Kidney stones analysis by ICP-OES

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Abstract. The elemental composition of 100 oxalate type (only whewellite, weddellite and their mixture) urinary stones was determined by ICP OES. The calcium content varies from 130 to 320 g/kg; phosphorus – from 1.6 to 28 g/kg; sodium – from 0.54 to 8.7 g/kg; Ba, Fe, K, Li, Mg, Sr and Zn – from 0.0002 to 2.0 g/kg. The correlation analysis (Spearman rank correlation) showed a very weak tightness of the relationship between the elements of Ca/Li; a weak bond tightness for the pairs of elements Ba/Ca, Ba/Mg, Ba/Sr; Ca/Fe, Ca/Na, Ca/Sr; Mg/Sr, Mg/Zn; average bond tightness for pairs of elements Ca/Mg, K/Na, Mg/P. All these correlations are positive.

1. Introduction
Urolithiasis takes one of the leading places among the urological diseases in Russia. Invasive technologies have made the surgical treatment of urolithiasis relatively safe and routine. However, simple removal of calculi or urinary stone without a subsequent diet correction and drug treatment in more than a half of cases leads to the urolithiasis relapse. Nowadays, the effective organization of the second stage of urolithiasis treatment is actual – conservative and drug metaphylaxis. It is a part of personalized patient management [1]. Since the choice of a metaphylactic strategy is impossible
without a thorough study of the disease causes, it is necessary to develop an individual approach to clinical diagnosis and study metabolomes of the uroithiasis [2].

At the present time the following problematic aspects are described in the scientific literature: (1) the phase composition study and frequency of the minerals presence [3-11]; (2) the urinary stone structure – phase composition in a core, middle zone and crust; (3) the relationship between the type of urinary stone (according to the main component) and territorial affiliation [12-14]; (4) the frequency of a mineral presence as an endemic characteristic [4, 11, 15-17]; (5) the relationship between a frequency of the mineral presence with age, gender, race and other characteristics [5-7, 18]; (6) modeling of crystallization processes of urinary stones [19, 20]. But it should be noted published data is scattered and researchers use different methods for determination of the elemental composition in a small number of urinary stones samples (20<n<50) [7, 11, 16]. There is an extremely small amount of data about dependencies between the phase and elemental composition of urinary stones in the published data.

It is important to determine at both elemental and phase compounds in calculi. This knowledge will help us to estimate the contribution of trace elements to the pathogenesis of kidney stones as well as to formulate future strategies for the treatment and to prevent stone formation. In the present study we investigated the elemental composition of oxalate kidney stones using inductively coupled plasma optical emission spectrometry (ICP OES) and performed inter-element correlation analysis.

2. Design, setting, and participants
We investigated calculi of Ob’ River watershed residents. A total quantity of patient’s calculi treated between 2011 and 2019, is 1155. At first, for these calculi, an X-ray analysis was performed [17]. 100 urinary stones consisting of calcium oxalate phase (only whewellite and weddellite or these mixture) were selected from previously analyzed stones for ICP-OES analysis. The sample size of the current analysis was defined by the inclusion of all patients who have medical intervention with the extraction of calculus from the urinary system. The calculi were collected and send for analysis to NIIC SB RAS by INVITRO-Siberia Laboratory.

3. Experimental
The elemental analysis was carried out using high-resolution spectrometer iCAP-6500 Duo (Thermo Scientific). For complete dissolution of investigated calculi concentrated hydrochloric (HCl) and nitric (HNO₃) acids previously purified by sub-boiling distillation were used. If it was necessary (in the case of sparingly soluble minerals, for example, SiO₂), the microwave system MARS was used.

4. Results and discussion
4.1. Elemental analysis
It is known that the spectral lines intensity of the elements by ICP-OES method can be affected by the elements presented in a sample in amount significantly exceeding of traces. In our case, the matrix component is calcium (Ca), which presence in solution substantially changes the parameters of ICP. The matrix influence on determined analytes was studied to eliminate the systematic error. The spike experiment showed that 100 mg/L Ca significantly distorts the found concentration for Li (an overestimation). The over concentrations were observed at the 1000 mg/L calcium for K, Li, Na, and Sr, while for Mg and Mn found concentrations were leveled. Figure 1 shows a diagram for a 1000 mg/L calcium concentration.

