CHARACTERIZATION OF EXOGEOUS PARTICLE CONTENT:

OF CANINE TISSUE URBAN VS. RURAL

INHALATION EXPOSURES

by

JAMELL KENNEDY

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTERS OF SCIENCE IN GEOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

May 2014

Acknowledgements

I would like to acknowledge Steven M. Lev, Jerrold L. Abraham and Andrew Hunt for their contributions to this research via data collection methods, preparation of sample material and guidance and direction.

April 21, 2014

Abstract

CHARACTERIZATION OF EXOGEOUS PARTICALE CONTENT:

OF CANINE TISSUE: URBAN vs. RURAL

INHALATION EXPOSURES

Jamell Kennedy, MSc

The University of Texas at Arlington, 2014

Supervising Professor: Andrew Hunt

Exogenous zinc (Zn) is emerging as a serious contaminant in the environment. Yearly deposition of zinc particles line heavily traveled inner city roadways and less travelled rural roadways. Particle size for zinc ranges from approximately PM10 to PM 2.5 µm or less. These fine particles contain microscopic solids or liquids that can cause serious health problems. PM10 are considered to be "thoracic" sized particles, with the mass fraction of inhaled particles penetrating beyond the larynx. Whereas, PM2.5 are considered to be "respirable" sized particles, with the mass fraction of inhaled particles penetrating to the unciliated airways. Exogenous zinc can be used as a quantifiable marker to contrast the differences in exposures in canines originating from urban and rural environments. These exposures are analyzed using a scanning electron microscope with energy dispersive X-ray spectrometry, and usage of a morphometric point counting method for a physical count and categorization of composition of inhaled retained particle content.

Table of Contents

Acknowledgements ... iii

Abstract ... iv

List of Illustrations ... vi

List of Tables ... vii

Chapter 1 Introduction: ... 1

Chapter 2 Research Methods: ... 8

Materials ... 8

Methods ... 11

Chapter 3 Results: ... 15

Chapter 4 Discussion and Conclusion: ... 29

Discusssion ... 29

Conclusion ... 42

Appendix A: Characterized Field and Diameter Views for Samples 1A-7B ... 45

References ... 181

Biographical Information ... 183

List of Illustrations

Figure 1-1 Relative Size of Particulate Matter 2

Figure 1-2 Types of Particles Found In Tissues 4

Figure 2-1 Field of View Image 13

Figure 2.2 Field of View Image 13

Figure 2-3 Magnification Image 14

Figure 2-4 Magnification Image 14

Figure 3-1 Total Exogenous Content 1A-7A 17

Figure 3-2 Total Exogenous Content 1B-7B 18

Figure 4-1 Tissue Image 30

Figure 4-2 Tissue Image 31

Figure 4-3 Tissue Image 31

Figure 4-4 Tissue Image 32

Figure 4-5 Total Exogenous Content: Urban and Rural Concentrations 40

Figure 4-6 Total Exogenous Content: Urban and Rural Total Concentrations 41

List of Tables

Table 2-1 Canine Data 9

Table 3-1 Total Exogenous Particles, Silica, AlSi, M-Silicate, and Metals:. 15

Table 3-2 Total Exogenous Particles, Silica, AlSi, M-Silicates, and Metals. 16

Table 3-3 Total Exogenous Content and AlSi Percent Concentration 1A-7A............. 19

Table 3-4 Total Exogenous Content and Silicate Percent Concentration 1A-7A 20

Table 3-5 Total Exogenous Content and Mixed Silicate Percent Concen 1A-7A...... 21

Table 3-6 Total Exogenous Content and Metal Percent Concentation 1A-7A 22

Table 3-7 Total Exogenous Content and Zinc Percent Concentation 1A-7A 23

Table 3-8 Total Exogenous Content and AlSi Percent Concentration 1B-7B 24

Table 3-9 Total Exogenous Content and Silicate Percent Concentration 1B-7B 25

Table 3-10 Total Exogenous Content and Mixed Silicate Percent Concen 1B-7B....... 26

Table 3-11 Total Exogenous Content and Metal Percent Concentration 1B-7B 27

Table 3-12 Total Exogenous Content and Zinc Percent Concentration 1B-7B 28

Table 4-1 Exogenous Particle Content Comparison: 1A vs. 2A 33

Table 4-2 Exogenous Particle Content Comparison: 3A vs. 4A 34

Table 4-3 Exogenous Particle Content Comparison: 6A vs. 7A 35

Table 4-4 Exogenous Particle Content Comparison: 5A vs. Sample set 36

Table 4-5 Exogenous Particle Content Comparison: 1B vs. 2B 37

Table 4-6 Exogenous Particle Content Comparison: 3B vs. 4B 37

Table 4-7 Exogenous Particle Content Comparison: 6B vs. 7B 38

Table 4-8 Exogenous Particle Content Comparison: 5B vs. Sample set 39

Chapter 1

Introduction:

Atmospheric particulate matter is a composite mixture of extremely small particles. The composition of such particulate matter (PM) includes a number of components: acids (such as nitrates and sulfates), organic chemicals, metals, soils and natural dust particles. The relative sizes of these particles are directly related to their potential for adverse health effects. Due to the range of sizes of airborne particles, inhalation of material can occur. And smaller particulate matter, can penetrate deep into the lungs. Numerous scientific studies have linked PM pollution exposures to a variety of problems like those listed below:

- Premature death in people with preexisting heart and lung disease.
- Nonfatal heart attacks,
- Irregular heartbeat
- Aggravated Asthma
- Decreased lung function
- Increased respiratory symptoms including irritation of the airways, coughing and or difficulty breathing.

Figure 1-1 Relative Size of Particulate Matter

Shows a depiction of the relative sizes of $PM_{2.5}$ and PM_{10}. Notice how both PM_{10} and $PM_{2.5}$ are smaller than a grain of fine beach sand. The image also so the relative sizes vs. a strand of human hair. Notice here that PM_{10} can fit along the surface of human hair, while $PM_{2.5}$ fits within the PM_{10} on the surface of a human hair stand (U. EPA).

PM_{10} is considered to be of thoracic sized (pertaining to the upper part (transport region) of the respiratory tract) particles, which are capable of penetrating beyond the larynx. But not into the gas exchange region of the bronchial tree. $PM_{2.5}$ is considered to be capable of penetrating to the uncilited airways. Ultimately the $PM_{2.5}$ particles, pose a significantly higher risk of health problems. This is due to the penetration depth in the bronchial tree of particles, that are <2.5 µm. Once inhalation has occurred, particles deposited at varying depths in the lungs, will either be removed by host defense mechanisms mediated by the immune system (e.g..macrophages phagocytosis), or returned up the bronchial tree via the muco-cilliary escalator (then expectorated or swallowed), or will remain lodged in tissue. Chronic exposure to $PM_{2.5}$ sized particles eventually causes the severe health effects listed above. Categorization of particle content, 10 µm or less fall into two size categories designated by the EPA:

- 1) Are the "Inhalable coarse particles," for example those found near roadways and industrial locations. These particles are generally larger than 2.5 micrometers but are smaller than 10 micrometers in diameter (EPA).

- 2) Are the "Fine particles," for instance those found in smoke and haze, are 2.5 micrometers in diameter and smaller. Particles of this diameter can be directly emitted from sources such as forest fires, or anthropogenic forms such as gases (that subsequently condense as particles), emitted from power plants, industry and automobiles (EPA).

In terms of direct human exposure, particles found in tissue (following inhalation) are of exogenous origin (Abraham, J. L. (1978). Some particles can form in tissue and these are designated exogenous. Figure 2 which is a chart showing particles found in tissue, illustrates a few examples of each type (Abraham, J. L. (1978). The exogenous particle content is by far the most significant from a diagnostically environmental- oriented perspective because of inhalation.

TYPES OF PARTICULATES FOUND IN TISSUES

Endogenous (e.g., hemosiderin, calcification, urates)

Exogenous

- Inhaled or Aspirated
- Ingested (e.g., $BaSO_4$)
- Absorbed and deposited (e.g., drugs, Ca, Pb, Ag)
- Implanted (e.g., wear particles from prostheses, tattoo pigments)

Contaminants (e.g., dirt, formalin pigment, fixative precipitate)

Figure 1-2 Types of Particulates found in Tissues

Shows both exogenous and endogenous examples

of non-fibrous particulate matter found in lung tissue (Abraham, J. L. (1978).

This research seeks to characterize anthropogenic (exogenous) particles in tissue from general environmental exposures (as opposed, for example, from a specific industrial exposure). Of most interest to this study are vehicular emission related respirable particles. Specifically, fine particulate zinc, which is potentially present in the urban aerosol, may pose an inhalation threat. This study has its origins in a hypothesis proposed by Dr. Edward Landa (USGS retired), who following the work of Swinth and Huth (1974), proposed that solid particulate zinc present in lymph nodes can be a marker of adverse health effects. The study by Swinth and Huth (1974), identified zinc in particles in lymph node tissue, and Ed Landa posited that zinc from tire wear in urban locations could be readily trans-located to peripheral lymph nodes following inhalation.

Zinc is a metallic element found in nature but is also produced by anthropogenic processes such as the metal industry, combustion exhausts and tire dust. Zinc has been recorded in ambient air at relatively higher levels than other metals (Adamson et al., 2000; Balachandran et al., 2000; Dye et al., 2000; Harrison and Yin, 2000; Kodavanti et al., 2000). Studies conducted on Utah Valley, UT, PM extracts identified a number of metals in the PM with significant amounts of zinc present. Investigations of the health effects of the Utah Valley PM extracts were found to be, mostly, due to the levels of zinc in the PM, which varied because of changes in the operation (in service vs. out of service) of the local steel mill (Dye et al., 2001; Soukup et al., 2000). This and other evidence has led zinc to be considered as not only an environmental pollutant, but also a potential risk to health.

Zinc exposure in experimental animal models has been the focus of several studies. For example, Kodavanti et al. (2002) investigated pulmonary injury in three strains of rat exposed to oil combustion emission PM containing bioavailable zinc. They showed that the concentration of zinc during intra-tracheal instillation may be critical in initiating neutrophilic inflammatory responses in the lung. With respect to urban environment sources of zinc, Councell et al. (2004) suggested that tread-wear from road surface abrasion is a source of zinc in the environment. In this research two approaches were used to assess the magnitude of this source of zinc in the U.S. during the period 1936 to1999. In the first approach, tread-wear rates were used in conjunction with vehicle-distance driven data to determine zinc releases, Councell et al. (2004). The second approach quantified this source in terms of volume of tread lost during the lifetime of tire wear.

This analysis showed that the quantities of zinc released by tire wear abrasion in the U.S in the mid-1990s were estimated to be between 10,000-11,000 metric tons. The study considered urban and rural areas that commonly had significant amounts of road traffic. In 1999 U.S. urban roads comprised 3,050,000 km of lanes or about 23% of the total lane distance (Bureau of Transportation Statistics 2002). However 60% of annual miles driven that year were on urban roadways(Bureau of Transportation Statistics 2002). In this study it was argued that if zinc were to be released uniformly over all roadways, then urban areas would receive more zinc from tires due to the high traffic volume and greater road density. This means that due to larger population densities in urban cites zinc concentrations should be elevated in these areas.

Another case where zinc emerged as an important exogenous particle type found in tissue samples were in those analyzed in autopsy samples of individuals who died during the London smog of 1952. The analysis of the tissue samples took into account people with preexisting respiratory and cardiovascular diseases (Hunt et al., 2002). Due, likely, to a large amount of fine particulate matter in the London aerosol in December of 1952, there was a spike in the morbidity and mortality rates for the older members of the London population (probably with preexisting health conditions) and the infant population. Sulfur dioxide, coal combustion dust and vehicular emitted particles were thought to be the primary causes for the air pollution episode. The study by Hunt et al. (2002) employed scanning electron microscopy (SEM) analysis of tissue sections to determine the composition of particles inhaled during the smog event that could have been a factor in the deaths of individuals studied. What the analysis showed was that these autopsy samples contained significant amounts of diesel particles in the airways and metal particles in macrophages in alveoli, interstitial tissue and in lymph nodes.

Inhalation of PM has long been recognized as a potential source of health problems in the U.S. but has often not elicited appropriate concern. In a survey of 10,000 physicians in the U.S. conducted by the National Institute of Occupational Safety and

Health (NIOSH) in 1976 (Abraham et al. 1990), the level of interest by physicians regarding lung tissue analyzed for inorganic particulates was assessed. From nearly 1,200 interpretable responses to the survey, an estimation of well over 10,000 cases annually in the U.S. are recognized as being of potentially relatable to particulate exposure. Abraham (1978) stated, that because of the lack of interest including particle content in the diagnosis of environmental lung disease led to many under diagnosed patients. This lack of interest would potentially lead to a large portion of lung related diagnoses being under diagnosed. It has been repeatedly suggested that the determination of the lung burden of particulate material may provide important clues to occupational and other exposures during a patient's life up to that time (Abraham et al. 1990). It is important to note that the analysis of lung tissue does not provide the complete record of exposures. But it does give insight to the type of environment a patient is exposed to via retained burden in the lung.

In the following sections, we try to answer some specific questions regarding inorganic particulate burden in tissue that has resulted from general environmental exposure: (1) Does zinc constitute an identifiable portion of elements found in canine tissue? (2) If zinc is not readily found in canine tissue samples what particle types constitute identifiable markers of exposure?

Chapter 2

Research Materials and Methods:

Materials:

The reason canine tissue samples are used in this research instead of human tissue is because of the many difficulties in procuring human tissue samples. With human tissue, the approval of the patient is needed if a biopsy sample is to be prospectively taken (not realistic) or if retrospectively biopsied material is to be analyzed. The acquisition of canine tissue samples is less problematic and much easier to obtain and work with. Here, samples of canine tissue were provide by Scott Fitzgerald, DVM, PhD, and DACVP of the College of Veterinary Medicine at Michigan State University.

Information regarding the location, age, breed, zip code, and size of human population of the location where the dog came from, sample can be found in Table 1 below:

Table 2-1 Canine Data

Sample #	Dog Info	Male/Female	Age Yr	Location	Zip Code	Population	Classification
Sample 1	Golden Retriever	F/S	7 yr	Tecumseh MI	49286	8,427	Rural
Sample 2	Great Dane	M/C	6 yr	Lansing MI	48919	113,996	Urban
Sample 3	Irish Wolfhound	M/C	10 yr	Lansing MI	48933	113,996	Urban
Sample 4	Mixed Breed	F/S	11 yr	Grand Blanc MI	48439	8,144	Rural
Sample 5	Mixed Breed	F/S	5 yr	Bay City MI	48706	34,521	Suburban
Sample 6	Siberian Husky	F/S	8 yr	Owosso	48867	14,852	Rural
Sample 7	Golden Retriever	?	15 yr	Owosso	48867	14,852	Rural

Shows Breed type, Location, sex, zip code, population, and classification of each sample.

To find the population of each location, access to the US Census Bureau was used. This data helped to identify urban populated areas vs. the smaller rural areas. Samples 1A and 1B were from a dog from Tecumseh Michigan. Tecumseh Michigan has a population of about 8,427. Samples 2A and 2B were from a dog from the Lansing Michigan area, with population estimated at 113,996. Samples 3A and 3B were also from a dog from the Lansing Michigan area. Samples 4A and 4B were from a dog from Grand Blanc Michigan which has a population estimate of about 8,144. Samples 5A and 5B were from a dog from the Bay City Michigan area that has a population total of 34,521. Samples 6A-6B-7A and 7B were from a dogs from Owosso Michigan with a population estimated of 14,852.

The urban size of the locations these dogs were was not large. Moreover, only 2 of the 7 samples were from a location that has a population over 50,000 people. The question of what is considered urban and what rural depends on location attributes. Based solely on population data, provided by the Census Bureau, Grand Blanc MI has the fewest number of people and is considered to be the most rural area. This is then followed by Tecumseh MI with a slightly larger population but is considered rural. Owosso MI has almost 2 times the population of either Tecumseh or Grand Blanc, and therefore is consider to be slightly more urban that both cites. Bay City MI has 2 times the population of Owosso and 4 times the population of Tecumseh and Grand Blanc and is generally considered more urban then the less populated areas. However, Lansing MI has roughly 14 times the population of Tecumseh and Grand Blanc and is considered to be the most urban of the location from the where the dogs resided.

Methods:

Abraham et al. (1994), conducted research which compared particle content in lung tissue, using quantification techniques. This research required the preparation of paraffin embedded lung tissues which were than sectioned at 5 µm thickness. These cut samples were then mounted onto a carbon substrate. The sectioned samples were then deparaffinized and examined by scanning electron microscopy (SEM in tandem with energy dispersive X-ray spectroscopy (EDX). Under standard operating conditions the particles burden in the sections was characterized using a morphometric point counting approach.

A morphometric point counting method is one that refers to a quantitative lung burred analysis and utilizes particle size and shape measurement. Each tissue sample that is analyzed, requires the analysis of fields of view (FOV) in the SEM that are selected at random (Figure 2-1-2.2). In each field the inorganic particles were identified, sized and analyzed. The FOVs are examined in the backscattered electron (BE) imaging mode (that identifies variations in the average atomic number of the tissue constituents), which permits exogenous particles to be identified on the basis of their composition (essentially denser than tissue particles are identified by their higher BE yield). In this method, data is collected until either 300 fields are reviewed (giving a minimum detection limit of 1 million particles per cubic centimeter of tissue) with, or without, particulate matter, and/or 100 particles in the tissue are analyzed (to determine [particle element composition]).

In this study 5 µm thick sectioned samples of tissue were imaged in the SEM at an operating magnification of 6000 times (Figure 2-3-2-4). During the SEM review of a tissue section, particle composition in each FOV was recorded until either 300 FOVs were examined or data on 100 particles was acquired. Once all the samples were analyzed the individual particle data was input into an excel spreadsheet. The results of each sample analysis were then compared showing total exogenous particle content which was summarized by a number of separate particle "types" These types were: mixed silicate particles (containing Al and Si plus other elements), aluminum silicate particles (containing Al and Si), silica particles (containing Si), and metal-bearing particles (containing first row transition or heavier metals). Total exogenous content were compared between samples, as were the particle "type" concentrations. The outcomes of this study can be compared to a similar study was conducted on 192 greyhounds and 5 pet dogs. The objective, of that study (Schoning et.al, (1996) was to asses, the amount of opaque dust in lung specimens with age of greyhounds with pet dogs as controls. The preparation of tissue samples in that study were the same as employed here. Lung tissue particles described in the Schoning study were separated into endogenous and exogenous groups that were subdivided into 3 groups- silica, silicates, and metals (Schoning et al., 1996).

