Athanasiadou et al. Int Arch Urol Complic 2017, 3:020
DOI: 10.23937/2469-5742/1510020
Volume 3 | Issue 1

International Archives of
Urology and Complications

ISSN: 2469-5742

Research Article: Open Access

New Insights into the Chemical and Isotopic Composition of Human-Body Biominerals. II: COM Kidney Stones from Greece

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Abstract

We have analyzed kidney stones from patients from Greece in which the mineral phase was calcium oxalate monohydrate (COM) as identified by powder-XRD and FTIR spectra. SEM-EDS analysis revealed microscale-COM crystal aggregates mixed with organic/biological matter. XRF revealed that in addition to Ca (max. ca. 21.4 wt.%; av. ca. 21 wt.%) and Sr (max. 35 ppm; av. 34 ppm), there were significant levels of heavy metal impurities, namely Zn (max. 214 ppm; av. 204 ppm), Pb (max. 149 ppm; av. 136 ppm), Fe (max. 136 ppm; av. 132 ppm) and Cu (max. 17 ppm; av. 15 ppm), as well as minor amounts of Br. As identified by IRMS, all three examined kidney stones presented a very light $\delta^{13}C$ signature (average $\delta^{13}C$ ca. -25.4‰ PDB) as compared to previously reported data on kidney stones from humans from different geographical locations. The $\delta^{18}O$ values averaged ca. -7.31‰ PDB. With regard to radioactive isotopes, HR γ-ray spectrometry demonstrated the existence of the natural radionuclides ^{214}Pb and ^{214}Bi due to ^{238}U-series, and also an additional amount of ^{40}K. We conclude that these kidney stones from southeastern Europe are enriched in essential biometals (Zn and Fe), and also contain a high content of harmful heavy metals such as Pb, and traces of U. This elemental composition may be related to a toxic diet and/or environmental pollution.

Keywords

Biominerals, Kidney stones, Calcium oxalate monohydrate (COM), Metals, Isotopes

Abbreviations

COM: Calcium Oxalate Monohydrate; SEM: Scanning Electron Microscopy; EDS: Energy Dispersive Spectrometry; XRD: X-ray Diffraction; FTIR: Fourier-Transform Infrared Spectroscopy; XRF: X-ray Fluorescence; IRMS: Isotope Ratio Mass Spectrometry; HR: High Resolution; γ-ray: Gamma Ray.

Introduction

Kidney stone disease (urolithiasis) is a serious health problem, involving more than 5% of the population, especially in developing countries [1,2]. Urolithiasis, the formation of urinary calculi in the kidney, bladder and/or urethra, can be attributed to several risk factors related to geographical region, gender and dietary habits [e.g. 3-5]. Urine ion supersaturation, and the effects of various mineralization promoters and inhibitors, in combination with genetic risk factors, plays a significant role in the crystallization process within kidney stones [5]. Understanding the elemental and isotopic composition of kidney stones is central to understanding their initiation and growth, and to developing treatments that prevent their formation.

Kidney stones vary in composition and contain a wide variety of different organic and inorganic compounds. They can be broadly classified into five types of stones depending on their major chemical composition: (a) calcium oxalate, (b) calcium phosphate, (c) uric acid, (d) struvite and (e) cystine [e.g. 3,6]. Calcium oxalate-containing stones are the most common type, where the mineral phase can exist as calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) and rarely calcium oxalate trihydrate (COT), the latter being the most thermodynamically unstable form of calcium oxalate. COM is the most stable form, and it predominates in more than 80% of total stones. Struvite, uric acid and cystine stones are found in a smaller percentage of cases, at 15%, 5-10% and 1% respectively of total kidney stones [3,5-7].

In previous studies by others, analytical techniques such as X-ray Fluorescence/XRF and Proton Induced X-ray Emission/PIXE were used to verify the concentrations of trace elements in COM stones [8-14]. Further analytical techniques, such as Inductively Coupled

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Citation: Athanasiadou D, Godelitsas A, Sokaras D, Karydas AG, Dotsika E, et al. (2017) New Insights into the Chemical and Isotopic Composition of Human-Body Biominerals. II: COM Kidney Stones from Greece. Int Arch Urol Complic 3:020. doi.org/10.23937/2469-5742/1510020

Received: October 17, 2016: **Accepted:** February 07, 2017: **Published:** February 10, 2017

Plasma -Optical Emission & -Mass Spectrometry/ICP-OES & ICP-MS, Atomic Absorption Spectrometry & Emission Spectrometry/AAS & AES, Instrumental Neutron Activation Analysis/INAA and Synchrotron-based methods were also used [9,11,15-25].

