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ORIGINAL PAPER

Trace elements in urinary stones: a preliminary investigation in Fars province, Iran

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Abstract In view of the high incidence rate of urinary stones in the south and southwest of Iran, this paper investigates trace elements content including heavy metals in 39 urinary stones, collected from patients in Fars province, Iran. The mineralogy of the stones is investigated using X-ray diffractometry. The samples are classified into five mineral groups (calcium oxalate, uric acid, cystine, calcium phosphate and mixed stone). Major and trace elements in each group were determined using ICP-MS method. P and Ca constitute the main elements in urinary stones with Ca being more affine to oxalates while other alkali and alkaline earths precipitate with phosphate. Significant amounts of trace elements, especially Zn and Sr, were found in urinary calculi (calcium oxalate and phosphates) relative to biominerals (uric acid and cystine). Among urinary calculi, calcium phosphate contains greater amounts of trace metal than calcium oxalate. Phosphates seem to be the most important metal-bearing phases in urinary stones. Results indicate that concentrations of elements in urinary stones depend on the type of mineral phases. Significant differences in elements content across various mineralogical groups were found by applying statistical methods. Kruskal–Wallis test reveals significant difference between Ca, P, K, Na, Mg, S, Zn, Sr, Se, Cd, and Co content in different investigated mineral groups. Moreover, Mann–Whitney test differentiates Ca, Na, Zn, Sr, Co, and Ni between minerals in oxalate and uric acid stones. This study shows that urinary stone can provide complementary information on human exposure to elements and estimate the environmental risks involved in urinary stones formation.

Keywords Urinary stone · Trace element · Mineralogy · Fars · Iran

Introduction

Urinary stone formation in the urinary tract of the human body is the most common and painful urological disorder throughout the world (Singh et al. 2009; Stamatelou et al. 2003). Urinary stones, which mostly form in kidneys (Evan 2010), are products of the pathological bio-mineralization processes in the

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urinary system (Kuta et al. 2012; Giannossi and Summa 2013). The major constituents of the stones include bio-minerals and different inorganic substances, with crystalline or amorphous structures (Hesse et al. 2009).

Urinary stone disease is a multi-factorial health problem with no simple solution from the medical prevention of stone recurrence (Robertson et al. 2010). Kidney stones generally form in response to environmental and/or metabolic risk factors (Brikowski et al. 2008). The following factors may play a role in kidney stone formation: environment, nutrition, gender, geography, fluid intake, climate, associated diseases, and socio-economic status (Golovanova et al. 2006; Safarinejad 2007; Pourmand and Pourmand 2012; Pearle and Lotan 2012). The prevalence of renal stone disease has been rising in both sexes, and is 2–3 times more common in males than in females (Dajani et al. 1988; Andrew and Chandru 2001; Stamatelou et al. 2003). Peak incidence rate occurs in the second or third decade of life (Shokouhi et al. 2008). The lifetime prevalence of kidney stone disease is estimated at 1–15 % (Pearle and Lotan 2012).

Genetic factors and dietary influences is said to outweigh climatic effects (Pearle and Lotan 2012). Dietary factors include a high intake of animal proteins and oxalates and a low intake of potassium-bearing citrus fruits and fluids (Tur et al. 1991). A person with a family history of kidney stones is more likely to develop urinary stones (Abboud 2008c).

Mineralogical studies of urinary stones and the involved crystallization processes are particularly important in understanding possible environmental and metabolic factors that lead to stone formation. Early studies on urinary stones have revealed some information on composition, mineralogy, structure, processes of formation and geo-environmental factors (Ackermann et al. 1988; Bellizzi et al. 1999; Kohri et al. 1989). Urinary stones consist of about 40 components (Bazin et al. 2012; Hesse 1988). Major chemical constituents of calculi stones are calcium phosphate (hydroxyapatite), calcium oxalates (whewellite and weddellite) and Urates (Nasir et al. 2004; Evan 2010). Struvite stone is a less common type caused by the urinary tract infection. Cystine is also a rare stone (Giannossi et al. 2013). Uric acid stone is a less common stone (Abboud 2008c). Physiologically, urinary calculus formation is generally caused by disturbed urinary compositions with altered urinary pH, and increased concentrations of lithogenic components as, e.g., calcium, oxalate, phosphate, and lack of inhibitoric substances such as citrate and magnesium (Beging et al. 2010). Calcium oxalate is the most frequent mineral phase found in uroliths with a frequency of approximately 70–75 % (Schubert 2006).

In recent years, the significance and role of trace elements in biological lithogenesis have received increasing attention. Although trace elements are necessary for the optimal development and metabolic functioning of all living organisms, many researchers believe that they are also essential mediators in the development and progression of various diseases (Mertz 1981; Fraga 2005; Failla 2003; Houtman 1996; Navarro and Rohan 2007). Trace elements also play a role in crystal formation and/or stone organization owing to their special affinity for some crystalline phases (Slojewski et al. 2010; Touryan et al. 2004; Bazin et al. 2008). Some trace elements may influence the external morphology of growing crystals and accelerate or slow down the crystallization process (Slojewski 2011; Fang et al. 2005; Munoz and Valiente 2005). However, metal content in urinary stones would reflect exposure of an organism to these metals analogous to other biological matrices used for human biomonitoring of metals (e.g., blood, hair, or urine; Esteban and Castaño 2009).