We established the optimal calcium concentration – 100 mg/L after investigating the matrix influence, the error of concentration determined in this case does not exceed 20% (see Table 1 – Recovery, %).

Being based on investigation of the matrix effect for found analyte concentrations, the analysis was carried out for the 100 urinary stones consisting of calcium oxalate phase. The calcium content in the studied urinary stones varies from 130 to 320 g/kg. That is associated with the presence of
crystallization water and unidentified X-ray amorphous organics. All analyzed oxalate stones contain phosphorus (1.6-28 g/kg). It is suggested that the urinary stones have phosphate-type minerals in an X-ray amorphous state, which is confirmed by published data [22-24]. The sodium content in the analyzed stones varies from 0.54 to 8.7 g/kg. The element concentration of Ba, Fe, K, Li, Mg, Sr and Zn lays in the range from 0.0002 to 2.0 g/kg (for some calculi).

![Figure1](image)

**Figure1.** The spike experiment for \( C_{Ca} = 1000 \text{ mg/L} \). The certain contradiction was found in the Ni and Ti contents when comparing the results with the earlier published data [21] on single-phase stones. The indicated elements were not detected by ICP-OES with limit of detection 4 mg/kg and 1 mg/kg, respectively, while the authors of [21] find more than 7 mg/kg of nickel and 4 mg/kg of titanium, by the X-ray fluorescence spectrometry. Perhaps this is due to the peculiarities of the analysis procedure.

**Table 1.** Spike experiment for matrix concentration 100 mg/L (n=4, P=0.95).

| Analyte | Concentration, mg/L | Recovery, % | Analyte | Concentration, mg/L | Recovery, % |
|---------|---------------------|-------------|---------|---------------------|-------------|
|         | Added               | Found       |         | Added               | Found       |
| Al      | 0.139               | 0.143±0.002 | 103     | Li                  | 0.0278      | 0.0318±0.0001 | 114 |
| B       | 0.139               | 0.138±0.001 | 99      | Mg                  | 0.139       | 0.139±0.001   | 100 |
| Ba      | 0.056               | 0.053±0.001 | 95      | Mn                  | 0.139       | 0.139±0.001   | 100 |
| Bi      | 0.139               | 0.142±0.003 | 102     | Na                  | 0.139       | 0.138±0.007   | 99  |
| Cd      | 0.139               | 0.140±0.002 | 101     | Ni                  | 0.139       | 0.138±0.002   | 99  |
| Co      | 0.139               | 0.138±0.003 | 99      | P                   | 0.139       | 0.147±0.006   | 106 |
| Cr      | 0.139               | 0.138±0.001 | 99      | Se                  | 0.139       | 0.14±0.01     | 101 |
| Cu      | 0.139               | 0.139±0.003 | 100     | Sn                  | 0.139       | 0.14±0.01     | 101 |
| Fe      | 0.139               | 0.139±0.005 | 100     | Sr                  | 0.056       | 0.053±0.001   | 95  |
| Ga      | 0.139               | 0.141±0.001 | 101     | Zn                  | 0.139       | 0.139±0.001   | 100 |
| K       | 0.139               | 0.154±0.017 | 111     | Zr                  | 0.139       | 0.136±0.002   | 98  |

4.2. *Correlation analysis*

In order to establish the inter-element correlation dependences, the elemental composition of calculi related to the oxalate type of minerals was studied. Oxalate type minerals contain only whewellite and weddelite or their combination according to the XRD data.
The Spearman rank correlation coefficient was calculated to establish the inter-element correlation dependencies. The results of the correlation analysis are shown in Table 2. A very weak positive correlation was found between the elements Ca/Li \( r = 0.200 \). A weak positive correlation was found for pairs of elements Ba/Ca, Ba/Mg, Ba/Sr; Ca/Fe, Ca/Na, Ca/Sr; Fe/Mg, Fe/P, Fe/Sr, Fe/Zn; Li/Mg; Mg/Sr, Mg/Zn; P/Zn \( r = 0.202 \cdots 0.399 \). An average positive correlation was found for pairs of elements Ca/Mg, K/Na, Mg/P \( r = 0.428 \cdots 0.494 \) (see Figure 2 for pair Mg/P). For these pairs the relationship between the two variables is considered statistically significant. The table also shows the Spearman coefficient values for the remaining pairs of elements which are highlighted in red. The relationship for these variables is statistically insignificant.

