

AMINO ACIDS AS CONTROLLING FACTOR FOR THE MINERALOGY AND THE CHEMISTRY OF SOME BIVALVE AND GASTROPOD SHELLS

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Key words: Amino acids, chemistry, mineralogy, bivalves, gastropods, shells calcification.

ABSTRACT

The present study entails with the role of amino acids in the mineralogical and chemical composition of some bivalve and gastropod shells. The common skeletal minerals reported for the studied shells were found to be aragonite, calcite, and Mg-calcite. The bivalve *Tridacna elongata* showed high level of calcite and low level of aspartic the acid. Aragonite recorded high levels in the other examined species.

Glycine, isoleucine and proline represented the extreme higher values among the neutral amino acids in all examined shells. The neutral amino acids can be quantitatively arranged in the following order; Glycine > Isoleucine > Proline > Alanine > Leucine > Valine > Serine > Threonine.

The chemical study of the investigated shells indicates that, copper recorded its maximum value ($33 \mu\text{g g}^{-1}$) in the bivalve *Tridacna elongata* and its minimum ($12 \mu\text{g g}^{-1}$) in the gastropod *Conus coronatus*. The maximum values of zinc, lead, iron and manganese were recorded in bivalve *Acanthocardium aculeata* whereas, the studied gastropods revealed lower values for these elements. This trend of the element distribution was observed also for calcium and magnesium. Acidic amino acids seem to play an important role in fixation of calcium, nucleation of CaCO_3 and growth of the mineral phase.

INTRODUCTION

The mineralogy and elemental composition of any carbonate is partly dependent upon the mode of precipitation. Most workers have supported an enzyme theory (the presence of various enzymes such as carbonic anhydrase or phosphorylase that determines the site of calcification). They suggested also the template theory (the nucleation and the form of the seed that related to the composition of the organic template or matrix upon which it forms). Some workers have favored crystal poison or electrochemical models (Milliman, 1974).

At present, many data favor the template theory of calcification, although not all organisms necessarily utilize this technique. Wilber *et al.* (1969) supported the template model and pointed to this fact that, all carbonates, even ooids, contain a matrix of protein and amino acids (Mitterer, 1968). According to this model, shell nucleation involves the coordination of Ca ions to free carboxyl groups of acidic amino acids residues in the matrix protein (Hare and Abelson, 1965; Degens, 1967; Degens *et al.*, 1967; Matheja and Degens, 1968; Jackson and Bischoff, 1971 and Degens, 1976). The spatial sequence of specific peptide chains and the nature of the organic matrix can determine the micro-architecture, (Hare and Abelson, 1965), as well as, the mineralogy of the precipitated shell. Matheja and Degens (1968) have explained this latter phenomenon by the influence of oxygen absorption by various proteinaceous compounds upon the coordination polyhedra; $\text{Ca}^{+2} \text{O}_6$ will form calcite and $\text{Ca}^{+2} \text{O}_9$ will favor aragonite. The differences in the ratios of protein compounds from one species to another also may have phylogenetic implications; (Hare and Abelson, 1965 and Ghiselin *et al.*, 1967).

In many primitive phyla, calcification is extracellular softening point that the concentration of trace elements is controlled by the physicochemical processes (Kinsman, 1969). In contrast, advanced organisms, mollusks being the best example, calcify by passing cations through cellular tissues. This process can be effective in partitioning various elements.

The two elements most easily related to physicochemical processes are strontium and magnesium, although other elements such as iron also may be dependent upon the complexity of calcification. Lowenstam (1963 and 1964a,b) demonstrated that, more

phylogenetically advanced organisms contain less strontium, the strontium being preferentially excluded by more complex calcification processes. Excluding mollusks from this picture, one can see a slight decrease in strontium with increasing physiologic complexity. Mollusks appear to be totally unique in their ability to selectively discriminate against strontium (Likins *et al.*, 1963).

The specific role of amino acids in calcified proteinaeous organic matter depends on their negatively charged carboxylic group (COO⁻) which may act as concentration sites for calcium ions (Hare, 1963). Similarly, carbonate or bicarbonate could be concentrated on positively charged (basic) side chains (Muller and Suss, 1977).

The present investigation entails with the role of the amino acids in the mineralogical composition of the studied shells. The investigated species include two bivalve species, as well as three gastropod species.

Hydrographic features of the study area:

According to Anon (1997), the Bitter Lakes exhibited the denser water over the whole Suez Canal, Egypt (Fig.1). A salt barrier of salinity greater than 65‰ was established in the Bitter Lakes which acted as an obstacle for the migration of marine organisms between Mediterranean and Red Sea due to the existence of the salt bed at the lakes bottom. Soliman and Morcos (1990) suggested that the salt bed was about exhausted and its effect on salinity was insignificant. Therefore, the salinity in the lakes is expected to fluctuate within the range $\pm 1.2\%$ about the mean value of 42.3‰. Accordingly, the migration of the marine organisms between the joined seas can occur at any time without any osmotic problem.

