African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 4, Issue 2, April - June 2025
Page No: 190-198
Website: https://aaasjournals.com/index.php/ajapas/index
ISI 2024: 0.877 **SJIFactor 2024: 6.752** **معامل التأثير العربي: 1.62**

Biodiversity and Chemical Composition Study of Shells in Mollusks from The Intertidal Zone of Apollonia, Susah Shores, Libya Using X-Ray Fluorescence (XRF) Spectroscopy

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دراسة التنوع البيولوجي والتركيب الكيميائي لأصداف الرخويات في المنطقة بين المد والجزر بمنطقة أبولونيا، سواحل سوسة، ليبيا باستخدام مطيافية الأشعة السينية الفلورية (*XRF*)

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Received: February 27, 2025	Accepted: April 19, 2025	Published: April 26, 2025

Abstract:
This research investigates the biodiversity and elemental composition of mollusk shells—specifically gastropods and bivalves—collected from the intertidal zone of Apollonia, Susah Shores, Libya. Shell samples (n = 456) were gathered between November 2019 and February 2020, and species identification was performed using standard taxonomic keys. Ten molluscan families were recorded, with Patellidae, Trochidae, Conidae, and Muricidae being the most prevalent. X-Ray Fluorescence (XRF) analysis revealed that calcium carbonate ($CaCO_3$) was the predominant shell constituent, accounting for 93.33% to 97.88% of the composition. Minor amounts of silica (SiO_2), aluminum oxide (Al_2O_3), and iron oxide (Fe_2O_3) were also present, suggesting both environmental influence and physiological processes in shell development. These findings contribute to the understanding of molluscan shell formation in coastal ecosystems and offer a foundation for further studies on their ecological roles and potential industrial utilization.

Keywords: Mollusk shells, XRF analysis, Biodiversity, Trace elements, Libya.

الملخص
تتناول هذه الدراسة التنوع الحيوي والتركيب العنصري لأصداف الرخويات، وبشكل خاص بطنيات القدم وذوات المصراعين، التي جُمعت من المنطقة بين المدّ والجزر في أبولونيا، شواطئ سوسة، ليبيا. تم جمع 456 عينة من الأصداف خلال الفترة من نوفمبر 2019 إلى فبراير 2020، وتم تحديد الأنواع باستخدام المفاتيح التصنيفية القياسية. وقد تم تسجيل عشر عائلات من الرخويات، وكانت العائلات الأكثر شيوعًا هي: Patellidae، Trochidae، Conidae، وMuricidae. أظهرت تحاليل التألق بالأشعة السينية (XRF) أن كربونات الكالسيوم ($CaCO_3$) كانت المكوّن الرئيسي للأصداف، بنسبة تراوحت بين 93.33٪ و97.88٪. كما كُشف عن وجود كميات ضئيلة من ثاني أكسيد السيليكون (SiO_2)، وأكسيد الألمنيوم (Al_2O_3)، وأكسيد الحديد (Fe_2O_3)، مما يشير إلى وجود تأثيرات بيئية وعمليات فسيولوجية أثناء تكوّن الأصداف. تسهم هذه النتائج في تعزيز فهم آليات تكوّن أصداف الرخويات في النظم البيئية الساحلية، وتوفّر أساسًا لأبحاث مستقبلية حول أدوارها البيئية وإمكانياتها في الاستخدامات الصناعية.

الكلمات المفتاحية: اصداف الرخويات، تحليل XRF، التنوع الحيوي، العناصر النزرة، ليبيا.

Introduction

Molluscs constitute approximately 23% of marine biodiversity in the Mediterranean Sea, highlighting their ecological significance[1,2]. The region's continental shelves and intertidal zones support around 2,100 species of gastropods and bivalves, either in isolation or coexisting with other marine organisms[3,4] . These species exhibit a wide range of morphological and behavioral adaptations across freshwater, marine, and terrestrial environments. A distinctive feature of molluscan anatomy is the mantle, which plays a crucial role in shell formation, respiration, excretion, and environmental sensing[2] .

Recent studies have emphasized the ecological and economic importance of molluscs, particularly in the context of emerging threats such as climate change and ocean acidification [5]. Shifts in environmental conditions within the Mediterraneanespecially along the Libyan coastline—are increasingly affecting molluscan biodiversity and shell development [6].

