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Applied methodology

Trace element investigations in urinary stones: A preliminary pilot case in Basilicata (Southern Italy)

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ABSTRACT

The role of trace elements in the lithogenesis of urinary stones is still debated. However, it is generally appreciated that urinary stones are one of the most common health problems in the world and are strongly associated with environmental factors. It is important to highlight that urinary stones containing trace elements could be considered a marker of environmental pollution.

A large set of urinary stones (48), collected among the Basilicata (southern Italy) inhabitants, was analysed by AAS and ICP for the content of specific chemical elements that were either involved in the crystallisation process of kidney stones (Ca, Mg, K, Zn, Fe, Cu, and Mn) or which were potentially toxic (Pb, Cr).

Three main findings emerge from the results:

(1) Most urinary stones had high concentrations of elements such as K, Cu and Mg and a low content of Fe when compared to the results of previous studies.
(2) Significant amounts were found in inorganic phases (calcium oxalate and phosphates), whereas only the Zn content was high in organic phases (uric acid and cystine).
(3) Among calcium-based stones, those that were calcium phosphate contained greater amounts of trace elements than those that were calcium-oxalate. Among the calcium oxalate stones, weddellite retained more trace elements than whewellite.

Furthermore, the results showed that the concentrations of Zn, Cu, Fe, Pb and Cr were greater than that of ingested from a standard diet. Consequently, varying amounts of these elements may have been attributed to their enrichment in the diet of the inhabitants of polluted areas.

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Introduction

In recent years, there has been growing interest in the analysis of trace elements in all fields of chemical, biochemical, geochemical, biological and environmental research.

Pathologies caused by microelements (Fe, Cu, Zn, Pb, etc.) have been studied for more than two decades. These elements have high affinity for essential Group II elements (Ca, Mg) and substitute for the latter in organic and inorganic structures. Thus, they form insoluble compounds that can contribute to the formation of the pathological mineral structures referred to as urinary stones.

Urinary stones may contain various combinations of chemicals. The most common types of stones are comprised of calcium in combination with either oxalate [1–3] or phosphate. Uric acid stones are also frequent. Struvite stones are less common and formed by infections in the urinary tract. In addition, cystine stones are rare.

While the bulk elemental composition of urinary stones has been the subject of many investigations, there is insufficient information with regard to the distribution of trace elements in these stones, especially within the different stone types.

However, it is of considerable medical importance to understand the role played by trace elements in the nucleation and crystallisation process. This knowledge will not only help to elucidate factors contributing to the initiation and growth of stones but may also facilitate treatment protocols, such as ultrasonic breakdown.

Materials and methods

We collected more than 100 urinary stones, which were either expelled or surgically extracted from 80 patients admitted to the San Carlo Hospital in Potenza (Basilicata, Southern Italy), during January 2007 to January 2008. Patient information was gathered

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from questionnaires asking for personal details (such as gender, age, marital status, address and educational level), socio-economic details (occupation) and medical history (such as weight, height, personal and family anamnesis).

The removed stones were placed in polyethylene dry bottles (bearing the name, sex and age of the patient) and transferred to the laboratory of Environmental and Medical Geology of the Institute of Methodologies for Environmental Analysis (IMAA-CNR, Italy).

All samples were washed several times with deionised water to free the stones of urine, blood debris and remnants of organic matter.

Optical observations were carried out by a stereomicroscope to determine the colour, shape, overall appearance, surface features and evidence of crystalline layers and/or organic matter on the surface.

Half of the sample was used for X-ray diffraction analysis (XRD). Urinary stones were crushed in an agate mortar such that a fine mesh powder was obtained suitable for X-ray diffraction. This technique was used to obtain the crystalline phase composition of the urinary stones.

The XRD measurements were performed with a microdiffractometer (D/max RAPID Rigaku), using a CuKα tube operating at 40 kV and 30 mA. The qualitative interpretation of the diffractometric profiles was achieved by comparing the peaks of reflection with those in the PDF card (Powder Diffraction File) [4].

Mineralogical and morphological data on the full stock of stone were the subject of a previous study [5].

