Coll. Antropol. **43** (2019) 3: 167–XXX
Short communication

Investigations of Renal Calculi Using New Methods

Sandra Tucak-Zorić[2], Sanda Horvatić[1], Sanja Njegovec[1], Emma Zimprich[1], Vesna Babić-Ivančić[2,3] and Vladimir Bermanec[1,2]

[1]University of Zagreb, Zagreb, Croatia
[2]Faculty of Medicine Osijek, Department of mineral metabolism, University J.J. Strossmayer Osijek, Croatia
[3]Ruđer Bošković Institute Zagreb, Croatia

ABSTRACT

Appearance of kidney stones causes numerous problems in functioning of urinary tract. It is necessary to identify its growth mechanism in order to find successful ways of prevention. The main components building kidney stones are already well known1. But research of bulk composition was not successful enough to develop useful and universal prevention methods. Such result asks for better and more precise methods for investigation of kidney stones. Established methods for investigation of renal calculi are IR spectroscopy2, X-ray diffraction and optical microscopy. These methods are sufficient and adequate to identify the most frequent and common substances, which can appear in human kidneys. Such identification was important to recognize the most important purposes and mechanisms for forming minerals inside the kidneys and to calculate the statistical abundance of each mineral in observed population. Development of different new methods for investigation of solid-state materials permit better insight in reasons for specific crystallization and mechanisms of its growth. Further research of renal stones would surely need implementation of additional methods like: scanning electron microscopy, electron dispersive spectroscopy, atomic force microscopy and tomography, which can identify new subordinate phases.

Key words: *kidney stones, formation of the stones, IR spectroscopy, scanning electron microscopy, X-ray diffraction*

Introduction

Earlier research show that the most abundant kidney stones are Ca salts of several acids. They are oxalates, phosphates, and urates. Single stone can have monomineral or polymineral composition[3]. It is assumed that around 70 and 80% of all stones are made of Ca-oxalate or Ca-phosphate. 5 to 10% are of uric acid origin, mostly correlating with some disease(s). Struvite composes around 10% of all stones and the rest (less than 1%) are made of cystine[4] (Figure 1).

Thousands of investigated samples resulted in fact that all of them could be classified as Ca-phosphates, Ca-oxalates, and urates. Accurate classification allows physicians to recommend possible corrections to patient's behaviour. It is still not straight and simple recommendation, but mostly helps. Considering different factors influencing the crystallization of kidney stones it is important to evaluate the age of patient, feeding, lifestyles, but also natural environment.

Anyhow, reappearance of kidney stones is not neglecting. That fact gives new ideas about causes for developing environment for crystallization of kidney stones. For that purpose, it is important to have better insight in composition and growing of kidney stones. There are many papers dealing with recognition of different conditions in urinary tract which are in favour crystallization or degradation of kidney stones[5]. Except preconcentration of urinary solution the presence of different bacteria is also important factor for forming kidney stones[6]. At the Ruđer Bošković Institute in Zagreb, investigations on the normal and pathological mineralization of tissues in humans, animals and plants since the late 1970s and mid 1980s has intensified[7]. Several institutions from Croatia, the Ruđer Bošković Institute, the Institute for Medical Research, the Rebro Clinical Hospital in Zagreb and the General Hospital Osijek in Osijek, researchs and collaboration were continued. Also, Croatian researchers have collaborated with colleagues from abroad, not only on basic research[8], but also a multidisciplinary approach to clarifying the formation of kidney and/or urinary stones, eg. Struvite[9]. These examinations were resulted in the publication of the book Urolitijaza[10]. The articles in the more chapters have presented a large part of epidemiological[11], physico-chemical

Received for publication June 19, 2019

and electrophysiological investigations[12]. The metabolic studies[13], prevalence of metabolic syndrome in northeastern of Croatia[14] and surgical methods of neprolithiasis also was given[15], as well as non-surgical removal of kidney stones (ESWL)[16]. In a separate issue of the scientific journal Medicinski vjesnik (2010)[17], researchers have published in their scientific and professional papers the results of research that not only relate to an interdisciplinary approach to the problem of mineralization in the human body, but also investigate urate urolithiasis[18], correlation of urolithiasis and osteoporosis[19], bone mineral density and urolithiasis[20], bone mineral density in adolescents[21]…, the encrustation of ureteral stents were detected[22]. The metabolic biochemistry parameters in the first urine and connections with composition of the stones were determined[23]. The knowledge about the formation of the urinary and/or kidney stones with molecular biology and medicine also was explained[24], as well as the correlation between nephrolithiasis and genes[25]. The microscopic observation of stones was also used, such as a microscopic method of FTIR spectroscopy analisis, to determine the composition of calculus[26]. Several chemical methods in the laboratory have been used to determine the ability of urine inhibition to precipitate calcium oxalate which is the most common ingredient calcium stones in the kidney. The mentioned methods were can be to distinguish the urine of healthy individuals from the urine of stone-forming individuals[27]. It is also assumed that potassium citrate is adequate inhibitor of calcium containing and uric acid kidney stones growth. Because potassium is replacing calcium and this process decreases calcium concentration in urine, which prevents crystallization. Similarly, increasing of Mg^{2+} removes oxalates from urine[28]. Modern methods for material investigation are not very common in the kidney stone researches, even if they are employed for study of other crystallized or less crystallized materials. This is a reason for using these nonconventional methods to explore the kidney stones.

Materials and Methods

Kidney stones from anonymous sources were collected in Osijek and Zagreb. Small number of samples is not crucial for the results of this research, because all earlier identified minerals are confirmed in this study (Table 1). Samples of kidney stones collected in northwestern part (signed K1 to K7) and northeastern part (signed OS1 to OS12) of Croatia. (Table 1)

Widely and most commonly used methods for identification of kidney stones are X-ray diffraction and IR spectroscopy. They became standard methods, because these methods are very accurate and fast. Employing these methods enabled good classification and statistical calculation attached to appearance of kidney stones. For X-ray powder diffraction (XRPD) Philips X+Pert PRO was employed using 40 kV and 40 mA current. It is assumed to be the best method for kidney stones research, because it can distinguish different mineral phases[30].

Except X-ray diffraction on powdered samples and IR spectroscopy for this study electron microscopy (SEM), and light optical microscopy were also important tools. This is a reason that figures are restricted to scanning electron microscopy images. Results of other methods are just reported in text or in tables. Vega Tescan scanning electron microscopy was employed to explore the surface for possible recognition of crystal habit and different phases. Due to fact that kidney stones are not conductive materials they are covered with thin layer of carbon to be prepared for electron microscopy[31].

TABLE 1

A LIST OF STUDIED SAMPLES*

SAMPLE	ICDD PDF29	XRPD	SEM
K1	OO-015-0762	Struvite +	Struvite apatite
K2	00-028-2016	Uric acid	n.a.
	00-019-1996	Uric acid dihydrate	
K3		n.a.	Whewelite ?
K4	01-075-1314	Weddellite	Weddellite
	00-020-0231	whewellite	
K5	00-020-0231	Whewellite +	
K6	00-001-1008	Apatite	
K7	01-075-1314	Weddellite, whewellite	
	00-016-0379		
OS1	01-077-2303	Struvite, Fluorapatite	Struvite fluorapatite EPS
OS2	00-016-0379	Whewellite	
	00-001-1008	Hydroxylapatite	
OS3	00-009-0077	Brushite, whewellite, weddellite	Weddellite, whewellite
	00-016-0379		
	00-020-0233		
OS4A	00-020-0231	Whewelite	Whewelite
	01-074-0565	Hydroxylapatite	Hydroxylapatite
	01-070-2064	Whitlockite	Whitlockite
		Brushite	
OS4B	00-020-0231	Whewellite	
	01-074-0565	Hydroxylapatite	
OS5	00-037-1802	Cistine	
OS6	01-071-2089	Struvite	Struvite, brushite apatite
OS7	00-028-2016	Uricite	
OS8	01-072-1240	Brushite	
	00-009-0077		
	00-026-1056	Calcium hydrogen-phosphate hydrate	
OS9	00-020-0231	Whewellite	
	00-025-0166	Hydroxylapatite	
OS10	00-001-1008	Hydroxylapatite	
	00-003-0240	Struvite	
OS11	01-071-2089	Struvite	
OS12	01-077-2303	Struvite	

*Composition of samples is not identical if different methods are employed. Samples not analysed signed as n.a.