Libya's coastline, characterized by both rocky and sandy shores, supports a diverse array of marine life, although it remains understudied. These coastal habitats are vital to numerous species and present significant opportunities for scientific exploration [7,8]. Molluscs contribute both ecologically and economically and are utilized in fields such as medicine and environmental monitoring[9,10] . Despite extensive documentation of Mediterranean molluscs[11,12] , data specific to Libyan waters are relatively scarce. Initial surveys recorded 139 molluscan species, and subsequent research has contributed to an expanded understanding of regional diversity [13,1415,16,17,18,19].Nonetheless, a comprehensive inventory of gastropods and bivalves inhabiting Libya's intertidal zones is still lacking.

Environmental factors such as temperature and seawater chemistry significantly influence the mineral composition of molluscan shells, which are primarily composed of calcium carbonate ($CaCO_3$).[20] Shell composition reflects prevailing oceanographic conditions and ecological changes, with biomineralisation processes generating complex structural layers[20,21,22]. Elevated atmospheric CO_2 concentrations reduce carbonate availability, thereby impairing shell integrity and threatening marine calcifying organisms [23,24] Despite the importance of shell geochemistry, research in this area along the Libyan coast remains limited. This study seeks to address this gap by analyzing the elemental composition of molluscan shells collected from the intertidal zones of Apollonia and Susah using X-ray fluorescence (XRF) spectroscopy. XRF provides a non-destructive and precise method for determining the elemental profiles of biological samples[25,26].The primary objectives of this research are to document molluscan diversity and assess shell composition, thereby providing valuable insights to support resource management and conservation initiatives along the Susah coast during the period from November 2019 to February 2020.

Material and methods

Study Area

The study was carried out in the Apollonia region, located near Susah Harbor (31°54' N, 21°58' E), approximately 25 km north of Al-Bayda, Libya. This coastal area is characterized by the presence of a protective reef barrier and relatively deep waters (>35 m) in close proximity to the shoreline[27,28,29,30].(Fig.1).

Figure 1: Study area, sampling and identifying families and species of gatropods and bivalves from Apollonia zone, Susah, Libya between November 2019 and February 2020

Sampling and Species Identification

A total of 456 empty shell specimens were collected by hand from the intertidal zone at shallow depths (5–10 m) within the archaeological site of Susah, Libya, between November 2019 and February 2020. Manual collection was performed using a spatula[31]. The shells were cleaned using a stiff brush or, when necessary, gently scraped with a needle, then stored in plastic bags and transported to the Marine Sciences Department Laboratory at Omar Al-Mukhtar University in Al-Bayda, Libya.Species identification was conducted using both reference collections and taxonomic literature[32,33,34.35]Each shell was measured, and representative specimens were photographed (Fig. 1).

X-Ray Fluorescence Scanning (XRF)

The collected samples were sieved and ground using a rock smasher to obtain a uniform powder. This powdered material was then pressed into steel rings for analysis. Elemental composition was determined using an X-ray fluorescence (XRF) spectrometer (S2 Ranger 2008 - Bruker Company) at the chemical laboratory of the Libyan Co-operative Company (LCC) in Dernah. The analytical procedure followed established methodologies for geochemical analysis[36,37].

Data Analysis

All data were processed and analyzed using Microsoft Excel 2010.

Results and discussion

Molluscan Family Diversity in the Apollonia Zone

This study identified ten families of gastropods and bivalves in the Apollonia region. Among these, Columbellidae, Cardiidae, Truncatellidae, Thyasiridae, Charoniidae, and Arcidae accounted for less than 15% of the total molluscan assemblage. In contrast, the families Patellidae (24.3%), Trochidae (22.4%), Conidae (21.1%), and Muricidae (17.8%) were the most dominant in the study area (Fig. 2). Bariche (2012) noted that bivalves typically burrow into soft sediments, whereas gastropods from the families Patellidae and Trochidae are often found attached to hard substrates or boring into rocks, the coloration of gastropod shells frequently resembles the substrate, likely serving as a camouflage mechanism against predators.[38].