Of the several aforementioned factors, environmental factors are suspected to have a direct relationship to the composition of urine, which is governed mainly by diet and drinking water composition (Chandrajith et al. 2006). Also, the geographic distribution of stone disease tends to roughly follow environmental risk factors; a higher prevalence of stone disease is usually found in hot, arid, or dry climates such as the mountains, desert, or tropical areas (Pearle and Lotan 2012; Zarasvandi et al. 2013). In certain areas of the world, such as the Afro-Asian stone belt, including the Middle East, and countries located in tropical and subtropical areas, a high incidence rate of urolithiasis is reported (Abboud 2008c).

The prevalence of renal stones in Iran is reported to be 5.7 % (Safarinejad 2007), and varies in different parts of the country (Zarasvandi et al. 2013). The regional distribution indicates a higher incidence rate in the southcentral (Kerman and Hormozgan) and southwest (Fars, Boushehr, Khouzestan, Ilam, and

Chaharmahal provinces) of the country (Pourmand and Pourmand 2012), with an increasing trend from west to east and from north to south (Safarinejad 2007).

Fars province, southwest of Iran (50–55°E and 27–31°N) have different climatic regions. These include north and northwest mountainous area with moderately cold winters and mild summers; the central regions, with relatively rainy mild winters and hot dry summers and south and southeast region with mild winters and very hot summers. Maximum annual temperatures ranges between 35 and 40 °C (in August), while minimum annual temperatures ranges from 2 to −7 °C (in December). Annual rainfall varies from 100 mm in the south to 200–400 mm in the north.

Careful study of elements association with minerals or mineral groups is therefore crucial in assessing the effect of other factors on trace metal content (Kuta et al. 2013). Urinary stone analysis is also important in determining the possible etiology and pathophysiology of stone formation, which in turn may help prevent urinary stone formation (Jing et al. 2010).

This paper describes the results of chemical analysis of 39 urinary stones samples. The main purpose is to determine associated elements with minerals in order to establish the most important factors affecting minor elements content in urinary stone.

Materials and methods

Eighty-three samples were collected between April 2013 and August 2013 from patients undergoing surgery for urinary stone removal (57 males and 26 females). By a single surgeon (D.I.) at Shahid Faghihi Hospital, Shiraz City. Each patient was asked to fill out a questioner prior to his/her surgery regarding personal details including gender, age, medical history (such as weight, personal and family anamnesis), address, and other relevant social information.

The removed stones were placed in polyethylene dry bottles and kept cool until analysis. All polyethylene bottles were labeled with the name, sex, and age of the patient and the date of surgery.

Thirty-nine urinary stone samples with different morphologies were washed several times with deionized water to free the stones of urine, blood stains, and remnants of organic matter, and finally were rinsed with distilled water. After drying, each stone was ground in an agate mortar and homogenized.

A fraction was sent to Kansaran Binaloud Lab, Tehran, for X-ray diffraction analysis (XRD) to obtain the crystalline phase composition of the urinary stones. The instrument used a nickel-filtered Cu-Kα with a generator voltage of 40 kV and generator current of 30 mA. Qualitative interpretation of the diffractometric profiles was achieved by comparing the peaks of reflection with PDF card (Powder Diffraction File). Oxalate, Phosphate, uric acid, and cystine minerals were detected in 36 renal, two bladders, and one ureteral stone samples.

Elements concentration was determined using an inductively coupled plasma-mass spectrometry (ICP-MS). A 0.25-g sample was heated in HNO_3–$HClO_4$–HF at 200 °C in hot box for 4–5 h. The residue was dissolved in HCl. Following digestion, urinary stones were completely dissolved. As the digestion was carried out in encapsulated vessels, it is assumed that there was no loss of volatile elements. In this method, QA/QC included reagent blanks and analytical duplicates, and analysis of the standard reference material (multi-element CRM such as OREAS 70b, OREAS 73b, GBW 07302, GBW 07305) are used. Data was processed statistically using SPSS statistical package version 19.0 for Windows. Mann–Whitney *U* test and Kruskal–Wallis ANOVA were used for descriptive statistics. The correlation between elements is described by Spearman correlation coefficient.

Results and discussion

The collected urinary stones are varied in terms of patients' age and may be divided into four groups: <40 years old (21 samples), 40–50 years old (20 samples), 50–60 years old (29 samples) and >60 years old (13 samples). Urinary stones were found to be more frequent in men, with increased prevalence in the 50–60 years old group. Also, the information taken from the questionnaires showed hypertension (9 samples), diabetes (4 samples) and family history of urinary stone (14 samples) the study group. Patients with a family history of the disease were infrequent. The most frequent co-existing diseases were hypertension and diabetes, as already shown by Basiri et al. (2010).