Results

After the identification of minerals building kidney stones it is possible to conclude that just known mineralisation is found (Table 1). 19 samples of kidney stones are used for this study. Monophase samples are classified in 3 groups: phosphates (7), oxalates (2), urate (1), and cysteine (1). All other samples are a mixture of two phases: oxalate/phosphate (4), oxalate/urate (1), and phosphate/urate (1). Combinations of oxalate/cysteine, phosphate/cysteine, and cysteine/urate were not recorded (Table 2).

As it can be seen in Figure 1, kidney stones are commonly composed of calcium phosphates and oxalates (36%). Calcium phosphates contribute with 26%, while calcium phosphate is infrequent (only 15%). 17% are made of struvite, 5% of uric acid, and only 1% are cysteine stones[4]. Figures 2 – 23 illustrate different variants of kidney stone composition found in our sample.

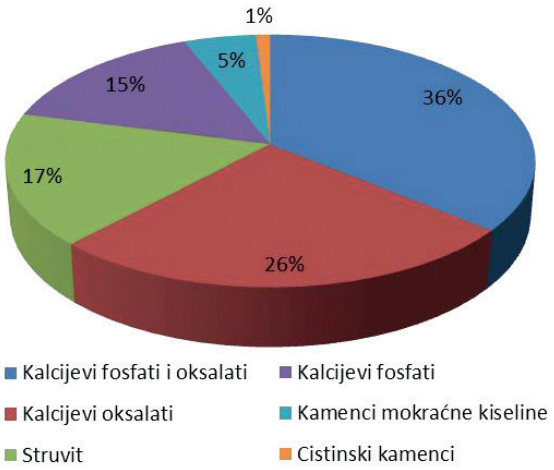


Fig. 1.

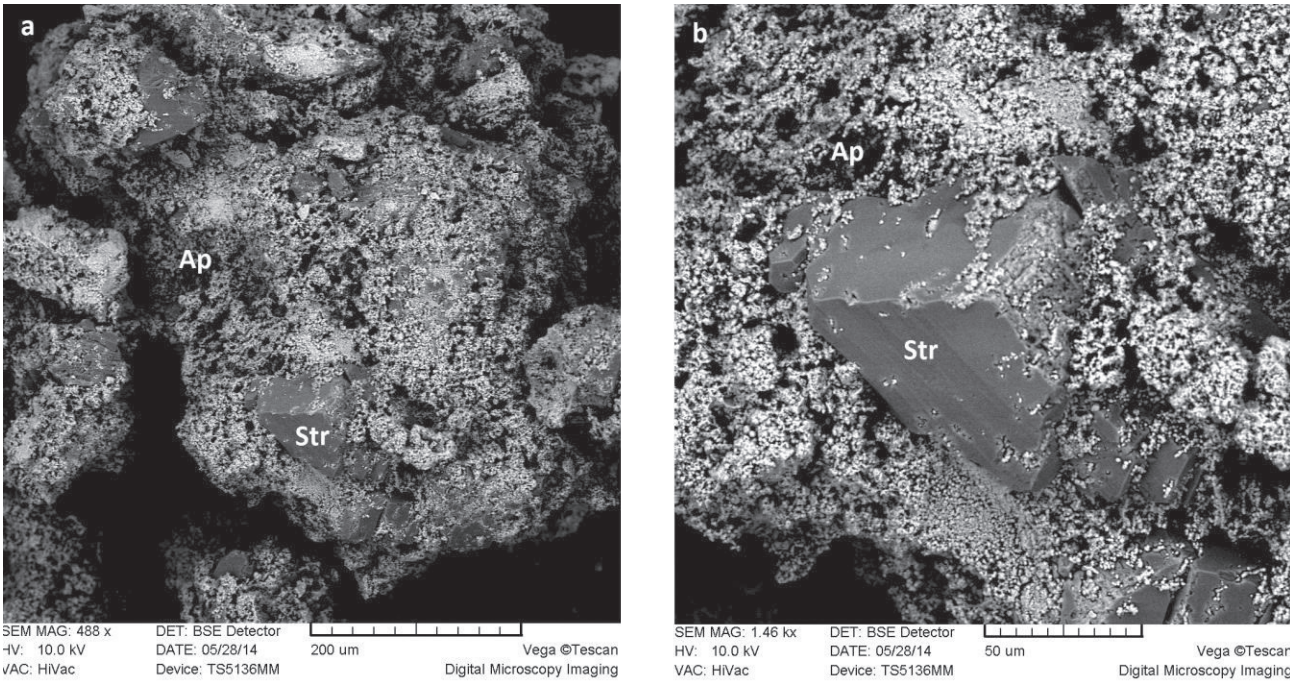


Fig. 2. SEM image of sample K1. Struvite (Str) crystals are big (up to 100 μm) and apatite crystalized as small conchoidal grains (up to 3 μm).

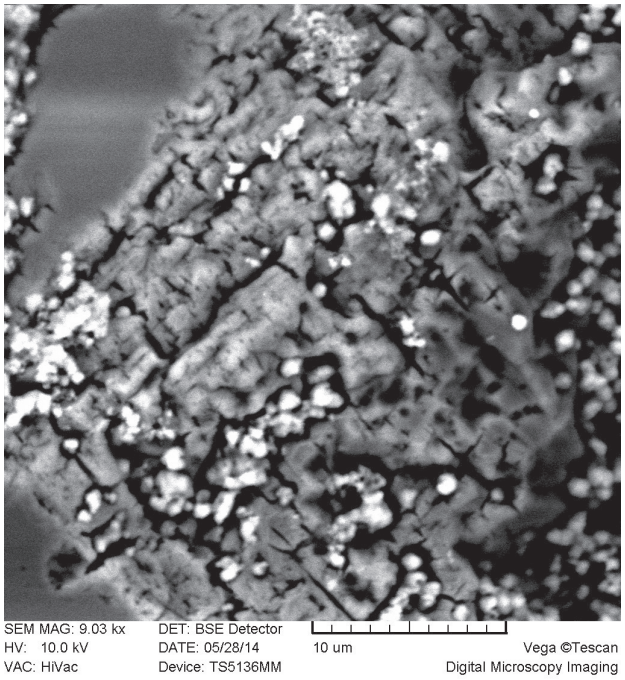


Fig. 3. SEM photograph of K1 shows that there is at least one more phase (white grains), except the big struvite crystal and apatite, containing much heavier elements present. This phase is represented as crystals smaller than 10 μm, and their quantity is too small to be recorded by X-ray diffraction.

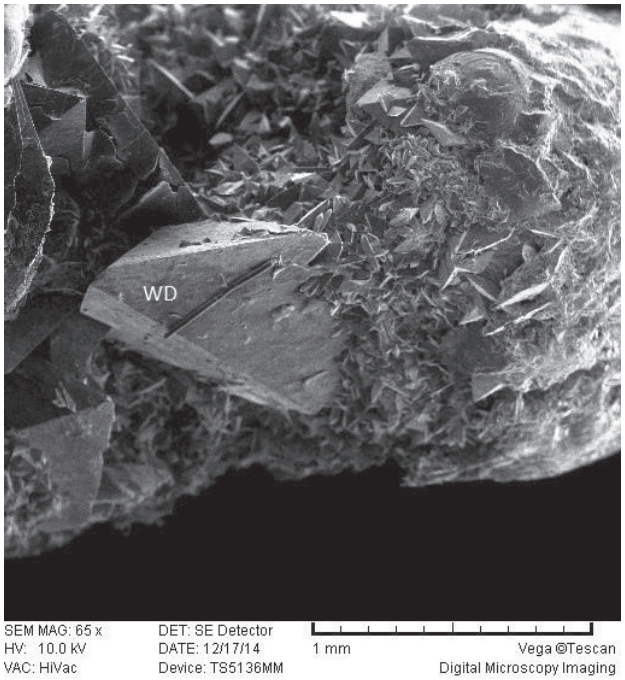
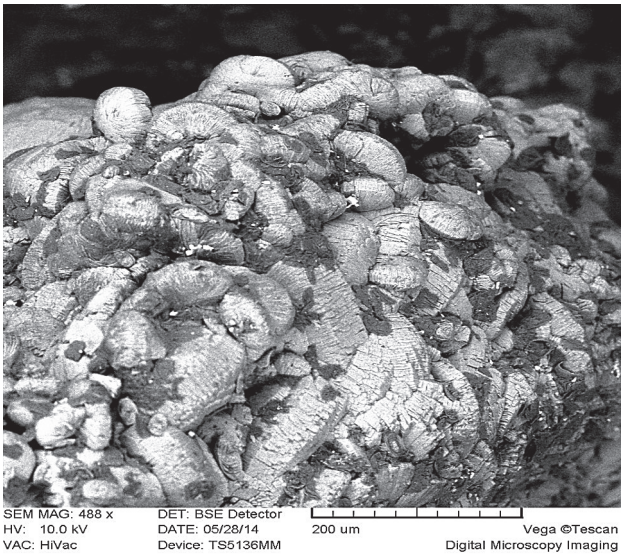


Fig. 5. Characteristic surface of sample K4 with big crystals of weddellite and small crystals of whewellite.