Analytical methods:

Representative specimens from each examined species have been collected from the area of study (Bitter Lakes of Suez Canal). For both heavy elements and mineralogical analyses, the shells were pulverized to pass through 44- μ mesh sieve-screen, after which the powder has been subjected to ultra-sonic to ensure its cleaning. The powdered samples were divided into two portions; the

first one was subjected to X-Ray diffraction analysis, using diffractometer model Philips (1840) to determine the mineralogical composition of the different species.

The conditions under which the measurements were taken were as follows; Wave length Cu K α = 1.54050.

Anode/strain/cathode/current=45K.v. /25m.A.Filter-Nickel

Sensitivity=4x100 and 10³ counts/second.

Measurements range (2 θ)=20-60°

The second portion was prepared for determination of amino acids according to the procedure modified by El-Sarraf *et. al.* (1984) using amino acid analyzer Beckman model 119 CL. Tubes containing about 50 mg dried, homogenous powder of the examined shells with 5 ml of 6 N Hcl were sealed under vacuum and hydrolyzed for 22-24 hours at 110 ° C.

RESULTS AND DISCUSSION

Amino acids analysis:

Analytical data of amino acids in the studied shell of both bivalves and gastropods are given in Table 1 and Figures 2 & 3. The total amino acids content in the investigated shells varies from 888.93 $\mu\text{g}\cdot\text{g}^{-1}$ in *Conus coronatus* to 4397.11 $\mu\text{g}\cdot\text{g}^{-1}$ in *Fusinus marmoratus*. The high levels of amino acids are believed to be due to the high concentration of organic matter in the matrix of the studied shells.

The ratio of basic amino acids to the total amino acids in the studied bivalve shells ranged from 6.9 % to 9.14 %, whereas in the gastropods varied from 7.8 to 11.4 %. In the fact, the ratio of basic amino acids to the total amino acids in the recent skeletal carbonate approximates to 10 % (Terashema and Tanaka, 1976).

Concerning acidic amino acids, they recorded higher concentrations in comparison with the basic in the bivalves. They ranged from 27 to 28.9 % relative to the total amino acids. The gastropods recorded values that varied from 21 to 33.1 %. Aspartic acid was the most abundant amino acid, ranging from 11 to 21.5 % relative to total amino acids. Terashema and Tanaka (1976), King (1977) indicated that aspartic acids are the most abundant in the calcareous microfossils.

The neutral amino acids fluctuate from 47.5 % in *Acanthocardium aculeatum* to 54.8 % in *Tridacna elongata*. In gastropods, it recorded 51.2, 52.3 and 53.3 % in *Cerithium caeruleum*, *Conus coronatus* and *Fusinus marmoratus* respectively.

Glycine, isoleucine and proline represent the extreme higher values among the neutral amino acids in all examined shells. The higher values of glycine could be owed to the decomposition of serine and threonine. The neutral amino acids can be quantitatively arranged in the following order;

Glycine > Isoleucine > Proline > Alanine > Leucine > Valine > Serine > Threonine.

Sulfur amino acids, cystine revealed higher values, but methionine recorded lower values.

The difference in the concentrations of the amino acids components may be interpreted on the basis of the decomposition pathways of serine and threonine via dehydration (Bada and Man, 1980; Bada *et al.* 1982). Aromatic amino acids (tyrosine and phenylalanine) revealed relatively moderate concentrations ranging between 2.2 and 4.95 % relative to the total amino acids.

Ammonia varied between 54.4 $\mu\text{g g}^{-1}$ in *Fusinus marmoratus* and 417.7 $\mu\text{g g}^{-1}$ in *Cerithium caeruleum*. Ammonia concentrations in bivalves are lower than that of gastropods.

The relatively higher values of both acidic and basic amino acids components in gastropods compared with those of bivalves may be due to phylogenetic implication, as well as, to differential skeletal composition.

Chemistry of the investigated shells:

Both quality and quantity of the elements required for building up the shell during the life span of an organism depend on the selective uptake of the organism, as well as, on the surrounding ecological conditions. Degens (1976) mentioned the ability of aspartic acid, serine and glycine to coordinate metal ions.

Copper recorded its maximum value ($33 \mu\text{g g}^{-1}$) in the bivalve *Tridacna elongata* and its minimum ($12 \mu\text{g g}^{-1}$) in the gastropod *Conus coronatus*. The maxima values of zinc, lead, iron and manganese were recorded in bivalve *Acanthocardium aculeata* (Table 2), whereas the studied gastropods reveal lower values for these elements. This trend of the element distribution was observed also for calcium and magnesium. Acidic amino acids seem to play an important role in fixation of calcium, nucleation of CaCO_3 and growth of the mineral phase.

The organic matrix recorded its maximum concentration (7.88 %) in the gastropod *Cerithium caeruleum* and the minimum (2.29 %) in *Conus coronatus*.

Mineralogical composition:

The common skeletal minerals that reported for the studied shells found to be aragonite, calcite and Mg-calcite (Table 3 and Figs. 4 a ,b). Aragonite ranged between 29.71 % in the bivalve *Tridacna elongata* and 95.78 % in the gastropod *Conus coronatus* (Table 3). Vise versa, calcite reveals its minimum value (2.11 %) in the gastropod *Conus coronatus* and its maximum (70.29 %) in the bivalve *Tridacna elongata*. However, high levels of calcite and low level of aspartic acid were shown in the bivalve *Tridacna elongata*. This could not be related to the high levels of both neutral and basic amino acid. Aragonite recorded high levels in the other examined species. This can be interpreted on that, glutamic and aspartic acids inhibited the transformation of aragonite into calcite. Degens (1976) mentioned that, the acidic amino acids form a protective overgrowth on aragonite surface.