All categories of the classification scheme proposed by Grases et al. [6], Grases et al. [7] were represented by selecting 48 urinary stones of different types (Table 1) from all of the stones collected.

After the mineralogical investigation, 60 mg of each sample was crushed and ground in an agate mortar for chemical analysis. Following a standard digestion process, the samples were studied. To test the validity of the techniques, the separately decomposed quantities of powder were divided into two portions.

Two 30 mg portions of the homogenised and powdered stones were dissolved in 3 mL of concentrated HNO_3 and 0.5 mL of $HClO_3$ (70%) at 70 °C in a water bath for at least 1 h. Following digestion, all of the urinary stones had completely dissolved.

Digested aliquots were diluted to a known volume and stored in polyethylene bottles for subsequent analysis by atomic absorption spectrophotometer (AAS spectra AA 200 Varian) and inductively coupled plasma-optical emission spectrometers (ICP-OES Vista mpx Varian).

The quantities of the trace metals Ca, Mg, K and Zn were analysed because of their indispensability in the crystallisation process. By contrast, Pb and Cr were analysed because of their known toxicity in humans.

Details of all the employed procedures are described elsewhere [8–14].

Results

Mineralogical stone analysis

The results of the mineralogical analysis of 48 selected stones are shown in Table 1, wherein 63% of the stone can be considered pure because only one mineralogical phase is shown.

Oxalates are often associated with other phases such as apatite. Stone types n. 4 and 9 (weddellite plus hydroxyapatite and weddellite plus uric acid, respectively) are considered mixed stone because they are made of the same quantity of more than one phase.

Patients: clinical indications

According to the interviews, urinary stones were more frequent in men than women, with increased prevalence in the 41–60 year-old age group (Table 2).

Patients with a family history of the disease were infrequent. In contrast, 30% of the patients presented with more than one stone episode.

The most frequent co-existing diseases were hypertension and diabetes. Generally, the frequency of important diseases, such as hypertension and diabetes, was greater among patients with urolithiasis as previously shown [15]. Regarding the location of the stones, 50% were found in the kidney, 16.7% in the upper ureter, 27.1% in the middle and lower ureter and 6.3% in the lower tract. Slight differences were observed between the male and female groups.

Trace elements in urinary stones and distribution of elements in mineral phases

The results of the chemical analysis were reported as the mean values with standard deviation (Table 3). In addition, the results of the trace element analyses of different powder portions were found to differ by no more than 5%.

In addition to calcium, not all detected elements were considered minor or trace elements that exceed 1% by weight of the sample. This is relevant in the case of K was a minor element for struvite stones, uric acid and some mixed stones (types 4 and 9). In addition, Mg, which is a major component of struvite stones, was a minor element for uric acid stones and mixed stone types (types 4 and 9).

One should note that the variability of the results is very high within the same group of stones.

An initial comparison was made by splitting the stones into two broad categories (Fig. 1):

- inorganic urinary stones (which has a prevalent calcium component: types 1, 2, 3, 4, 6, 9); and
- organic urinary stones (uric acid and cystine: type 8 and type 10).

Inorganic urinary stones contain a larger proportion of Cu, Mn, Fe, Pb and Cr and a lesser proportion of Zn compared organic stones (Fig. 1).

Differences were observed in the distribution of the elements within the group of inorganic urinary stones.

Mixed stones made up of oxalates in combination with uric acid (type 9) and hydroxyapatite (type 4) contained greater amounts of K, Mn and Zn compared with stones consisting exclusively of oxalate (types 1–3).

Although in lower quantity, Pb and Cr are found in all types of stones.

The high amount of magnesium in struvite stones is justified by the fact that this element is a major component of this mineral phase. The struvite stone also has high values of K, Fe, Zn and Mn.

Among the organic urinary stones, which are often pure stones, there is a greater amount of K and Cu in the uric acid stones compared with those of cystine, which show high values of Zn and Mg. However, manganese is not observed in the cystine stones.

Small detectable amounts of Pb and Cr are present in both types of stones.