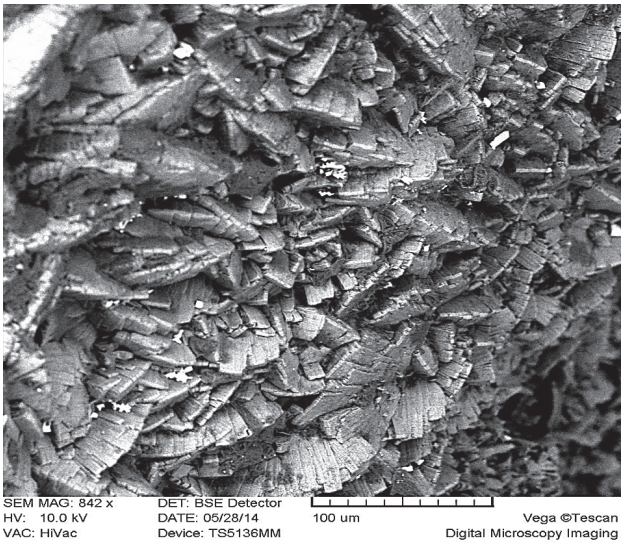


Fig. 4. Crystals building sample K3 not identified by XRPD show very sharp edges and mosaic texture. Based on crystal shape and texture it is possible to assume that it is whewellite. It is also possible to find small patches of other component, probably connected to bacteria (light gray).

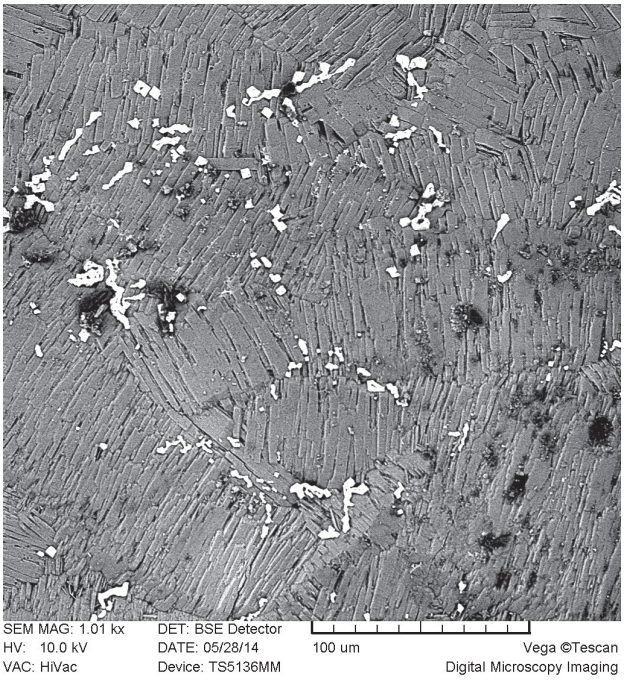


Fig. 6. Study of sample K5 by XRPD method gave just whewellite as composing phase. SEM photograph shows that whewellite is mixed with unidentified phase. This phase is composed of heavier elements, measured up to 30 μm in size.

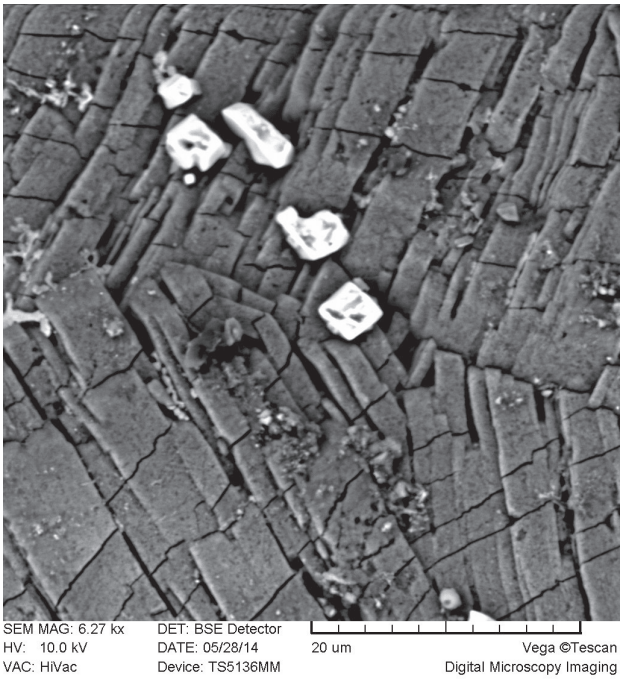


Fig. 6a. Detail of Fig. 6.

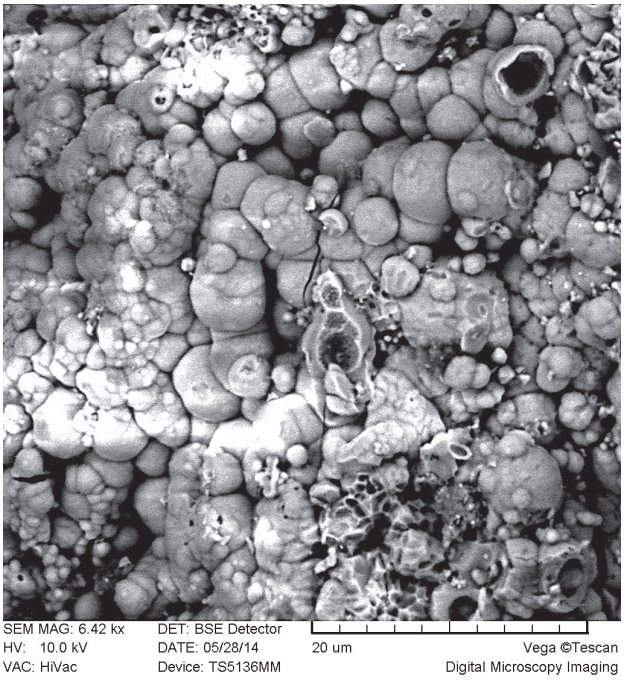


Fig. 7. On the other part of the same kidney stone K5 small quantity of different phase, the most probably apatite is found. It is another phase not seen by XRPD.

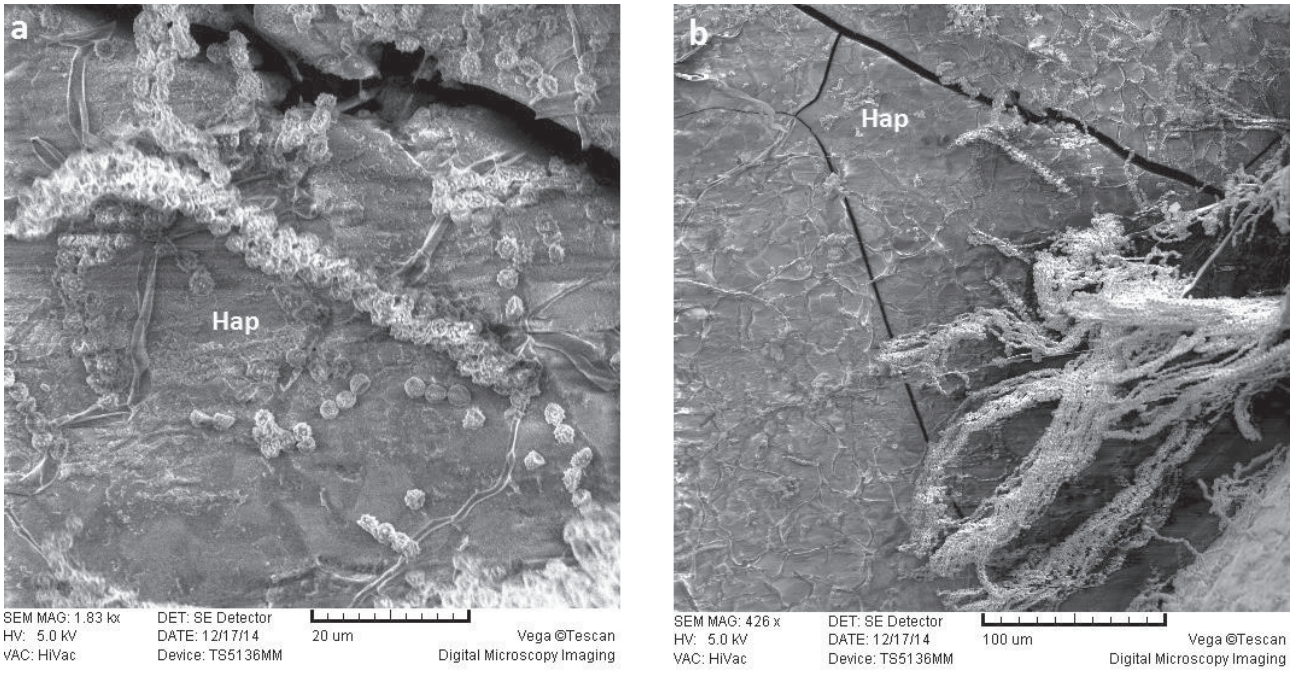


Fig. 8. Bacteria on sample K6. Streptococci make long chains on this sample, leaving also extracellular polysaccharide (EPS) fibres on the surface.