Table 1 Mineralogical composition of urinary stones using X-ray method

Sample number	Sample type	Major phase(s)	Minor phase(s)
MP*-1	Renal	Whewellite	–
MP-2	Renal	Whewellite	–
MK-1	Renal	Whewellite	–
MPo-1	Renal	Whewellite	–
MPo-2	Renal	Whewellite	–
MK-2	Renal	Whewellite	–
MP-3	Renal	Whewellite	–
MP-4	Renal	Uricite	–
FP-1	Renal	Uricite	–
MP-5	Renal	Uricite	–
FK-1	Renal	Uricite	–
MK-3	Renal	Uricite	–
MK-4	Renal	Uricite	–
MK-5	Renal	Uricite	–
MB-3	Bladder	Uricite	–
FK-2	Renal	L-Cystine	–
FU	Ureter	L-Cystine	–
FP-2	Renal	Hydroxylapatite	–
MPo-3	Renal	Whewellite and uricite	–
MPo-4	Renal	Uricite and whewellite	–
FPo-1	Renal	Uricite	Whewellite
MK-6	Renal	Uricite and whewellite	–
MK-7	Renal	Whewellite and uricite	–
MP-6	Renal	Uricite and whewellite	–
MP-7	Renal	Uricite	Whewellite
MPo-5	Renal	Whewellite	Uricite
MPo-6	Renal	Uricite	Whewellite
FP-3	Renal	Whewellite	Uricite
MP-8	Renal	Uricite and whewellite	–
FK-3	Renal	Uricite and whewellite	–
MP-9	Renal	Whewellite and uricite	Weddellite
FPo-2	Renal	Uricite	Whewellite
MP-10	Renal	Uricite and weddellite	Whewellite
MB-15	Bladder	Uricite and whewellite and weddellite	–
MPo-7	Renal	Whewellite	Weddellite
MK-8	Renal	Whewellite	Weddellite
FK-4	Renal	Whewellite	Weddellite
MP-11	Renal	Hydroxylapatite	Weddellite
FP-4	Renal	Whewellite and uricite	Calcium urate

MP*-1: man-pelvice

Table 1 represents the mineralogical composition of 39 samples according to mineralogical composition. The samples may be classified into five mineral groups (Table 2). The most common group (44 %) comprises mixed stones involving a mixture of calcium oxalate and uric acid. The second largest group is calcium oxalate stones (26 %), while (20 %) belong to uric acid (uricite) group. Each of the phosphate (as hydroxyapatite) and cystine group of stones also comprises 5 % of the urinary stones. Figures 1, 2, 3, and 4 show X-ray diffraction pattern of some urinary stone samples.

Table 3 displays the results of the chemical analysis and mean concentrations of each calculi type. Calcium has the highest concentration, ranging between 0.04 and 43.46 % and averaging 14.76 %. Calcium is the main constituent in all types of stones. The high Ca content in urinary stones probably reflects a Ca-rich diet such as diary and milk products, red meat, tea, egg, and hard water as already noted by Robertson et al. (1980) and Sobhi (2006), or pathologic conditions such as renal leak hypercalciuria, absorptive or resorptive and idiopathic hypercalciuria.

Phosphorous is the major constituent in phosphate stones. The P content in all stone samples ranges from 0.01 to 14.45 % and averages 0.95 % (Table 3). Also, the reason for high P content in some urinary stones can be the excessiveness of drinking milk and eating cheese, egg, and some vegetables with high phosphorous content is high concentration of P in water (Abboud 2008a), and pathologic conditions like renal tubular acidosis (RTA) type 1, hyperparathyroidism, medullary strong kidneys, and any condition causing metabolic acidosis and alkaline urine. The highest and

Table 2 Classification of analyzed urinary stones

Group minerals	Mineral	Number of stones	%
1. Calcium oxalate	Whewellite/weddellite	10	26
2. Uric acid	Uricite	8	20
3. Cystine	L-Cystine	2	5
4. Calcium phosphate	Hydroxylapatite	2	5
5. Mixed stone	Whewellite/uric acid/ weddellite	17	44

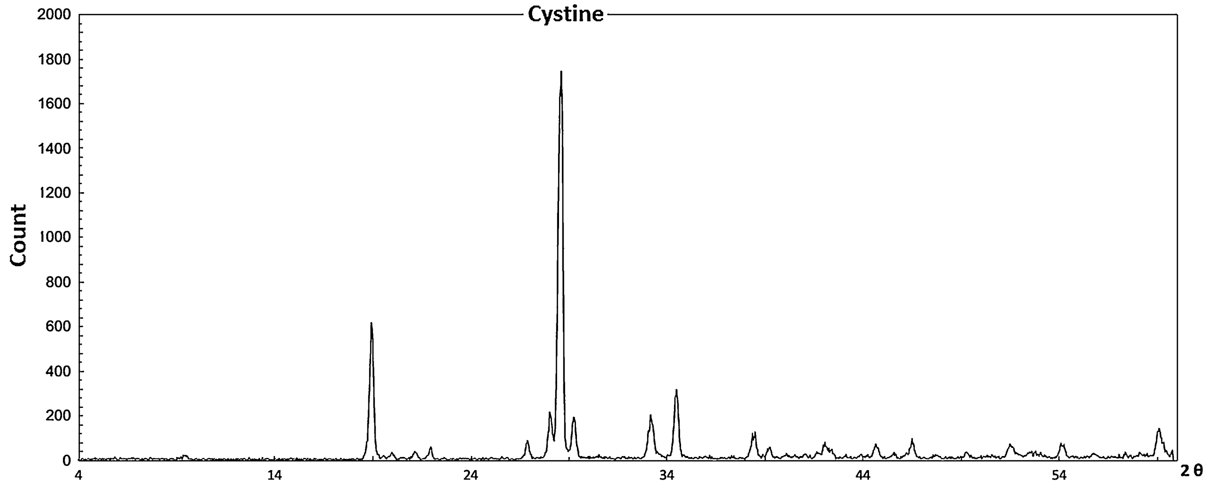

Fig. 1 X-ray diffraction pattern of cystine stone (sample FK-2)