The correlation patterns of the individual amino acids are demonstrated by various connections with a type of skeletal matrices. Threonine, serine, glycine and alanine concentrations can be interpreted on the basis of the decomposition pathways of serine and threonine. The decomposition reactions of both serine and threonine could be useful as geochronological indicator (Bada and Man, 1980).

The relations between glycine and aspartic acid also threonine with glycine and proline with glycine, aspartic against threonine and glutamic acid with proline reveal linear relationships (Figs.5a-f). This

may suggest that one of the amino acid fractions in the shell matrices be related to the type of protein.

The negative correlation between the measured copper in the investigated species and aragonite ($r = -0.98$) from one hand and the positive with calcite ($r = 0.90$) from the other hand reveals the incorporation of copper into the crystal lattice of calcite. The correlation matrix shows no effective interrelation between copper and the determined amino acid fractions. Lead shows positive correlation with zinc and manganese (Figs. 6a and b), whereas all are adsorbed on the calcite surface. The negative correlation between lead and Cystine (Fig. 7a) reveals that, cystine may follow various metabolic pathways; -SH- group is oxidized and then removed as sulfate or lead sulfate. The positive correlation between manganese and tyrosine (Fig. 7b) illustrates that; Mn^{+2} is an essential micronutrient for many enzymes that activated by manganese (Fostner & Wittmann, 1981). Magnesium is positively correlated with Mg-calcite ($r = 0.95$). The positive correlation of magnesium with both aspartic acid and proline ($r = 0.87$) could be interpreted on the basis that, the catabolism of amino acids required enzyme cofactors such as magnesium during irreversible reactions. The negative correlation between calcium and tyrosine ($r = -0.94$) may be related to that, magnesium inhibited the calcification processes in the living organisms, also tyrosine may reduce the calcium precipitation in the skeletal matrix. The positive correlations between aspartic acid with Proline and tyrosine acid with the organic carbon ($r = 0.87, 0.92, 0.94$) indicate that, the organic carbon contains protein fractions in the shell matrix Magnesium is positively correlated with organic carbon reflecting its adsorption onto the organic matrix. The negative correlation between calcite and cystine ($r = -0.91$) may be related to that, cystine inhibits the calcite formation. The positive correlation between Mg-calcite and tyrosine ($r = 0.93$) may be attributed to their association in the same phase during the skeletal calcification of organisms.

Conclusion

The common skeletal minerals reported for the studied shells were found to be aragonite, calcite, and Mg-calcite. *Tridacna elongata* showed high levels of calcite and low level of aspartic acid. This could not be related to the high levels of both neutral and basic amino acid. Aragonite recorded high levels in the other examined species.

This can be interpreted on that, glutamic and aspartic acids inhibit the transformation of aragonite into calcite.

The relatively higher values of both acidic and basic amino acids components in gastropods in comparison with those of bivalves may be due to phylogenic implication, as well as, to differential skeletal composition. Glycine, isoleucine and proline represent the extreme higher values among the neutral amino acids in all examined shells. The higher values of glycine could be owed to the decomposition of serine and threonine. The neutral amino acids can be quantitatively arranged in the following order;

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Table (1): Amino acids constituents in the skeletal material of the studied bivalve and gastropods species ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight).

Amino acids	Bivalves		Gastropods		
	<i>Acanthocardium aculeata</i>	<i>Tridacna elongata</i>	<i>Cerithium caeruleum</i>	<i>Comus coronatus</i>	<i>Fusinus marmoratus</i>
1) Basic amino acids					
Lysine	54.00	36.80	85.08	34.53	207.40
Histidine	83.97	31.42	126.10	66.74	173.40
Arginine	N.D.	N.D.	N.D.	N.D.	N.D.
2) acidic amino acids					
Aspartic acid	227.88	111.22	579.20	97.61	700.00
Glutamic acid	208.60	154.5	312.90	88.83	481.50
3) Neutral amino acids					
Glycine	153.80	91.95	251.60	101.55	352.10
Alanine	85.54	57.90	185.00	60.83	248.30
Valine	97.94	57.45	131.20	38.27	231.80
Serine	53.78	61.30	169.00	46.10	223.72
Threonine	69.82	44.30	149.60	43.60	235.30
Proline	119.70	83.10	191.90	67.16	202.60
Isoleucine	77.70	79.22	150.10	60.04	626.60
Leucine	58.66	64.28	149.70	49.74	425.50
4) Sulfur					
Cystine	53.46	39.60	66.82	68.20	81.90
Methionine	15.65	17.69	17.38	12.17	17.38
5) Aromatic					
Tyrosine	108.70	15.30	20.97	13.09	16.31
Phenylalanine	40.88	37.30	106.85	40.47	173.30
Total amino acid	1510.08	983.88	2693.40	888.93	4397.11
Ammonia	227.30	181.30	417.70	160.93	54.40
Total	1737.38	1165.18	3111.10	1049.86	4451.51

Amino acids as controlling factor for the mineralogy and the 159
chemistry of bivalve and gastropod shells

Table (2): Chemical constituents in the skeletal material of the studied bivalve and gastropod species ($\mu\text{g.g}^{-1}$ dry weight).