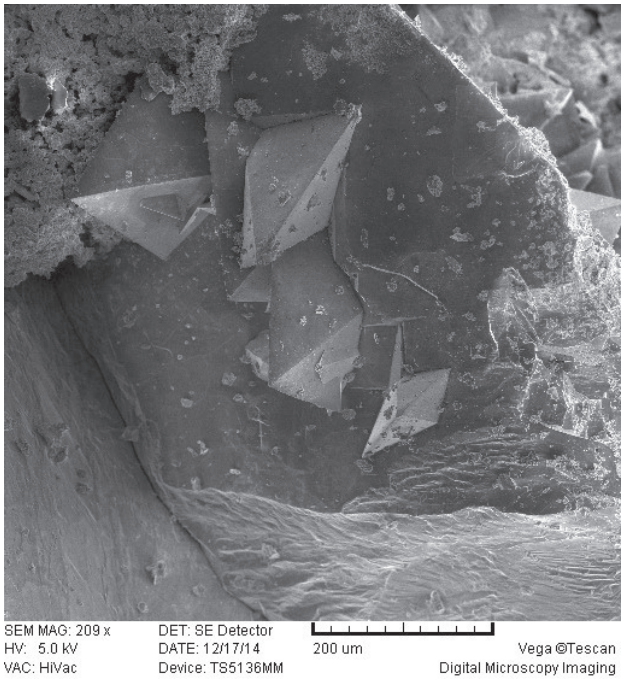


Fig. 9. Sample K7 is built of two phases, one is massive and another is represented with big crystals (up to 150 μm).

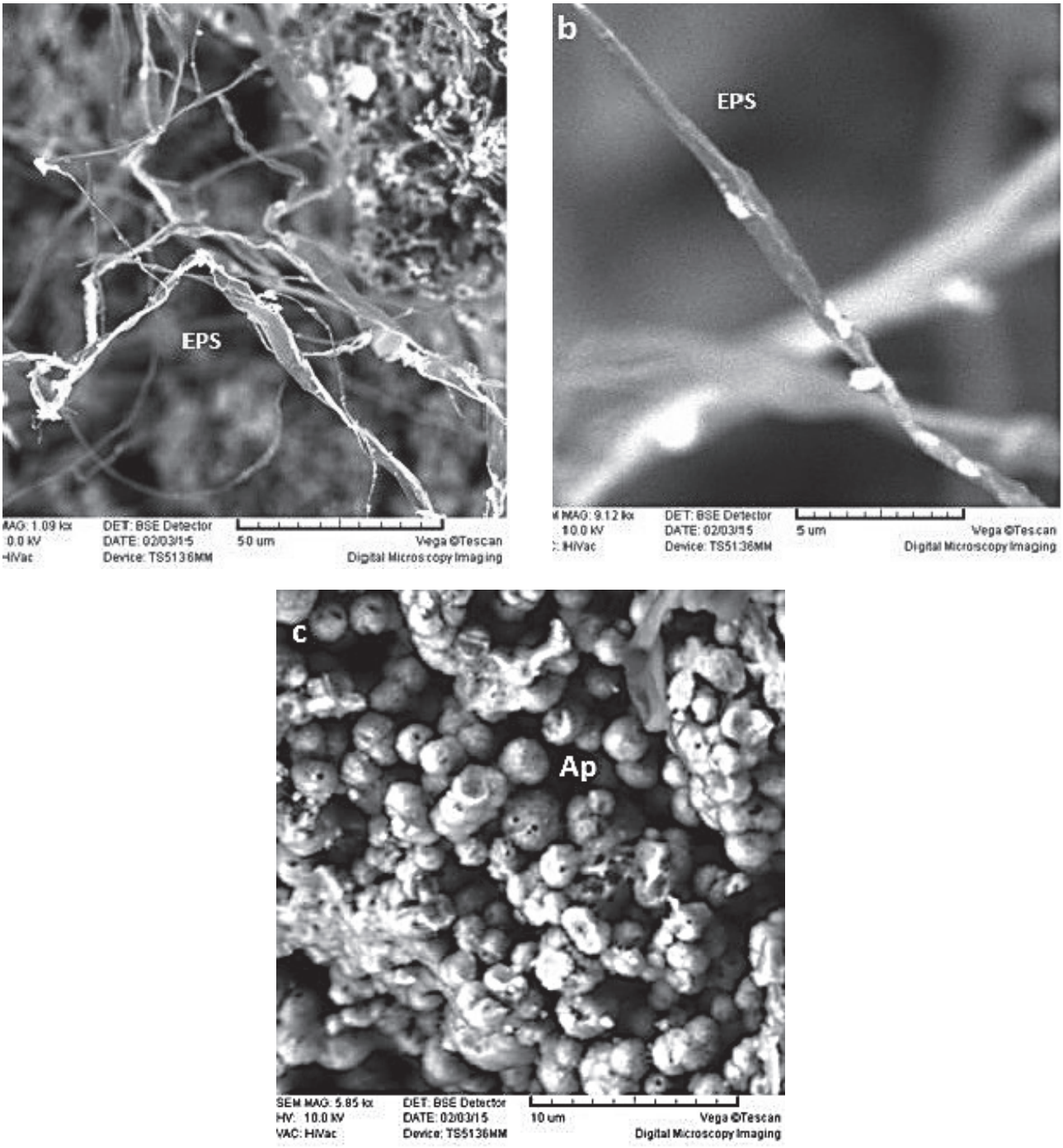


Fig. 10. a) and b) Extracellular polysaccharide (EPS) fibres from biofilm in sample OS1; c) apatite (Ap) developed as spaerulites up to 3 mm in size.

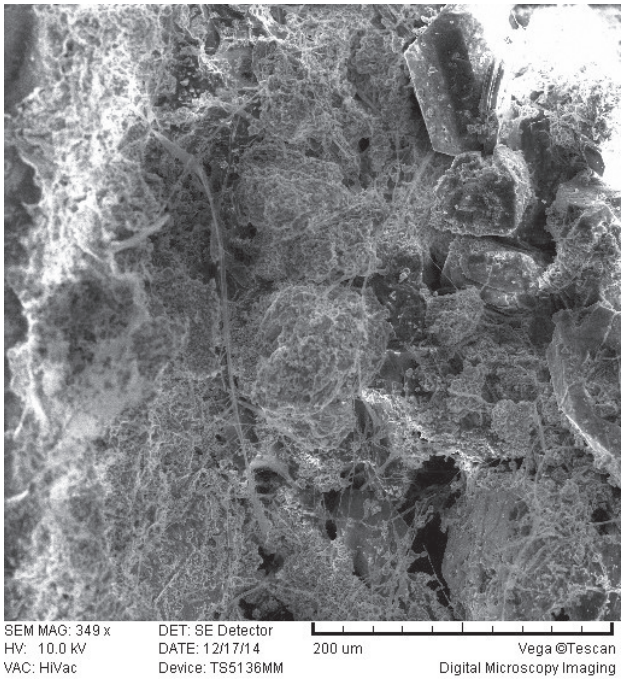


Fig. 11. Struvite crystals from kidney stone OS1 are wrapped in fine biofilm.