The type 3 stones are composed predominantly of weddellite and show higher levels of Zn, Mn, Fe and Cu compared with stones made predominantly of whewellite (types 1 and 2) [16,17] (Fig. 2).

This is more evident when the average content of elements found in the weddellite stones is plotted against that found in the

Table 1
Urinary stones selected for the analysis.

Patient number	Stone type	Description	Mineralogical composition					
			Whew	Wedd	Ap	Stru	UA	Cys
1	6	Struvite				100		
2	6	Struvite				100		
3	6	Struvite				100		
4	10	Cystine						100
5	1	Whew ellite papillary stone	100					
6	1	Whew ellite papillary stone	100					
7	1	Whew ellite papillary stone	100					
8	1	Whew ellite papillary stone	100					
9	1	Whew ellite papillary stone	100					
10	2	Whew ellite stone in cavity	100					
11	2	Whew ellite stone in cavity	100					
12	2	Whew ellite stone in cavity	100					
13	2	Whew ellite stone in cavity	100					
14	2	Whew ellite stone in cavity	100		tr.			
15	2	Whew ellite stone in cavity	100		tr.			
16	2	Whew ellite stone in cavity	100		tr.			
17	2	Whew ellite stone in cavity	100		tr.			
18	2	Whew ellite stone in cavity	100		tr.			
19	3	Weddellite stone		100				
20	3	Weddellite stone		100				
21	3	Weddellite stone		100				
22	3	Weddellite stone		100				
23	3	Weddellite stone		100				
24	3	Weddellite stone	10	90				
25	3	Weddellite stone	30	70				
26	3	Weddellite stone	5	95				
27	3	Weddellite stone		100				
28	3	Weddellite stone	12	88				
29	3	Weddellite stone		100				
30	3	Weddellite stone		100	tr.			
31	3	Weddellite stone		100	tr.			
32	3	Weddellite stone		100	tr.			
33	4	Mixed stone: weddellite + hydroxyapatite	2	44	54			
34	4	Mixed stone: weddellite + hydroxyapatite	2	54	44			
35	4	Mixed stone: weddellite + hydroxyapatite	3	58	39			
36	8	Uric acid					100	
37	8	Uric acid					100	
38	8	Uric acid					100	
39	8	Uric acid					100	
40	8	Uric acid					100	
41	8	Uric acid					100	
42	8	Uric acid					100	
43	8	Uric acid					100	
44	8	Uric acid					100	
45	8	Uric acid					100	
46	9	Mixed stone: whewellite + uric acid	56				44	
47	9	Mixed stone: whewellite + uric acid	89	2			9	
48	9	Mixed stone: whewellite + uric acid	47	3			50	

whewellite stones. As an element deviates from the diagonal, the greater is the difference in the accumulation of the two minerals.

One should note that there is no perfect 1:1 relationship between the elements. However, weddellite (dihydrate oxalate) contains a greater amount of trace elements.

Even between the two types of whewellite stones, there are differences in the concentration of the trace elements (Fig. 3).

Whewllite stones formed in renal cavities display greater abundance of K, Mg and Zn.
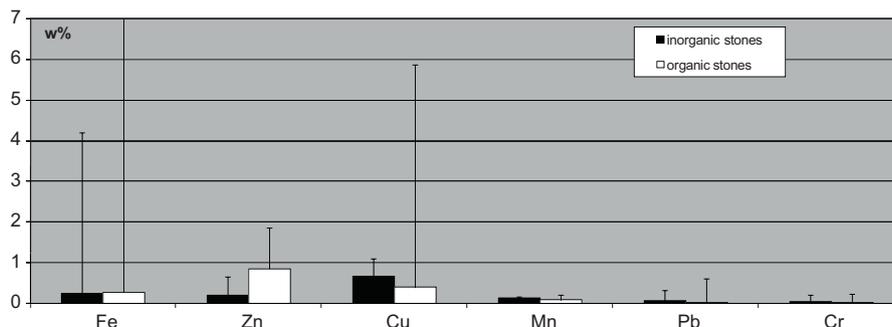