The main purpose of the present work was to contribute to information on the chemical composition of European kidney stones, specifically those from southeastern Europe (Greece). Here, XRF was also used for the quantitative analysis of COM kidney stones which were previously characterized using multiple techniques (Scanning Electron Microscopy/SEM, X-ray Diffraction/XRD, and Fourier-Transform Infrared Spectrometry/FTIR). Also, carbon and oxygen isotopic ratios ($\delta^{13}C$ and $\delta^{18}O$) were measured using Isotope Ratio Mass Spectrometry/IRMS whereas high-resolution (HR) Gamma (γ)-ray spectrometry measurements provided with data of natural radionuclides (^{238}U-series, ^{232}Th-series, ^{40}K) in COM kidney stones from the human body.

Materials and Methods

Kidney stones of different sizes were examined after surgical removal from patients at the Laikon General Hospital in Athens (Greece). Three kidney stones were subjected to further detailed analysis after optical examination.

Microscopic analysis was performed using a Jeol JSM-5600 microscope equipped with an Oxford EDS microanalytical system. XRD patterns were acquired by a Siemens D5005 (Bruker AXS) X-ray diffractometer using Cu *Kα*-radiation. FTIR spectra were collected using a Perkin Elmer Spectrum One spectrometer operating in the frequency range 4000-450 cm^{-1} with a 2 cm^{-1} nominal energy resolution. KBr discs were prepared for each measurement from 1.3 mg of powdered sample. An in-house developed transferable milli-beam XRF spectrometer was used for the XRF analysis. This XRF