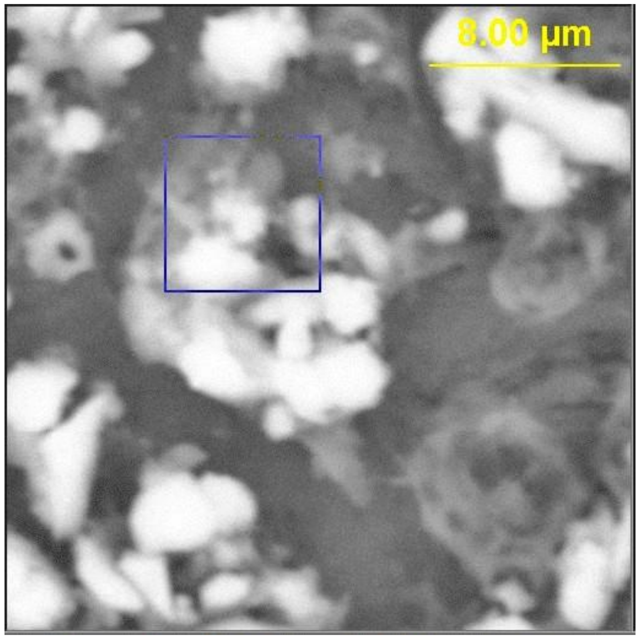


Figure 2-1 Field of View Image

Example of a field of view (FOV)

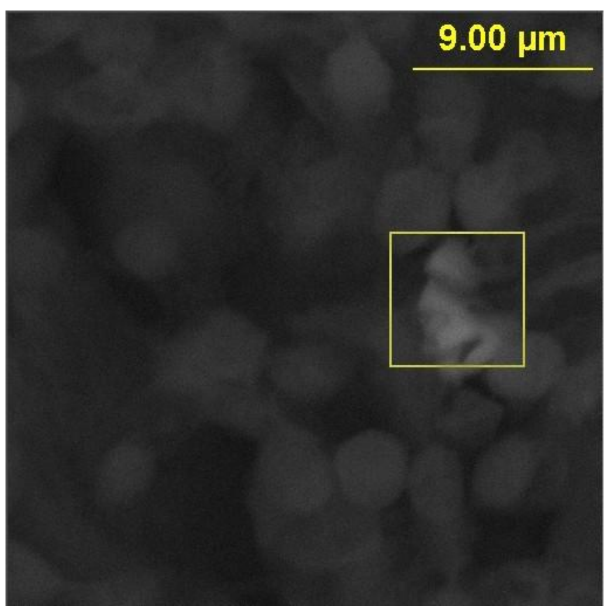


Figure 2-2 Field of View Image

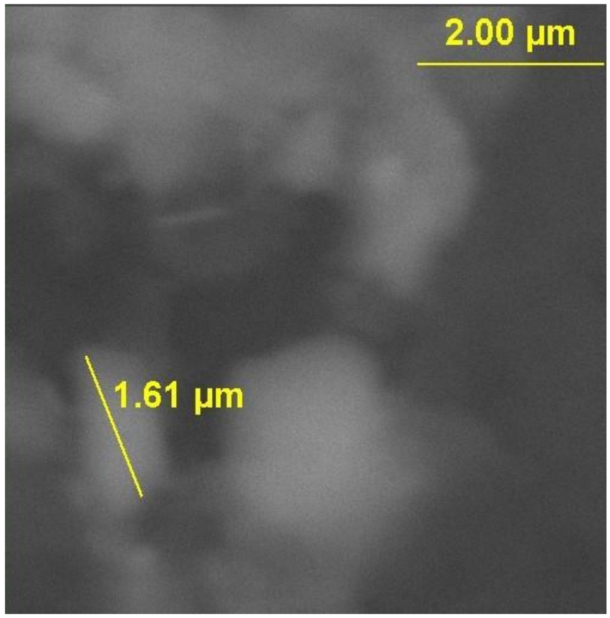


Figure 2-3 Magnification Image

Example of particle size measurement in the SEM.

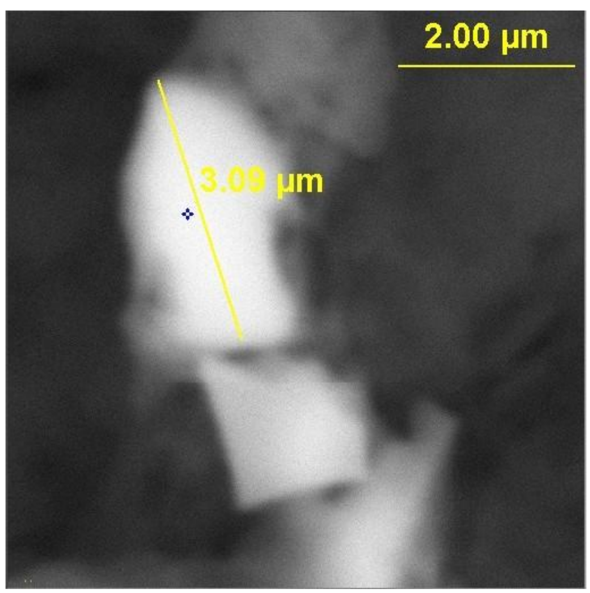


Figure 2-4 Magnification Image

Example of particle size measurement in the SEM.

Chapter 3

Results:

The analyses of the samples show a large concentration of mixed silicates, aluminum silicates, silica, and various metals. However, zinc was not prevalent in any sample analyzed. Analysis shows that mixed silicates are the most prevalent particle accounting for 51% of the total exogenous particle content in the study of samples 1A-7A. Aluminum silicates accounted for 22% of the total exogenous particle concentration in all samples. The exogenous particle content of silica accounted for 21% of the particle concentration in all samples. The metal exogenous content accounted for only 5% of the total. The test target zinc only accounted for 1% of the total exogenous content in all samples shown below in Tables 3-1-12 and Figures 3-1-3-2.

Table 3-1 Total Exogenous Particles, Silica, AlSi, M-Silicate, and Metals

Sample#	Si	AlSi	Mixed Si	Metals	Zinc	Total
1A	0	15	76	0	0	
2A	1	8	16	6	2	
3A	3	37	58	2	0	
4A	27	11	52	8	1	
6A	0	40	57	5	0	
7A	79	5	12	7	1	
ToT Exo Part content	110	116	271	28	4	
ToT % Exo Part content	21%	22%	51%	5%	1%	529

Shows the Total Exogenous Particles content

in all samples analyzed via SEM-EDX.

The analysis of sample 1B-7B once again shows the mixed silicate exogenous concentration to be the most prevalent in canine tissue. The concentration of mixed silicates accounted for 40% of the exogenous content in all samples. It was found that silica content accounted for 26% of the total exogenous particle content. In samples 1A-7A silica only account for 21% of the total particle content. Aluminum silicate showed an increase in particle concentration at 24%. In sample A, aluminum silicate concentration account for 22% of the total exogenous particle content. The exogenous metal concentration accounted for 10% of the total particle content vs. the 5% concentration in sample A. The comparison of sample A and B showed that sample B contained a higher percentage of silica (26% vs. 21%) exogenous particle content. The concentration of metals in sample B showed a 5% difference in exogenous content than sample A (10% vs. 5%). However, sample A shows a larger concentration of mixed silicates versus the B samples. Zinc concentration was not prevalent in sample B and only accounted for 1% of the total exogenous particle content in sample B.

Table 3-2 Total Exogenous Particles, Silica, AlSi, M-Silicate, and Metals

Sample#	Si	AlSi	Mixed Si	Metals	Zinc	Total
1B	1	2	32	1	0	
2B	0	9	66	9	0	
3B	0	8	1	5	0	
4B	55	9	17	17	0	
6B	0	61	33	5	0	
7B	54	12	22	4	0	
ToT Exo Part content	110	101	171	41	0	423
ToT % Exo Part content	26%	24%	40%	10%	0%	100%

Shows the Total Exogenous Particles content

in all samples analyzed via SEM-EDX.

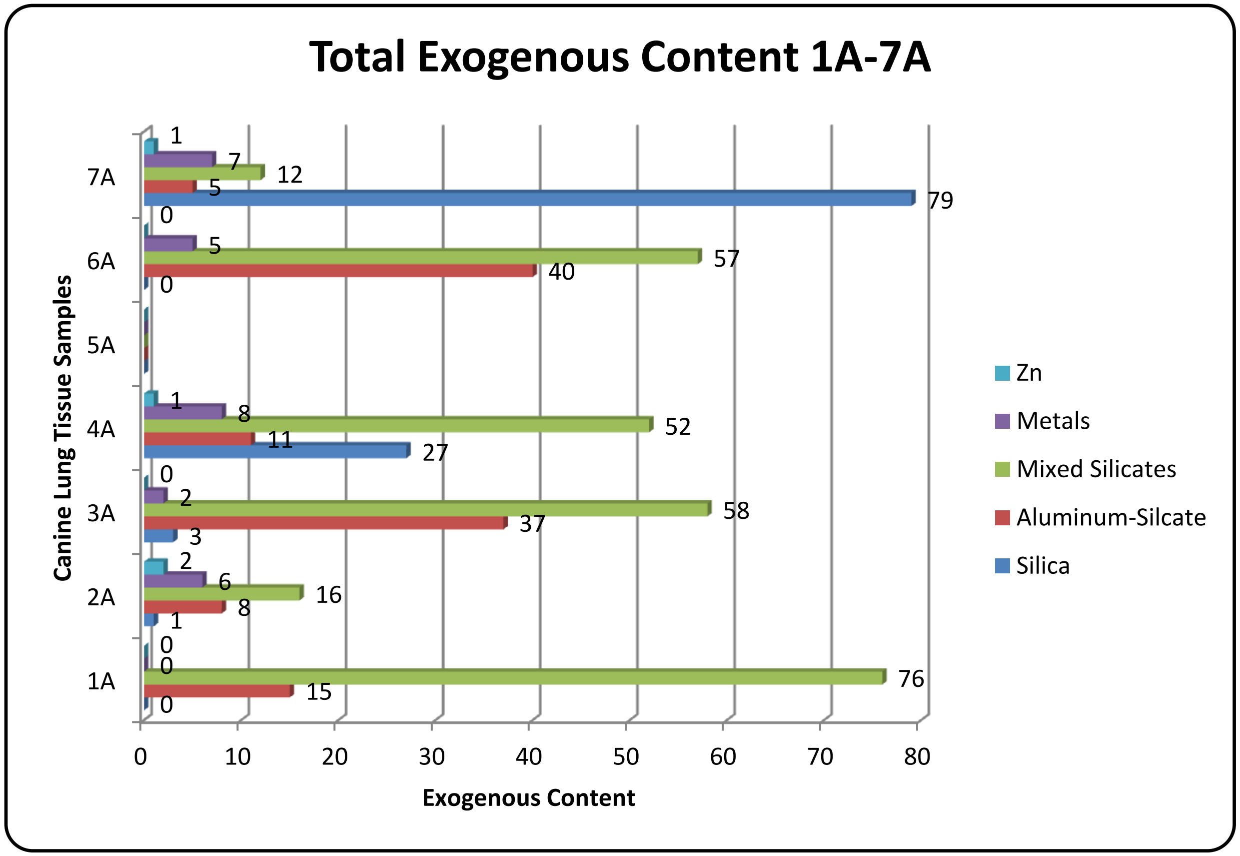


Figure 3-1 Shows a Bar chart representation of Total Exogenous Content 1A-7A

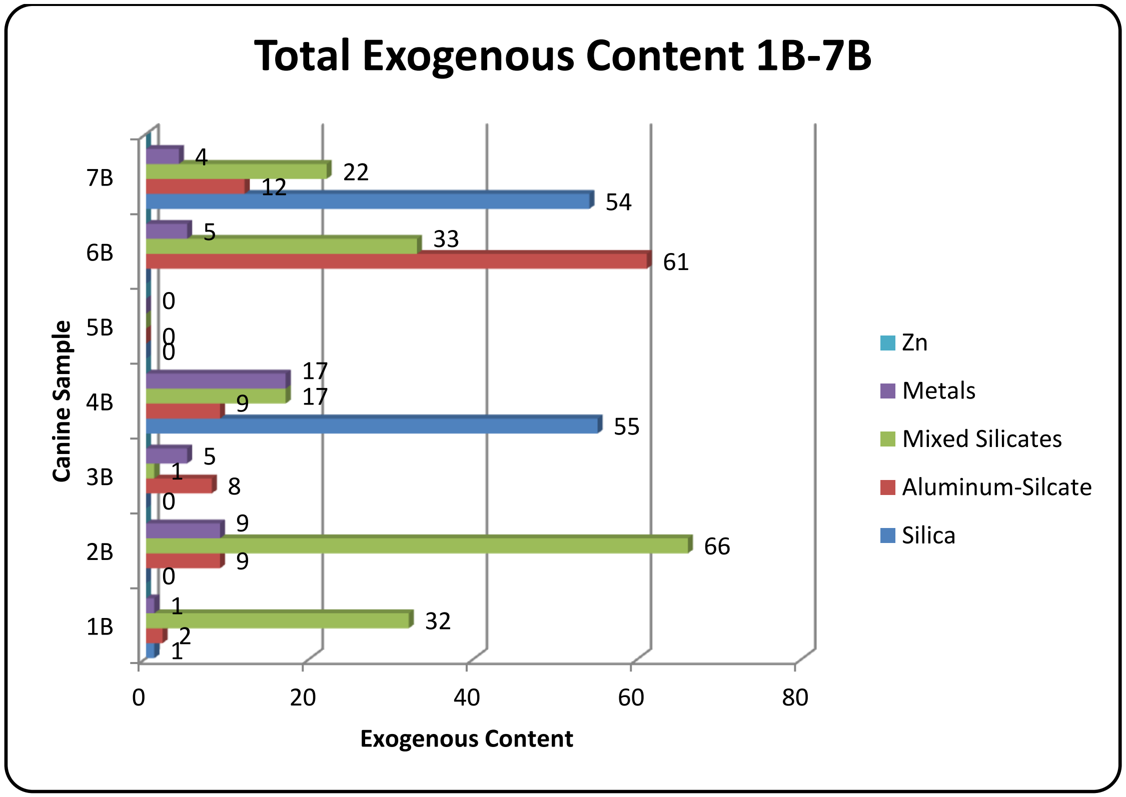


Figure 3-2 Shows a Bar chart representation of Total Exogenous Content 1B-7B

Table 3-3 Total Exogenous Content and Aluminum Silicate Particle Concentrations for Sample 1A-7A

Total Exogenous Particles		Aluminum Silicate Particles	
Sample#	In Tissue	In Tissue	% Concentration AlSi
1A	91	15	3%
2A	33	8	2%
3A	100	37	7%
4A	99	11	2%
5A	0	0	0%
6A	102	40	8%
7A	104	5	5%

Shows the total Aluminum silicate exogenous particle content for samples 1A-7A

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-4 Total Exogenous Content and Silicate Particle Concentrations for Sample 1A-7A

Total Exogenous Particles		Silicate Particles	
Sample#	In Tissue	In Tissue	% Concentration Si
1A	91	0	0%
2A	33	1	0.2%
3A	100	3	1%
4A	99	27	5%
5A	0	0	0%
6A	102	0	0%
7A	104	79	15%

Shows the total silicate exogenous particle content for samples 1A-7A

Percentages were calculated using the sum of all particle content then

dividing the specific value for each sample by the total value.

Table 3-5 Total Exogenous Content and Mixed Silicate Percent Concen 1A-7A

Total Exogenous Particles		Mixed Silicate Particles	
Sample#	In Tissue	In Tissue	% Concentration Mixed Silicate
1A	91	76	14%
2A	33	16	3%
3A	100	58	11%
4A	99	52	10%
5A	0	0	0%
6A	102	57	11%
7A	104	12	2%

Shows the total mixed silicate exogenous particle content for samples 1A-7A

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-6 Total Exogenous Content and Metal Particle Concentrations for Sample 1A-7A

Total Exogenous Particles		Metal Particles	
Sample#	In Tissue	In Tissue	% Concentration Metal
1A	91	0	0%
2A	33	6	1%
3A	100	2	0%
4A	99	8	2%
5A	0	0	0%
6A	102	5	1%
7A	104	7	1%

Shows the total Metal exogenous particle content for samples 1A-7A

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-7 Total Exogenous Content and Zinc Particle Concentrations for Sample 1A-7A

Total Exogenous Particles		Zinc Particles	
Sample#	In Tissue	In Tissue	% Concentration Zinc
1A	91	0	0%
2A	33	2	0.4%
3A	100	0	0%
4A	99	1	0.2%
5A	0	0	0%
6A	102	0	0%
7A	104	1	0.2%

Shows the total zinc exogenous particle content for samples 1A-7A

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-8 Total Exogenous Content and Aluminum Silicate Particle Concentrations for Sample 1B-7B

Total Exogenous Particles		Aluminum Silicate Particles	
Sample#	In Tissue	In Tissue	% Concentration AlSi
1B	36	2	0%
2B	84	9	2%
3B	14	8	2%
4B	98	9	2%
5B	0	0	0%
6B	99	61	14%
7B	92	12	3%

Shows the total Aluminum silicate exogenous particle content for samples 1B-7B

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-9 Total Exogenous Content and Silicate Particle Concentrations for Sample 1B-7B

Total Exogenous Particles		Silicate Particles	
Sample#	In Tissue	In Tissue	% Concentration Si
1B	36	1	0%
2B	84	0	0%
3B	14	0	0%
4B	98	55	13%
5B	0	0	0%
6B	99	0	0%
7B	92	54	13%

Shows the total silicate exogenous particle content for samples 1B-7B

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-10 Total Exogenous Content and Mixed Silicate Particle Concen 1B-7B

Total Exogenous Particles		Mixed Silicate Particles	
Sample#	In Tissue	In Tissue	% Concen. Mixed Si
1B	36	32	8%
2B	84	66	16%
3B	14	1	0%
4B	98	17	4%
5B	0	0	0%
6B	99	33	8%
7B	92	22	5%

Shows the total mixed silicate exogenous particle content for samples 1B-7B

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-11 Total Exogenous Content and Metal Particle Concentration for Sample 1B-7B

Total Exogenous Particles		Metal Particles	
Sample#	In Tissue	In Tissue	% Concentration Metal
1B	36	1	0%
2B	84	9	2%
3B	14	5	1%
4B	98	17	4%
5B	0	0	0%
6B	99	5	1%
7B	92	4	1%

Shows the total exogenous metal particle content for samples 1B-7B

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value.

Table 3-12 Total Exogenous Content and Zinc Particle Concentration for Sample 1B-7B

Total Exogenous Particles		Zinc Particles	
Sample#	In Tissue	In Tissue	% Concentration Zinc
1B	36	0	0%
2B	84	0	0%
3B	14	0	0%
4B	98	0	0%
5B	0	0	0%
6B	99	0	0%
7B	92	0	0%

Shows the total zinc exogenous particle content for samples 1B-7B

Percentages were calculated using the sum of all particle content then dividing the specific value for each sample by the total value

Chapter 4

Discussion and Conclusion:

Discussion:

Here we investigate the exogenous particle content of canine lymph node (LN) tissue from sacrifice dogs that had lived in two types of environmental setting. The intention was to investigate the PM content of tissue from dogs that had resided in urban versus rural locales. We hypothesized that if zinc were persistent in lymph node tissue (to where it had been transported through the lymphatic system following inhalation) then respirable particulate zinc may induce important adverse health effects. Given the prevalence of Zn in urban dusts it was anticipated that Zn would be found in higher concentrations in the tissue samples from dogs that resided in more urban locations.

