METAL CONTENT OF SEASHELLS FROM BLACK SEA'S ROMANIAN COAST

Alexandra Ioana Bucur[1], <u>Mihai-Cosmin Pascariu</u>[1,2], Bogdan-Ovidiu Taranu[1], Raul Bucur[1], Iosif Hulka[3], Radu Banica[1*]

[1]National Institute of R&D for Electrochemistry and Condensed Matter – INCEMC, 144 Aurel Păunescu-Podeanu, RO-300569, Timişoara, Romania
[2]„Vasile Goldiş" Western University of Arad, Faculty of Pharmacy, 86 Liviu Rebreanu, RO-310414 Arad, Romania
[3]Research Institute for Renewable Energies – ICER, Politehnica University of Timişoara, 138 Gavril Musicescu, RO-300501, Timişoara, Romania
*e-mail: radu.banica@yahoo.com

Abstract
The main metals from various species of seashells, commonly found on the Black Sea's Romanian coast, were determined by using microwave plasma atomic emission spectroscopy (MP-AES) and energy-dispersive X-ray spectroscopy (EDX). As expected, calcium was the main component, followed by magnesium, aluminum, iron and manganese.

Introduction
Although seashells are generally regarded as waste products with little commercial value, they can be chemically modified to give useful materials by using simple technologies. For example, they can find applications as support for growing biomass, for heavy metal decontamination and for hydroxyapatite fabrication [1].
Various authors studied the elemental distribution in marine organisms and sediments from the Black Sea coast. By using atomic absorption spectroscopy (AAS), Cadar et al. determined the heavy metal (Cd, Cu, Pb and Zn) concentrations in marine water, sediments and algae from the Romanian coast in 2017 and 2018 and found some contamination related to the harbor activities (Constanța and Mangalia) [2]. Heavy metals (Cd, Co, Cr, Hg, Ni, Pb, Sn) from tellina (*Donax trunculus*) on the Turkish coast were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and it was found that temperature changes affected the metal accumulation in the species [3]. Trace elements (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) were determined by ICP-OES in the Black Sea mussel (*Mytilus galloprovincialis*) and rapa whelks (*Rapana venosa*) from Bulgarian coast and they were found not to exceed the maximum residual levels prescribed for seafood [4]. Also, the chemical composition (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Ti, V, Zn) of *Rapana thomasiana* from the Romanian coast was studied with ICP-OES by Sereanu et al. in order to assess the degree of shells mineralization [5].
During our efforts to find practical applications for seashells as biomass support and hydroxyapatite precursors [6-8], we made some preliminary analysis regarding the elemental distribution in molluscan shells collected from the Black Sea's shore.

Experimental
Reagents (65% HNO_3, 28-30% NH_3) were analysis grade from Merck.
Raw seashells were collected in 2020 and at the beginning of 2021 from a Black Sea beach (43.819222 N, 28.589222 E) in the city of Mangalia, Romania (Figs. 1 and 2). They were dried naturally for seven days before further processing.

Figure 1. Seashell collection site in Mangalia, Romania

Figure 2. General aspect of the collection site

For MP-AES analyses, some of the most common types of seashell species found in the Black Sea were selected: *Mytilus galloprovincialis* (sample 1), *Mya arenaria* (sample 2) and *Cerastoderma edule* (sample 3). Dry seashells were washed using an ultrasonic bath and were carefully cleaned on the surfaces of the exoskeleton with a plastic brush. The shells were crushed by pressing them, after which particles smaller than 2 mm were separated using a sieve. These were dried at 115 °C for 20 minute after which 2.000 g of each sample was calcined at

550 °C for 10 h in alumina crucibles with a temperature increase of 5 °C/min. The crucibles are brought to room temperature and the samples are weighted, after which they are kept in a desiccator in the crucibles with concentrated HNO_3 until complete digestion. After dilution with double-distilled water, the samples were analyzed using an Agilent MP-AES 4100 spectrometer, with the following parameters for all samples and wavelengths: 0 degree viewing position, 120 kPa nebulizer pressure (nitrogen as carrier gas), 30 seconds sample uptake time, 15 seconds stabilization time, 10 seconds read time and 3 replicates for all measurements. All glassware was washed with 2% HNO_3 and rinsed with double-distilled water. Solid $CaCO_3$, Fe_2O_3 and metallic magnesium were dissolved in HNO_3 in order to prepare the three standard stock solutions (1000 ppm calcium, 1000 ppm iron and 100 ppm magnesium). Calibration used five levels (0.00, 0.80, 2.00, 10.00, 20.00 ppm for calcium and 0.00, 0.60, 1.50, 7.50, 15.00 ppm for magnesium and iron). For maximum sensibility, calibration and data recording used the top five most intense emission lines offered by the spectrometer software, leaving aside those lines that interfere with sodium and potassium (393.366, 396.847, 422.673, 430.253, 445.478 nm for calcium, 279.553, 280.271, 285.213, 383.829, 518.360 nm for magnesium and 259.940, 302.064, 358.119, 371.993, 385.991 nm for iron). Two dilutions were used for the three samples which were analyzed: for magnesium and iron, present in much lower quantities, the samples were 50 times more concentrated then those for calcium.