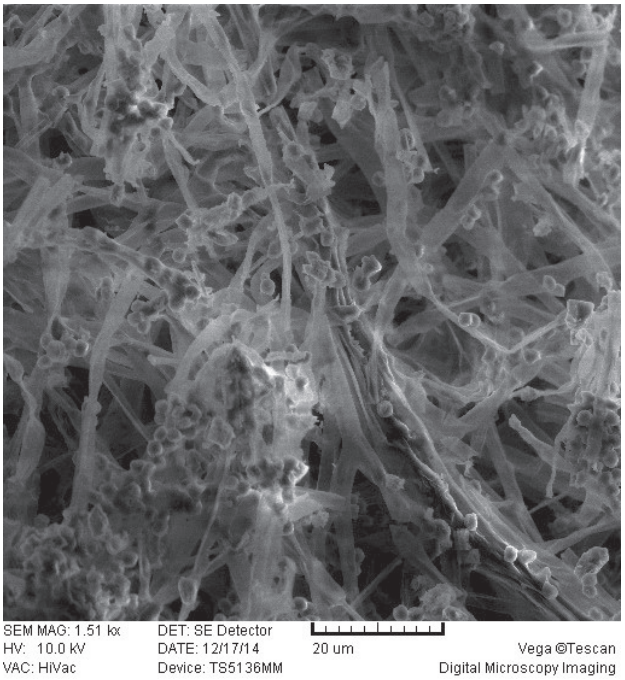


Fig. 12. Bacteria (cocci) on sample OS2 produce EPS (fibres).

Fig. 13. Sample OS3 is composed of sharp crystals of weddellite.

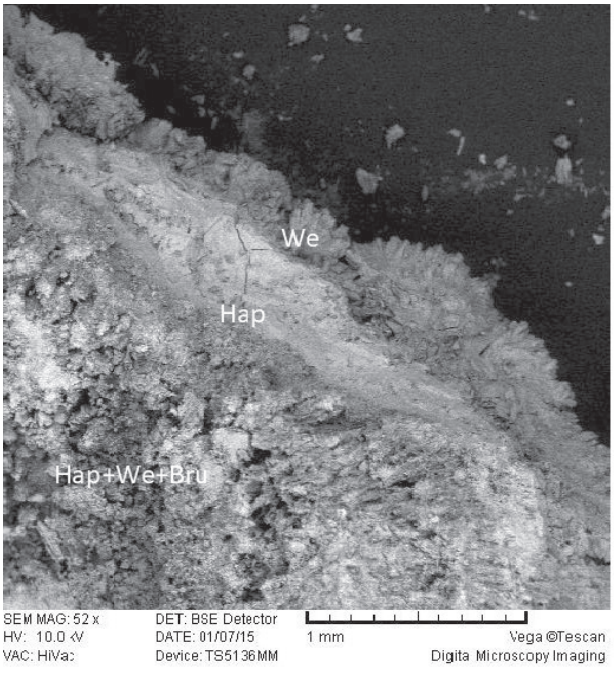


Fig. 14. Cross section of sample OS4 show zonation. There is mixture of hydroxylapatite, weddellite and brushite inside the sample. Second layer is built of massive hydroxylapatite and the surface is made of weddellite.

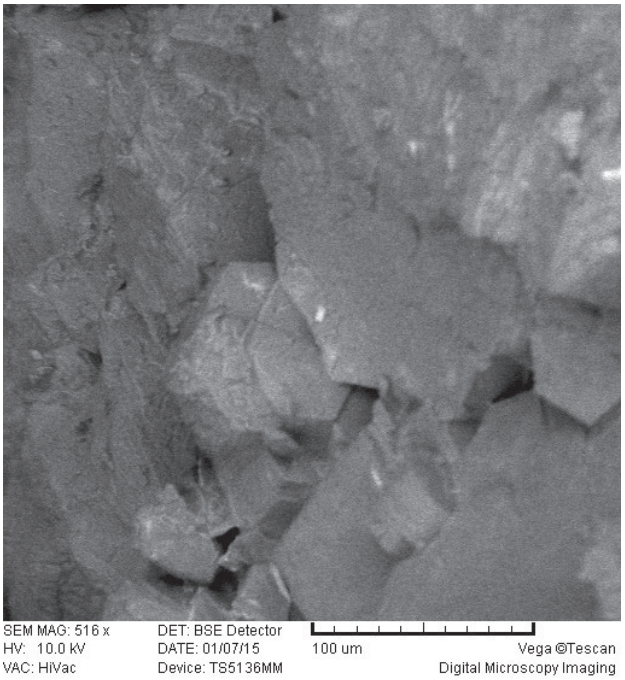


Fig. 15. L-cisteine in sample OS5 is single mineral phase, but fibres of bacterial origin could also be seen using SEM.

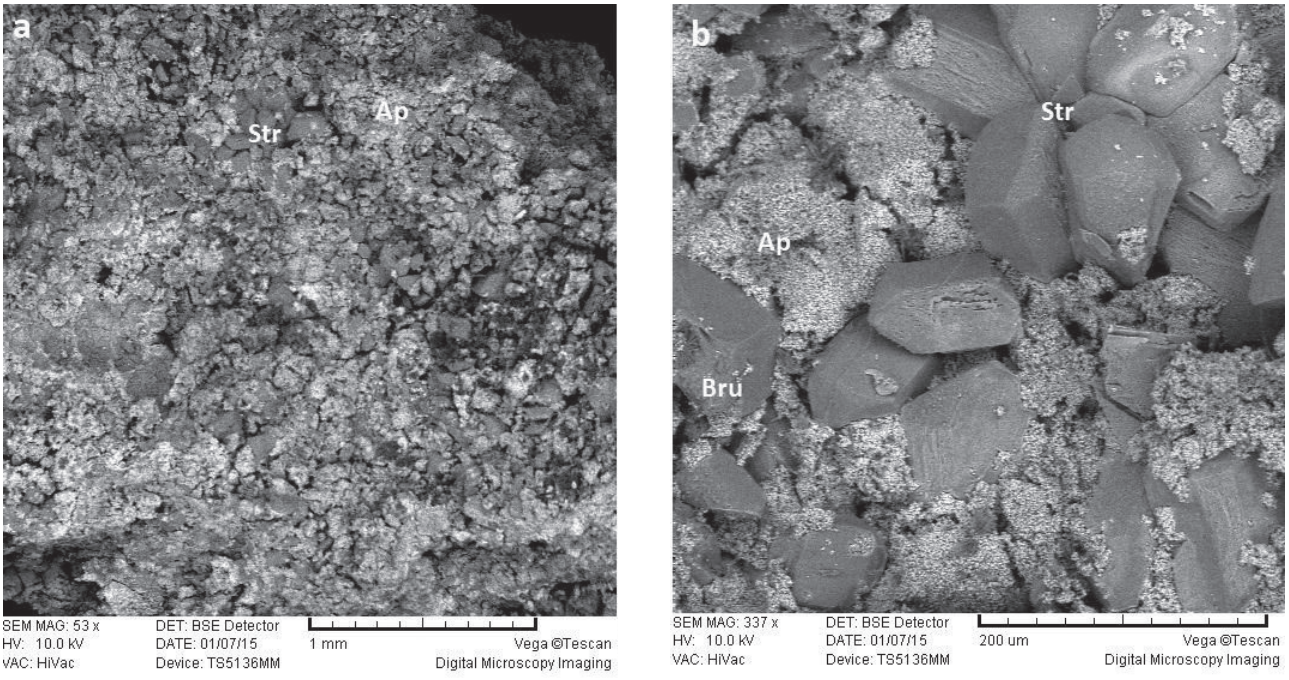


Fig. 16. Struvite (Str) crystals mixed with apatite (Ap) and brushite (Bru) in sample OS6.