Fig. 1. Comparison between trace element concentration in inorganic and organic urinary stones.
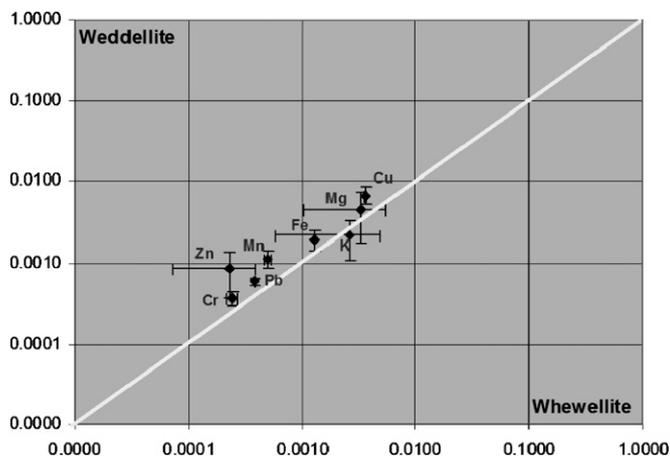
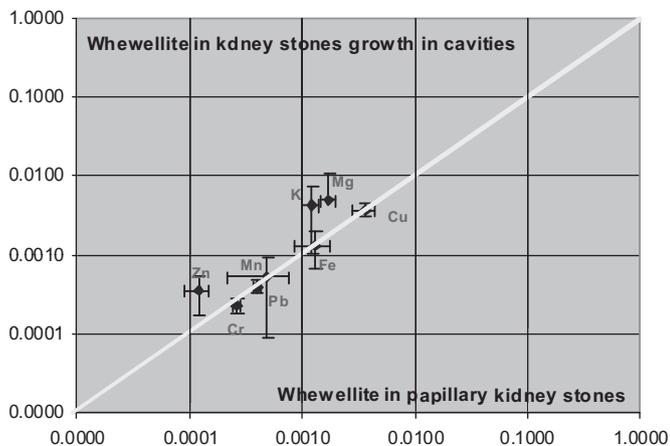**Fig. 2.** Comparison between the average element concentrations in oxalate urinary stones.

Fig. 3. Comparison between the average element concentrations in the two types of whewellite prevalent urinary stones.

Table 2
Characteristics of 48 patients with urinary stones.

Characteristics of the 48 patients with urinary stones		
Characteristics	No.	%
Medical conditions		
Diabetes	3	6.3
Fibroma	1	2.1
Inflammatory bowel disease	3	6.3
Hypertension	5	10.4
Hyperthyroidism	2	4.2
Arthrosis	2	4.2
Chronic renal failure	1	2.1
Family history of urinary stones	19	39.6
Recidivisms	30	62.5
Gender		
Male	25	52.1
Female	23	47.9
Age		
<20	1	2.1
21–40	13	27.1
41–60	22	45.8
61–80	12	25.0

Location of kidney stones	%	M	F
Renal	50.0	13	11
Ureter upper	16.7	4	4
Ureter middle and lower	27.1	7	6
Lower urinary tract	6.3	1	2

Trace elements ingestion from the food chain

The literature often refers to an entire group of urinary stones without any classification or simply divides them into a few groups [13,14,18,19].

Thus, it was necessary to calculate the average concentration of each element, irrespective of the type of analysed stones. Here, we consider studies that are more recent where the results were expressed as a percentage by weight (Table 4).

Considering only the mean values, the differences observed in the stones of the Basilicata inhabitants showed a far lower content of Fe and a higher level of Cu.

To assess the contribution of trace elements obtained from the food chain, the average content of elements derived from a standard diet (daily dietary intake) was taken into consideration [20,21] (Table 5).

To compare the levels of elements found in urinary stones and those which are introduced daily into the human body, the normalisation of the value of each element relative to the quantity of calcium (which was the most abundant element) was performed.

In the mixed stone types (types 4 and 9), the distribution of elements is altered during the association between the principal component (usually oxalate) with another mineral phase (Fig. 4).

Table 3
Average element concentration in different urinary stone types.