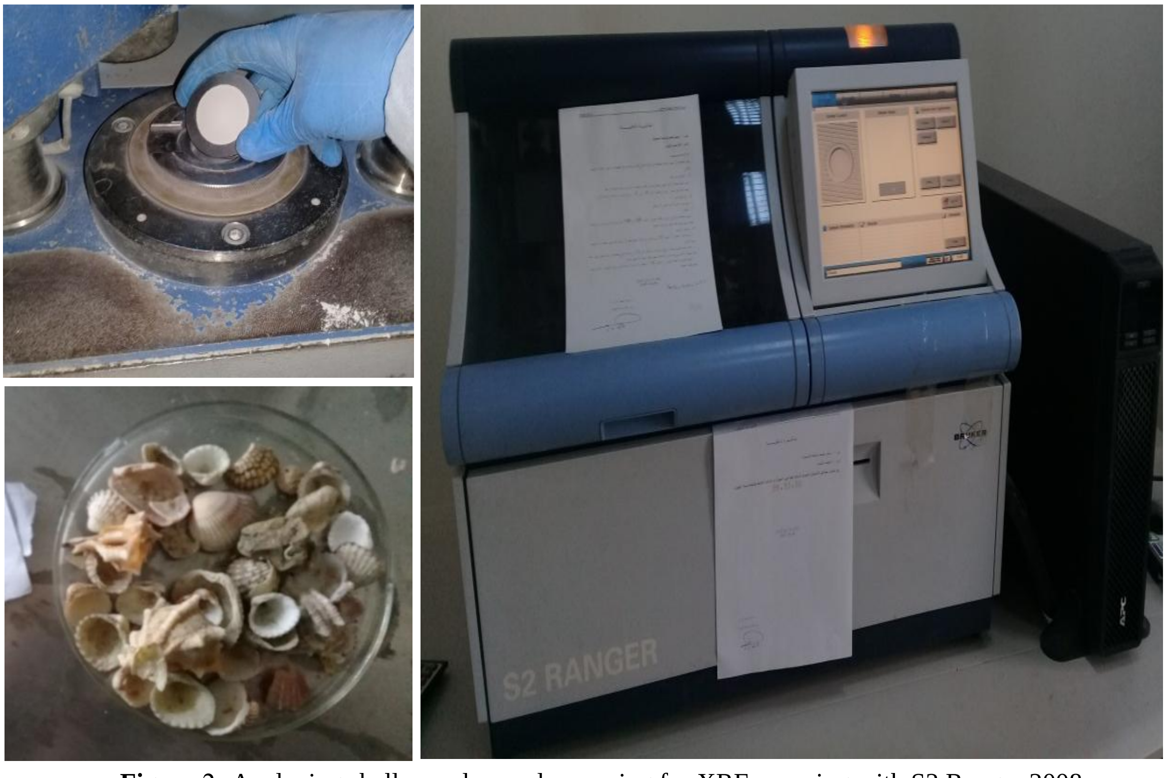

Figure 2**:** Analyzing shell samples, and preparing for XRF scanning with S2 Ranger 2008.

Recent studies have emphasized the influence of environmental stressors on molluscan biodiversity, especially in intertidal ecosystems[39] (Martinez et al., 2021; Thompson et al., 2023). These findings highlight the necessity of monitoring species composition as an indicator of ecosystem health. The increasing sea temperatures in the Mediterranean have caused shifts in gastropod distribution, favoring thermophilic species. This finding supports the observed dominance of *Conus mediterraneus* and *Phorcus turbinatus* in the Apollonia zone[40].
The prevalence of *Patellidae* and *Trochidae* in rocky intertidal habitats may be attributed to their ecological adaptations, such as strong substrate adhesion and resistance to wave action[41]

Species Composition: Dominance of Gastropods and Bivalves
The taxonomic survey revealed nine gastropod and three bivalve genera within the study area. *Conus mediterraneus* emerged as the most abundant species with 96 individuals (21.1%), followed by *Phorcus turbinatus* (87 individuals, 19.1%) and *Hexaplex trunculus* (81 individuals, 17.8%). In contrast, the least abundant species were *Charonia tritonis* and *Truncatella subcylindrica* (each representing 1.3%), along with *Acanthocardia tuberculata* (2.0%), *Thyasira flexuosa* (1.3%), and *Arca noae* (0.7%). several gastropods, including *Charonia* spp. and *Conus* spp., which feed on echinoderms, other mollusks, and fish[42].