Species	Cu	Zn	Pb	Fe	Mn	Mg	Ca%	CaCO ₃ %	O. C%	T.O.M %
I) Bivalves										
a) <i>Acanth. aculeata</i>	26.0	120	206	369	56	646	26.87	67.18	2.82	5.08
b) <i>Tridacna elongata</i>	33	74	113	192	5.3	47.0	26.89	67.22	1.79	3.21
II) gastropod										
a) <i>Cerithium Cuerculeum</i>	18	25	76	204	8.05	956	20.56	51.41	4.38	7.88
b) <i>Conus coronatus</i>	12	17	86	64	2.3	270	26.51	66.26	1.27	2.29
c) <i>Fusinus marmoratus</i>	22	33	81	118	3.6	597	25.60	63.97	3.27	5.89

Table (3): Mineralogical composition % constituents in the skeletal material of the studied bivalve and gastropod species ($\mu\text{g.g}^{-1}$ dry weight).

Species	Aragonite	Calcite %	Mg-calcite
<i>Acanth. aculeata</i>	86.17	8.51	5.32
<i>Tridacna elongata</i>	29.71	70.29	—
<i>Cerithium Cuerculeum</i>	68	3.1	18.14
<i>Conus coronatus</i>	95.78	2.11	2.11
<i>Fusinus marmoratus</i>	70.1	5.1	5.9

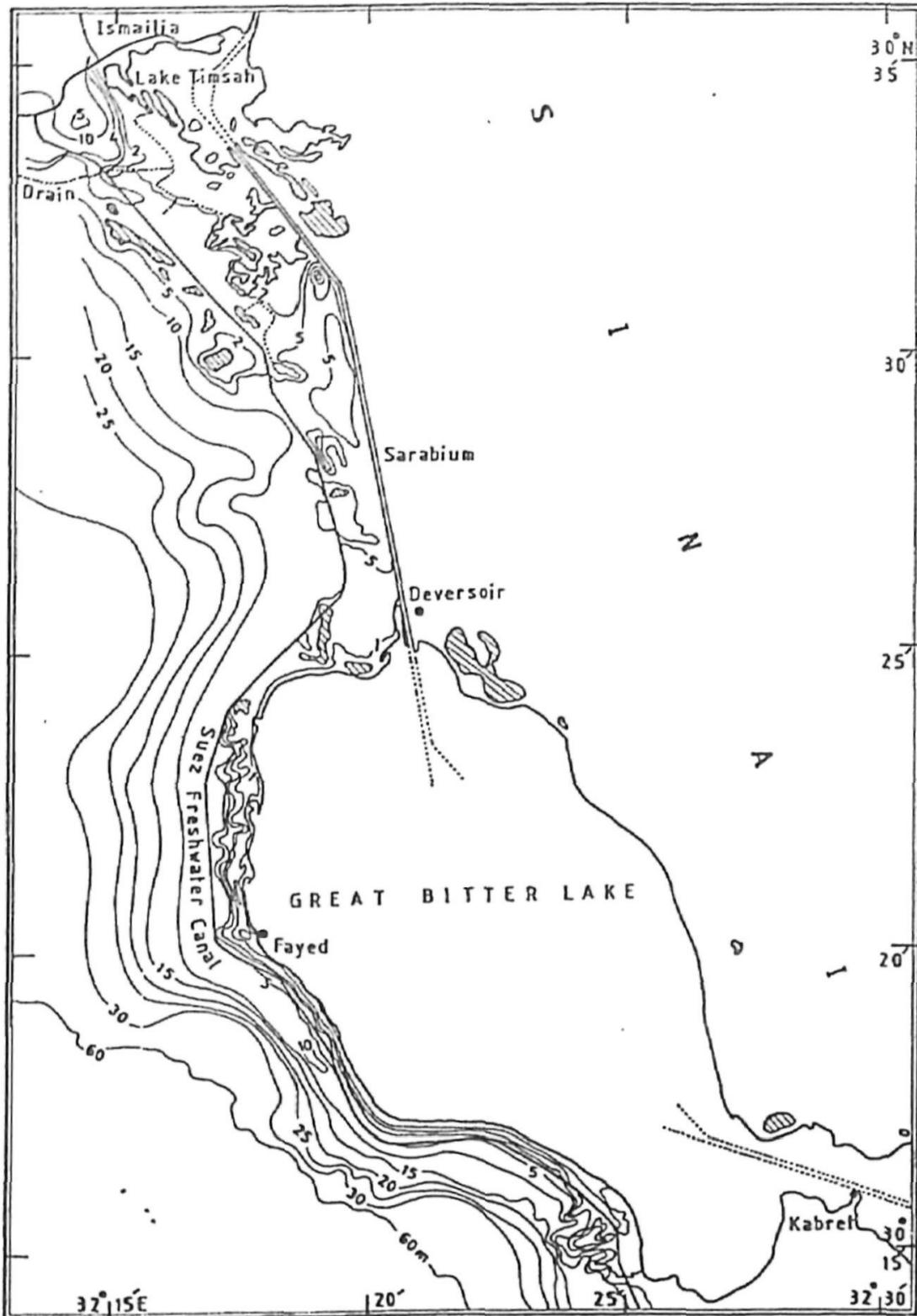


Fig.(1): Area of study.