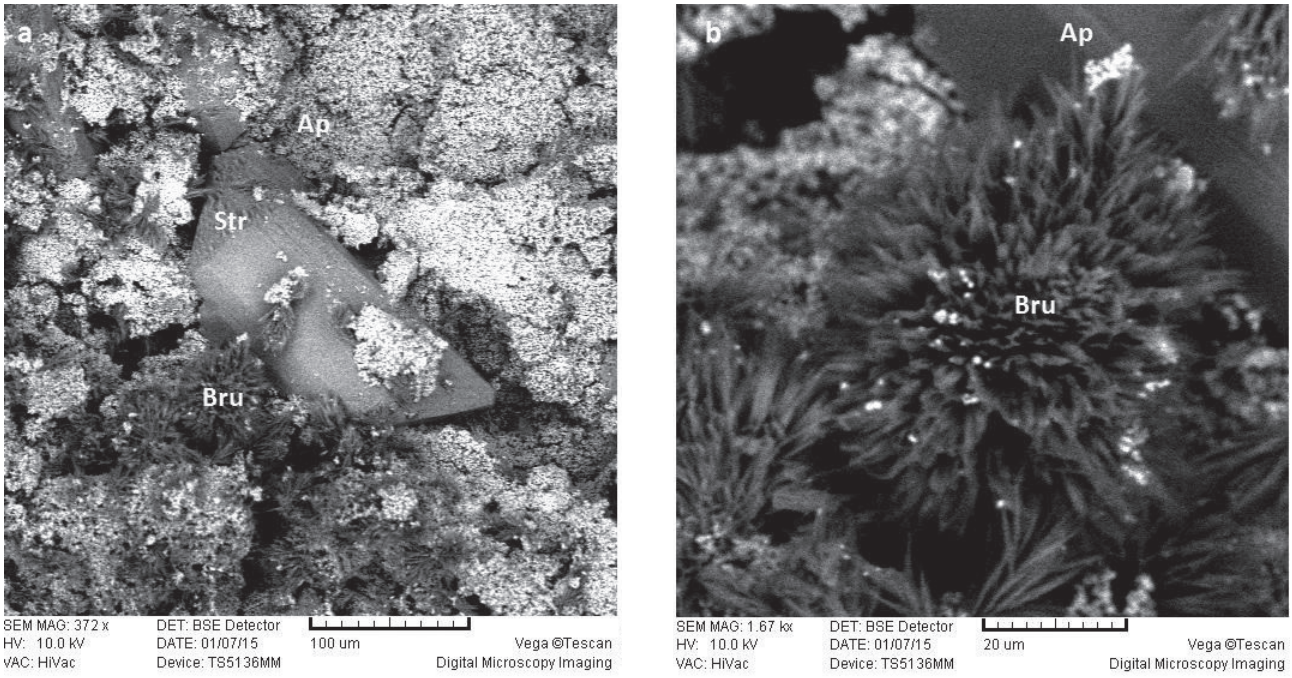


Fig. 17. same as figure 16.

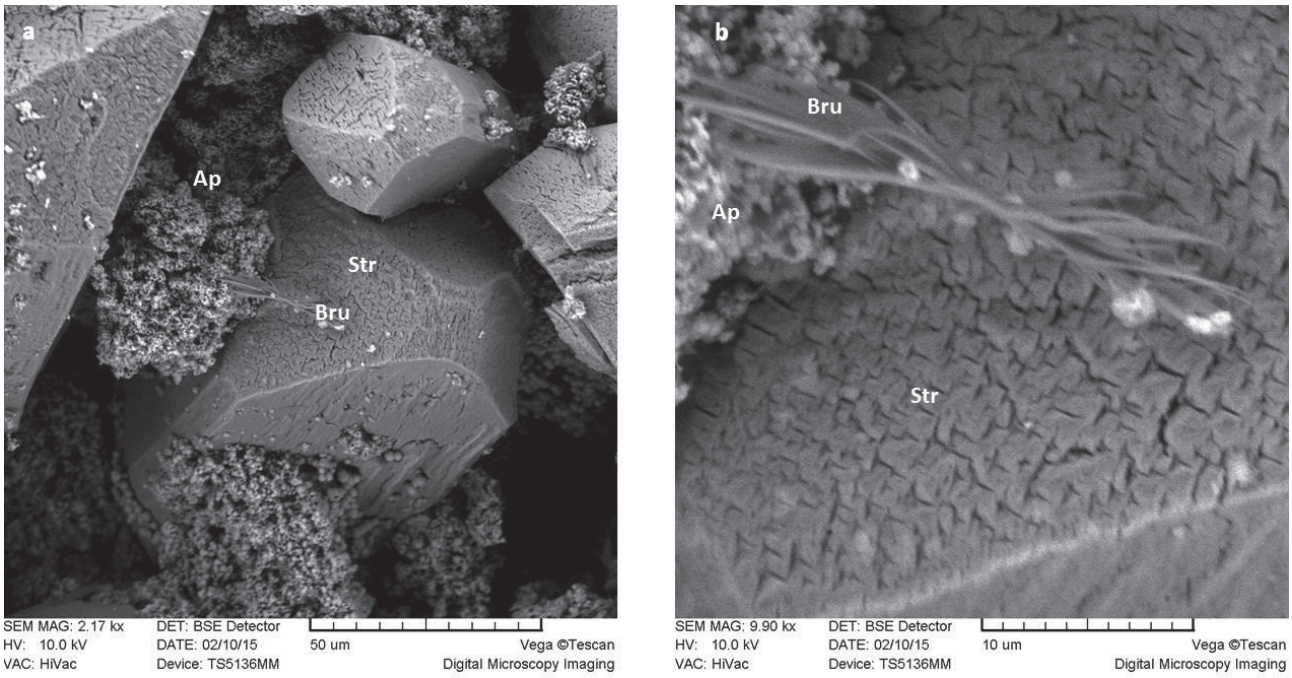


Fig. 18. Fine fibres of brushite (Bru) between struvite crystal (Str) and apatite (Ap) b) detail of image a)

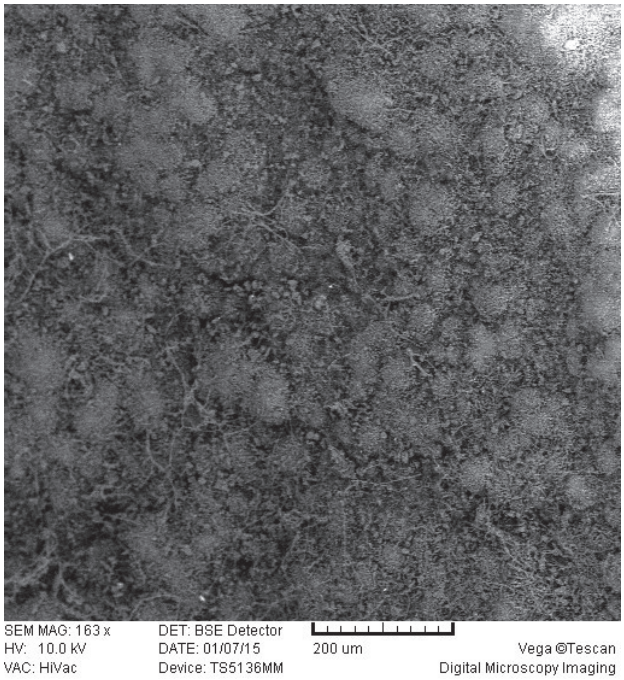


Fig. 19. Uricite crystals are completely covered with bacteria which also penetrate inside the sample OS7.

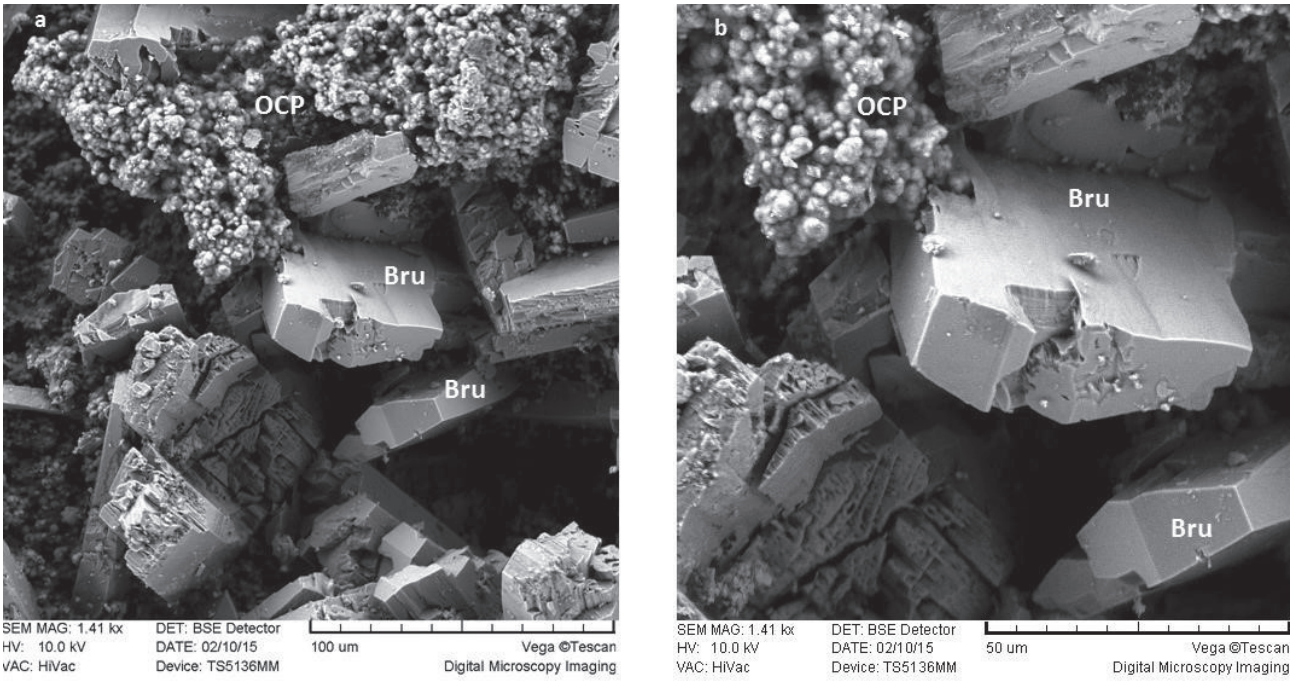


Fig. 20. Brushite crystals (Bru) with small spherules octacalcium phosphate, Ca8H2(PO4)6 x 5H2O in sample OS8. Octacalcium phosphate is not recorded by XRPD.