Mineral Composition of Mollusk Shells: Calcium Carbonate and Trace Elements
X-ray fluorescence (XRF) analysis indicated that calcium carbonate ($CaCO_3$) was the predominant mineral in mollusk shells, comprising between 93.33% in *Conus mediterraneus* and 97.88% in *Acanthocardia tuberculata*. Trace elements such as silica (SiO_2), aluminum oxide (Al_2O_3), manganese oxide (MnO), and iron oxide (Fe_2O_3) were also detected, with species-specific variability. For instance, *Alvania geryonia* exhibited the highest concentrations of silica and aluminum oxide, whereas *Mysia undata* showed elevated iron content. Sulfur (S) levels varied among species, with *A. tuberculata* registering the lowest concentration (0.421%). These results align with earlier research on molluscan biomineralization and the role of organic matrices in shell construction[43,44] .
Recent advances in XRF spectroscopy have improved its utility for studying shell mineralogy, providing insights into environmental variability and anthropogenic impacts[45,46] .The trace elements in molluscan shells are reliable bioindicators of marine pollution, especially in industrialized coastal regions. These findings reinforce the potential application of molluscan shell analysis in environmental assessment programs[47].
Moreover, the presence of trace elements such as silica, aluminum, and iron in mollusk shells may provide valuable information about local environmental conditions. Zhang et al. (2015, 2024) proposed that these elements are incorporated through sediment interaction or metabolic uptake, offering additional context for environmental monitoring. For example, the elevated iron concentration in *Mysia undata* may reflect localized redox conditions or dietary factors, while the high silica content in *Alvania geryonia* could indicate incorporation of siliceous sediment.

Conclusion
This study provides new insights into the biodiversity and chemical composition of mollusk shells in the intertidal zone of Apollonia, Libya. The results demonstrate that calcium carbonate is the primary component of the shells, with minor variations in trace elements reflecting environmental and biological interactions. These findings contribute to a better understanding of molluscan environmental adaptation mechanisms and provide a foundation for future research on the impact of climate change on marine environments. Additionally, this study can serve as a reference for industrial applications leveraging the mineral properties of shells, such as their use in biomaterials and environmental monitoring.

Table 1. List, Information, count, percentage and XRF results of concentrations of and minerals and mineral oxides in gastropods and bivalve species in Apollonia zone, Susah, Libya from November 2019 to February.