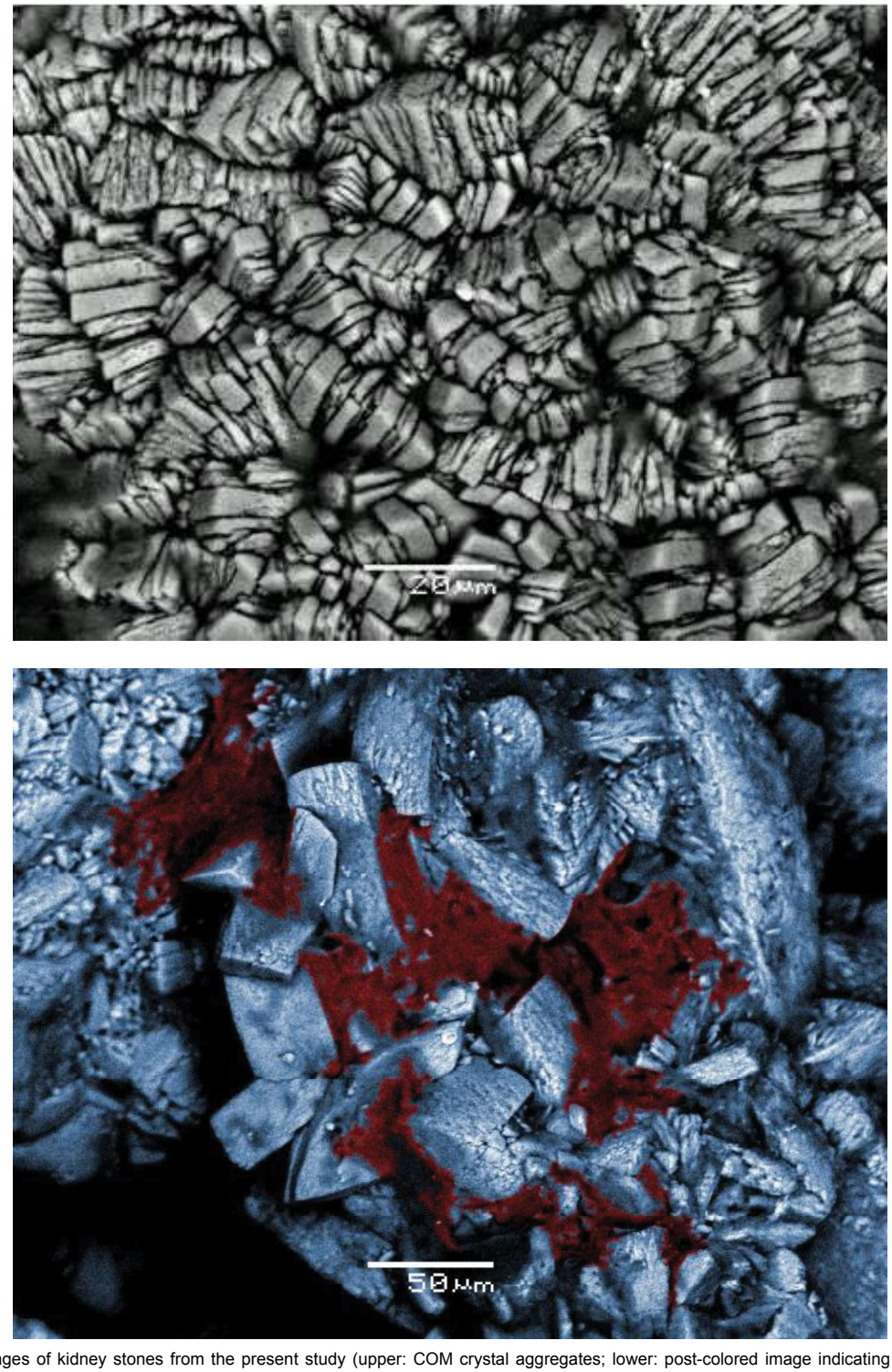


Figure 1: SEM images of kidney stones from the present study (upper: COM crystal aggregates; lower: post-colored image indicating COM crystals partly covered by organic/biological matter).

spectrometer involves a Rh-anode side-window low power X-ray tube (50 W, 50 kV maximum high voltage, 75 μm Be window) encompasses a Si–PiN diode X-ray detector (500 μm nominal crystal thickness, energy resolution of about 165 eV (FWHM) at Mn-*Kα*). For this study, the X-ray tube was functioned at 40 kV with hard filtering (i.e., Ni: 42.5 mg/cm^2, V: 33.0 mg/cm^2) to improve peak-to-background ratio for the determination of metallic trace elements. Quantitative analysis was based on elemental calibration factors obtained experimentally using a set of thin mono-elemental targets with endorsed areal density. Thin pellets of a 1.3 cm diameter each were prepared by compressing the homogenized sample powder using a 5 tonnes hydraulic press.

Stable carbon and oxygen isotopes were measured by Isotope Ratio Mass Spectrometry/IRMS (Thermo Scientific Delta V Plus equipped with a FlashEA 1112 elemental analyser). For evaluating the $\delta^{13}C$ and $\delta^{18}O$ values of the studied kidney stones, 150-200 mg of each sample was used. Finally, the radionuclides contained in the COM kidney stones were determined by high-resolution γ-ray spectrometry using a Canberra high-purity Ge detector (HPGe).

Results and Discussion

Representative morphology and aggregations of COM micro-crystals comprising human kidney stones from Greece, as recorded by SEM, are shown for one sample in Figure 1. *In vitro* studies have shown that carboxylate-rich urinary macromolecules, as well as anionic molecules, can serve as adhesives that stimulate the aggregation of COM crystals [26]. The powder XRD patterns of all three samples disclosed characteristic peaks corresponding to COM [27,28] whereas the FTIR spectra included characteristic bands in the region between 4000-450 cm^{-1} attributable to the vibrations of O–C–O, C–O and C=O, at 781, 1316 and 1621 cm^{-1}, arising from oxalates of COM [29,30] (Figure 2). All the above bulk data confirmed that the exam-

