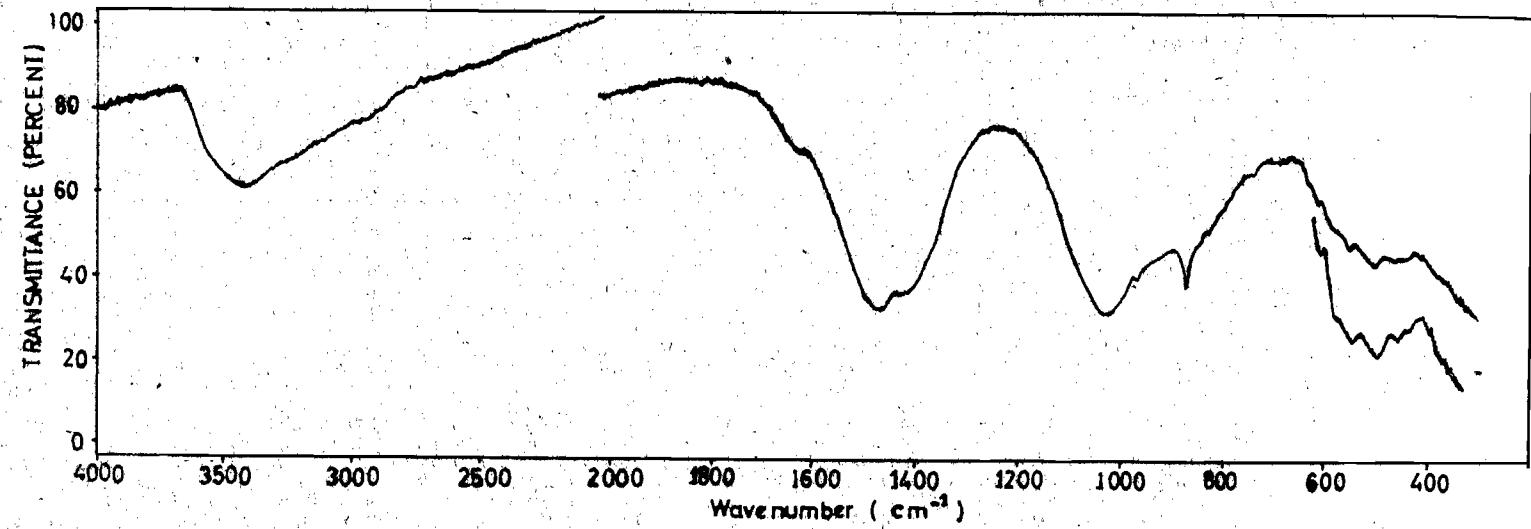


Figure 3. IR spectrum of Ardiçli mineral (KBr disk). In order to obtain higher intensity at 600-400 cm⁻¹ region, the spectrum was recorded again with a thicker sample.

TABLE 2. Vibrational wavenumbers of Ardiçlı mineral

wavenumber		Assignment
Ca. 3400	m br	$\nu_{\text{H}_2\text{O}}$
1620	w	$\delta_{\text{H}_2\text{O}}$
1470 1420}	s	$\nu_a \text{CO}_3$
1025	s	$\nu_a \text{SiO}_4$
965	w	
870	m	γCO_3
820	vw	$\nu_s \text{SiO}_4$
602	w	$\nu_{\text{La-O}}$
573	w	
543	m	
492	s	δSiO_4 and $\nu_{\text{Ln-O}}$
452	m	
434	m	

ν, δ, γ : indicate valence stretching, in plane angle bending and out of plane bending vibrations respectively.

s : strong, m : medium, w : weak, vw : very weak, br : broad.

REFERENCES

1. ASTM Index of X-ray diffraction data, 11-301, and 11-459. American Society for testing materials Philadelphia 1979.
2. Denoyer E., Van Greken R., Adams F., Natusch D.F.S., "Laser micro-probe mass spectrometry", 1. Basic principle and performance characteristics", Anal.Chem., 54, (1982), 26A-41A.
3. Hughson M.R. and Sen Gupta J.G., "A thorian intermediate member of the Britholite-Apatite series", Am. Miner., 49, (1964), 937-951.
4. Lazarev A.N., "Vibrational Spectra and Structure of Silicates", Translations Ed. Farmer V.C., Consultants Bureau New York-London 1972.
5. Rao C., "Chemical applications of infrared spectroscopy", Academic press, 1963, London.
6. Saltoğlu T., "The Th-REE mineralization in Eastern Turkey, Systematics and Properties of the Lanthanides", Ed. Sinha S.P., NATO ASI Ser.C. Math. and Phys. Sci. No 109, 1982, Reidel Pub.Com. Holland p.628.

[†] Dept. of Chemistry, Univ. of Antwerpen (UIA), 2610 Wilrijk Belgium.

* Hacettepe Univ. Dept.of Physics, Beytepe Ankara Turkey.

** Mineral Research and Exploration Institute (MTA), Balgat Ankara Turkey.