FAMILY	SPECIES	Max. length (cm)	HABITAT AND BIOLOGY	n	%	XRF Analysis results								
						$CaCO_3$	CaO	P_2O_5	MnO	Cl	SO_3	Fe_2O_3	Al_2O_3	SiO_2
Charoniidae	*Charonia tritonis* (Linnaeus, 1758) [Variegated triton]	40.0 **[a]**	Demersal, on soft and hard bottoms. 10 - 50 m depth. Nocturnal. Carnivorous; feeds mainly on mollusks and echinoderms, particularly sea stars **[a]**	6	1.3	62.788	35.131	0.052	0.005	0.346	0.279	0.126	0.344	0.836
Truncatellidae	*Truncatella subcylindria* (Linnaeus, 1767) Cylinder Sea Snail]	0.8 cm Ranged 0.4 to 0.5cm **[b]**	Benthic. Subtropical **[2]**. Members of the family Truncatellidae feed on plant detritus and algae **[b]** **[c]**.	6	1.3	63.832	35.713	0.045	0.005	0.079	0.235	-	0.007	-
Columbellidae	*Columbella rustica* (Linnaeus, 1758) [Rustica dove shell]	1.2 - 2.5 cm	Benthic. Subtropical **[4]** The species displays poecilogonony as its reproductive strategy **[e]**.	36	7.9	63.744	35.325	0.056	0.006	0.412	0.346	0.013	-	0.007
Conidae	*Conus mediterraneus* (Bruguière, 1792) [Mediterranean Cone]	1.3 – 6.3 cm.	Benthic. Subtropical **[b]**	96	21.1	63.660	35.672	0.053	0.007	0.298	0.391	0.020	0.040	-
Muricidae	*Hexaplex trunculus* (Linnaeus, 1758) [Banded Murex]	8.3 **[a]**	Demersal, over soft and hard bottoms. Found usually from the surface down to 100 m depth. Carnivorous species, feeds mainly on mollusks **[a]**	81	17.8	62.510	36.679	0.060	0.013	0.397	0.417	0.020	0.400	-
Patellidae	*Patella ulyssiponensis* (Gmelin, 1791) [Rough Limpet]	7 (5 – 6) **[a]**	Demersal, over rocks and hard grounds. Found usually from the surface down to 5 m depth. Feeds by grazing on algae living on rocks **[a]**	51	11.2	63.110	35.780	0.052	0.006	0.110	0.371	0.120	0.340	-
	Patella rustica (Linnaeus, 1758) [Rustic Limpet]			60	13.2	63.484	35.263	0.045	0.007	0.110	0.270	-	0.007	0.692
Trochidae	*Phorcus turbinatus* (Born,1780) [Turbinate Monodont]	3.5 (2 - 3) **[a]**	Demersal, over hard bottoms. Found usually from the surface down to 5 m depth. Feeds by grazing on algae living on rocks **[a]**	87	19.1	63.237	36.265	0.049	0.005		0.278	0.012	0.032	-
	Gibbula (Magulus) ardens (Salis Marschlins, 1793) [Gibbula Sea snail]	0.8–1.6 cm	Benthic. Subtropical **[b]**	15	3.3	62.617	36.372	0.050	0.006	0.286	0.451	0.119	0.007	-
Cardiidae	*Acanthocardia tuberculata* (Linnaeus, 1758) [Morrocan cockle]	7.7 and 3.08 g **[f][g]**	Benthic; depth range 0 - 100 m **[h]**. Subtropical; 59°N - 7°S, 26°W - 36°. Found in fine and well sorted sand, suspension and deposit-feeder **[i][j]**.	9	2.0	63.344	35.943	0.052	0.005	0.139	0.418	-	-	-
Thyasiridae	*Thyasira flexuosa* (Montagu, 1803) [Flexuose cleftclam]	1.2 **[l]**	Benthic; brackish; depth range 80 - 250 m **[l]**. Temperate. Found in estuarine and inshore areas, in mud, silt, and fine sand substrate. Associated with rich organic sediment. A microvore that feeds on organic detritus **[m]**	6	1.3	62.413	36.020	0.047	0.005	0.340	0.331	-	0.043	0.701
Arcidae	*Arca noae* (Linnaeus, 1758) [Noah's Ark shell]	10.0 **[o]**	Benthic; depth range 125 - 200 m, Subtropical **[p]**. Common. Offshore **[m]**	3	0.7	64.536	34.531	0.005	0.007	0.253	0.428	-	0.146	--
				456	**100**									