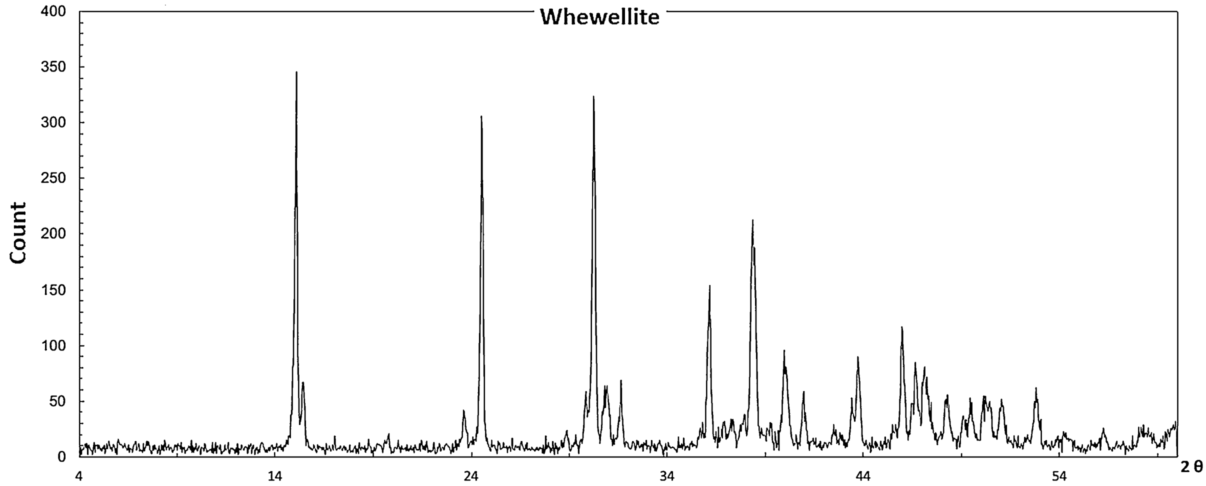

Fig. 2 X-ray diffraction pattern of calcium oxalate stone (sample MP-2)

lowest P content occurs in phosphate and cystine calculi minerals, respectively.

Na content ranges between 0.01 and 0.86 % while K comprises 0.11–0.22 %, respectively. Average Na and K content is 0.15 and 0.13 %, respectively (Table 3). Na and K normally occur at high concentrations in urine (approximately 150 mmol l^{-1} for Na and 50 mmol l^{-1} for K in urine; Kuta et al. 2013).

Calculated correlation coefficients are presented in Table 4. Na ($r = 0.572$) is positively correlated with phosphorous, indicating the strong affinity of alkali metals to phosphate in urinary stones (Chandrajith et al. 2006). Close association of Na to hydroxylapatite is quite natural. A high correlation coefficient between Na and Ca ($r = 0.633$) reflects that Na replaces Ca in many rock-forming minerals, for example, plagioclases (albite-anorthite series $NaAl_3Si_3O_8$-$CaAl_2Si_2O_8$), because of their very similar ionic radius (Shannon and Prewitt 1969). The low correlation between K and Ca ($r = 0.194$) in urinary stones reflects the fact that K and Ca have different geochemical properties.

Mg content in the analyzed stones ranges from 0.004 to 0.79 % averaging 0.06 % (Table 3). A positive correlation for Mg and P ($r = 0.767$) indicates the tendency of Mg to enter phosphate minerals

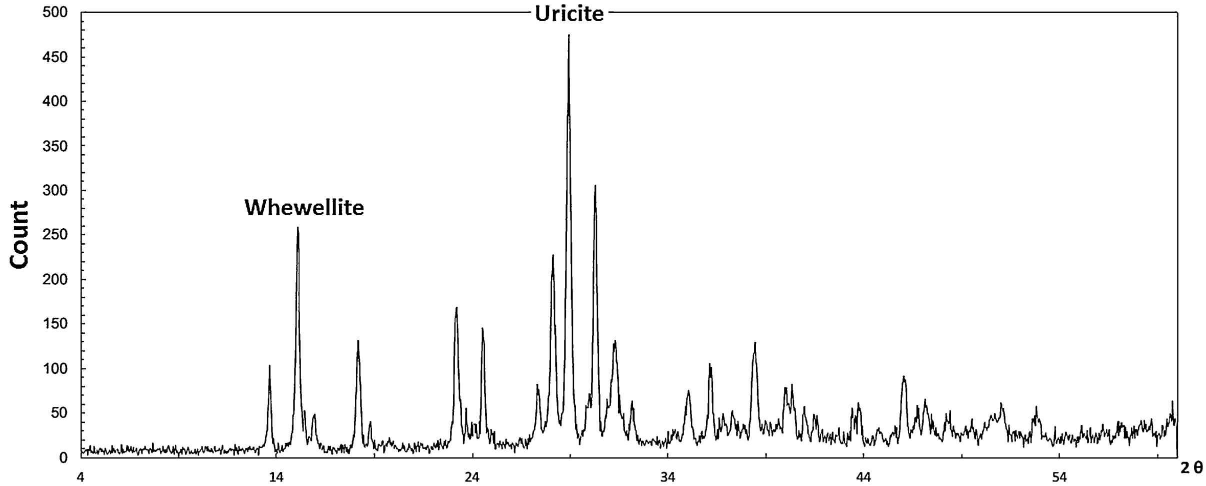

Fig. 3 X-ray diffraction pattern of mixed urinary stone (containing whewellite and uricite minerals, sample MP-8)