Urinary stone type		Ca (wt.%)	K (wt.%)	Mg (wt.%)	Fe (wt.%)	Zn (wt.%)	Cu (wt.%)	Mn (wt.%)	Pb (wt.%)	Cr (wt.%)
1		26.778	0.207	0.293	0.227	0.021	0.622	0.084	0.068	0.046
	σ	±1.747	±0.037	±0.036	±0.079	±0.006	±0.161	±0.048	±0.006	±0.004
2		24.682	0.715	0.814	0.222	0.059	0.630	0.088	0.066	0.038
	σ	±1.724	±0.535	±0.984	±0.111	±0.029	±0.145	±0.072	±0.012	±0.006
3		23.524	0.322	0.611	0.269	0.119	0.996	0.158	0.082	0.051
	σ	±4.081	±0.161	±0.389	±0.072	±0.065	±0.333	±0.040	±0.013	±0.013
4		23.558	1.083	1.104	0.215	0.567	0.593	0.151	0.083	0.026
	σ	±1.907	±0.009	±0.054	±0.059	±0.352	±0.015	±0.078	±0.040	±0.000
6		16.109	2.145	9.629	0.421	0.953	0.338	0.205	0.039	0.033
	σ	±3.927	±0.412	±1.272	±0.349	±0.364	±0.117	±0.273	±0.016	±0.021
8		2.645	1.374	0.051	0.216	0.201	0.635	0.063	0.005	0.019
	σ	±1.951	±0.404	±0.023	±0.104	±0.051	±0.102	±0.082	±0.002	±0.009
9		16.523	1.163	0.145	0.265	0.154	0.530	0.194	0.047	0.019
	σ	±2.129	±0.215	±0.072	±0.156	±0.039	±0.246	±0.187	±0.014	±0.003
10		6.190	0.144	0.218	0.197	1.405	0.220	0.000	0.008	0.011
	σ	–	–	–	–	–	–	–	–	–

Table 4
Comparison between trace element concentration in Basilicata urinary stones and other world geographical areas.

Element	Quantity (wt.%)							
	Basilicata (n. 48)		Giordania (n. 110) [8]		Nord Giordania (n. 35) [18]		Mauritius (n.12) [13]	
	Average	Range	Average	Range	Average	Range	Average	Range
Ca	17.234	2.64–26.77	20.33	6.8–38.25	60.88	24.7–88.8	16.13	0.16–32.81
K	0.902	0.14–2.14	0.9	0.002–1.57	1.33	0.19–3.02	0.217	0.03–0.56
Mg	1.632	0.05–9.63	3.08	0.009–6.95	1.47	0.39–2.83	0.84	0.0004–4.6
Fe	0.262	0.19–0.42	1.17	0.09–3.85	1.094	0.17–2.05	0.67	0.001–5.23
Zn	0.448	0.02–1.40	0.7	0.005–1.56	0.34	0.1–0.58	0.132	0.002–0.8
Cu	0.574	0.22–0.98	0.19	0.002–1.05	0.285	0.24–0.33	0.0013	
Mn	0.119	0.06–0.20	0.029	0.005–0.06	0.13		0.07	
Pb	0.049	0.008–0.085	–		–		–	
Cr	0.031	0.011–0.053	0.146	0.003–0.77	–		0.0065	0.0015–0.017

The calcium concentration was also shown to inhibit the absorption of Pb, Cd, Zn, and Cr. Thus, the concentrations of these elements relative to calcium may be more significant than taking their absolute values into account [22].

The quantity of elements derived from the diet ($Q_{(E)}$) were related to the quantity of daily calcium derived from food and water ($Q_{(Ca)}$) and compared with the quantity calculated in this study and normalised against the amount of calcium measured.