Fig.(2):compartive study for amino acids in the studied bivalve

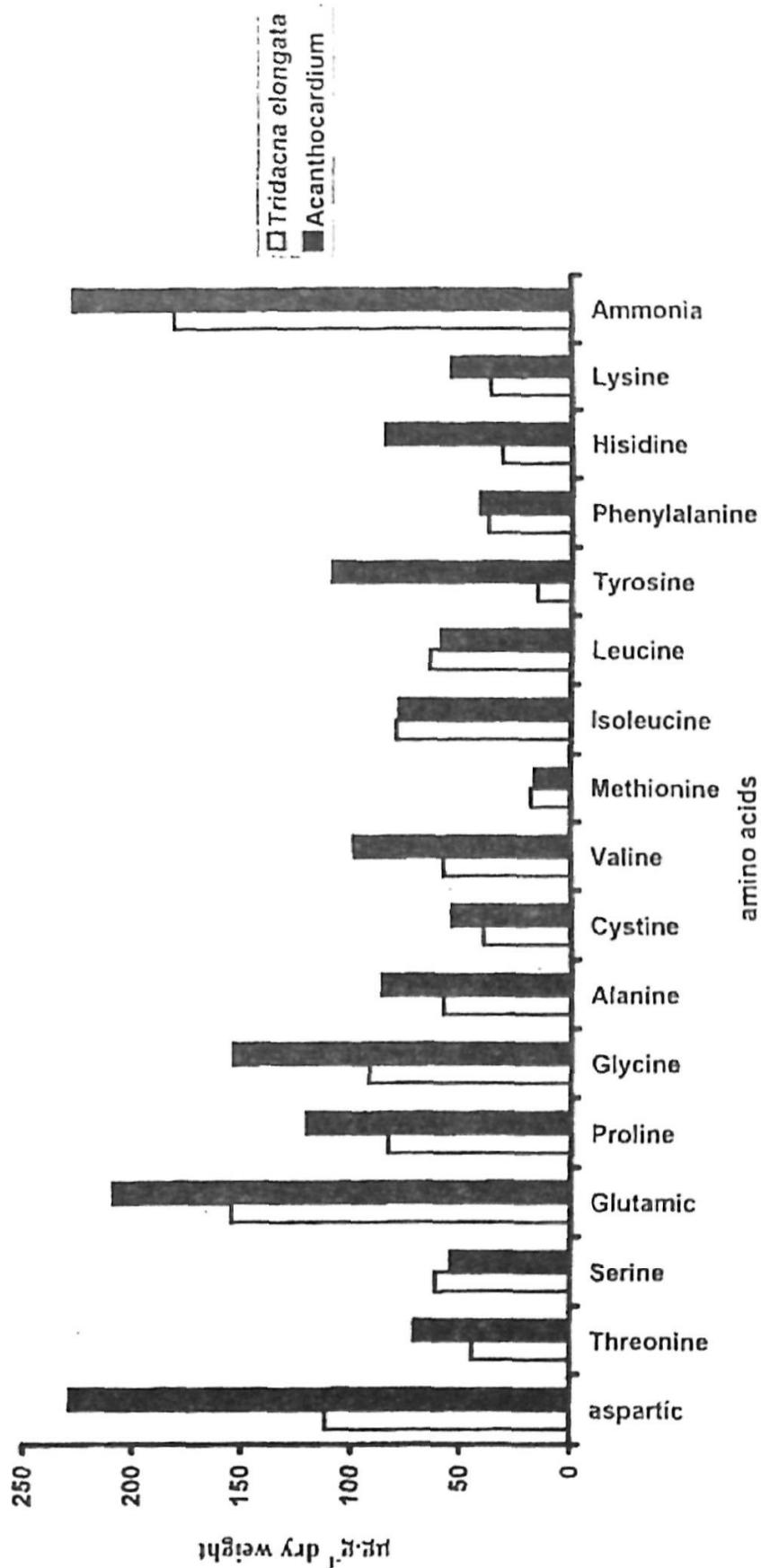


Fig.(3):comparive digram for the amino acid in the studied gastropods