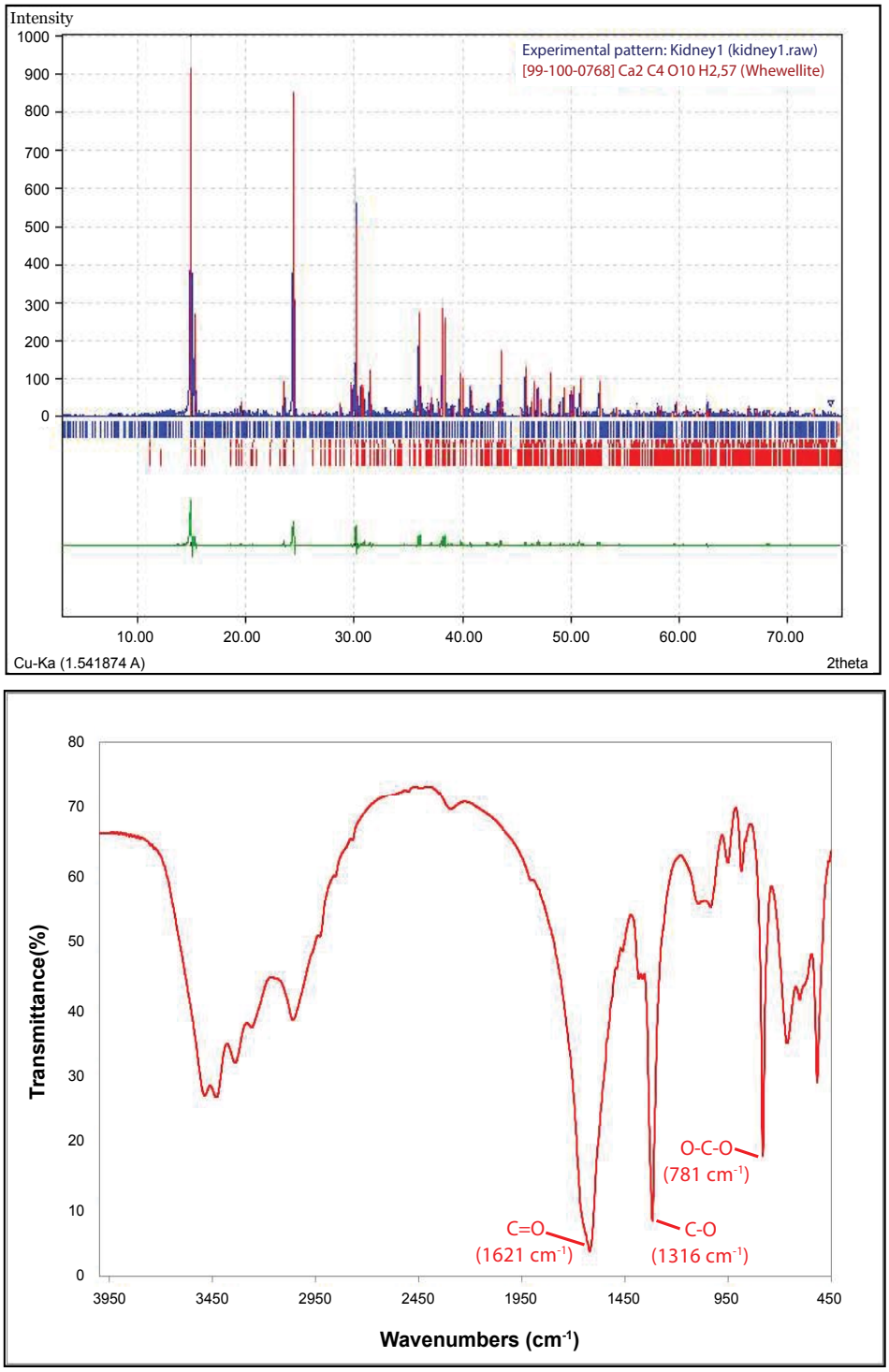


Figure 2: Powder-XRD pattern (upper) and FTIR spectrum (lower) of COM kidney stones from the present study.