However, it was found that in both A&B samples analyzed that zinc was not present as an maker in any tissue sample. This finding can be viewed in numerous ways. Potentially, the canines analyzed might not have been exposed to areas that were heavily contaminated with road dust. If the dogs did not live near roads that were trafficked heavily in urban cities, then the results will likely show little to no zinc. The rural samples are assumed to have low concentrations of zinc particles because rural areas have less travelled roadways in comparison to urban roadways.

Alternatively, these canines might have been exposed to appreciable quantities of zinc particles but these particles might have been bio-soluble and thus will not be present in the lymph node. It may also be the case that even though zinc is used in vulcanization processes which strengthen rubber, tire dust zinc is not released to the environment in a respirable form (the zinc is in particles too large to be retained in the lung). It is likely that the contamination by zinc is due to large quantities of tire wear, and it should not be discounted that studies (e.g., Hunt et al. 2002) have shown that inhaled zinc lung tends not to be bio-persistent. Irrespecitve of the results obtained for zinc, the analysis of the tissue did suggest an abundance of crustal elements like silica (Si) and aluminum (Al). The samples contained a large quantity of mixed silicates, aluminum-silicates and silica. Metals only constituents a small portion of the elements found. Examples of each broad group are listed below in figures 4-1-4-4.

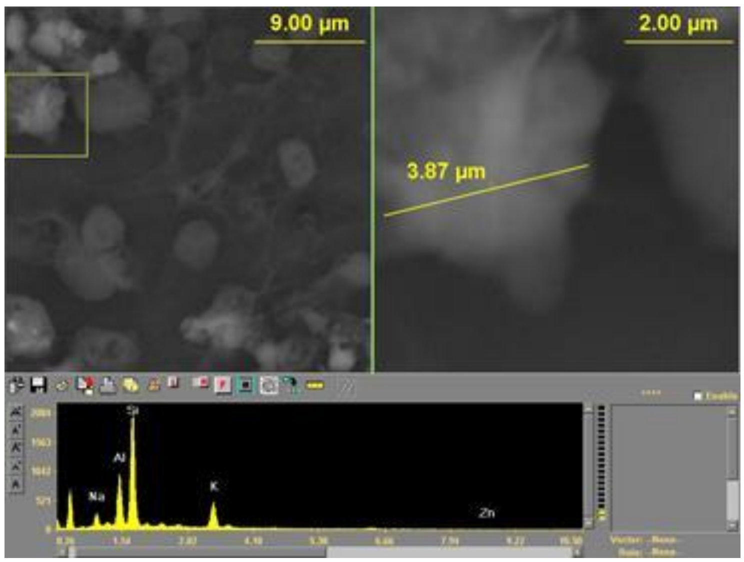


Figure 4-1 Tissue Image

Shows an example of a mixed silicate particle in the tissue of a canine specimen. The figure also shows the relative size of the particle in the (far right corner), the composition of the particle (bottom-middle) and the field of view (far left). The image was taken at a magnification of 6000x.

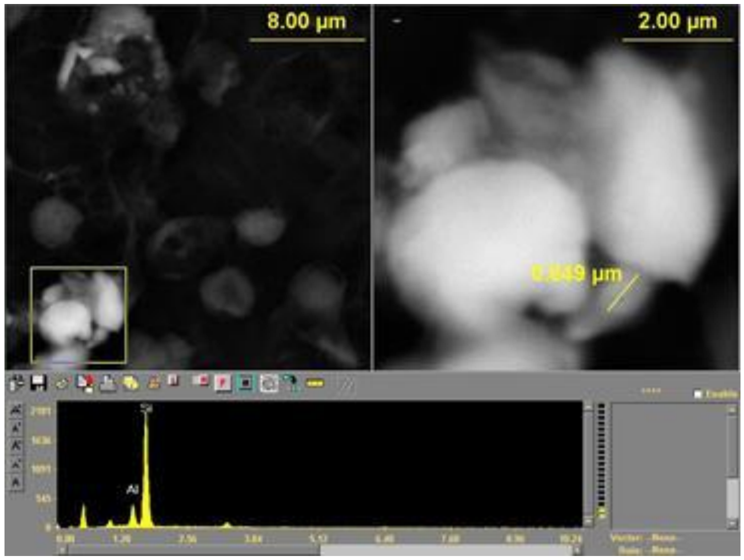


Figure 4-2 Tissue Image

Shows an example of an aluminum-silicate particle in the tissue of a canine specimen.

The figure also shows the relative size of the particle in the (right corner),

the composition of the particle (bottom-middle) and the field of view (far left).

The image was taken at a magnification of 6000x.

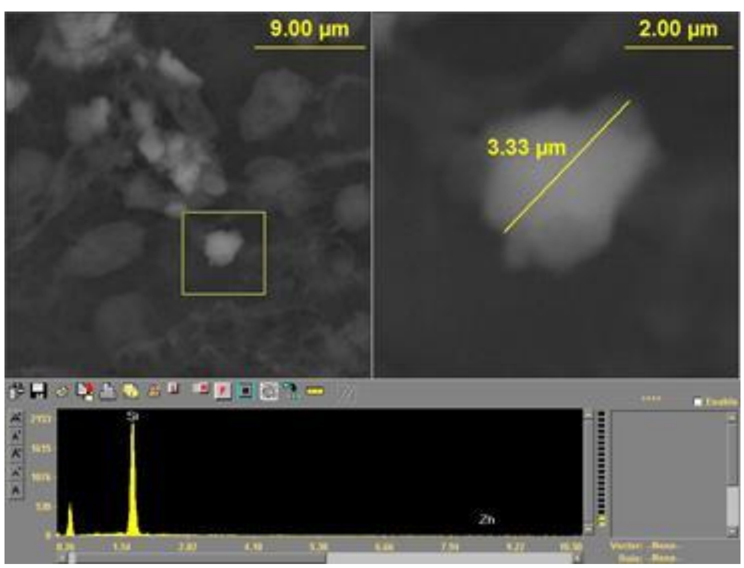


Figure 4-3 Tissue Image

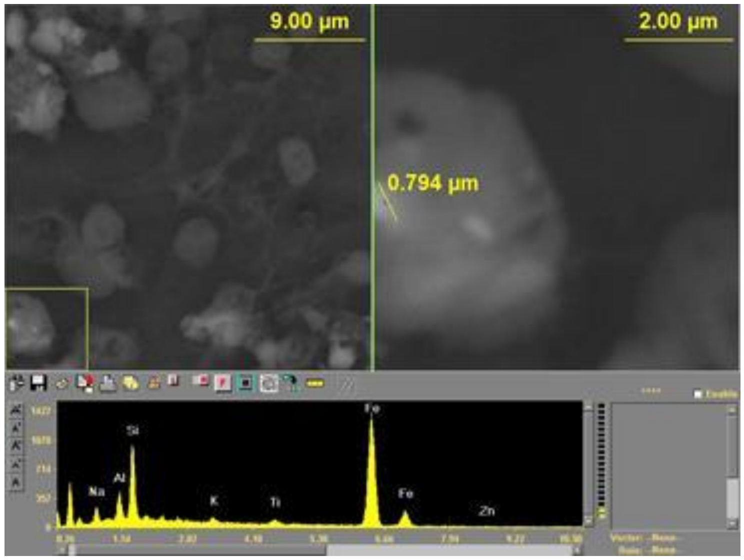


Figure 4-4 Tissue Image

Shows an example of a metal particle in the tissue of a canine specimen.

The figure also shows the relative size of the particle in the (right corner),

the composition of the particle (bottom-middle) and the field of view (far left).

The image was taken at a magnification of 6000x.

A close examination of each figure shows the composition in each case. It also shows the composition vs. the test target zinc. In fact, each figure listed does not have a trace of zinc in the composition section. Since zinc was not prevalent in the tissue, a comparison of the total exogenous particle content was investigated. This comparison in combination with the location data provided insights into the urban vs. rural comparison shown below.

Table 4-1 Exogenous Particle Content Comparison: 1A vs. 7A

	1A	2A	
Silica (Si)	0	1	Silica (Si)
Aluminum-Silicate (AlSi)	15	8	Aluminum-Silicate (AlSi)
Mixed Silicate	76	16	Mixed Silicate
Metals	0	6	Metals
Zn	0	2	Zn
Total Exogenous content	91	33	Total Exogenous content
Location MI	Tecumseh	Lansing	Location MI

Shows the total exogenous

particle content in each sample analyzed 1A compared to 2A.

The data from table 14 shows that sample 1A has significantly more exogenous material than sample 2A. This data seems to fit the location of the samples. Tecumseh MI where sample 1A was collected has a population of 8,427. Sample 2A collected from Lansing MI has a population of 113,996 and shows less exogenous content when compared with sample 1A. It follows that a larger population should have more content shown in the tissue. However, this is not the case. An explanation as to why sample 1A has more particle content than sample 2A is because of the location. Sample 1A is considered to be a rural environment with relatively easy access to dirt and crustal elements. Looking at the data collected from sample 2A, it suggests that this canine probably spent more time in concreted areas. This would result in significantly less exogenous particle content being inhaled.

Table 4-2 Exogenous Particle Content Comparison: 3A vs. 4A

	3A	4A	
Silica (Si)	3	27	Silica (Si)
Aluminum-Silicate (AlSi)	37	11	Aluminum-Silicate (AlSi)
Mixed Silicate	58	52	Mixed Silicate
Metals	2	8	Metals
Zn	0	1	Zn
Total Exogenous content	100	99	Total Exogenous content
Location MI	Lansing	Grand Blanc	Location MI

Shows the total exogenous particle

content in each sample analyzed 3A compared to 4A.

Sample 3A is another canine from the same city as sample 2A (Lansing MI). However, sample 3A as shown in Table 4-2 has significantly more exogenous particle content. The city of Lansing with a population of 113,996 fits in the urban classification. This suggests that a larger amount of exogenous material should be present in canines from urban environments. However, sample 4A has about the same amount of particles present in the analysis. Interestingly, sample 4A is considered a rural environment with the city of Gran Blanc containing a population of 8,144. The only question with this data is the amount of crustal elements found in the tissue analysis of sample 3A. An explanation for this finding could be that sample 3A had access to areas in Lansing MI that were not heavily concreted. An example would be a junk yard dog or a dog on the outskirts of the city limits. Sample 4A shows data that is characteristic of rural environments. The large concentration of crustal elements suggests that this canine had access to a dirt oriented environment.

Another interesting data point is the difference in metal concentration, sample 4A has a higher concentration than sample 3A shown above in Table 15. This is interesting because sample 4A is the characteristically rural environment. Sample 3A should have more metal concentration because it was collected from an urban environment. The population of Lansing MI is approximately 14 times the population of Grand Blanc. With this large difference in population size, one would agree that a larger concentration of metals should be present in the urban environment.

The question here is why does sample 4A have more metal concentration than sample 3A? An explanation for this could be that the canine representing sample 4A is a guard dog for a scrap metal yard. This type of environment would fit the elevated metal exposure. However, there are numerous answers to this question.

Table 4-3 Exogenous Particle Content Comparison: 6A vs. 7A

	6A	7A	
Silica (Si)	0	79	Silica (Si)
Aluminum-Silicate (AlSi)	40	5	Aluminum-Silicate (AlSi)
Mixed Silicate	57	12	Mixed Silicate
Metals	5	7	Metals
Zn	0	1	Zn
Total Exogenous content	102	104	Total Exogenous content
Location MI	Owosso	Owosso	Location MI

Shows the total exogenous particle content in each sample analyzed 6A compared to 7A.

The comparison of samples 6A and 7A are both from the same environment in Owosso MI. Owosso MI has a population of about 14, 852 and is considered to be a rural type environment. The data collected from both samples seem to fit the characteristic rural environment, which means access to more dirt-oriented environments.

This data is not a surprise because we should see more crustal elements in rural environments that in urban environments. In Table 4-3 we see this trend, as the data shows a high concentration of crustal elements in both samples.

Table 4-4 Exogenous Particle Content Comparison: 5A vs. Sample set

	5A	1A	2A
Silica (Si)	0	0	1
Aluminum-Silicate (AlSi)	0	15	8
Mixed Silicate	0	76	16
Metals	0	0	6
Zn	0	0	2
Total Exogenous content	0	91	33
Location MI	Bay City		

Shows the total exogenous

particle content 5A vs. any other samples analyzed.

Sample 5A vs. any other sample collected showed no exogenous content. This sample was collected for Bay City MI with a population of 34,521 which would be considered more of a sub urban city. The interesting find here is that no exogenous particle content was present during the analysis of the tissue. A likely explanation for the absence of exogenous material would be that sample 5A was an inside dog with relatively minute exposures to crustal elements, which were removed by the lungs defense mechanisms. The comparison of samples 1B-7B shows an interesting twist in the trend.

Table 4-5 Exogenous Particle Content Comparison: 1B vs. 2B

	1B	2B	
Silica (Si)	1	0	Silica (Si)
Aluminum-Silicate (AlSi)	2	9	Aluminum-Silicate (AlSi)
Mixed Silicate	32	66	Mixed Silicate
Metals	1	9	Metals
Zn	0	0	Zn
Total Exogenous content	36	84	Total Exogenous content
Location MI	Tecumseh	Lansing	Location MI

Shows the total exogenous

particle content in each sample analyzed 1B compared to 2B.

The Data collected from both samples shows that sample 2B has more exogenous content than 1B. This data seems to fit the idea that urban environments should contain more exogenous (metal) content. As expected of urban environments with elevated road traffic and access to numerous metals. This data compare with Table 4-1 is consistent with the elevated metal content.

Table 4-6 Exogenous Particle Content Comparison: 3B vs. 4B

	3B	4B	
Silica (Si)	0	55	Silica (Si)
Aluminum-Silicate (AlSi)	8	9	Aluminum-Silicate (AlSi)
Mixed Silicate	1	17	Mixed Silicate
Metals	5	17	Metals
Zn	0	0	Zn
Total Exogenous content	14	98	Total Exogenous content
Location MI	Lansing	Grand Blanc	Location MI

Shows the total exogenous particle content in each

sample analyzed 3B compared to 4B.

Samples 4B collected from Grand Blanc MI has a larger concentration of exogenous particle content. Interestingly the concentrations of metal particles are significantly elevated in 4B than in 3B. 4B collected from Grand Blanc MI, which has a population of 8,144 is considered a rural environment. The question that should be asked is why does Grand Blanc with a population 14 times less than that of Lansing have significantly more exogenous content? An answer to this question could be that sample 4B is characteristic of a rural environment. This dog could have been exposed to metals in some other way.

Table 4-7 Exogenous Particle Content Comparison: 6B vs. 7B

	6B	7B	
Silica (Si)	0	54	Silica (Si)
Aluminum-Silicate (AlSi)	61	12	Aluminum-Silicate (AlSi)
Mixed Silicate	33	22	Mixed Silicate
Metals	5	4	Metals
Zn	0	0	Zn
Total Exogenous content	99	92	Total Exogenous content
Location MI	Owosso	Owosso	Location MI

Shows the total exogenous particle content in each

sample analyzed 6B compared to 7B.

The comparison of samples 6B and 7B are both from the same environment in Owosso MI. Owosso MI with a population total of about 14,852, is considered to be a rural area. The data collected from both samples seem to fit the characteristic rural environment, which means access to more dirt-oriented environments. This data is consistent with data expressed in Table 4-3 when compared. This data is not a surprise because we should see more crustal elements in rural environments than in urban environments. In Table 4-3 we see this trend, as the data shows a high concentration of crustal elements in both samples as seen in Table 4-7.

Table 4-8 Exogenous Particle Content Comparison: 5B vs. Sample set

	5B	1B	2B	
Silica (Si)	0	1	0	Silica (Si)
Aluminum-Silicate (AlSi)	0	2	9	Aluminum-Silicate (AlSi)
Mixed Silicate	0	32	66	Mixed Silicate
Metals	0	1	9	Metals
Zn	0	0	0	Zn
Total Exogenous content	0	36	84	Total Exogenous content
Location MI	Bay City			Location MI

Shows the total exogenous particle content 5B vs. any other samples analyzed

Sample 5B vs. any other sample collected showed no exogenous content.

Sample 5B is analogous to sample 5A, which was thought to be an inside dog because of the lack of exogenous content. Figure 4-5 shown below, demonstrates the relationship between all non-fibrous exogenous material which includes (Silica, Aluminum-Silicates, Mixed-Silicates, and Metals) in comparison of non-fibrous content in both urban and rural areas.

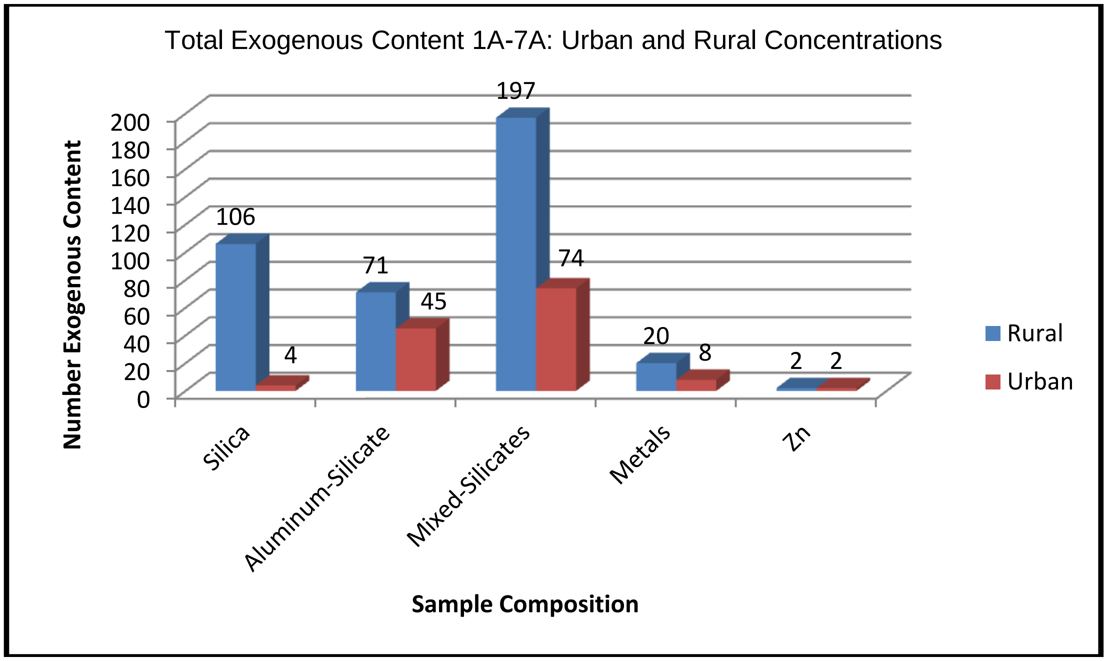


Figure 4-5 Total Exogenous Content 1A-7A: Urban and Rural Concentrations

Shows the Total exogenous relationship between the urban and rural study. Notice that the rural area contains more crustal elements than in the urban study.