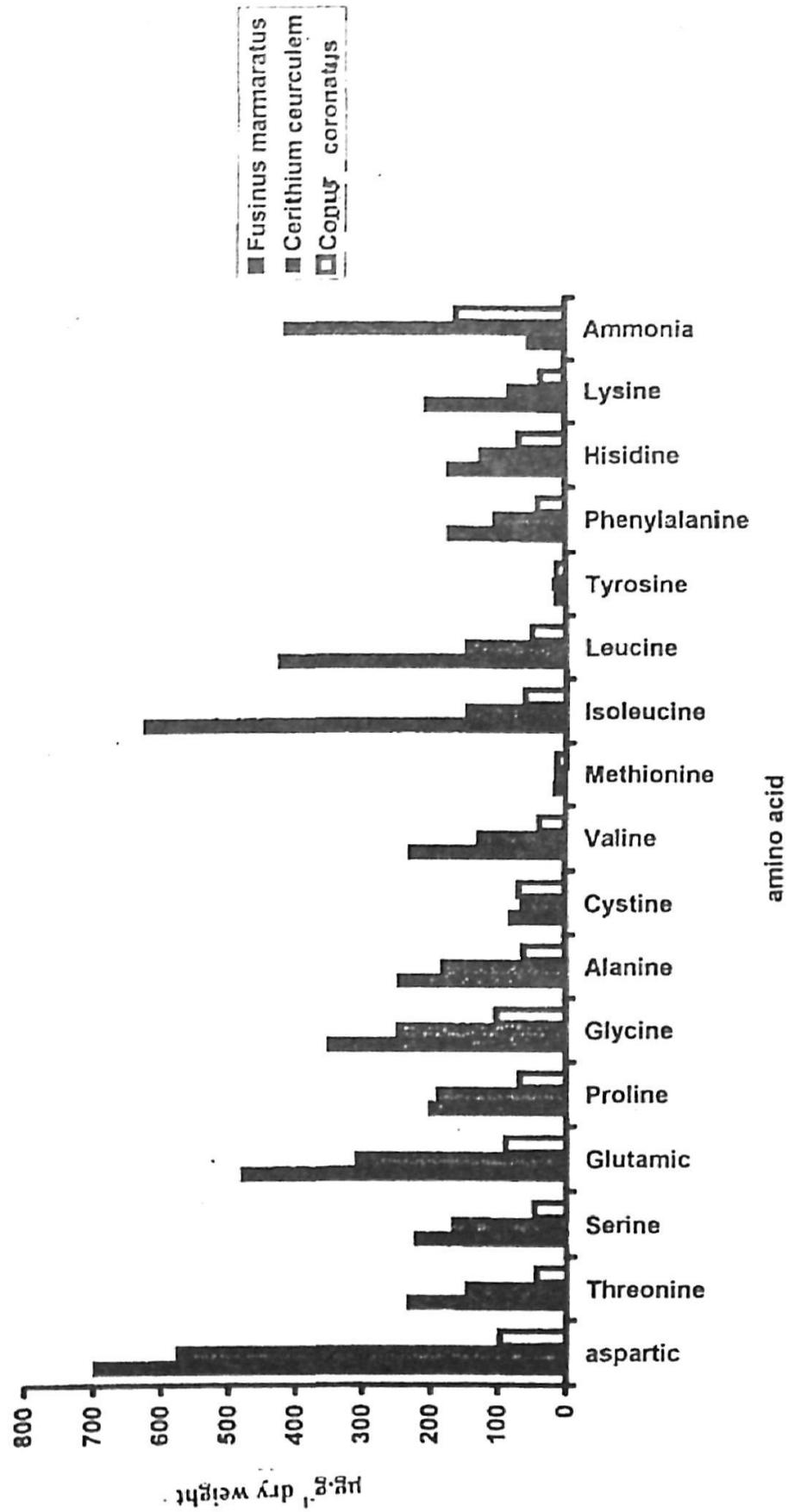




Fig. (4a): X-ray diffraction patterns for the studied shells; 1- *Cerithium Caerculeum*, 2- *Conus coronatus*-, 3- *Fusinus marmoratus*, 4- *Acanthocardium aculeatum*