**Table 2.** Spearman correlation coefficient for oxalate type urinary stones.

| Element | Ba  | Ca  | Fe  | K   | Li   | Mg   | Na   | P    | Sr   | Legend        |
|---------|-----|-----|-----|-----|------|------|------|------|------|--------------|
| Ca      | 0.264 | -   | -   | -   | -    | -    | -    | -    | -    |              |
| Fe      | 0.105 | 0.295 | -   | -   | -    | -    | -    | -    | -    | Very weak    |
| K       | 0.049 | 0.144 | 0.032 | -   | -    | -    | -    | -    | -    | positive     |
| Li      | -0.007 | 0.200 | 0.188 | 0.187 | -    | -    | -    | -    | -    |              |
| Mg      | 0.399 | 0.424 | 0.262 | 0.034 | 0.245 | -    | -    | -    | -    | Weak         |
| Na      | 0.180 | 0.202 | 0.049 | 0.477 | 0.089 | 0.157 | -    | -    | -    | positive     |
| P       | 0.150 | 0.094 | 0.231 | 0.095 | 0.134 | 0.494 | 0.104 | -    | -    |              |
| Sr      | 0.255 | 0.347 | 0.315 | 0.125 | 0.063 | 0.303 | 0.137 | 0.132 | -    | Av.          |
| Zn      | 0.194 | 0.193 | 0.213 | 0.099 | 0.058 | 0.303 | -0.017 | 0.376 | 0.072 | positive     |

It should be noted that the positive correlations for Ca/Mg, Mg/P, Mg/Sr and Mg/Zn pairs could be explained by the participation of magnesium in many vital processes in human body, including participation in the synthesis and metabolism of proteins and nucleic acids. Magnesium also acts as a cofactor of many enzymatic reactions. It is known that a phosphates excess in the body leads to a decrease in the absorption of magnesium and zinc. A positive correlation for K/Na can be explained by the functioning of the Na+/K+ adenosine triphosphatase protein (Na+/K+-ATPase pumps) [25]. Moreover potassium directly affects urination. The relationship between excess potassium in the body and renal failure has been established. However, the found correlation dependencies may have a deeper physiological basis, the study of which was not included in the scope of current study.

**Figure 2.** Inter-element Spearman correlation for Mg-P.

The researchers have no common opinion about fundamental reasons determining the trace element composition of urinary stones. Some associate the composition with the patient's region of residence...
or the chemical composition of drinking water [26]. The literature for comparable data in this area is difficult due to the differences in the methods for investigating the composition and spectrum of trace elements analyzed.

5. Conclusions
In the recent study we established that the optimal concentration of Ca in the solution was found to be 100 mg/L; the accuracy in this case does not exceed 20% for Al, B, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, P, Se, Sn, Sr, Zn, Zr by ICP-OES analysis of oxalate-type stones.

The elemental composition of 100 oxalate type (only whewellite, weddellite and their mixture) urinary stones have been determined by ICP-OES. The calcium content varies from 130 to 320 g/kg; phosphorus – from 1.6 to 28 g/kg; sodium – from 0.54 to 8.7 g/kg; Ba, Fe, K, Li, Mg, Sr and Zn – from 0.0002 to 2.0 g/kg (for some urolites).

Correlation analysis (Spearman rank correlation) showed a very weak tightness of the relationship between the elements of Ca/Li; a weak bond tightness for pairs of elements Ba/Ca, Ba/Mg, Ba/Sr; Ca/Fe, Ca/Na, Ca/Sr; Fe/Mg, Fe/P, Fe/Sr, Fe/Zn; Li/Mg; Mg/Sr, Mg/Zn; P/Zn; an average bond tightness for pairs of elements Ca/Mg, K/Na, Mg/P. All correlations are positive.

The revealed time trends and inter-element correlation dependences for the studied urinary stones will allow us to come closer to understanding their formation processes. These studies will expand the fundamental knowledge base on the mineral composition of renal calculi, which will contribute to the development of strategies for personalized methods of metaphylaxis in the urolithiasis disease treatment.

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