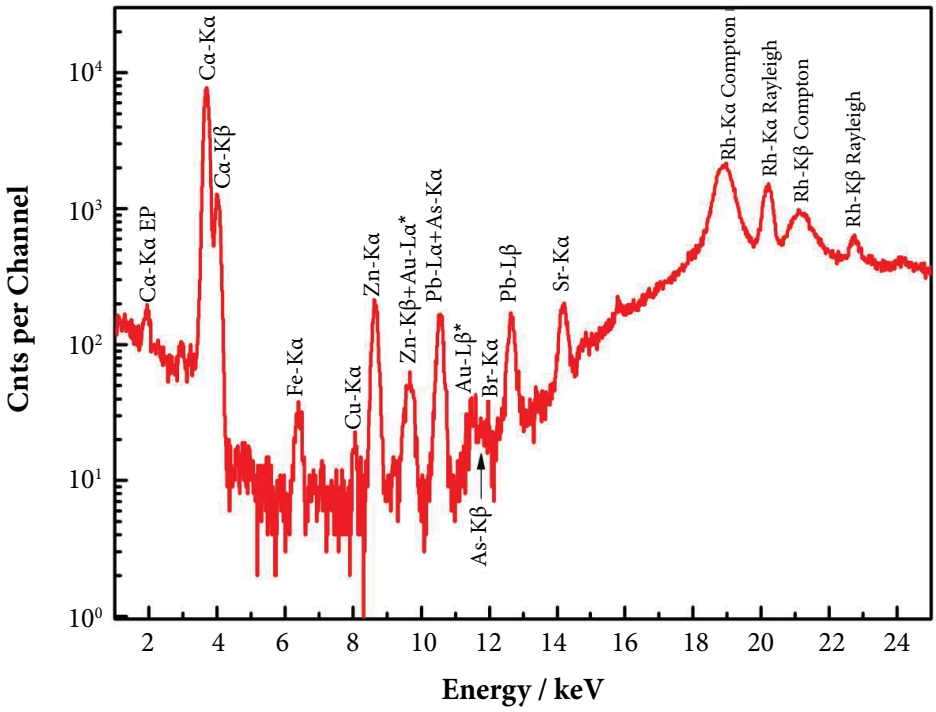
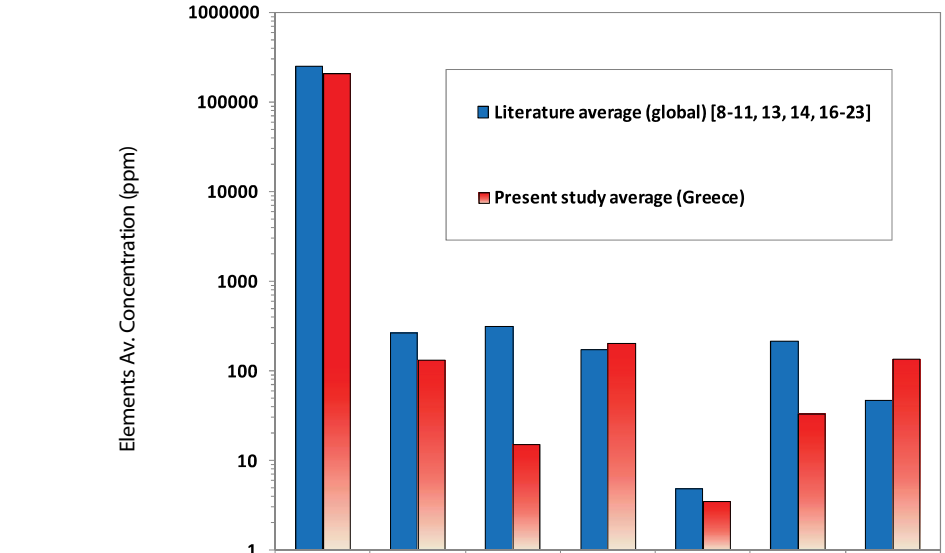


Figure 3: Representative XRF spectrum concerning the COM kidney stones from Greece. The presence of Au *L*-characteristic X-rays is due to interference by the XRF spectrometer materials, namely the X-ray detector (contact electrode).



	Ca	Fe	Cu	Zn	Br	Sr	Pb
Literature average (global) [8-11, 13, 14, 16-23]	254060	268	316	171	5	216	47
Present study average (Greece)	209830	132	15	204	4	34	136

Figure 4: Average concentration of elements in calcium oxalate kidney stones (average of global values from the literature [8-11,13,14,16-23] and average of Greece from the present study).

ined biominerals belong to the COM type of kidney stones. XRF analysis (Figure 3) revealed the presence of essential biometals (Ca, Fe, Cu, Zn) within the stones, as well as potentially toxic metals, alkali earth metals and halogens (Pb, Sr, Br). The presence of Au *L*-characteristic X-rays in the XRF spectrum is attributable to contamination by the XRF spectrometer component materials, namely the X-ray detector (contact electrode). Calcium was found to be the major nonorganic constituent in all kidney stones (max. ca. 21.4 wt.%; av. ca. 21 wt.%).