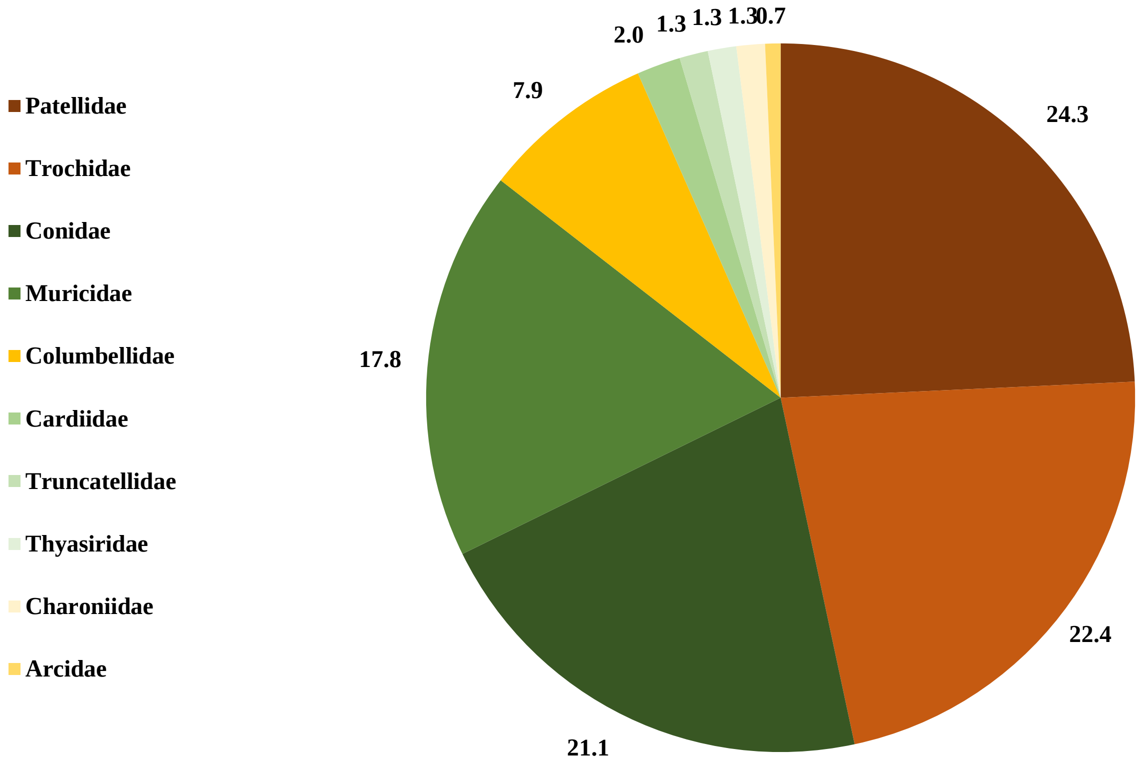

Figure 3**:** Percentage of families of gatropods and bivalves from Apollonia zone, Susah, Libya between November 2019 and February 2020.

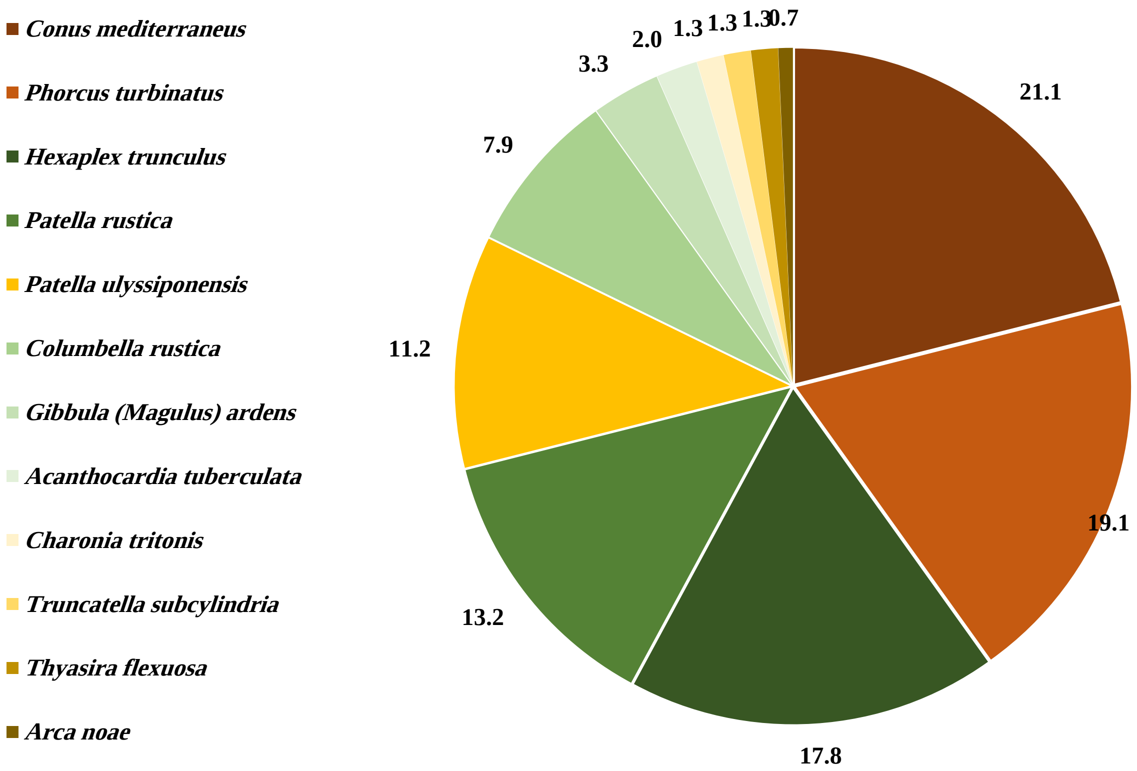

Figure 4**:** Percentage of species of gatropods and bivalves from Apollonia zone, Susah, Libya between November 2019 and February 2020.