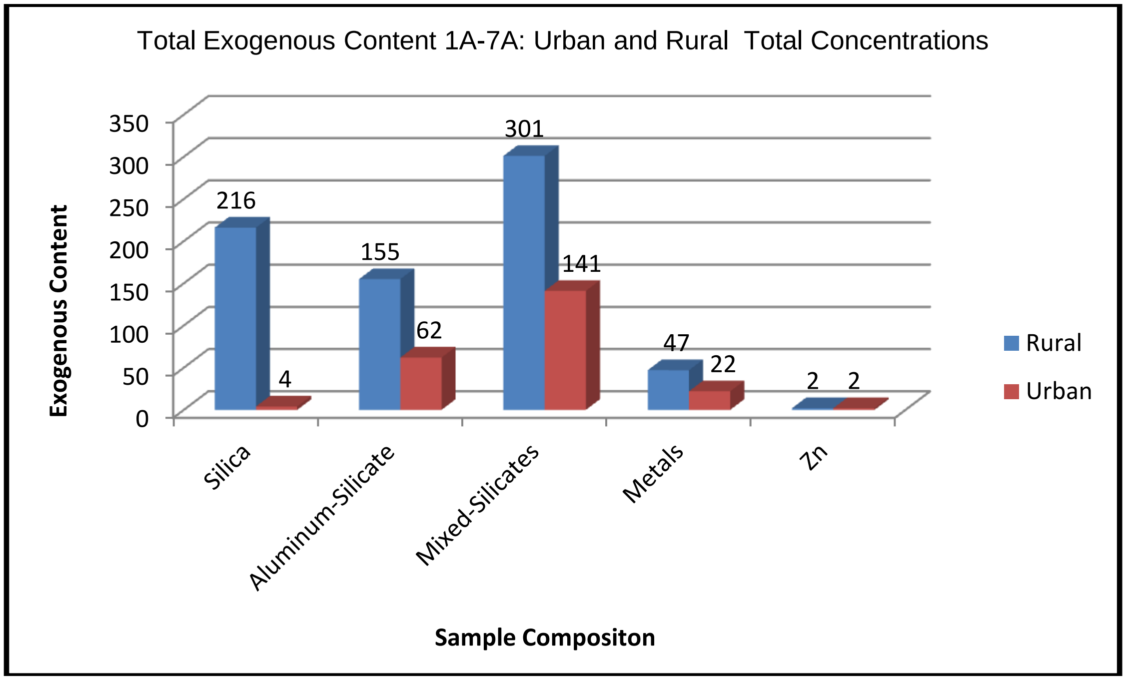


Figure 4-6 Exogenous Content 1A-7A: Urban and Rural Total Concentrations

Shows the Total exogenous relationship between the urban and rural study combining both A and B samples analyzed. Notice that the rural area contains more crustal and metal elements than in the urban study.

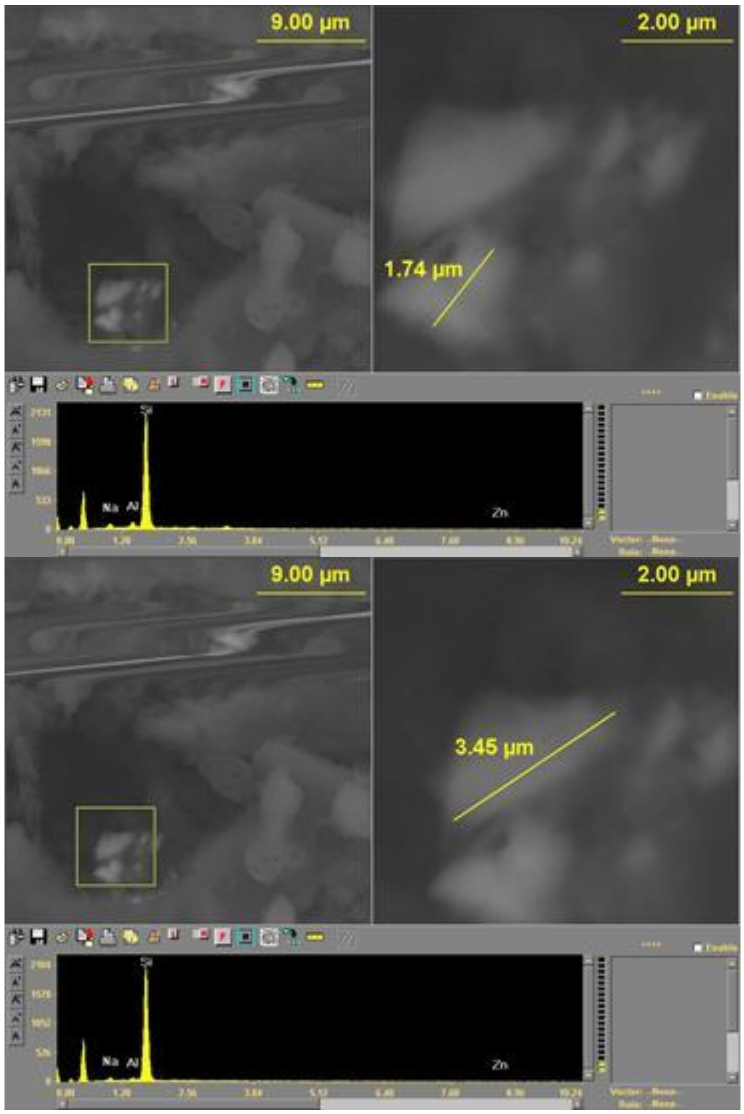
Conclusion:

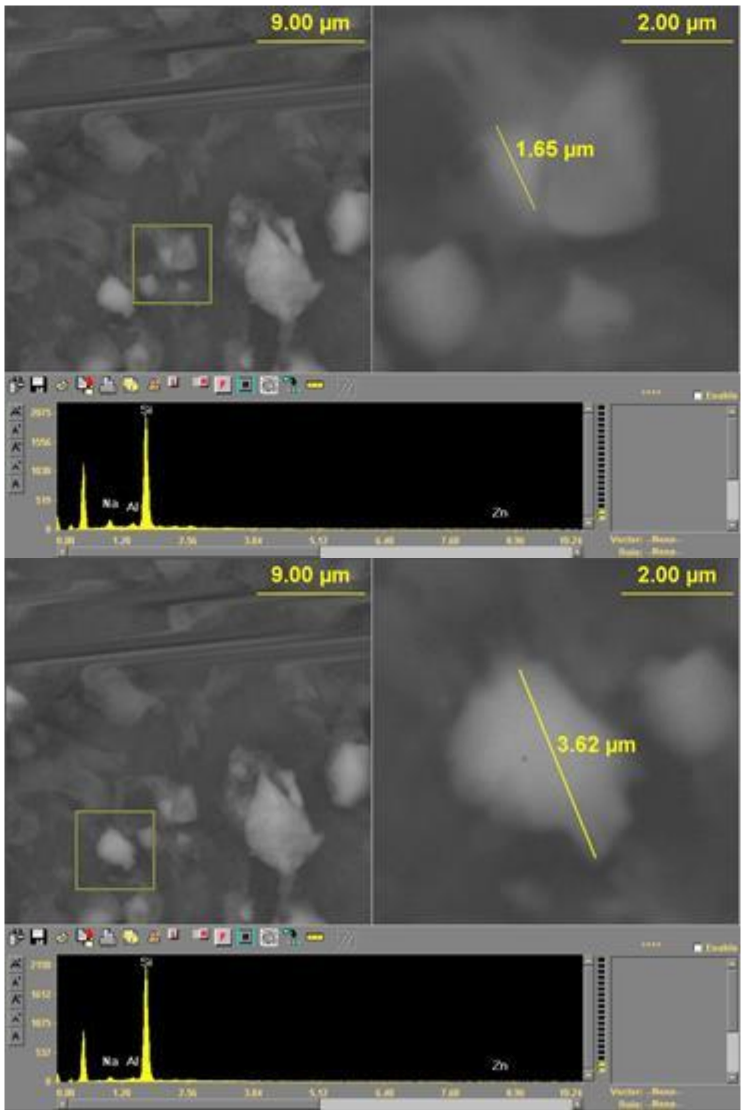
In conclusion, zinc is considered to be an emerging contaminant because of the different pathways by which anthropogenic sources release it the environment. Because zinc is used in the strengthening of rubber tires it was suggested by Councell et al. (2004), that the source of zinc contamination in the environment is from tread-wear due to surface road abrasion. The total exogenous zinc concentration found in the analysis of urban and rural canines, constituted only 1% of the total exogenous material analyzed. Since larger populations have additional vehicles travelling major and non-major highways in urban cities, the concentration of zinc would presumably, be elevated in these areas. However, the analysis in Figure 4-5 and 4-6 illustrate the prevalence of zinc in the environment that does not fit this hypothesis. An explanation for the lack of zinc concentration in tissue analysis could be that zinc is easily bio-soluble. This would be consistent with previous work by Hunt et al. (2002), who found that while particulate zinc could be identified in aggregates of PM in airways, it could not be identified in the lymph nodes of the same individual. This find would, therefore, argue against the original hypothesis proposed by Ed. Landa (see above).

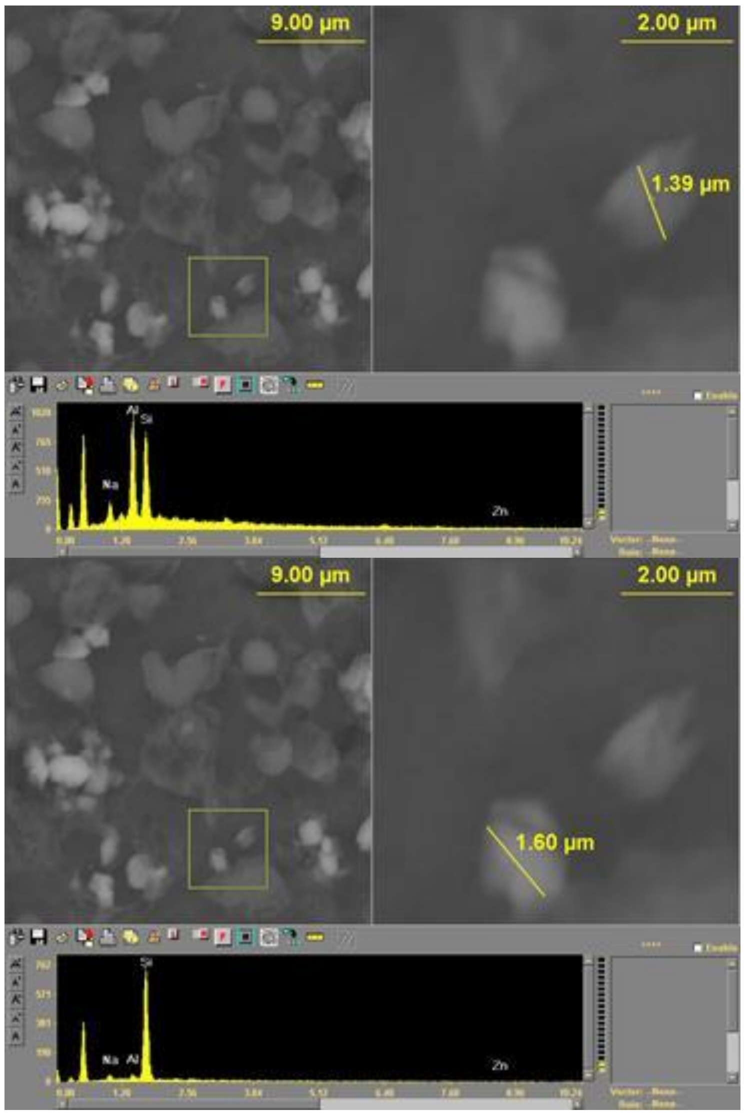
The analysis of tissues 1A-7A showed that mixed silicates were the most prevalent particle in the tissue samples, accounting for 51% of the total exogenous particle content. Aluminum silicates accounted for 22% of the total exogenous particle concentration in all samples. The exogenous particle content of silica accounted for 21% of the particle concentration in all samples and the concentration of metals accounted for only 5%. These values were consistent with values found for samples 1B-7B explained in the results section. The three most common metals analyzed were iron, chromium, and titanium.

Appendix A

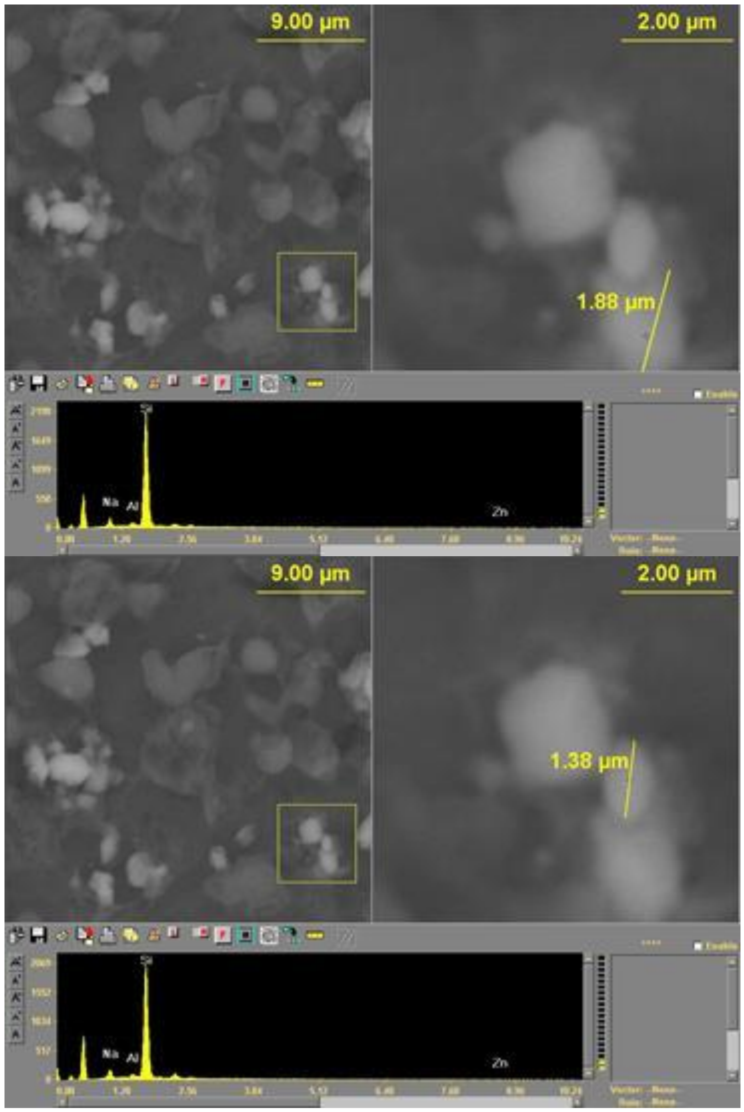
Characterized Field and Diameter Views for Samples 1A-7B

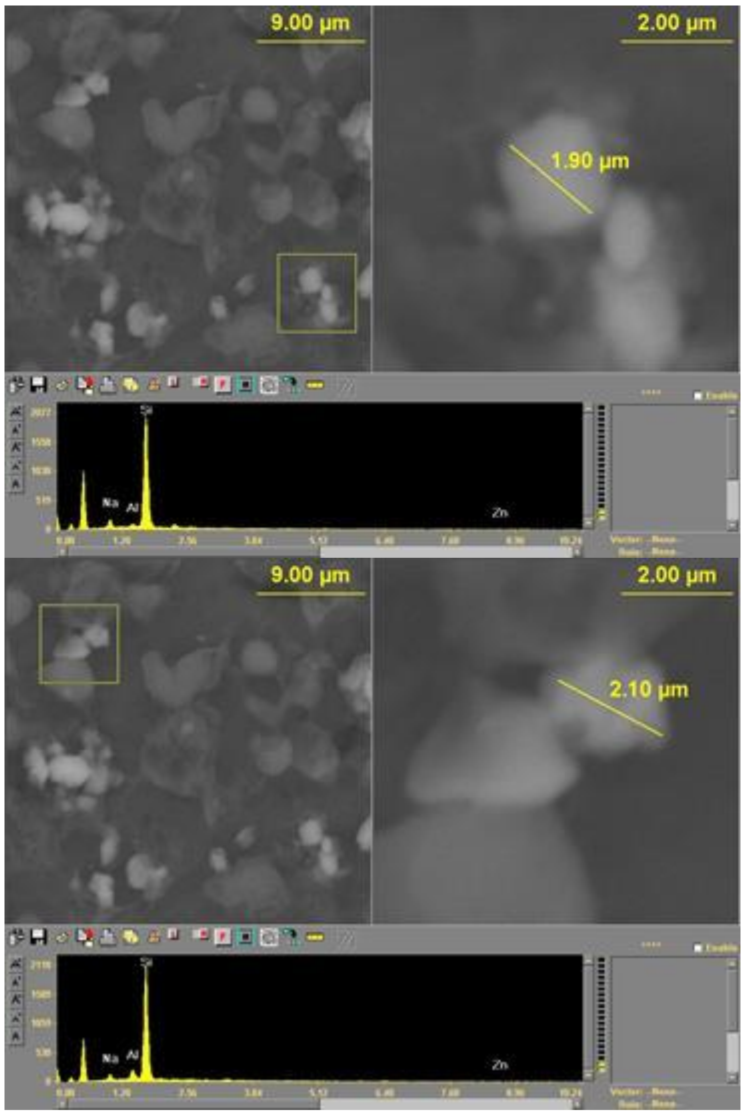

9.00 µm
2.00 µm
1.74 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
3.45 µm
Si
Na
Al
Zn