Dairy and milk products, eggs, water and tea, amongst others, influences Ca content in humans [11]. Strontium (max. 35 ppm; av. 34 ppm) might substitute for Ca due to its valence and similar ionic radius [31]. The precipitation of bromide salts could explain the existence of Br (av. 4 ppm) in the studied kidney stones, but the role of Br in calcium oxalate stone formation needs further investigation [16]. Iron and copper were present in all examined samples, with a maximum concentration of 136 ppm and 17 ppm, respectively (av. 132 and 15 ppm). Beans, meats and water are the main source of Fe, whereas green foods, meats, flour and milk products are rich in Cu [11]. The role of Fe in stone formation has not been elucidated completely. According to Muñoz, et al. [32], ferric ions affect the development of calcium oxalate stones by forming stable chemical interactions with the oxalate ions on their crystal surface. Nevertheless, Meyer, et al. [33] reported that Fe does not influence calcium oxalate crystal growth. The relative low concentration of Cu compared to the global average could be related to its low excretion

in urine [14]. In addition, Zn (max. 214 ppm; av. 204 ppm) was found to occur in concentrations slightly higher than the global average (Figure 4). Similar to Sr, Zn can substitute for Ca in the crystal lattice of calcium oxalate stones [14,34]. The presence of Zn could also be associated with levels of atmospheric pollution [35]. In comparison to other geographic areas around the world, these Greek kidney stones contained comparatively high amounts of Zn and Fe, and less Cu (max. 17 ppm; av. 15 ppm), as illustrated in Figure 5.

It is evident that the kidney stones examined here are highly enriched in potentially toxic metals such as Pb, as compared to the global average (Figure 4). The presence of Pb (max. 149 ppm; av. 136 ppm) may be natural and/or industrial, as well as unexpected (e.g., home-made wine [36]), whereas high concentrations of Pb are found to be related to Ca-containing stones rather than organic phases [14]. It is notable that no concentrations of As were observed, even though high As concentrations have been observed in other biominerals such as cholesterol gallstones from England and Greece [37].

The carbon and oxygen stable isotopic composition of the kidney stones was found to be in the same range. The average $\delta^{13}C$ and $\delta^{18}O$ values of the samples were calculated at -25.4‰ PDB and ca. -7.31‰ PDB, respectively. The $\delta^{13}C$ values are low compared with findings from other studies [38,39]. These low values could be attributable to remaining organic material trapped between the calcium oxalate crystals (Figure 1). The isotopic composition of carbon reflects the dietary habits of the patients. From the $\delta^{13}C$ values the kidney stones are enriched in the light isotope ^{12}C. In a previous study, it was showed that biogenic calcium oxalate was enriched in the light isotope ^{12}C compared to geological calcium oxalate which was enriched in the heavy isotope ^{13}C [40]. DeNiro and Epstein [41] and Jim, et al. [42] were able to determine the influence of various food animals (pigs and rats) in the isotopic composition of carbon. However, the accurate determination of food influence in the isotopic composition of humans is difficult to establish because of the variety of dietary habits. Likewise, the isotopic composition of oxygen reflects the living geographical location of the patients. Dotsika, et al. [43] showed that $\delta^{18}O$ values around -7.5‰ PDB are typical values corresponding to areas near the sea.

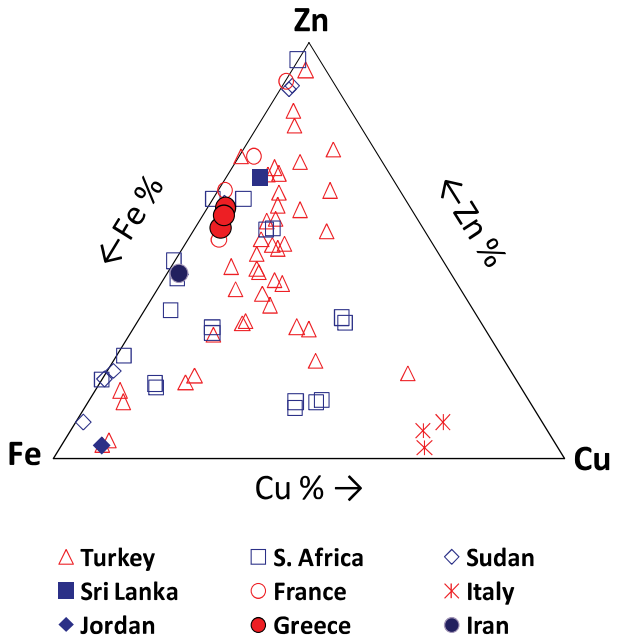


Figure 5: Ternary plot showing the main heavy metal (Fe, Cu, Zn) composition of calcium oxalate kidney stones from different countries of the world ([8-11,13,14,16-23] and Greece from the present study).