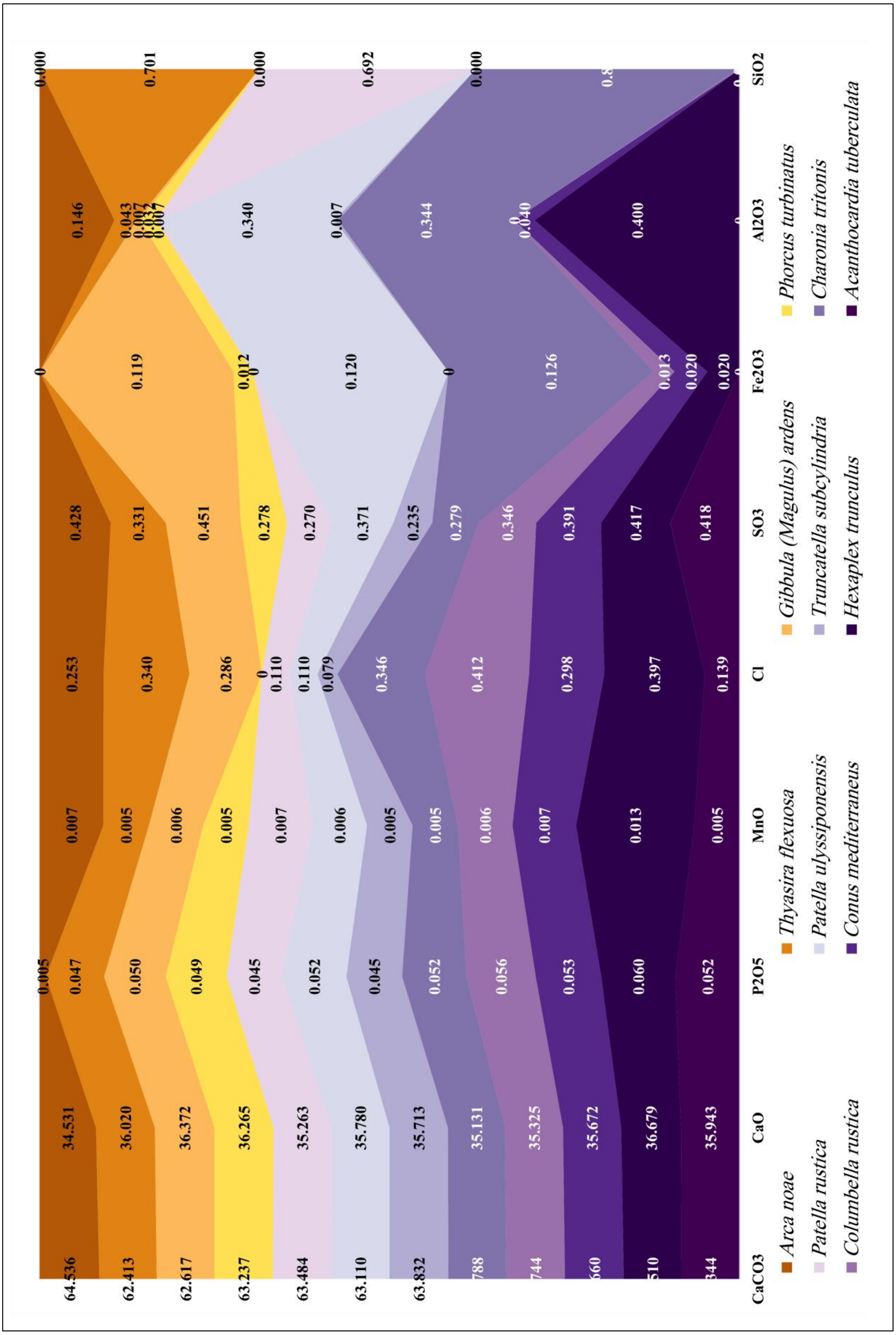

Figure5: Concentrations of and minerals and mineral oxides in shells samples of coastal locations in Cyrenaica, Libya.

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