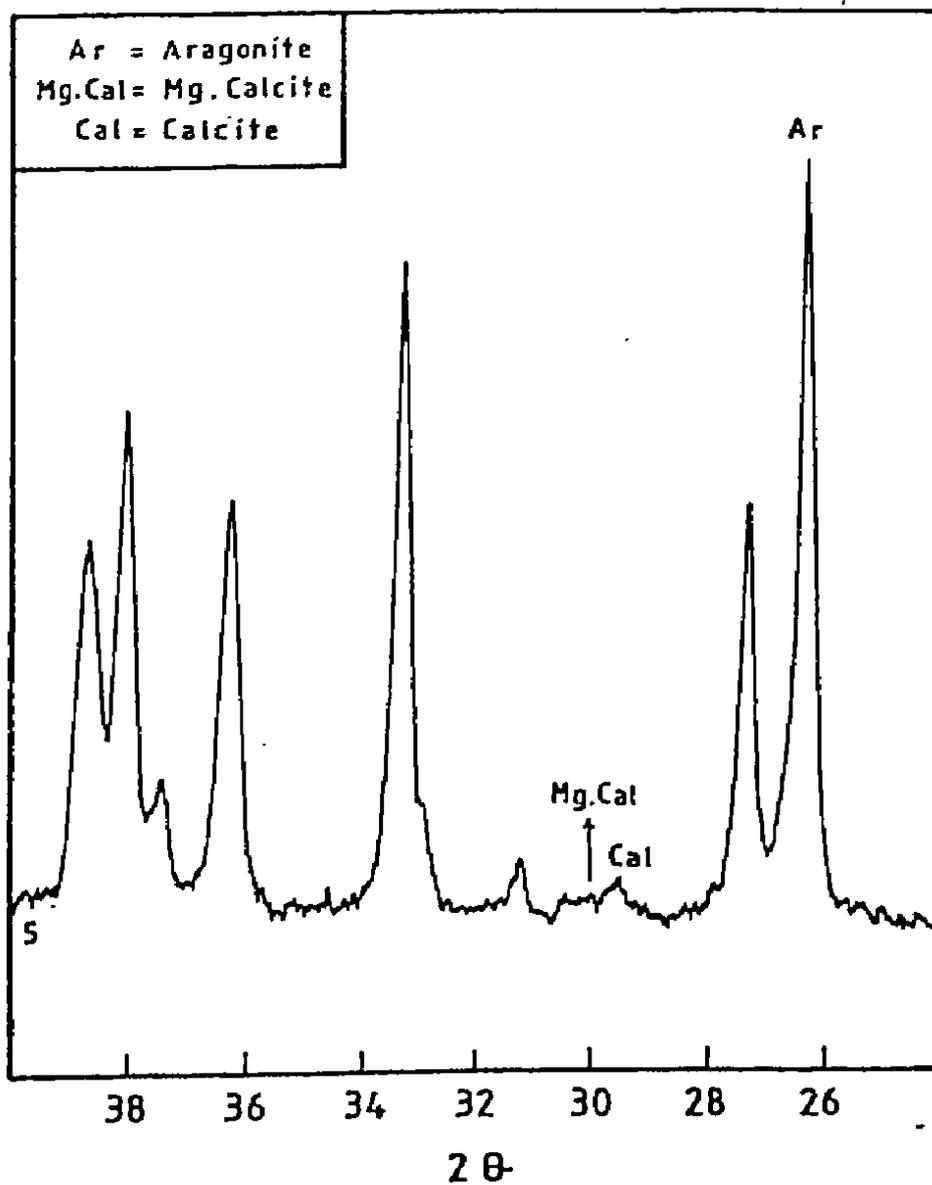


Fig. (4b): X-ray diffraction patterns for the studied shells; 5- *Tridacna elongata*.

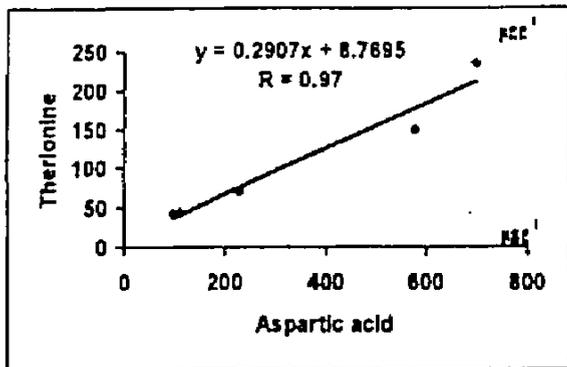


Fig. (5a): Aspartic acid-Threonine correlation in the studied species.

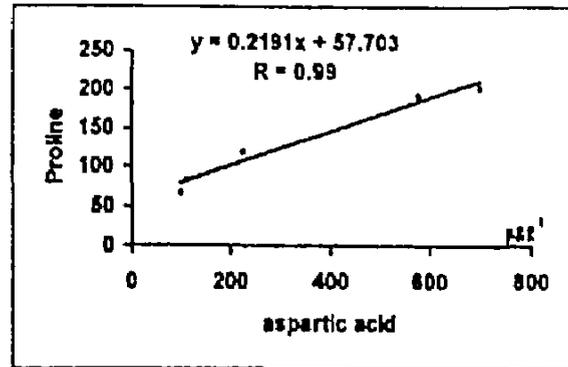


Fig.(5b): Aspartic acid-Proline correlation in the studied species.

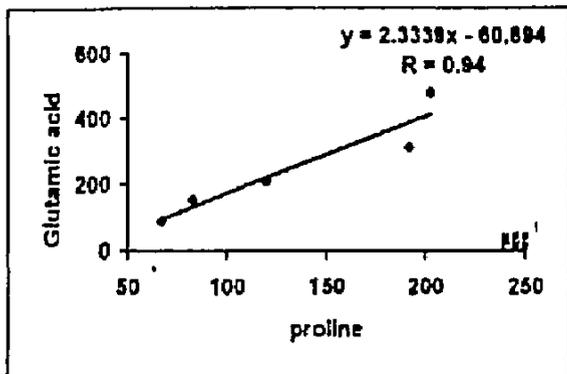


Fig.(5c): Proline-Glutamic acid correlation in the studied species.

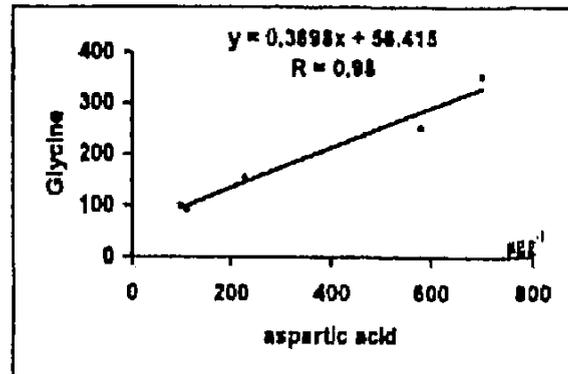


Fig. (5d):Aspartic acid-Glycine correlation in the studied species.