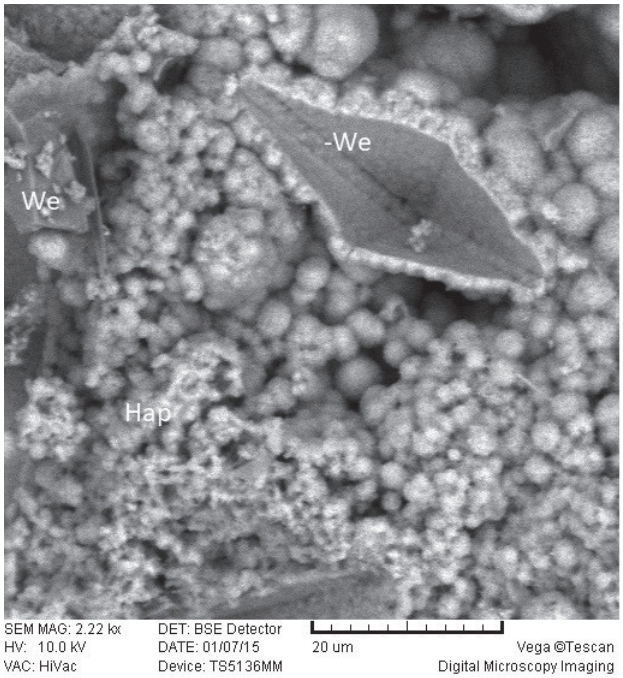


Fig. 21. Spherical growth of hydroxylapatite (Hap) and mold of whewellite (-We) crystal.

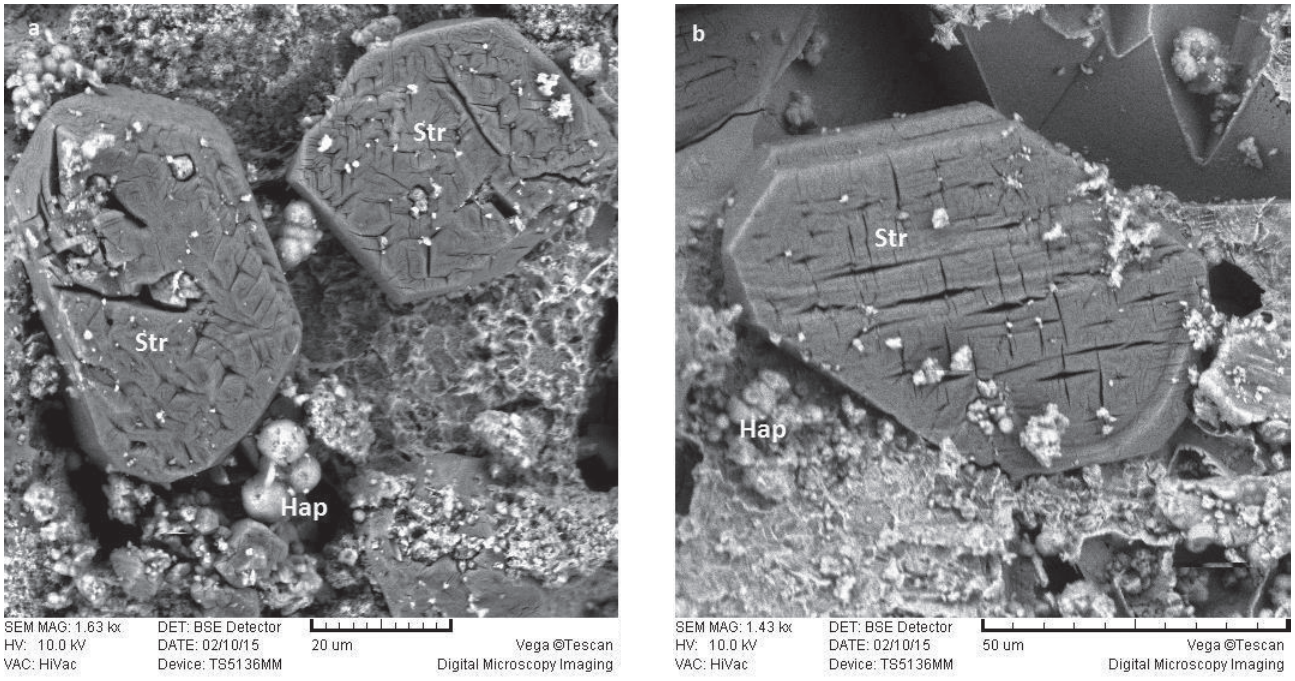


Fig. 22. Spheres of hydroxilapatite (Hap) are cementing crystals of struvite (Str) on sample OS10. Struvite crystals are cleaved due to dehydration process.

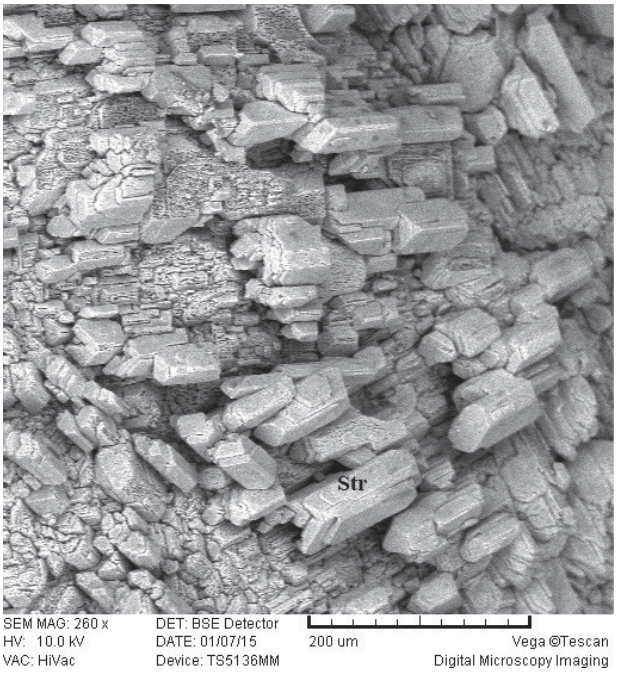


Fig. 23. Semiparallel growth of struvite (Str) crystals on sample OS11.

Discusion

It is obvious that using common methods, XRD PD and IR spectrometry, can provide information about bulk composition of kidney stone. But, sensitivity of these methods is not good enough to be sure that bulk composition can show all present phases. Moreover, using SEM one can define growth properties and distribution of different coexisting phases. Possible presence and type of bacteria or EPS could be also recorded. Recognition of crystal morphology can give us important information about shape and distribution of phases form kidney stone. There are two possibilities which can appear through research using SEM. There could be additional phases present in small quantity, with phase(s) containinng heavier elements.

This option is observed in samples K1 and K5 (Figures 2, 6, and 6a). It will be possible, using EDX spectrometry, to identify elemental composition of such phase. Another possibility is to find additional phase(s), like in samples K3 and OS6, which is (are) composed of lighter elements (Figures 3, 16, and 17). It is also possible to recognize microorganisms or biofilms like on samples: K6, OS1, OS2, and OS7 (Figure 8, Figures 10 - 12, Figures 15 and 19). Shape, dimmensions, and type of aglomeration could be interesting properties which could help to distinguish bacteria and to decide how to prevent further development of kidney stones. Additional information about bacterial cause for forming kidney stone could come from the quantity and shape of EPS, easily seen on samples K6, OS1, OS2, and OS7. Detailed examination of samples K3 and K5 could also discover microbial activity.

This research shows that there is almost no human kidney stone built from single mineral composition. They are commonly if not always mixed from two or more mineral phases. The role of bacterial metabolism is also confirmed as very important in their development. Applying several other methods and techniques could be also important in the future. These are particularly methods like electron back scattering diffraction, tomography, atomic force microscopy, and computer imaging. Such information could be very important for the treatment of these dangerous crystals in the kidney.