$$\frac{Q_{(E)}}{Q_{(Ca)}}$$

The comparison was performed between organic and inorganic stones and between calcium oxalate stones (Fig. 5).
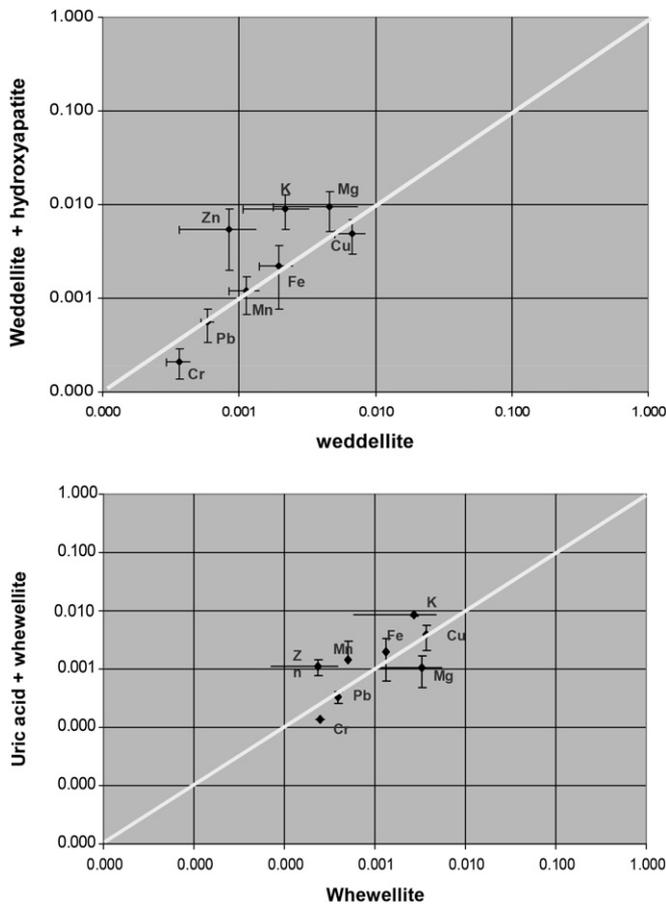

Fig. 4. Elemental distribution in weddellite urinary stones (type 3) vs. weddellite and hydroxyapatite stones (type 4); uric acid and whewellite kidney stones (type 9) vs. whewellite stones (types 1 and 2).
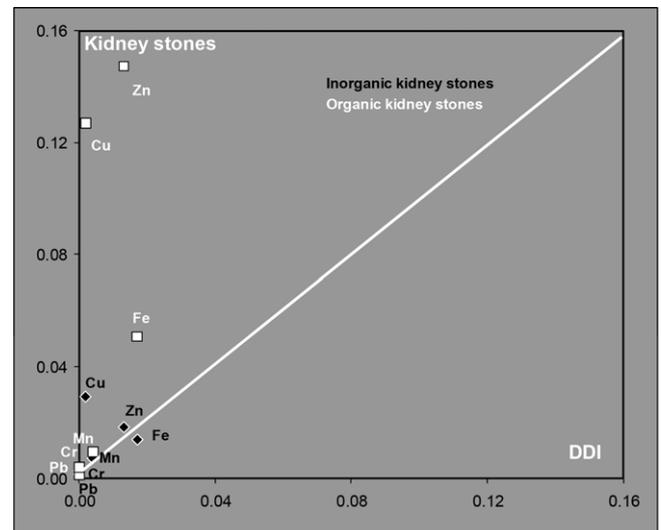
Table 5
Amounts of elements from the daily diet ([20] in [21]).

Daily dietary intakes (DDI) (mg)	
Al	4.5
B	1.6
Ba	0.3
Ca	760
Cl	5670
Cr	0.05
Cs	0.0088
Cu	1.5
F	1.5
Fe	13
K	2670
I	0.19
Li	0.038
Mg	300
Mn	3.1
Na	3510
Ni	0.15
P	1300
Pb	0.054
Rb	0.0022
Se	0.06
Sn	0.65
Sr	1.5
V	0.016
Zn	10

Fig. 5. Comparison between trace elements in urinary stones and those transferred by daily diet.

Some elements are present in the body in greater quantities with respect to the average contribution of trace elements derived from a standard diet (Fig. 5). Organic stones accumulate greater levels of Cu, Zn and Fe than inorganic stones.

In addition, for all types of stones, the quantities of Mn, Pb and Cr are slightly above the average contributed from a standard diet.