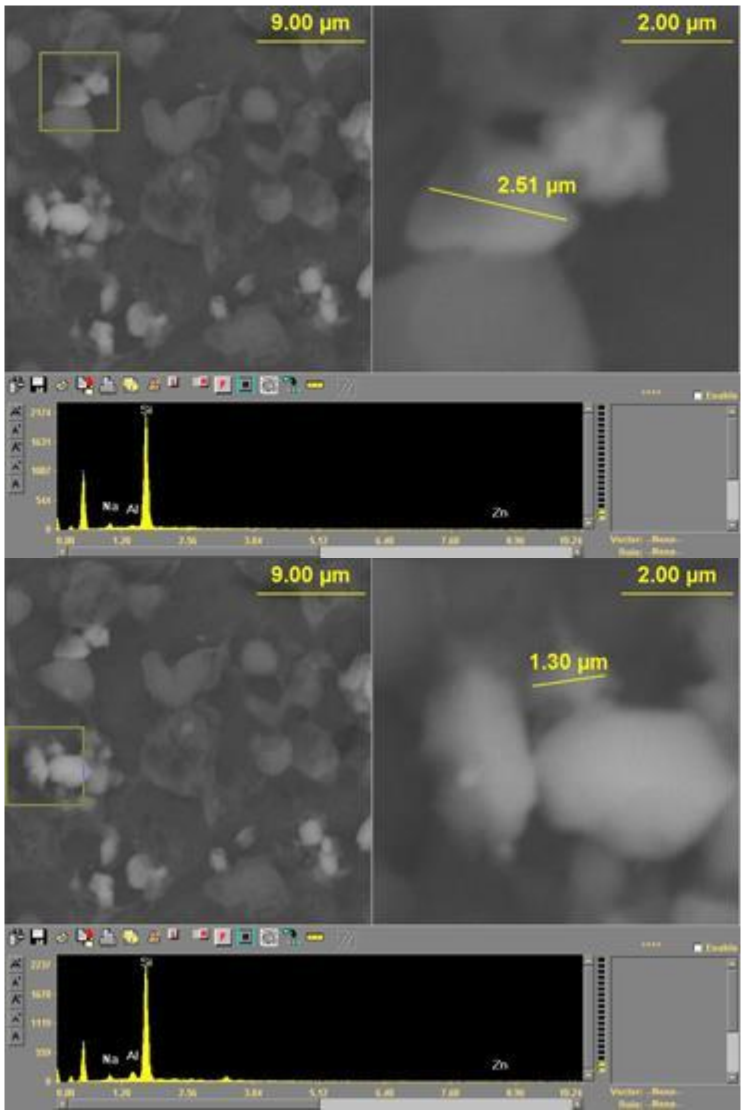

9.00 µm
2.00 µm
1.65 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
3.62 µm
Si
Na
Al
Zn

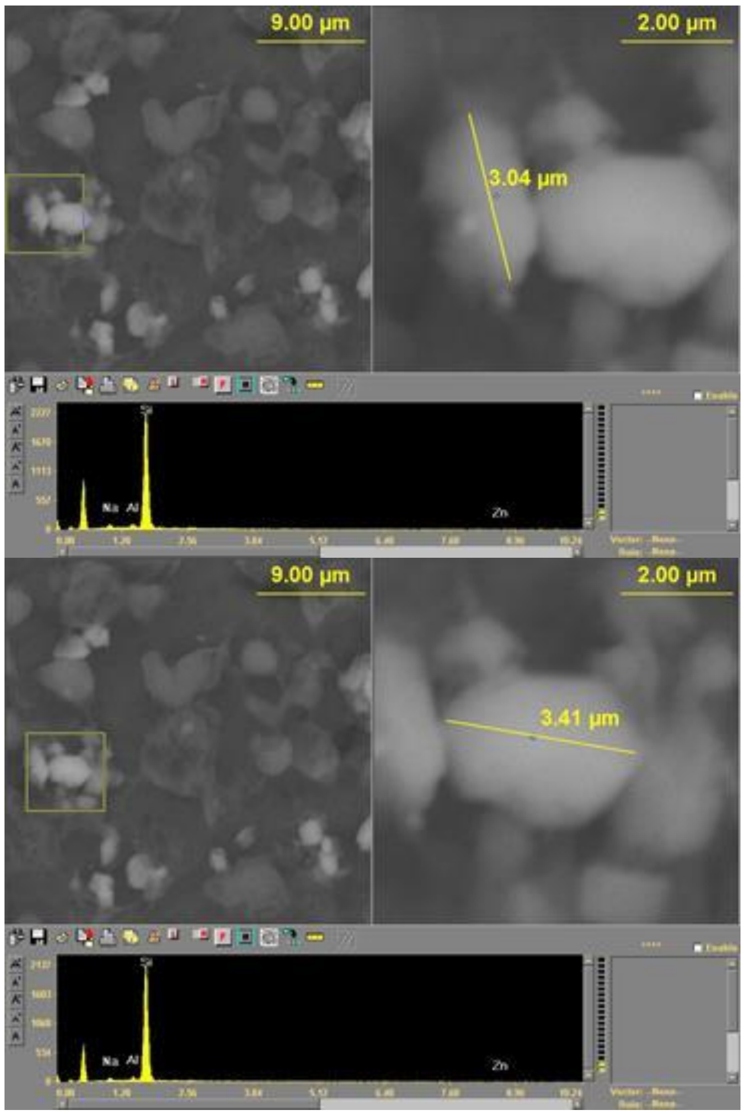


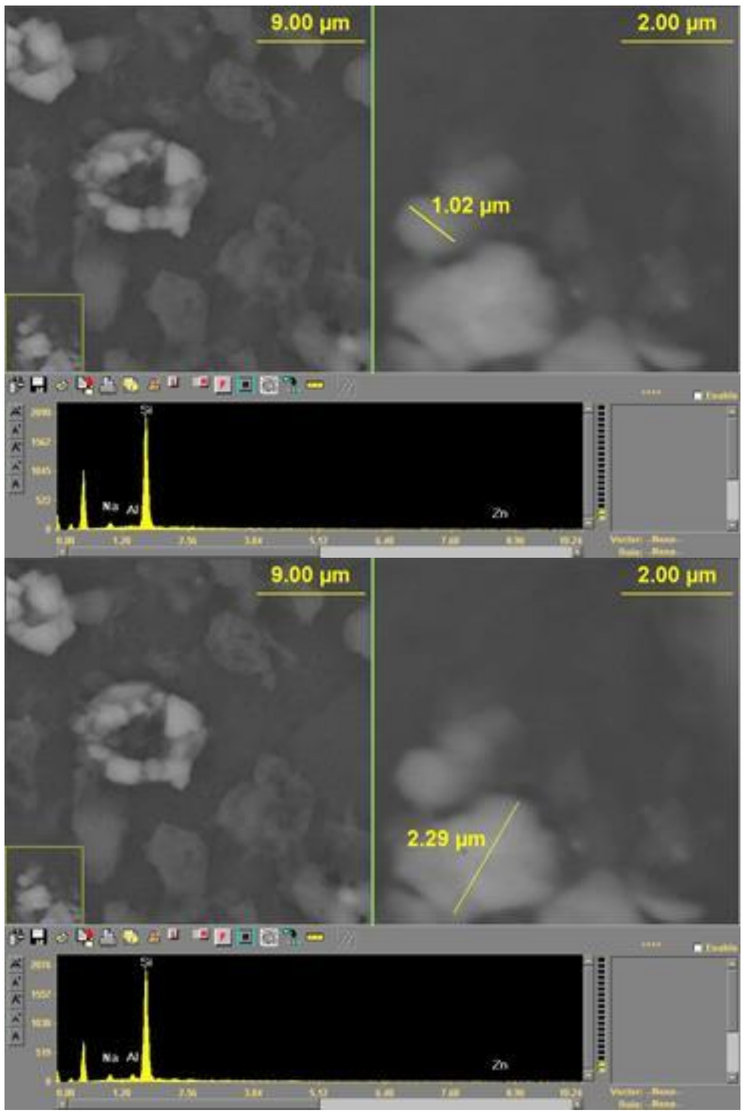
9.00 µm
2.00 µm
1.39 µm
Al
Si
Na
Zn
9.00 µm
2.00 µm
1.60 µm
Si
Na
Al
Zn

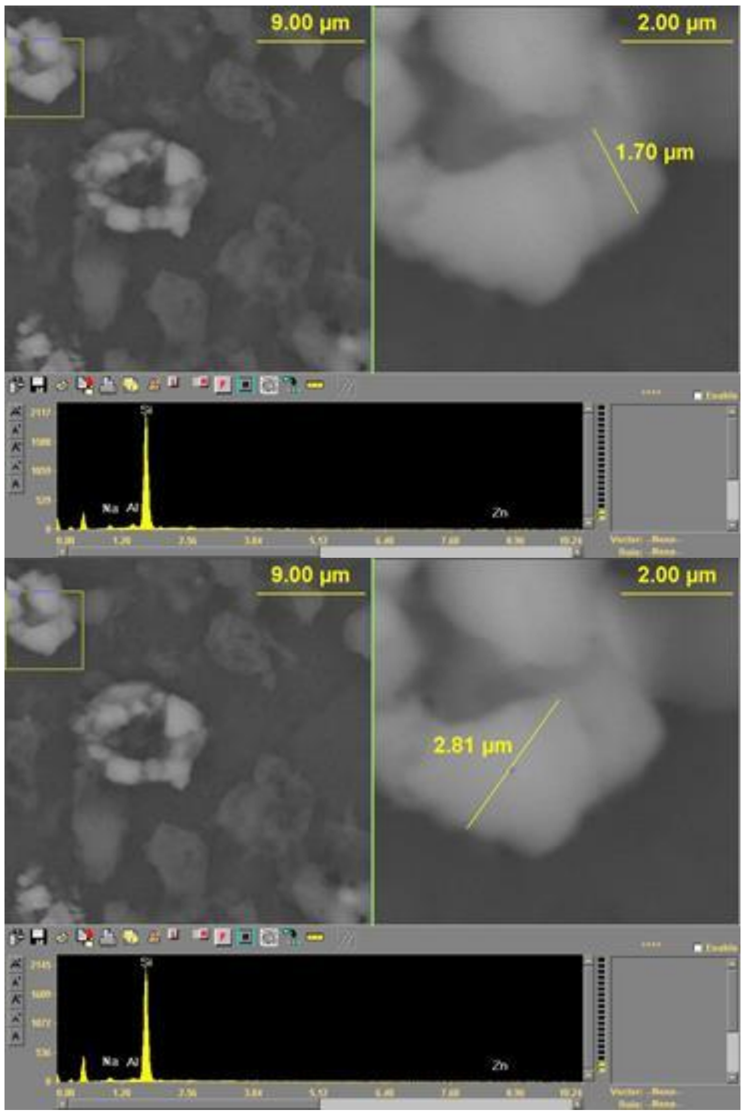

9.00 µm
2.00 µm
1.88 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
1.38 µm
Si
Na
Al
Zn

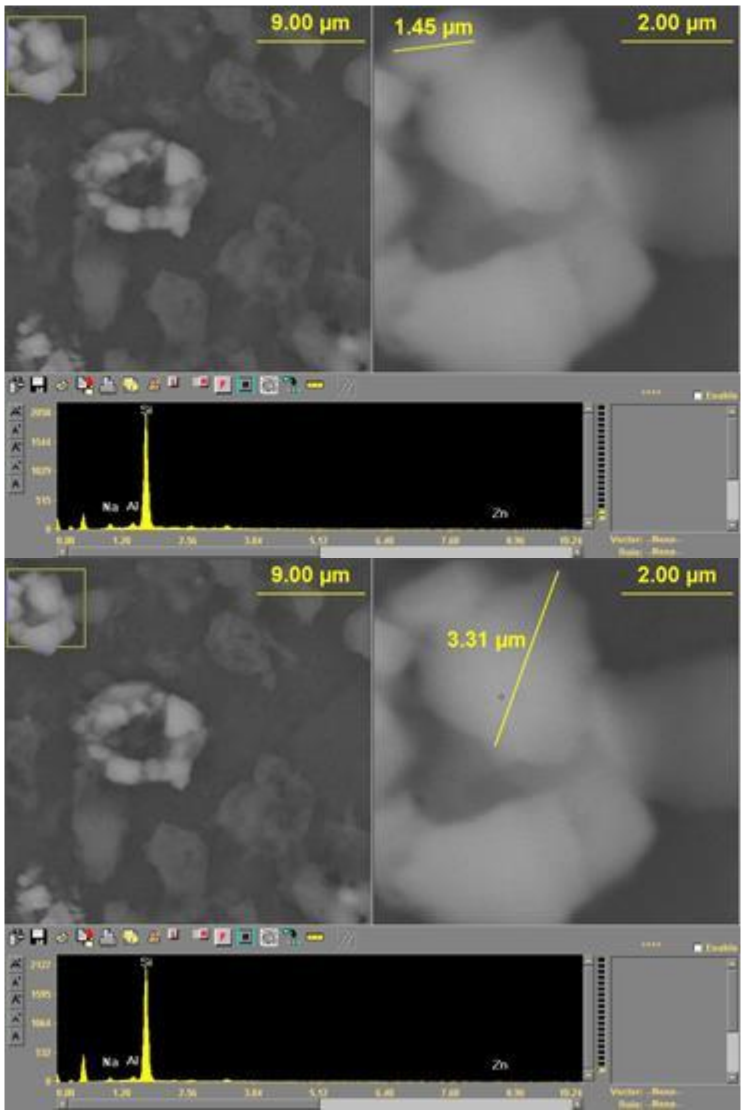

9.00 µm
2.00 µm
1.90 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
2.10 µm
Si
Na
Al
Zn

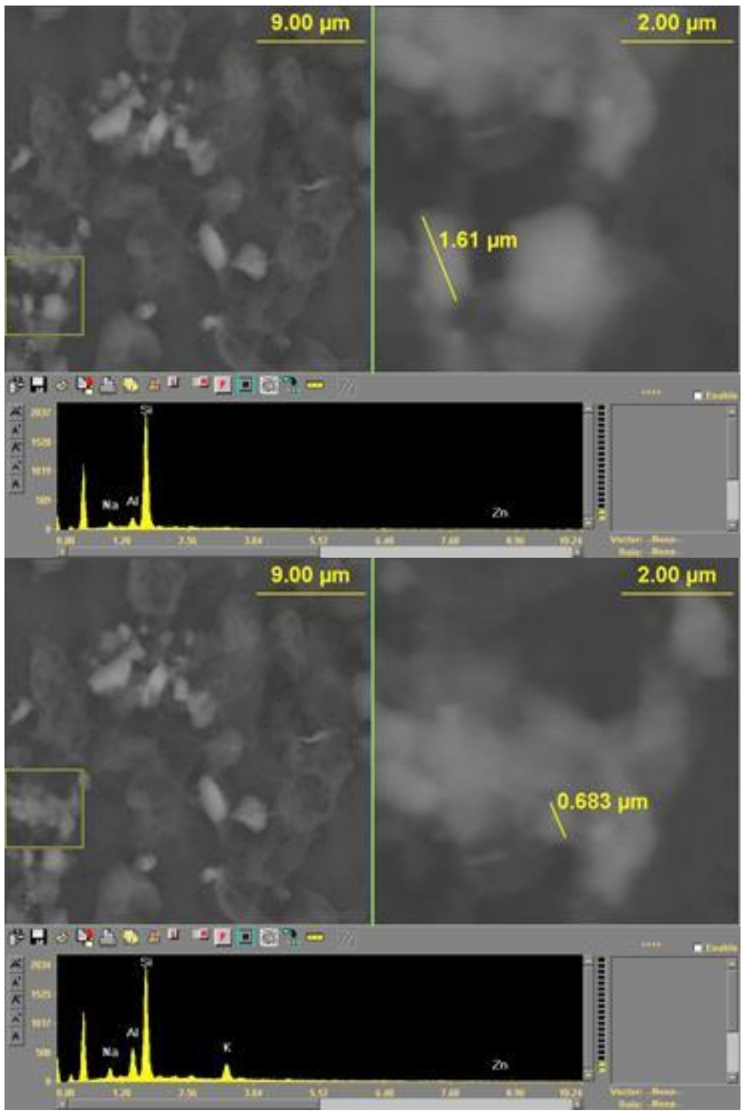

9.00 µm
2.00 µm
2.51 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
1.30 µm
Si
Na
Al
Zn

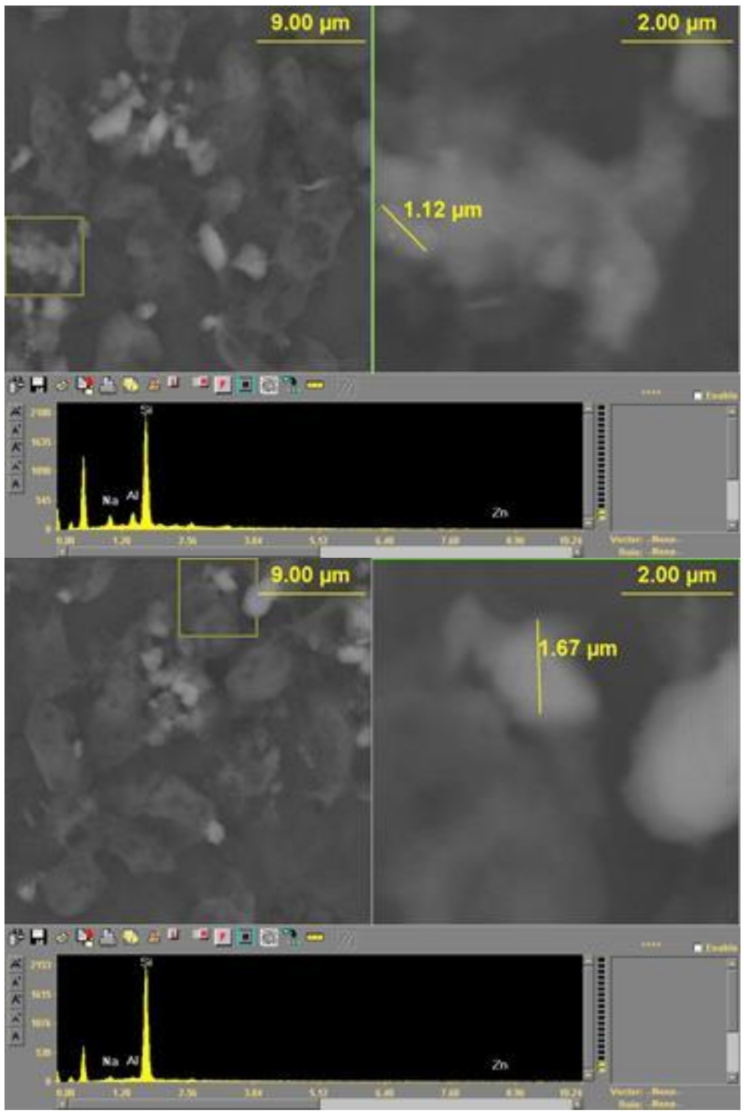

9.00 µm
2.00 µm
3.04 µm
Si
Na Al
Zn
9.00 µm
2.00 µm
3.41 µm
Si
Na Al
Zn

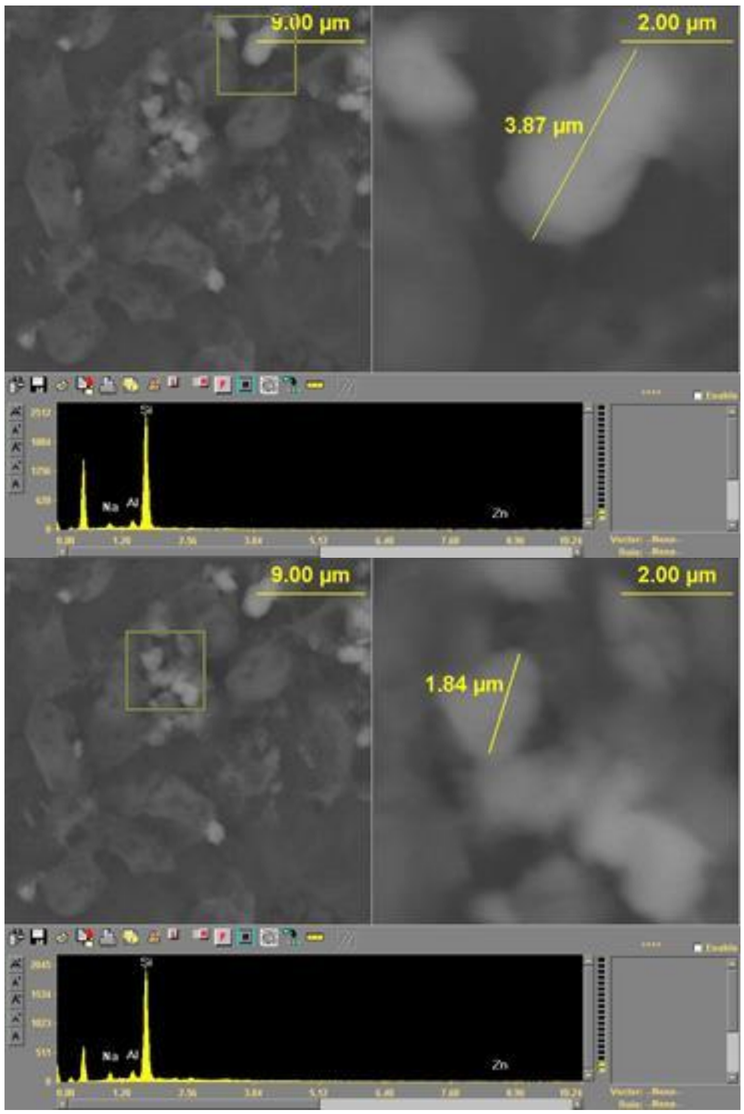

9.00 µm
2.00 µm
1.02 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
2.29 µm
Si
Na
Al
Zn