REFERENCES

1. BASIRI A, TAHERI M, TAHERI F, Urology Journal, Iran, (2012) 445. – 2. MOENKE HHW, Vibrational Spectra and the Crystal-chemical Classification of Minerals. In: FARMER VC (Ed): The infrared spectra of minerals (Mineralogical Society, London, 1974). – 3. COE FL, PARKS JH, ASPLIN JR, The New England Journal of Medicine, 327 (17) (1992) 1141. – 4. SHAFIEE MA, Urinary composition and stone formation (MSc Thesis, University of Toronto, Toronto 2010). – 5. BASAVARAJ DR, BIYANI CS, BROWNING AJ, CARTLEDGE JJ, European Association of Urology, United Kingdom, (2007) 126. – 6. MILOŠEVIĆ D, BATINIĆ D, Paediatr Croat, 46 (Supl 1) (2002) 33. – 7. FÜREDI-MILHOFER H, BABIĆ-IVANČIĆ V, BREČEVIĆ LJ, FILIPOVIĆ-VINCEKOVIĆ N, HLADY V, KOMUNJER Lj, MARKOVIĆ M, ŠKRTIĆ D, Normalna i patološka mineralizacija tkiva u ljudskom organizmu. Stanovište fizikalnog kemičara. U: IV Znanstveni Sabor Slavonije i Baranje, Osijek, Zbornik radova, (1984) 718-730. – 8. BABIĆ-IVANČIĆ V, FÜREDI-MILHOFER H, BROWN WE, GREGORY TM, J Crystal Growth, 83 (1987) 581. – 9. BABIĆ-IVANČIĆ V, KONTREC J, BREČEVIĆ LJ, Urological Research, 32 (2004) 350. – 10. TUCAK A, RADONIĆ M, FUREDI-MILHOFER H, DEKANIĆ D, ČEČUK LJ (Eds) Urolitijaza (IC Revija Osijek, Osijek, 1989). – 11. KLARICA J, POLIĆ V, Epidemiološka istraživanja urolitijaze. Osvrt na Istraživanja u SR Hrvatskoj. In: TUCAK A et al (Eds): Urolitijaza (IC Revija Osijek, Osijek, 1989). – 12. BABIĆ-IVANČIĆ V, UZELAC M, MARKOVIĆ M, FÜREDI-MILHOFER H, Utjecaj kalcijevih, oksalatnih i fosfatnih iona na svojstva taloga kalcij-oksalata i kalcij-fosfata. In: TUCAK A et al (Eds): Urolitijaza (IC Revija Osijek, Osijek, 1989). – 13. DEKANIĆ D, Metaboličke studije kod nefrolitijaze. In: TUCAK A et al (Eds): Urolitijaza (IC Revija Osijek, Osijek, 1989). – 14.TUCAK-ZORIĆ S, BILIĆ-ČURČIĆ I, MIHALJ H, DUMANČIĆ I, MAJETIĆ-CETINA N, ZELIĆ Ž, KUTLE A, TUCAK A, RUDAN P, Coll Antropol, 32 (2008) 659. – 15. GABRIĆ V, DEREŽIĆ D, MAREKOVIĆ Z, Kirurška terapija nefrolitijaze. In: TUCAK A et al (Eds): Urolitijaza (IC Revija Osijek, Osijek, 1989). – 16. TUCAK A, KOPROLČEC D, ESWL – prikaz prvih 500 bolesnika Extracorporeal Shock Wave Lithotripsy (ESWL). In: TUCAK A et al (Eds): Urolitijaza (IC Revija Osijek, Osijek, 1989). – 17. BABIĆ-IVANČIĆ V, TUCAK A, MARKOVIĆ M, ŠERIĆ V, CVIJETIĆ AVDAGIĆ S, FÜREDI-MILHOFER H, Med Vjesn, 42/3-4 (2010) 19. – 18. MARIĆ I, MILAS-AHIĆ J, Med Vjesn, 42/3-4 (2010) 269. – 19. BILIĆ-ČURČIĆ I, KIZIVAT T, MILAS-AHIĆ J, SMOLIĆ M, SMOLIĆ R, MIHALJEVIĆ I, TUCAK A, Med Vjesn, 42/3-4 (2010) 273. – 20. CVIJETIĆ S, FÜREDI-MILHOFER H, BABIĆ-IVANČIĆ V, TUCAK A, GALIĆ J, DEKANIĆ-OŽEGOVIĆ D, Arch Med Res, 33 (2002) 152. – 21. CVIJETIĆ AVDAGIĆ S, SLAVIČEK J, KARAČIĆ I, PURETIĆ Z, KES P, Med Vjesn, 42/3-4 (2010) 241. – 22. KUVEŽDIĆ H, BABIĆ-IVANČIĆ V, ŠERIĆ V, TUCAK A, Med Vjesn, 42/3-4 (2010) 247. – 23. ŠERIĆ V, DUTOUR-SIKIRIĆ M, MIHALJEVIĆ I, TUCAK-ZORIĆ S, BILIĆ-CURCIĆ I, BABIĆ-IVANČIĆ V, Coll Antropol, 33 (2009) 85. – 24. OPAČAK-BERNARDI T, BABIĆ-IVANČIĆ V, MARIĆ I, Med Vjesn, 41/1-2 (2009) 29. – 25. POLAŠEK O, GUNJAČA G, KOLČIĆ, I, ZGAGA L, DŽIJAN S, SMOLIĆ R, SMOLIĆ M, MILAS-AHIĆ J, ŠERIĆ V, GALIĆ J, TUCAK-ZORIĆ S, TUCAK A, RUDAN I, LAUC G, Croatian Medical Journal, 51 (2010) 48. – 26. ŠERIĆ V, BABIĆ-IVANČIĆ V, KUVEŽDIĆ H, TUCAK A, Med Vjesn, 42/3-4 (2010) 285. – 27. OPAČAK-BERNARDI T, BABIĆ-IVANČIĆ V, ŠERIĆ V, MARKOVIĆ M, FÜREDI-MILHOFER H, MARIĆ I, SMOLIĆ R, SMOLIĆ M, TUCAK A, Archivio italiano di urologia, andrologia, 83 (2011) 37. – 28. GUPTA M, BHAYANA S, SIKKA SK, Int J of Research in Pharmacy and Chemistry, 1 (2011) 793. – 29.

THE INTERNATIONAL CENTRE FOR DIFFRACTION DATA, Powder diffraction file, http://www.icdd.com/pdfsearch/. – 30. GIANNOSSI ML, SUMMA V, InTechOpen, (2012) 123. – 31. MUKHOPADHYAY SM, Sample preparation for microscopic and stereoscopic characterization of solid surfaces and films. In: MITRA S (Ed): Sample Preparation Techniques in Analytical Chemistry (John Wiley & Sons Inc., Hoboken, New Jersey, 2003).

S. Tucak-Zorić

Faculty of Medicine Osijek, Department of mineral metabolism, University J.J. Strossmayer Osijek, Croatia
sandra.tucak-zoric@zagreb.hr

ISTRAŽIVANJA BUBREŽNIH KAMENACA NOVIM METODAMA

S A Ž E T A K

Pojava bubrežnih kamenaca izaziva brojne probleme u mokraćnom sustavu. Potrebno je utvrditi njihov mehanizam rasta kako bi prevencija bila što uspješnija. Glavne komponente u sastavu bubrežnih kamenaca već su dobro poznate1. Opsežna istraživanja stvaranja kamenaca, njihovog sastava, nisu bila dovoljno uspješna za razvoj korisnih i univerzalnih metoda prevencije. Takvo stanje zahtijeva bolje i preciznije metode za istraživanje bubrežnih kamenaca. Uhodane metode ispitivanja bubrežnih kamenaca su IR spektroskopija2, rendgenska difrakcija i optička mikroskopija. Ove su metode dostatne i odgovarajuće za prepoznavanje najčešćih i zajedničkih komponenata u kamencu koje se mogu pojaviti u bubregu čovjeka. Takva identifikacija bila je važna kako bi se prepoznalo najvažnije uzroke i mehanizme za stvaranje minerala unutar bubrega te izračunala statistička gustoća, pojavnost svakog minerala u promatranoj populaciji. Razvoj različitih novih metoda za ispitivanje čvrstog stanja tvari omogućuje bolji uvid u uzroke specifične kristalizacije i mehanizme njihovog rasta. Za buduća istraživanja bubrežnih kamenaca sigurno će trebati primijeniti dodatne metode kao što su: skenirajuća pretražna mikroskopija, elektronska disperzijska spektroskopija, mikroskopija atomskih sila i tomografija, koje mogu identificirati nove slojevite faze kamenca.