Discussion and conclusion

In considering the results, it is clear that all types of urinary stones contain several trace elements. The stone type, however, affects the quantity of those elements.

The most dominant element appears to be Ca and influences the distribution of trace elements.

The different distribution of trace elements between inorganic and organic stones is related to the affinity of Ca with some ions (especially bivalent ions such as Mg) in terms of size and charge, which favours the cationic substitutions in the crystal structure [23].

Zn has an abnormal behaviour, in that it displays greater affinity for Ca and accumulates more frequently in organic stones. This result is in disagreement with many previous studies [16,17,24,25].

In calcium-phosphate stones, Zn is more often detected with respect to oxalate stones. The higher affinity of Zn with P may explain this result [14,26–28]. Indeed, the levels of Zn in the mixed weddellite–hydroxyapatite stones exceed those found in the stones exclusively made of weddellite.

The higher content of Zn in the weddellite compared to the whewellite may be linked to the fact that weddellite is formed during the first phase of the crystallisation process and subsequently transforms to whewellite. It is thus likely that Zn is released during this process.

Zn is a very common element in the human body and more abundant than Cu [17]. It is interesting to note that the amount of copper stored in the stones is more relevant when compared to zinc, especially among oxalate stones.

Zn is considered as one of the most important inhibitors of crystallisation [29–31], and low levels of Zn that are observed in some types of stones may represent an inhibitory effect on oxalic lithiasis calcium [27,32–34].

The content of Fe, which is most abundant among the inorganic stones, is not in agreement with previous studies. Fe is considered as an inhibitor of oxalate and thus its occurrence in these stones should be negligible (Elliot and Ribeiro [41]).

The increased presence of Pb in the inorganic stones and its near complete absence in uric acid and cystine stones are reported by other studies [16,17].

The excretion of Pb occurs mainly through the kidneys but is also partly excreted through the bile and is thus found in faeces. This percentage of Pb represents the portion not absorbed during passage through the gastrointestinal tract (Alessio and Foa [42]; Goyer [43]). Therefore, the highest values of Pb are a consequence of high levels of body absorption.

The presence of this element is strongly associated with environmental pollution, and it could be of great interest to determine the presence of Pb in other stones of patients from the same geographical areas. Bazin et al. [24] observed decreased levels of Pb in stones, which correlated with a decrease in the available Pb in the environment.

The varying amounts of all of the elements are attributed to an incorrect diet (food and water) and unintended ingestion through the food chain in polluted environments [8,9,16,17,24,29,35].

Toxic elements, such as Pb and Cr, of which 80% of the amount absorbed is excreted in the urine, may trigger disorders of the renal papilla. This is the reason why slightly higher values are observed in papillary whewellite stones.

Other elements accumulate more in the urine, and more contact time between the urine and the whewellite stones in the renal cavity explains the highest accumulation.

The most abundant cation appears to be Ca in almost all types of urinary stones. It is obvious that the calcium content depends on the type of ingested food (e.g., milk, eggs) and water (e.g., hard) [36–38].

The presence of Mg in the stones is usually an indicator of an increased concentration of this element in the human body (Deeming and Webu [40]). Many foods and hard water are responsible for Mg content as well as some medicines and drugs.

The concentrations of potassium are due to dietary habits.

The presence of some metals (Fe, Cu, Mg and Mn) in urinary stone structures is usually very low; however, it was detected at appreciable levels in the analysed samples. The ingestion of meat and beans is largely responsible for the ingestion of Fe. Vegetables, meat, milk and flour increase the Cu content and Mn results from the consumption of beans, tea and vegetables.

The results show that some elements have a concentration that exceeds the average contribution arising from the diet. Thus, it can be assumed that not only do these elements have a different origin, but they also have an important role in the process of biomineralisation, in combination with either the inorganic or the organic portions [10,39].

Trace elements could also be a feature of other events that occur in these patients. This may include the presence of co-existing diseases, such as hypertension and diabetes. However, no conclusion could be drawn from the current data, and further studies are required.

More detailed studies may permit identification of the factors that may correlate the effect of the environment and the diet. Such studies could therefore identify areas of environmental risk for nephrolithiasis.

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