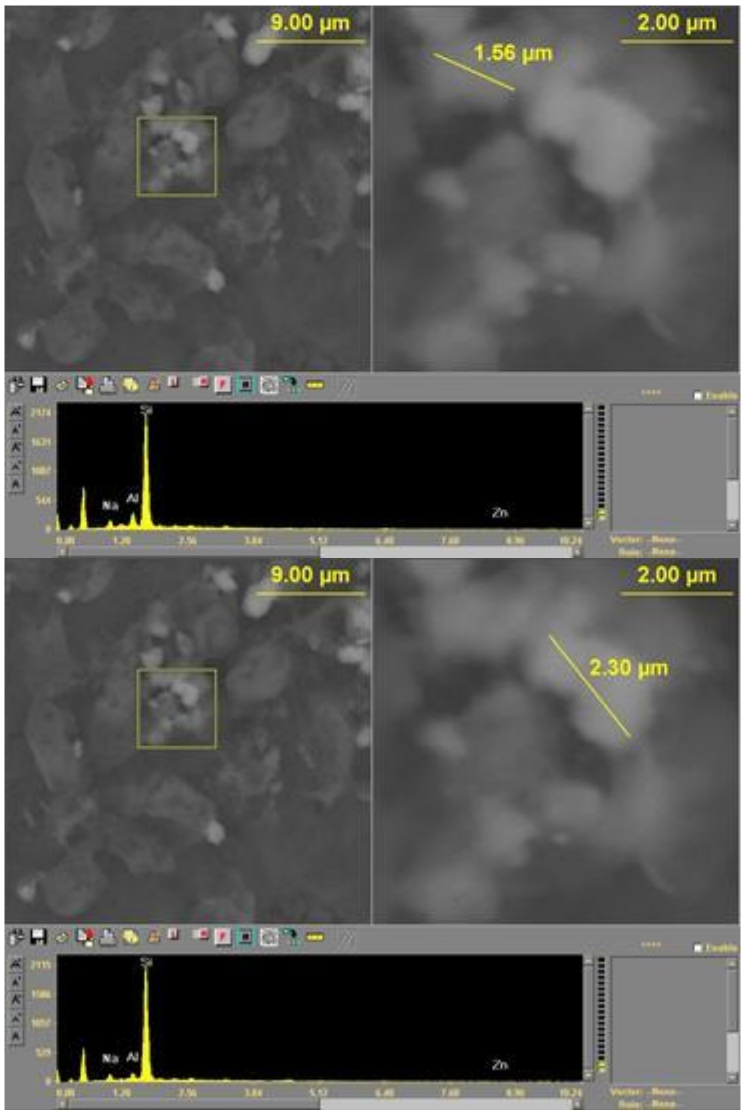

9.00 µm
2.00 µm
1.70 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
2.81 µm
Si
Na
Al
Zn

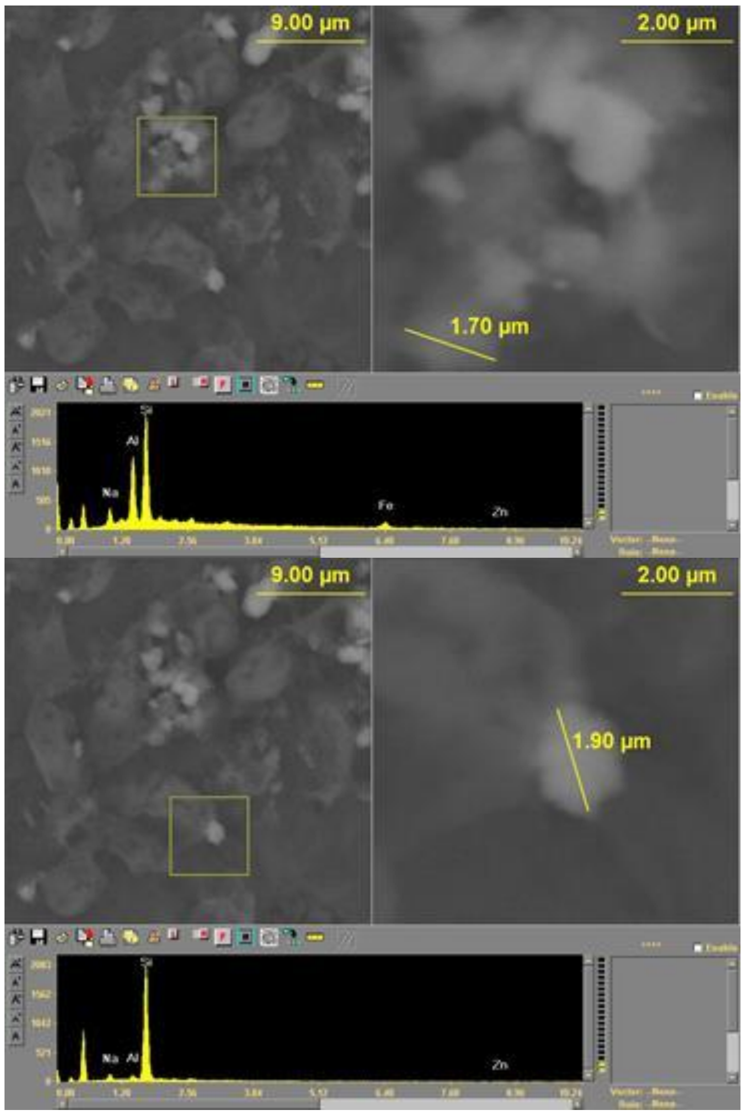

9.00 µm
1.45 µm
2.00 µm
Si
Na Al
Zn
9.00 µm
2.00 µm
3.31 µm
Si
Na Al
Zn

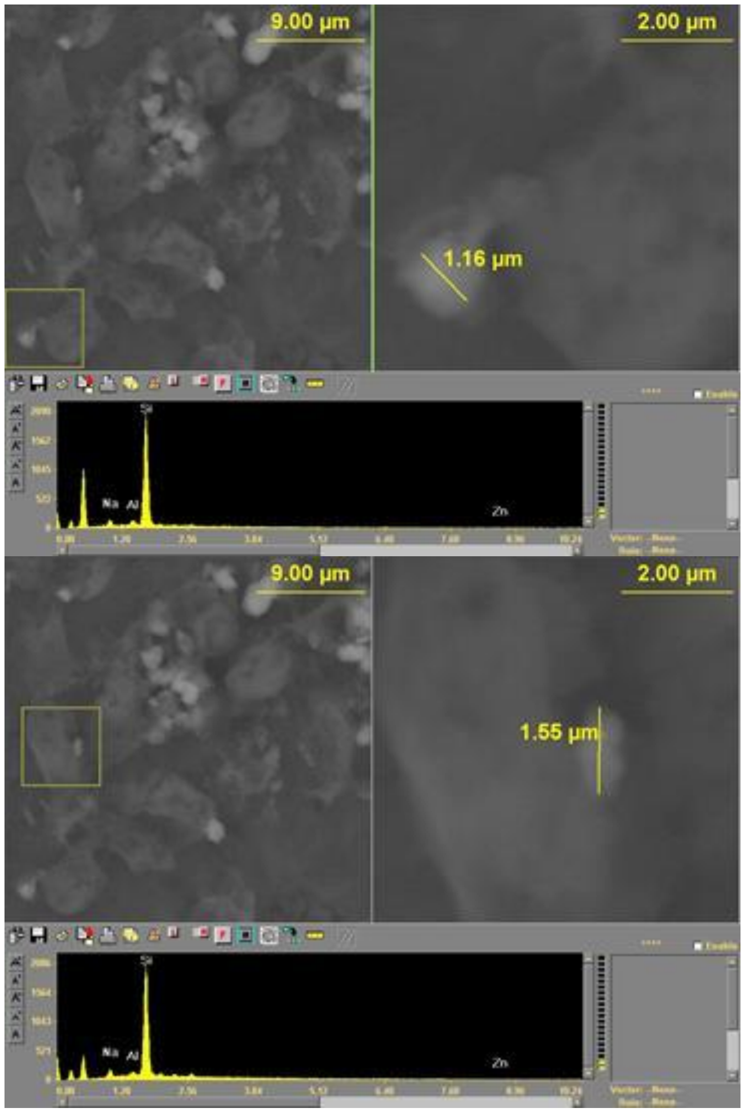

9.00 µm
2.00 µm
1.61 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
0.683 µm
Si
Al
Na
K
Zn

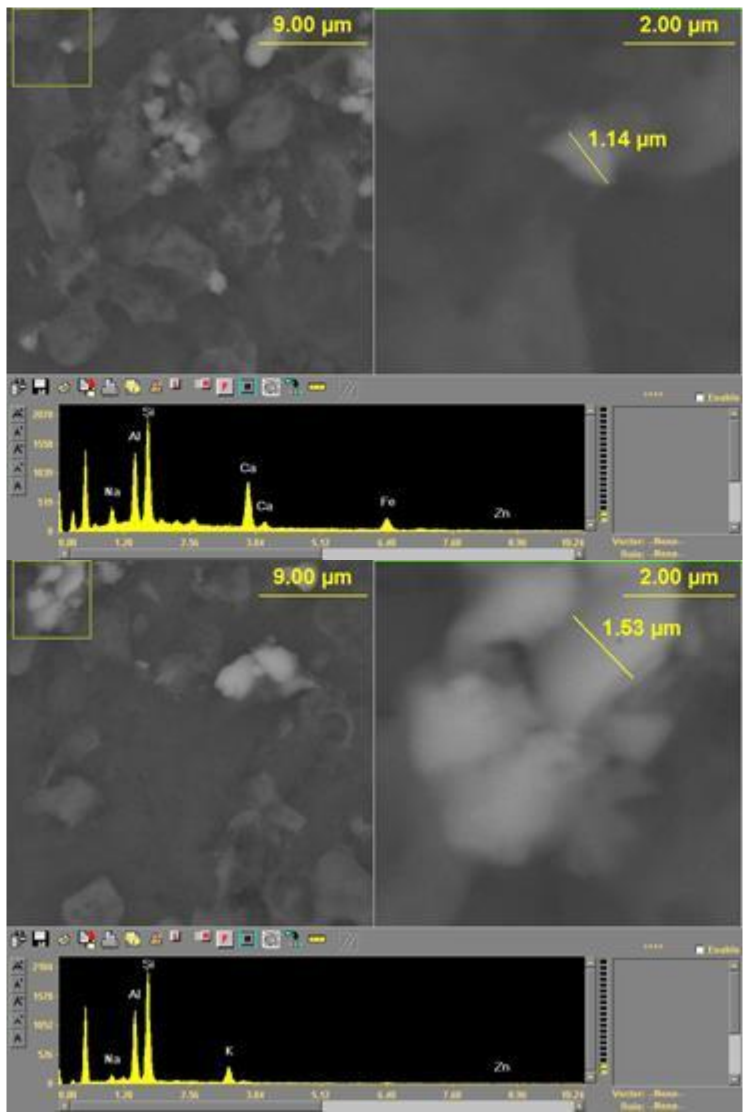

9.00 µm
2.00 µm
1.12 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
1.67 µm
Si
Na
Al
Zn

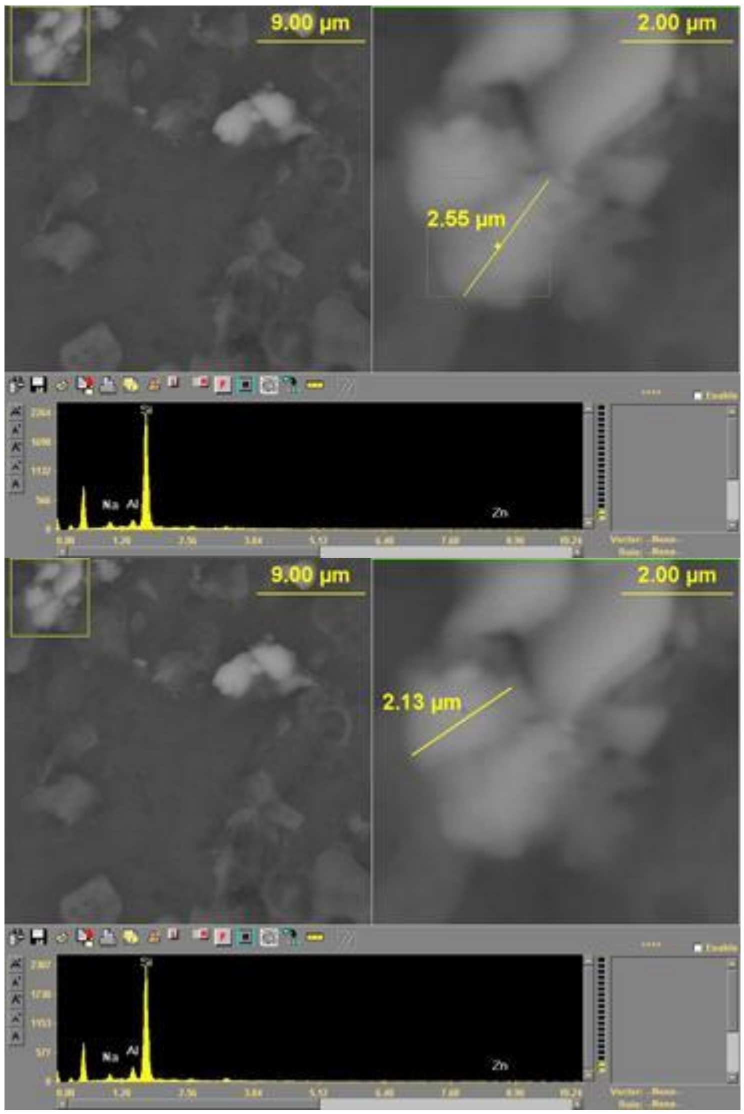

9.00 µm
2.00 µm
3.87 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
1.84 µm
Si
Na
Al
Zn

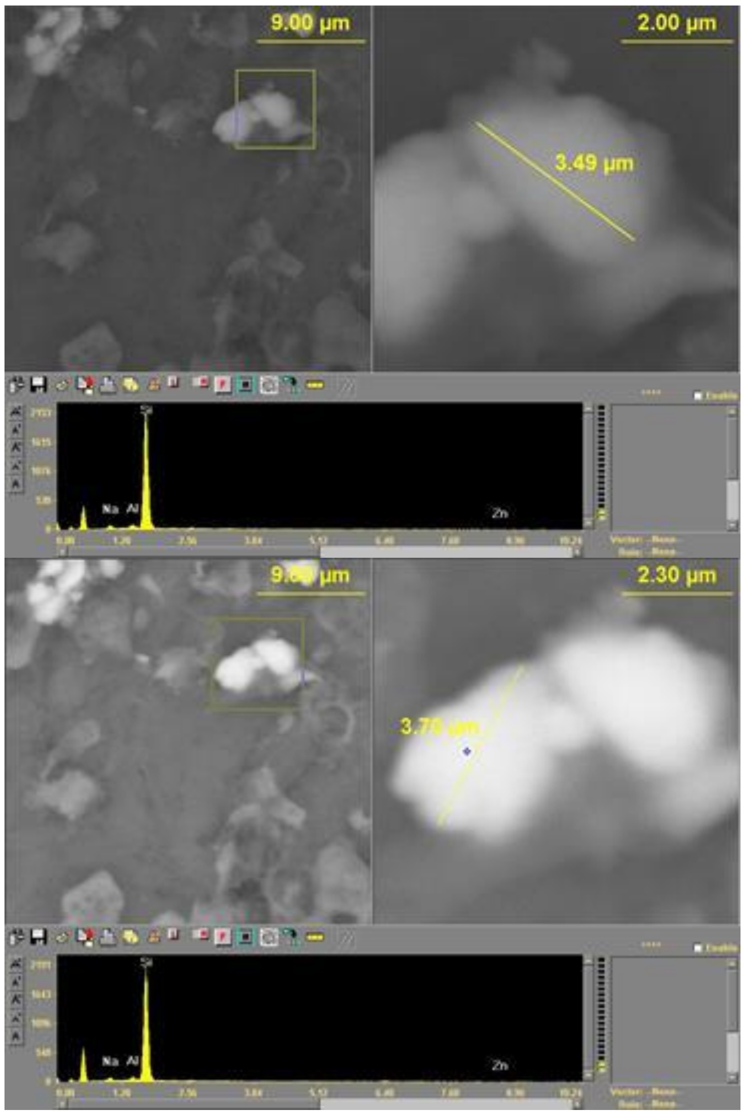

9.00 µm
2.00 µm
1.56 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
2.30 µm
Si
Na
Al
Zn