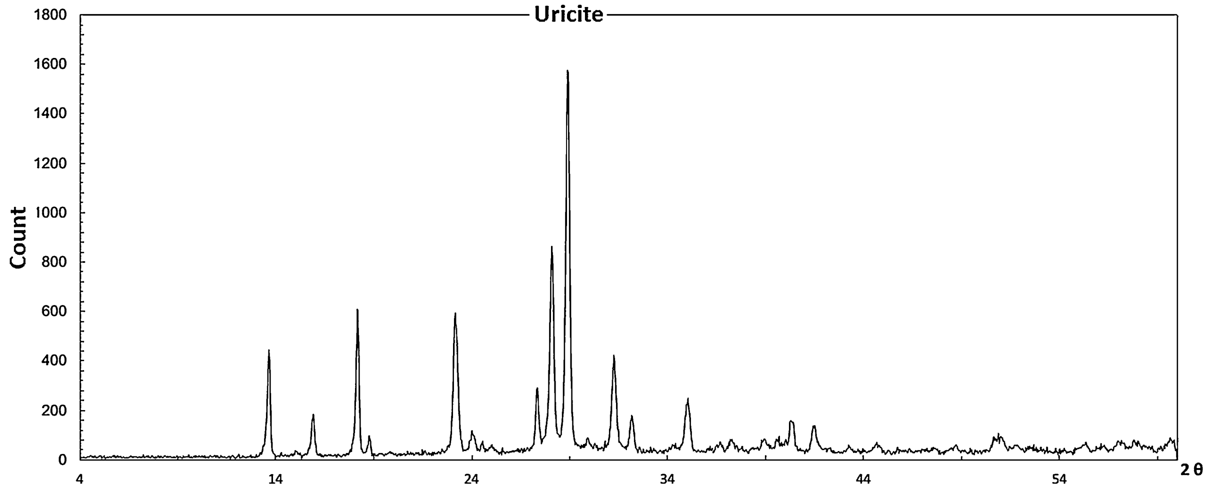

Fig. 4 X-ray diffraction pattern of uric acid stone (sample MP-4)

structure. Mg is an important element in biological calcification (Atakan et al. 2007) and its presence in urinary stones is usually an indicator of an increase in concentration in the human body (Deeming and Weber 1977). The exact role of Mg in kidney stone formation is yet to be fully explained (Singh et al. 2009; Kohri et al. 1988). Apparently, food and hard water are responsible for Mg content along with some medicines and drugs (Giannossi et al. 2013), and Mg is a major component of struvite (infected) stones. In this stone, mg is precipitated with ammonium and phosphate in alkaline urine.

S content (0.03–35.90 %) is characteristic of the cystine group of stones. Concentrated Fe in urine usually comes from cells in the human urine channel (Lentner 1981), and increases in percentage in the urinary system and allows to be incorporated in oxalate and calcium phosphate stones (Wandt and Underhill 1988). Fe ranges between 37 and 204 ppm, averaging 82.33 ppm. Fe content is the result of the inhibitory properties of Fe^{3+} on calcium oxalate crystallization as suggested by Meyer and Thomas (1982) and (Munoz and Valiente 2005).

Trace elements such as Zn and Sr are present in high concentrations, averaging 83 and 141 ppm, respectively (Table 3). The highest Zn and Sr concentrations occur in phosphatic calculi minerals. Table 4 shows the relationship between Sr and Zn ($r = 0.823$).