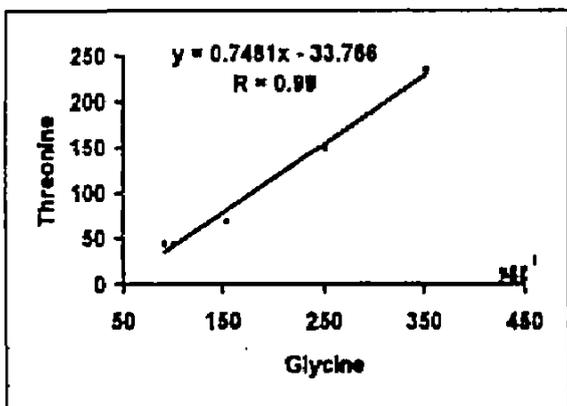


Fig. (5e): Glycine-Threonine correlation in the studied species

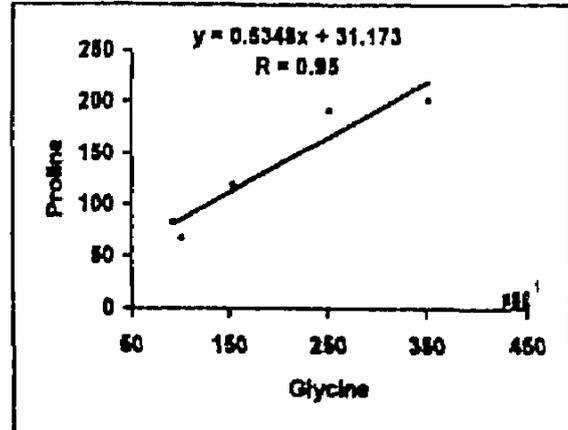


Fig. (5f):Glycine-Proline correlation in the studied species.

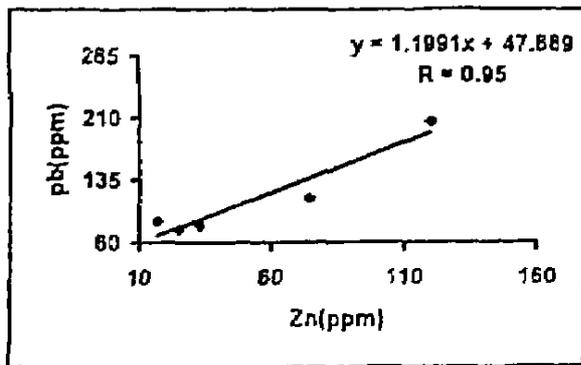


Fig. (6a): lead-zinc correlation in the studied species species

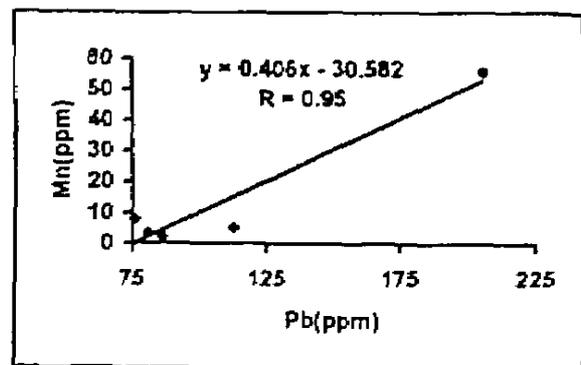


Fig.(6b): lead-manganese correlation in the studied studied sp

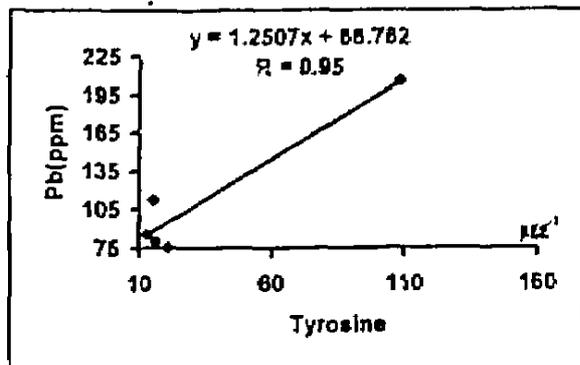


Fig. (7a): Pb-Tyrosine correlation in the studied species.

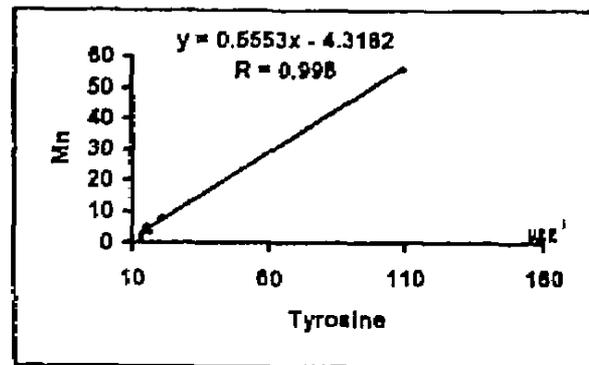


Fig. (7b): Mn-Tyrosine correlation in the studied species.