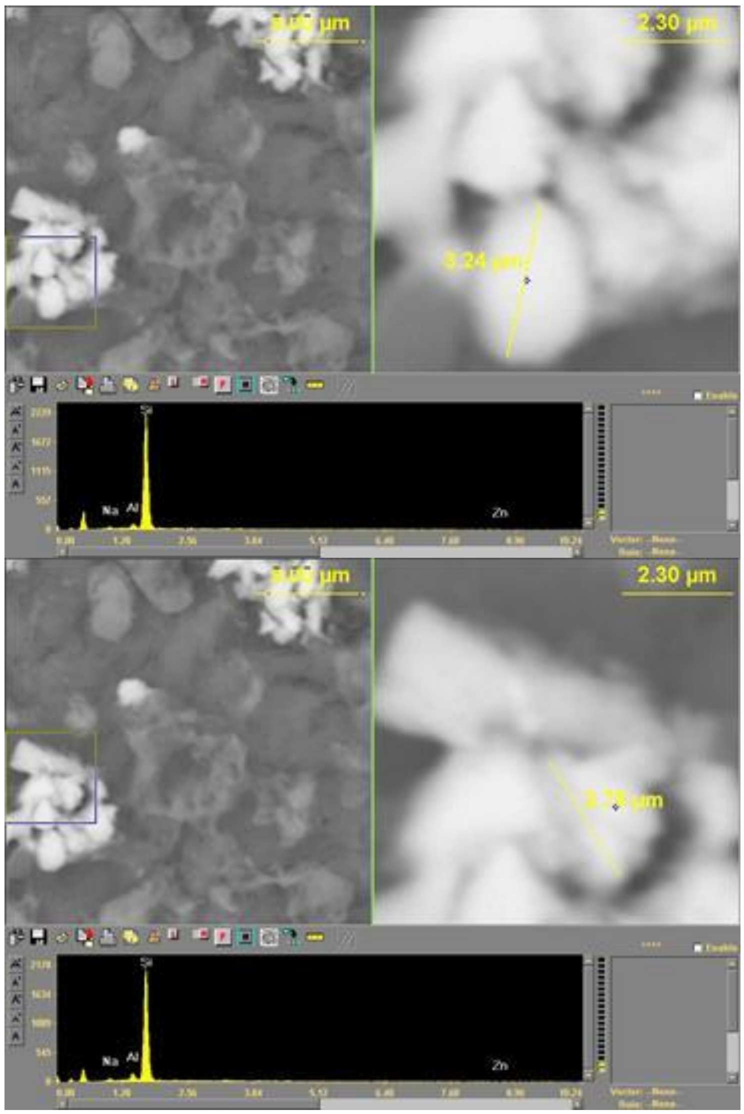

9.00 µm
2.00 µm
1.70 µm
Si
Al
Na
Fe
Zn
9.00 µm
2.00 µm
1.90 µm
Si
Na
Al
Zn

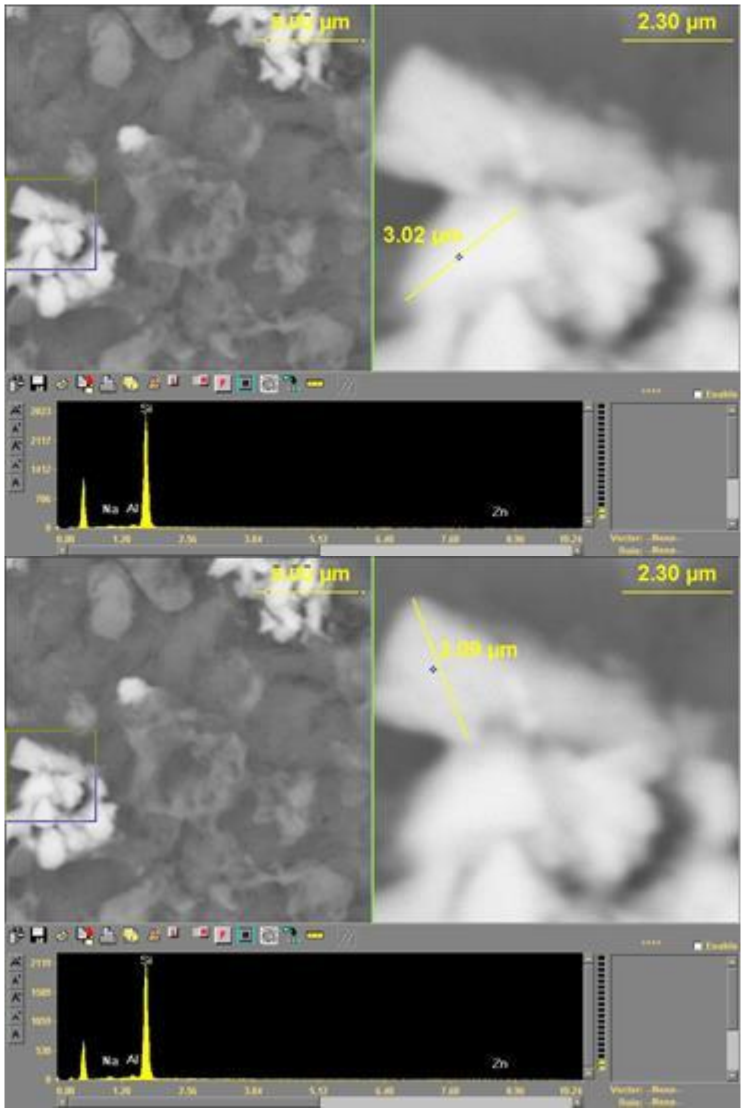

9.00 µm
2.00 µm
1.16 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
1.55 µm
Si
Na
Al
Zn

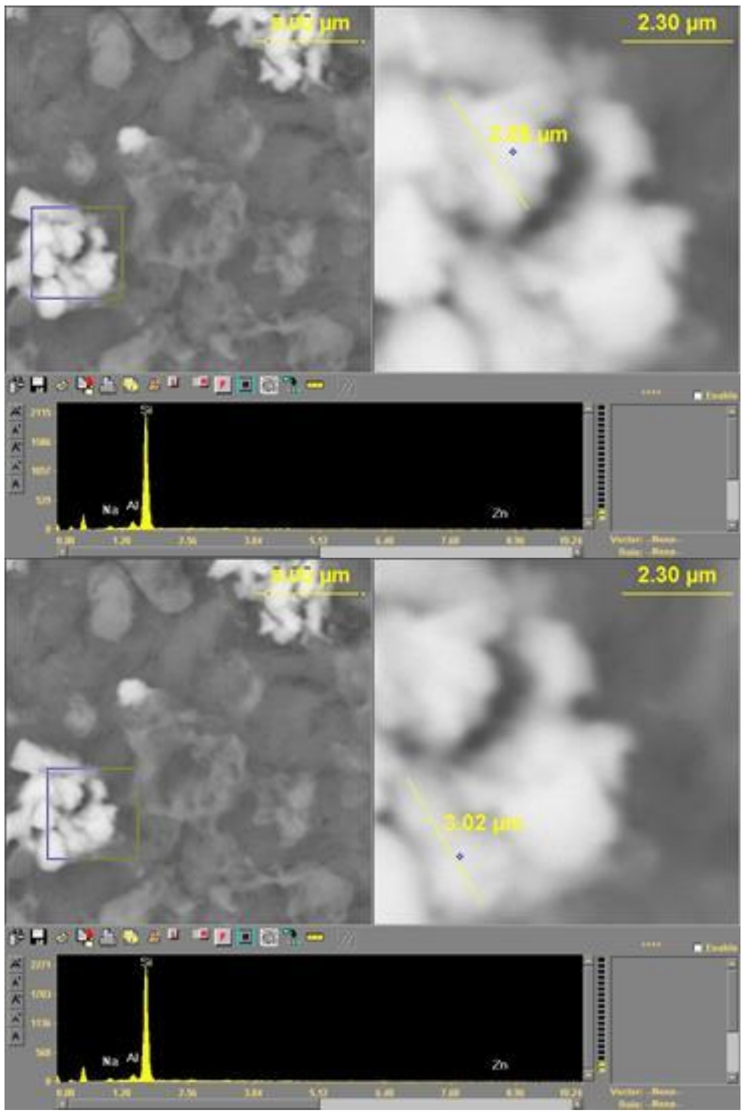

9.00 µm
2.00 µm
1.14 µm
Si
Al
Na
Ca
Ca
Fe
Zn
9.00 µm
2.00 µm
1.53 µm
Si
Al
Na
K
Zn

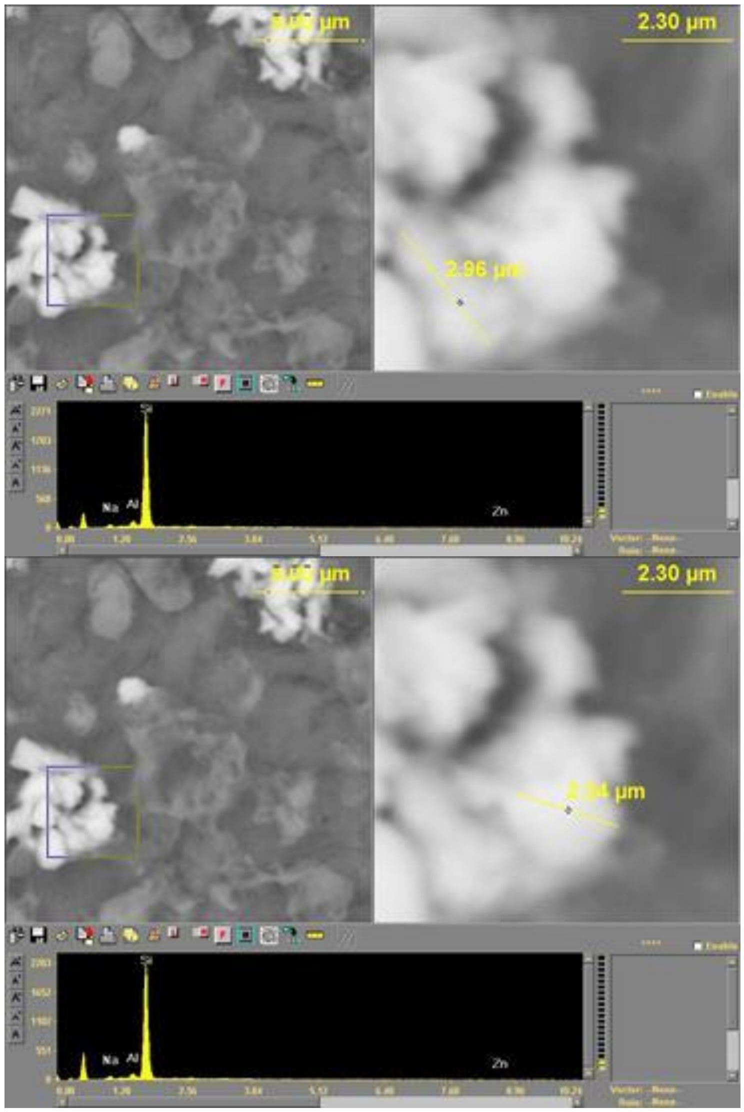

9.00 µm
2.00 µm
2.55 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
2.13 µm
Si
Na
Al
Zn