Table 3 Element contents in the analyzed urinary stones

Element	Group 1 ($n = 10$)			Group 2 ($n = 8$)			Group 3 ($n = 2$)			Group 4 ($n = 2$)			Group 5 ($n = 17$)			Total ($n = 39$)			
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	SD
Element content (%)																			
Ca	0.34	37.97	28.17	0.04	7.58	1.17	0.08	0.52	0.30	0.85	43.46	22.16	0.09	43.46	14.06	0.04	43.46	14.76	15.96
P	0.16	1.98	0.61	0.01	0.11	0.03	0.01	0.03	0.002	9.77	1.44	12.11	0.03	2.10	0.38	0.01	14.45	0.97	2.73
K	0.12	0.15	0.13	0.12	0.13	0.12	0.11	0.11	0.11	0.17	0.22	0.20	0.12	0.16	0.13	0.11	0.22	0.13	0.02
Na	0.12	0.21	0.16	0.01	0.10	0.03	0.02	0.02	0.02	0.65	0.86	0.76	0.04	0.77	0.14	0.01	0.86	0.17	0.21
Mg	0.03	0.07	0.07	0.004	0.01	0.01	0.01	0.01	0.01	0.39	0.79	0.59	0.01	0.21	0.03	0.004	0.79	0.07	0.14
S	0.08	0.21	0.14	0.03	0.08	0.04	32.70	35.90	34.30	0.08	0.10	0.09	0.03	0.16	0.09	0.03	35.90	1.88	7.65
Element content (ppm)																			
Fe	39	187	94	46	110	76	60	98	79	56	65	61	37	204	82	37	204	82	37
Zn	17.0	218.0	78.7	0.5	18.0	6.6	4.0	8.0	6.0	525.0	979.0	752.0	4.0	301.0	51.7	0.5	979.0	83.0	178.1
Sr	130	286	196	2	42	9	2	7	5	690	775	732	9	452	118	2	775	141	171
Pb	4.0	23.0	12.0	2.0	35.0	11.0	17.0	22.0	19.5	14.0	18.0	16.0	2.0	39.0	11.4	2.0	39.0	12.1	8.4
Al	83	285	158	89	504	157	93	203	148	125	125	125	82	299	154	82	504	154	81
Cu	3.0	6.0	4.3	4.0	6.0	5.0	3.0	4.0	3.5	3.0	15.0	9.0	3.0	11.0	5.3	3.0	15.0	5.1	2.3
Rb	2.0	4.0	2.5	2.0	4.0	3.0	2.0	3.0	2.5	2.0	3.0	2.5	2.0	5.0	2.6	2.0	5.0	2.6	0.8
Ni	8.0	16.0	10.4	5.0	12.0	7.3	11.0	16.0	13.5	9.0	12.0	10.5	6.0	18.0	8.8	5.0	18.0	9.2	3.1
Sb	2.0	7.1	4.3	3.1	4.7	3.8	5.6	11.6	8.6	2.8	3.9	3.4	2.1	12.3	4.5	2.0	12.3	4.4	2.3
V	7.0	10.0	8.2	6.0	10.0	7.9	9.0	9.0	9.0	10.0	10.0	10.0	7.0	10.0	8.4	6.0	10.0	8.3	1.4
Mn	2.0	19.0	4.3	2.0	8.0	3.6	3.0	7.0	5.0	2.0	6.0	4.0	2.0	9.0	4.2	2.0	19.0	4.2	3.2
Mo	0.05	2.35	1.04	0.05	1.20	0.43	1.30	3.40	2.35	0.60	0.80	0.70	0.05	5.60	1.01	0.05	5.60	0.95	1.07
Se	0.24	12.15	2.15	0.05	40.01	5.09	0.05	0.93	0.49	0.42	0.89	1.31	0.05	2.67	0.46	0.05	40.01	1.85	6.56
As	0.05	6.30	1.48	0.05	4.20	1.98	0.05	0.05	0.05	0.05	0.10	0.05	0.05	7.40	1.56	0.05	7.40	1.47	1.94
Ba	0.5	5.0	1.8	0.5	6.0	2.3	5.0	8.0	6.5	9.0	10	9.5	0.5	16.0	2.7	0.5	16.0	2.8	3.6
Cd	0.05	6.30	0.93	0.05	2.36	0.50	0.32	0.84	0.58	0.05	1	0.53	0.05	0.18	0.07	0.05	6.30	0.43	1.10
Co	1.1	1.7	1.4	0.5	1.3	0.8	1.4	2	2.3	1.6	2	1.6	0.5	2.8	1.2	0.5	32.8	1.2	0.5
Cr	0.5	13.0	6.9	0.5	9.0	4.6	13.0	25.0	19	9.0	10.0	10	0.5	27.0	7.2	0.5	27.0	7.3	6.0

n number of stones

Table 4 Correlation coefficients between the main and trace element-analyzed urinary stones

	Ca	P	K	Na	Mg	S	Fe	Zn	Sr	Pb	Al	Rb	Ni	Mo	Se	As	Ba	Cd	Co
Ca	1																		
P	.475**	1																	
K	.194	.310	1																
Na	.633**	.572**	.399*	1															
Mg	.665**	.767**	.378*	.689**	1														
S	.358*	.155	−.031	.324*	.375*	1													
Fe	.069	−.004	.230	.037	−.031	.038	1												
Zn	.638**	.761**	.379*	.705**	.792**	.283	.078	1											
Sr	.753**	.748**	.384*	.746**	.879**	.374*	.071	.823**	1										
Pb	−.141	.321*	.003	−.060	.150	.277	.148	.112	.111	1									
Al	.133	.202	.131	.114	.119	.097	.530**	.149	.162	.267	1								
Rb	−.340*	−.159	.323*	−.237	−.221	−.083	.577**	−.231	−.196	.274	.331*	1							
Ni	.236	.318*	.099	.150	.301	.461**	.498**	.342*	.333*	.642**	.366*	.384*	1						
Mo	.111	.182	−.022	.128	.279	.657**	.166	.261	.217	.487**	.283	.268	.666**	1					
Se	.350*	.519**	.174	.397*	.601**	.522**	.027	.386*	.542**	.387*	−.013	.015	.497**	.398*	1				
As	.088	−.337*	.202	.064	−.159	−.235	.147	−.026	.019	−.650**	−.044	.200	−.275	−.290	−.234	1			
Ba	−.215	.253	−.020	−.033	.056	.188	.034	.080	−.062	.774**	.046	.110	.514**	.397*	.187	−.825**	1		
Cd	−.157	.099	.008	−.193	.022	.131	.106	.037	−.097	.468**	.048	.211	.432**	.271	.326*	−.186	.303	1	
Co	.261	.403*	.219	.288	.389*	.501**	.406*	.407*	.410**	.711**	.385*	.380*	.914**	.669**	.497**	−.324*	.595**	.356*	1

** Correlation is significant at the 0.01 level (two-tailed)

* Correlation is significant at the 0.05 level (two-tailed)

Wandt and Underhill (1988) already indicated that Zn positively correlations with Sr, and predominantly associates with hydroxylapatite, a fact substantiated by positive correlations between Zn and P content of the stones (Abboud 2008a; Zarasvandi et al. 2013).

In the current study, Zn accumulations in hydroxylapatite support the relationship between Zn and P ($r = 0.761$) in the urinary stones. However, a positive correlation of Sr and P ($r = 0.748$) reflects the close natural relation between Sr and P (Abed and Abdalla 1998). Zn and Sr content in mixed stones also reflects the presence of the respective phases in urinary stones. For example, mixed calcium oxalate/uric acid stones contain moderate amounts of heavy metals (Bazin et al. 2007). Al content (82–504 ppm) is also relatively high when compared with other elements.