For EDX analyses, dry raw seashells were first sieved through a 1 cm mesh sieve. The larger fraction was kept on the sieve and the small foreign bodies were disregarded by blowing compressed air. The samples were then washed in the ultrasonic bath for half an hour, after which they were immersed in 6% sodium hypochlorite for 3 x 24 h to remove the outside organic matter. After this treatment, the samples were repeatedly washed with tap water and finally with double-distilled water. After drying in the oven at 80 °C, the samples were immersed in 1 M NaOH for 24 h in order to remove any organic residues from the surface of the exoskeletons [9]. They were again repeatedly washed with tap water and finally with double-distilled water, then they were dried at 80 °C. The dried samples were mechanically broken and the 4-7 mm fragments were isolated using a custom made sieving device. Heavy metals from seashells were concentrated for EDX analysis by using sulfide precipitation. In order to achieve this, the samples were digested by using 65% HNO_3 (200 mL of acid for 100 grams of seashells). The yellow suspension was boiled until the volume was reduced to half, during which the liquid became almost clear. The pH was adjusted to 10 by using ammonia and the mixture was diluted with double-distilled water until the volume was doubled. Sodium sulfide was added until the reaction with silver nitrate gave a positive result (0.3 mL of 0.1 mol L^{-1} Na_2S). The dark-green precipitate that formed was subjected to centrifugation and washing with double-distilled water (two cycles), after which it was dried in an oven at 105 °C and subjected to EDX analysis by using an accelerating voltage of 25 kV.

Results and discussion
In the case of MP-AES analyses, after inspecting the correlation coefficient (r) and calibration errors, the 422.673 nm line (r=0.99981) was selected for calcium and the 279.553 nm line (r=1.00000) for magnesium. By comparing the amount of calcium determined with MP-AES and the mass of the samples it was confirmed that the exoskeletons are made almost entirely of calcium carbonate, with only traces of other elements. The weight ratios between calcium and magnesium obtained for the three samples were 224.8:1 for the first sample, 1180.8:1 for the second sample and 1067.2:1 for the third sample, with a mean value of 824.3:1. Iron was below the detection limit of all monitored wavelengths and for all samples, even though the calibration correlation coefficient was very good (r=0.99997 for 259.940 nm and r=0.99999 for 385.991 nm) and the calibration errors were minimal (under 3.33%) for the selected wavelengths.

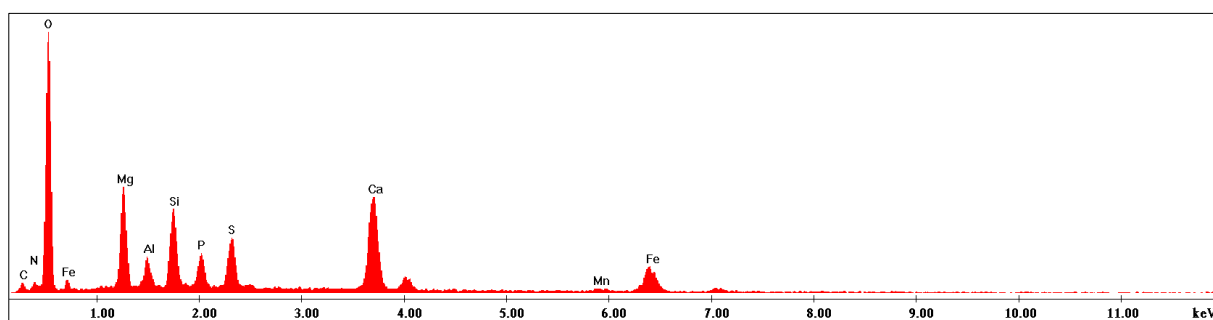The EDX analyses results are given in Fig. 3 and Table 1.

Figure 3. Peaks in EDX analysis of sulfide residue

Table 1. Percentages of each element in EDX analysis of sulfide residue

Element	Weight %	Atom %
O K	60.46	66.52
Mg K	9.41	6.81
C K	6.35	9.30
N K	5.93	7.46
Si K	5.90	3.70
Ca K	3.06	1.34
S K	2.73	1.50
Al K	2.57	1.68
P K	2.22	1.26
Fe K	1.23	0.39
Mn K	0.15	0.05
Total	100.00	100.00

In the EDX spectrum, the presence of C, N, O and P elements was observed, which may originate from the organic matter existing between the aragonite lamellae from the exoskeletons. Due to the fact that these elements were not quantitatively isolated in the analyzed sample, no conclusions can be drawn regarding their relative abundance in the shells. Sulfur can originate from both protein mass and sulfide added to precipitate heavy metals. Also, the residual calcium and magnesium salts originate from the incorporation in the mass of the precipitate. The largest amount of calcium and magnesium was removed during the heavy metal separation protocol. Finally, the ratio of the elements extracted quantitatively from the sample is Si : Al : Fe : Mn = 63.6 : 28.9 : 6.7 : 0.9 (atomic %) or Si : Al : Fe : Mn = 59.9 : 26.1 : 12.5 : 1.5 (mass %). The other elements are below the spectrometer detection limit.

Conclusion
EDX and MP-AES analyses revealed calcium to be by far the main metallic component, followed by traces of magnesium, aluminum, iron and manganese. Although some nonmetals, namely oxygen, carbon, nitrogen, silicon, sulfur and phosphorus were also identified in EDX analyses, only silicon can be quantified with the applied protocol.

Acknowledgements

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