Here we reveal for the first time using high-resolution γ-ray spectrometry that the kidney stones of urolithiasis patients may contain radioactive isotopes. Natural radionuclides such as ^{214}Pb (475 Bq/Kg) and ^{214}Bi (375 Bq/Kg) due to ^{238}U- series and ^{40}K (4 Bq/Kg) were determined (Figure 6). This also reveals that traces of U are present in the kidney stones. Finally, it is noticeable that artificial radionuclides such as ^{137}Cs (arising from nuclear accidents including Chernobyl) were not detected.

Conclusions

The present study characterized calcium oxalate monohydrate human kidney stones from Greece by means of microscopic, diffraction and spectroscopic techniques (SEM-EDS, XRD, FTIR), as well as by XRF, IRMS and high-resolution γ-ray spectrometry techniques, to elucidate their chemical and isotopic composition. XRF analysis indicated that Ca is the principal metal in the COM aggregates, along with Zn, Pb and Fe. According to IRMS results, all kidney stones showed a similar $\delta^{13}C$ signature (average $\delta^{13}C$ ca. -25.4‰ PDB) which is rather low compared to values reported in the literature, whereas the $\delta^{18}O$ values were found to have an average of ca. -7.31‰ PDB. Concerning radioactive isotopes, γ-ray spectrometry revealed the existence of ^{214}Pb and ^{214}Bi, due to ^{238}U- series, and also a detectable amount of ^{40}K. From these results, we can conclude that the examined Greek kidney stones are enriched in metals essential for the normal functioning of the body such as Zn (max. 214 ppm; av. 204 ppm), but they also contain excess harmful elements such as Pb (max. 149 ppm; av. 136 ppm) and traces of U. This might be related to a potentially toxic diet and/or environmental pollution.

Acknowledgements

We would like to thank Prof. M. D. McKee (Faculty of Dentistry, McGill University, Canada) for comments and reading of the manuscript, as well as Prof. E. Iatrou (Dept. of Chemistry, University of Athens, Greece) for collaboration during FTIR measurements.

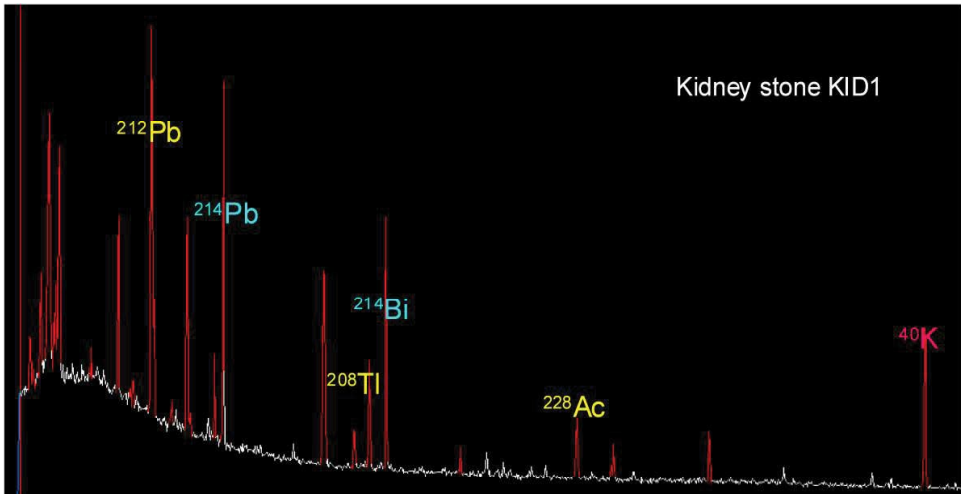


Figure 6: Representative HR γ-ray spectrum, indicating natural radioactivity, concerning the studied COM kidney stones from Greece.

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