Sutor (1969) found that a small amount of Al^{3+} can remove oxalate ions and thus prevent crystallization of calcium oxalate. Low concentrations of toxic trace elements, including Pb and V, range between 2 and 39 ppm, and 6 and 10 ppm, respectively. Average Pb and V content is 12.1 and 8.3 ppm, respectively. Pb is a consequence of high levels of Pb in the body. The presence of this element strongly indicates environmental pollution (Giannossi et al. 2013). Bazin et al. (2007) reported decreased levels of Pb in urinary stones, correlating with decreased available Pb in the environment. The role of Pb in biolithogenesis is not yet fully known, but some authors have found a correlation between lead in stones and urine, which may lead to the conclusion that Pb may also play some role in the crystallization of calculi stones in the urinary tract (Słojewski et al. 2010).

Selenium occurs only in trace amounts (<12.15 ppm) in analyzed stones, with the exception of Uricite stones in which selenium ranges from 0.05 to 40.01 ppm and averages 5.09 ppm. The role of Se in biolithogenesis is poorly documented. However, it is suggested that like other semimetal, selenium may have some interactions with stone constituents, or be captured in the structure of crystals incidentally (Bazin et al. 2007; Sakly et al. 2003). The highest Se content (40.01 ppm) was found in the patient who suffers from diabetes. It has been suggested that higher levels of selenium taken from supplements or received naturally are associated with an increased risk of diabetes (Stranges et al. 2007). Cadmium is also suggested as a risk factor in urinary tract stone formation (Perk et al. 2002). Only a few studies in the medical literature deal with the role of Cd in biolithogenesis. It is suggested that cadmium might have some inhibitory effect on calcium oxalate crystallization (Hofbauer et al. 1991). Furthermore, some heavy metals including Mo, Cd, Co, Rb, Ni, Cu, Mn, and As also display considerable concentration (Table 3).

Values below detection limits were assumed to be 1/2 of detection limits to compare the differences in elements content in Kruskal–Wallis and Mann–Whitney tests and also calculate correlation coefficients.

Statistical comparison of elemental content, and minerals groups was carried out using Kruskal–Wallis ANOVA. *P* values lower than 0.05 are considered to indicate significant differences. For measured elements (Ca, P, K, Na, Mg, S, Zn, Sr, Ni, Se, Ba, Cd, Co), obvious differences with particular mineral components, and a confidence level of 95 %, was observed. Other elements did not show significant differences (Table 5). However, some differences between mineral groups are obvious.

Mann–Whitney U, $p < 0.05$ test was used to confirm the results of Kruskal–Wallis test. This test shows the differences between the elements and different mineral groups. For instance, Na, Zn and Sr ($p = 0.00$), Ca ($p = 0.001$), Co ($p = 0.003$), and Ni ($p = 0.009$) show significant differences between mineral groups, with high concentrations in oxalate stones. Se ($p = 0.009$) also shows a significant difference between the two mineral groups of oxalate and uric acid, whereas, no significant differences were found between K ($p = 0.46$), Cd and Ba ($p = 0.573$).

Table 5 Kruskal–Wallis test between the elements content and groups of minerals ($p < 0.05$)

Element	p	Element	p
Ca	0.001	Rb	0.521
P	0.000	Ni	0.020
K	0.028	Sb	0.267
Na	0.000	V	0.357
Mg	0.000	Mn	0.712
S	0.000	Mo	0.065
Fe	0.737	Se	0.007
Zn	0.000	As	0.435
Sr	0.000	Ba	0.042
Pb	0.323	Cd	0.028
Al	0.712	Co	0.009
Cu	0.348	Cr	0.100

Table 6 Statistical comparison of elements and mineral groups using Mann–Whitney test ($p < 0.05$)

Element	P	Ca	K	Na	Mg	S	Zn	Sr	Se	Co	Ni	Cd	Ba
Groups	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2
p	0.00	0.001	0.46	0.00	0.00	0.00	0.00	0.00	0.01	0.003	0.01	0.573	0.573
Groups	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3
p	0.03	0.061	0.03	0.03	0.03	0.03	0.03	0.03	0.27	0.273	0.18	0.273	0.03
Groups	1–4	1–4	1–4	1–4	1–4	1–4	1–4	1–4	1–4	1–4	1–4	1–4	1–4
p	0.03	0.758	0.03	0.03	0.03	0.121	0.03	0.03	0.36	0.182	0.91	1.00	0.03
Groups	1–5	1–5	1–5	1–5	1–5	1–5	1–5	1–5	1–5	1–5	1–5	1–5	1–5
p	0.018	0.04	0.902	0.074	0.00	0.015	0.035	0.003	0.00	0.093	0.05	0.027	0.675
Groups	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3
p	0.889	0.889	0.044	0.889	0.18	0.044	0.889	0.889	0.71	0.044	0.09	0.40	0.089
Groups	2–4	2–4	2–4	2–4	2–4	2–4	2–4	2–4	2–4	2–4	2–4	2–4	2–4
p	0.044	0.178	0.044	0.044	0.04	0.089	0.044	0.044	0.18	0.044	0.18	1.00	0.044
Groups	2–5	2–5	2–5	2–5	2–5	2–5	2–5	2–5	2–5	2–5	2–5	2–5	2–5
p	0.000	0.000	0.754	0.000	0.000	0.001	0.002	0.000	0.175	0.175	0.157	0.157	0.887
Groups	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4
p	0.333	0.333	0.333	0.333	0.33	0.333	0.333	0.333	1.00	1.00	0.67	1.00	0.333
Groups	3–5	3–5	3–5	3–5	3–5	3–5	3–5	3–5	3–5	3–5	3–5	3–5	3–5
p	0.023	0.07	0.012	0.012	0.01	0.012	0.047	0.012	0.84	0.105	0.11	0.012	0.105
Groups	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4–5
p	0.012	0.842	0.012	0.023	0.01	0.749	0.012	0.012	0.19	0.14	0.23	0.421	0.47

1: Calcium oxalate
2: Uric acid
3: Cystine
4: Calcium phosphate
5: Mixed stone

Most Cd differences ($p = 0.01$ and $p = 0.027$) occur between the cystine and oxalate groups (Table 6). These differences may be due to the presence of Ca in the structure of calcium oxalate minerals such as whewellite and weddellite. High Cd concentration (0.93 ppm) also occurs in oxalate stones.

The results of this study confirm the conclusions previously described by other authors. Among calcium stones, calcium phosphate calculi contained a greater amount of metals than calcium-oxalate calculi (Giannossi et al. 2013). This is especially true for Sr and Zn, when compared with calcium oxalate stones. Fe, Rb, and Pb are also more abundant in calcium stones than in uric acid or cystine stones (Słojewski et al. 2010). Bazin et al. (2007), showed high Zn and Sr content in phosphatic stones, and contrary to Joost and Tessadri (1986) a lower proportion in calcium oxalate stones. Levinson (Levinson et al. 1978) observed a considerably higher Zn and Sr content in calcium oxalate and calcium phosphate stones than in uric acid and cystine urinary stones. Strong correlations between Zn, Sr, Ni, and P content are also observed in the work of Abboud (2008a).

Table 7 compares the mean concentration of elements in the present study with previous ones carried out in different geological areas (Kuta et al. 2013; Abboud 2008b; Zarasvandi et al. 2013; Chandrajith et al. 2006; Giannossi et al. 2013). The difference between elements distribution in the clusters of urinary stones collected from different regions is most likely associated with geology, drinking water quality, diet, and therapeutic facilities (Golovanova et al. 2006; Abboud 2008b). Therefore, it is not surprising that collected urinary stones from Fars province have their own differences with other parts of the world such as Jordan, Sri Lanka, and Italy.

Table 7 Comparison of average elemental concentrations in Fars province urinary calculi with the other regions

Element	Present study ($n = 39$)	Kuta et al. 2013 ($n = 614$)[a]	Abboud 2008a ($n = 110$)[b]	Zarasvandi et al. 2013 ($n = 20$)[c]	Chandrajith et al. 2006 ($n = 69$)[d]	Giannossi et al. 2013 ($n = 48$)[e]
Major element	(%)	(%)	(%)	(%)	(%)	(%)
Ca	14.76	24.80	20.33	15.21*	31.92	17.23
P	0.95	0.55	10.35	4.22*	1.93*	0.90
K	0.13	0.03	0.90	0.08*	0.03	–
Na	0.15	0.12	1.56	0.13*	0.13	–
Mg	0.06	0.04	3.08	0.52*	0.14	1.63
S	1.85	–	1.88	1.14*	–	–
Trace element	ppm	ppm	(%)	ppm	ppm	(%)
Fe	82	48	3.08	–	258	0.26
Zn	83.0	67	0.70	36.95	675	0.45
Sr	141	49	0.31	108.5	–	–
Pb	12.1	4.41	–	5.17*	69	0.05
Al	154	6	0.49	0.04	–	–
Cu	5.1	1.1	0.19	39.80	67	0.57
Rb	2.6	0.22	–	4.40	–	–
Ni	9.2	0.2	0.014	3	–	–
V	8.3	<LOD	–	–	4.75	–
Mn	4.2	0.12	0.029	8.39	–	0.12
Mo	0.95	0.22	0.20	–	–	–
Cr	7.3	0.05	0.15	–	–	0.03

* content of the elements oxide (%)

[a] Czech Republic

[b] Jordan

[c] Khouzestan, Iran

[d] Sri Lanka

[e] Basilicata, Italy

Conclusions

Essential trace elements for specific metabolic processes are temporally stored and excreted via the kidneys. In this process, the elements may result in the formation of urinary stones in the urinary tract. Ultimate analysis of urinary stones has previously been used to identify metal content in urinary stones. However, the results of this study show that the content of elements is significantly affected by mineralogical composition of the calculi. Calcium stones contain the highest proportion of metals, especially Sr and Zn. Trace amounts of metals are also found in uric acid or cystine stones. The reason for the observed difference in the metal content in different types of stones is apparently the similarity between the charge and size of Zn and Sr ions with calcium ion, which allows these elements to substitute for calcium in the crystal lattices. Se content in uric acid stones is also significant. Also, Zn and Sr accumulation in phosphatic stones supports the observed correlation between these elements and P in the urinary stones. However, geochemical similarity between various elements is only one effective factor in the formation of urinary stones. The results of statistical tests revealed significant elemental differences between the five assigned mineralogical groups of urinary stones. A high concentration of one or more elements in urinary stones with different mineralogical is probably due to various reasons such as different chemical behavior of elements, along with metabolic and environmental factors. Thus, the evaluation of elements content in different types of urinary stone should always be considered along with their mineralogical composition. Such data could help the urologist to understand the causes of the formation of urinary stones and ultimately to plan safer methods to avoid their formation and suggest dietary regimes to stop their inception.

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