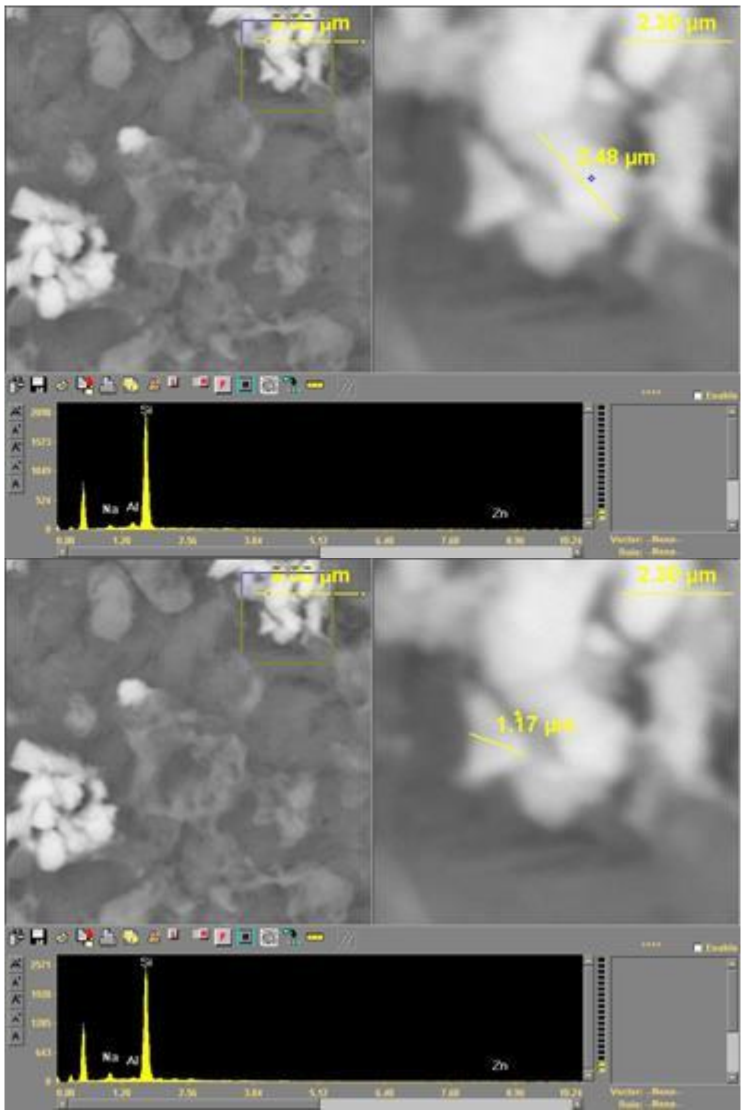

9.00 µm
2.00 µm
3.49 µm
Si
Na
Al
Zn
2.30 µm
Si
Na
Al
Zn


2.30 µm
3.24 µm
Si
Na
Al
Zn
2.30 µm
Si
Na
Al
Zn

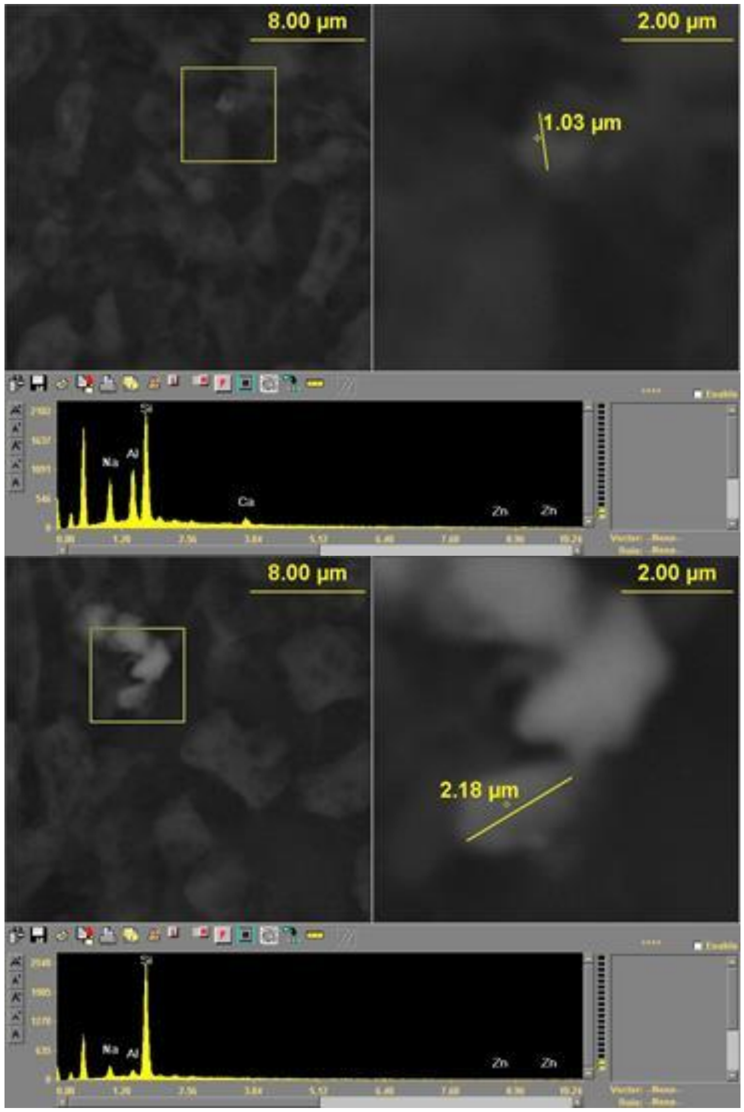

2.30 µm
3.02 µm
Si
Na
Al
Zn
2.30 µm
Si
Na
Al
Zn

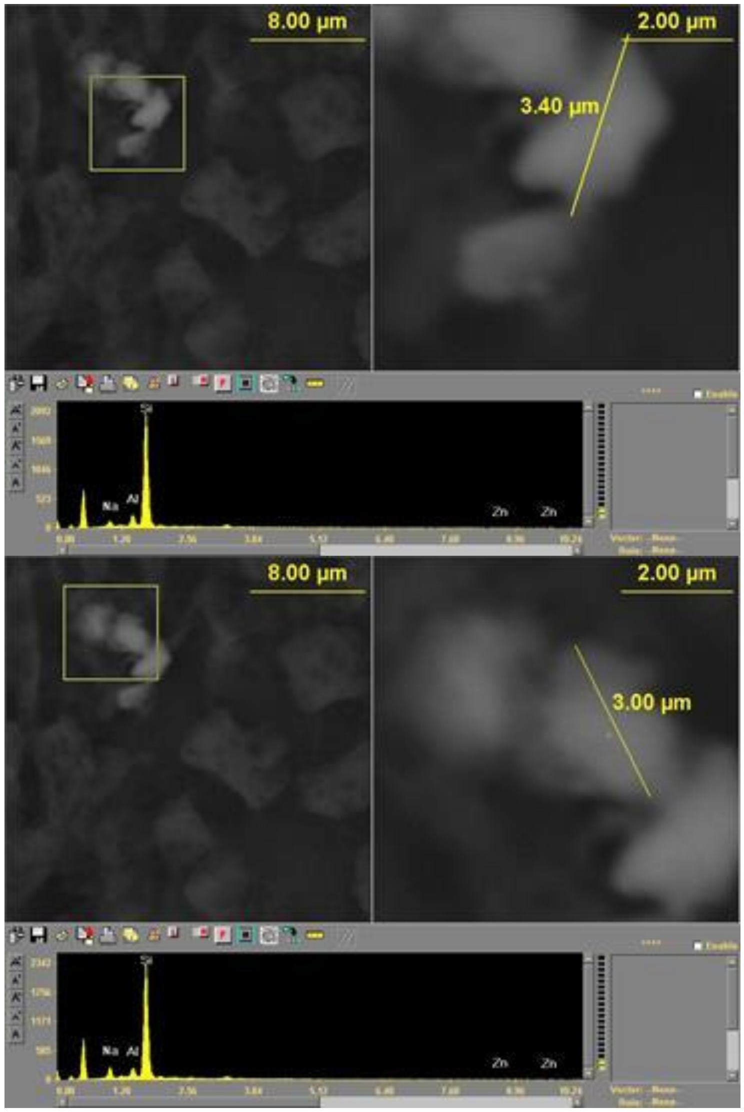

2.30 µm
Si
Na
Al
Zn
2.30 µm
3.02 µm
Si
Na
Al
Zn

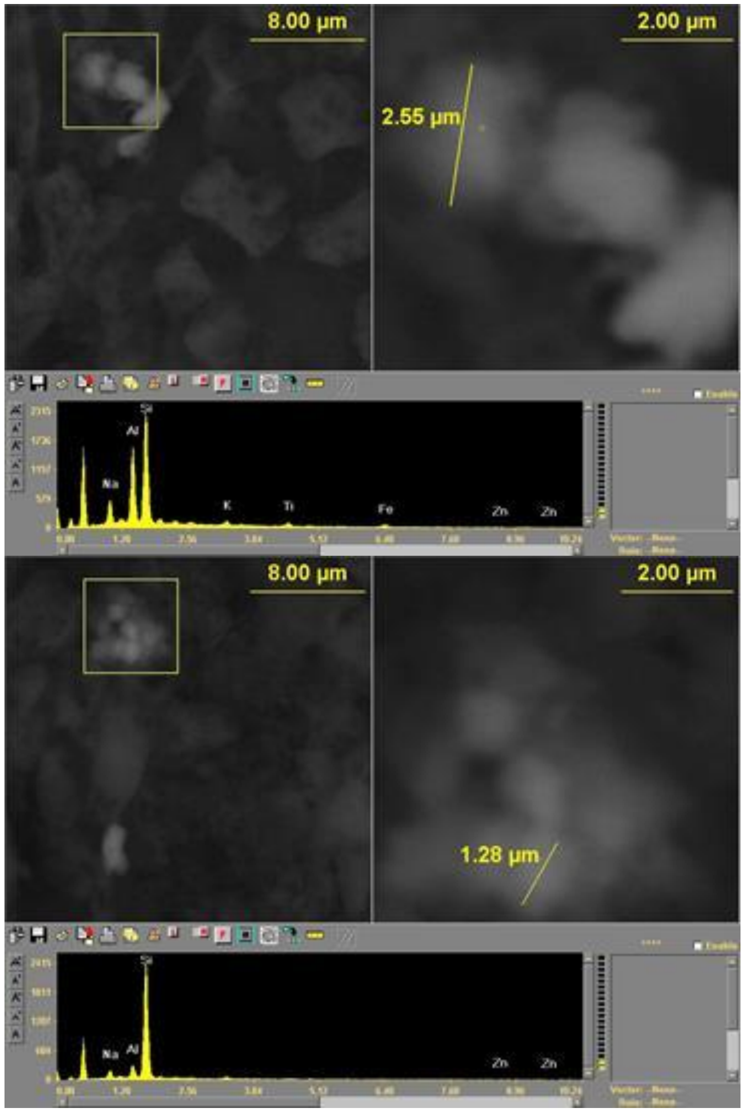

2.30 µm
2.96 µm
Si
Na
Al
Zn
2.30 µm
Si
Na
Al
Zn

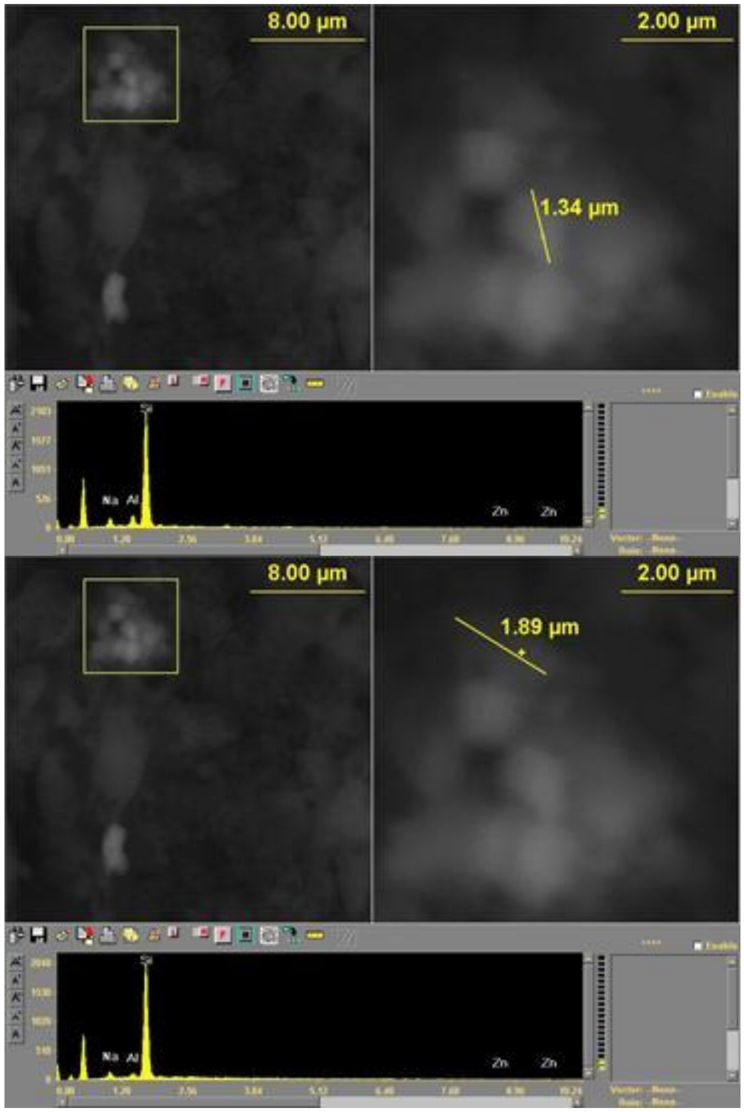

2.30 µm
2.48 µm
Si
Na
Al
Zn
2.30 µm
1.17 µm
Si
Na
Al
Zn

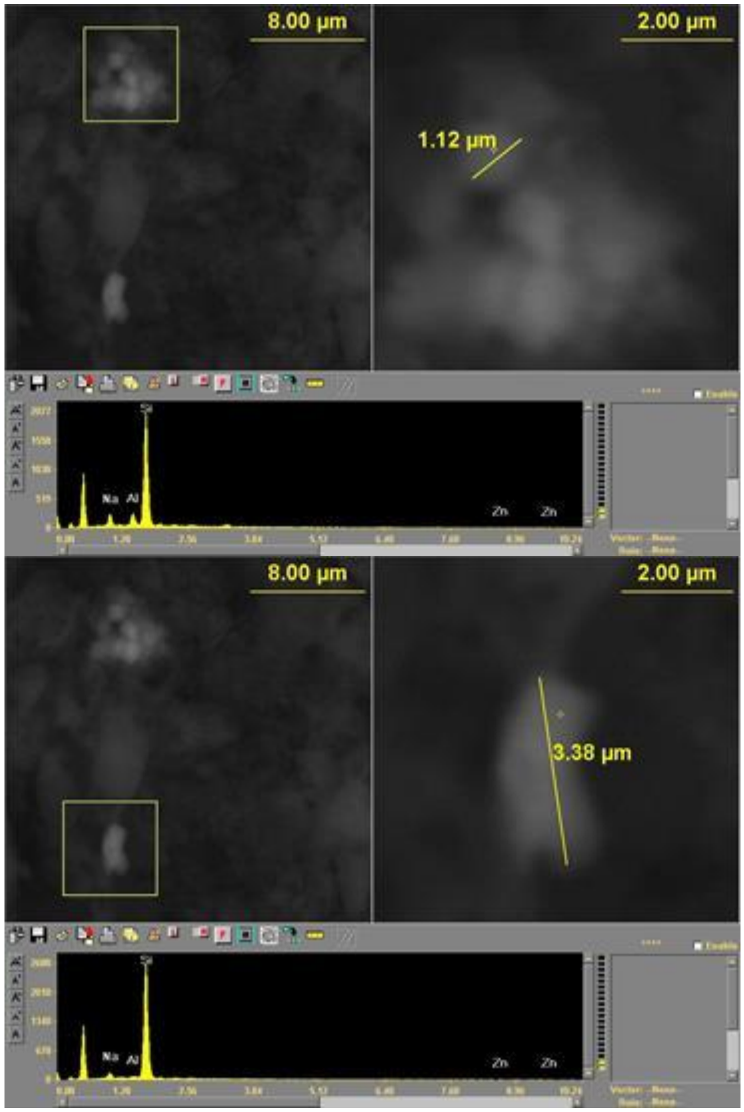
Sample 1B

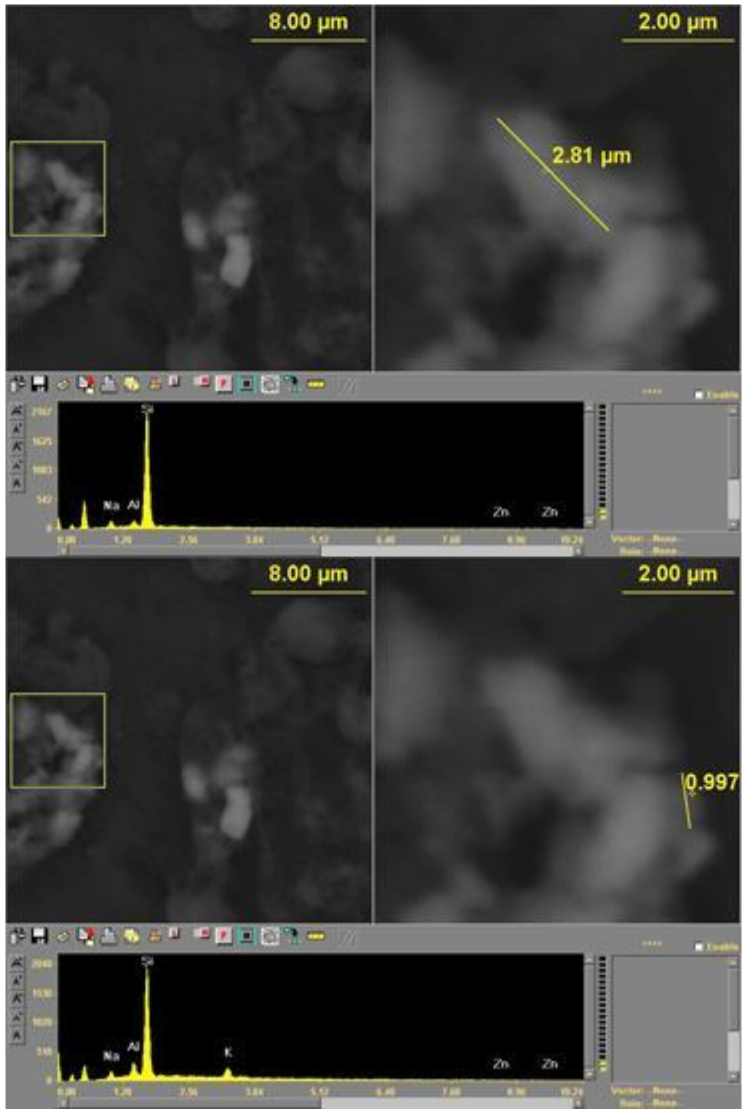

8.00 µm
2.00 µm
1.03 µm
Si
Na
Al
Ca
Zn
Zn
8.00 µm
2.00 µm
2.18 µm
Si
Na
Al
Zn
Zn

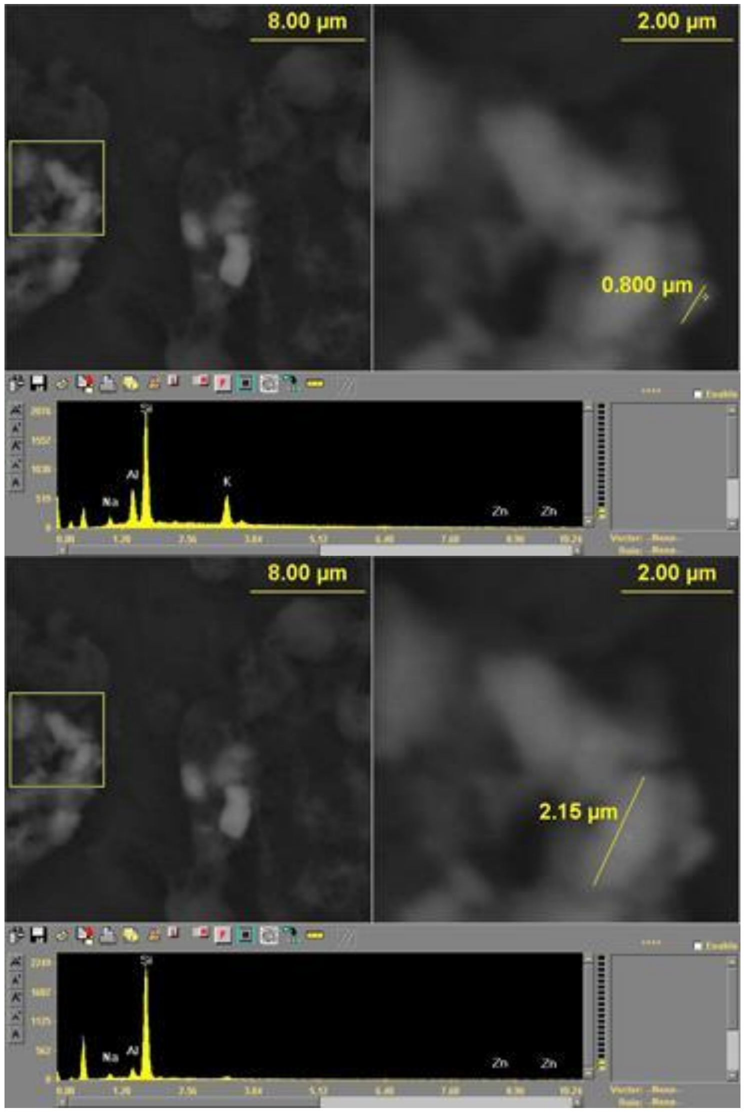

8.00 µm
2.00 µm
3.40 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
3.00 µm
Si
Na
Al
Zn
Zn

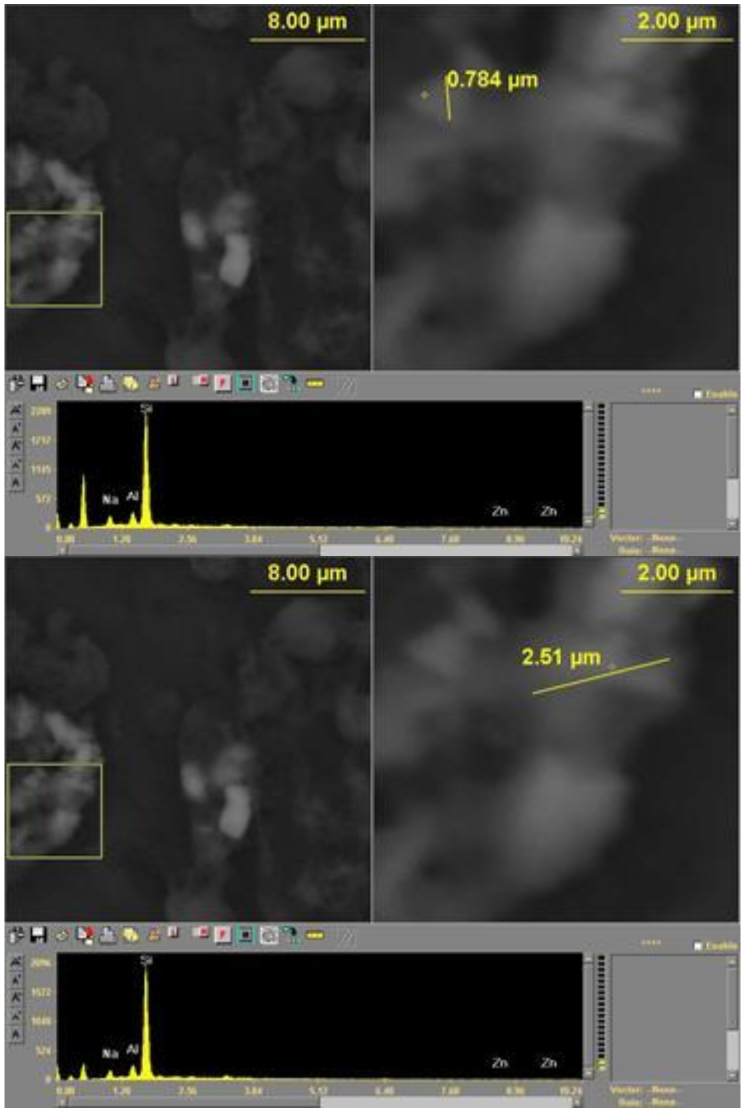

8.00 µm
2.00 µm
2.55 µm
Na
Al
Si
K
Ti
Fe
Zn
Zn
8.00 µm
2.00 µm
1.28 µm
Si
Na
Al
Zn
Zn

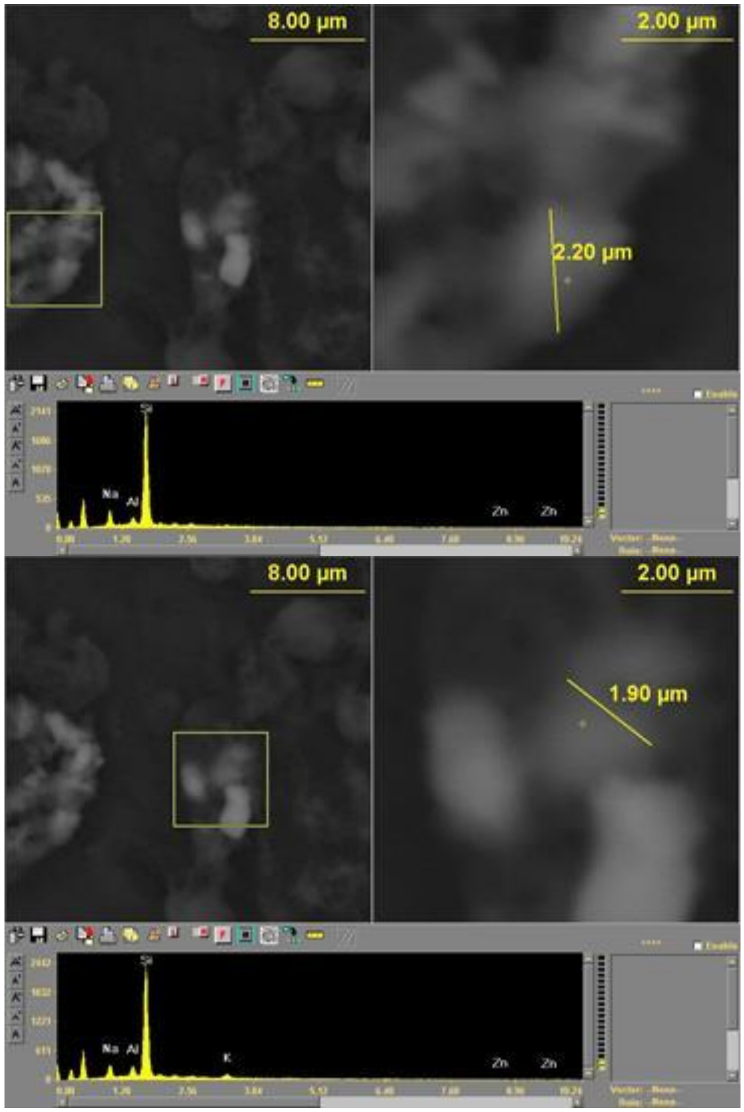

8.00 µm
2.00 µm
1.34 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
1.89 µm
Si
Na
Al
Zn
Zn

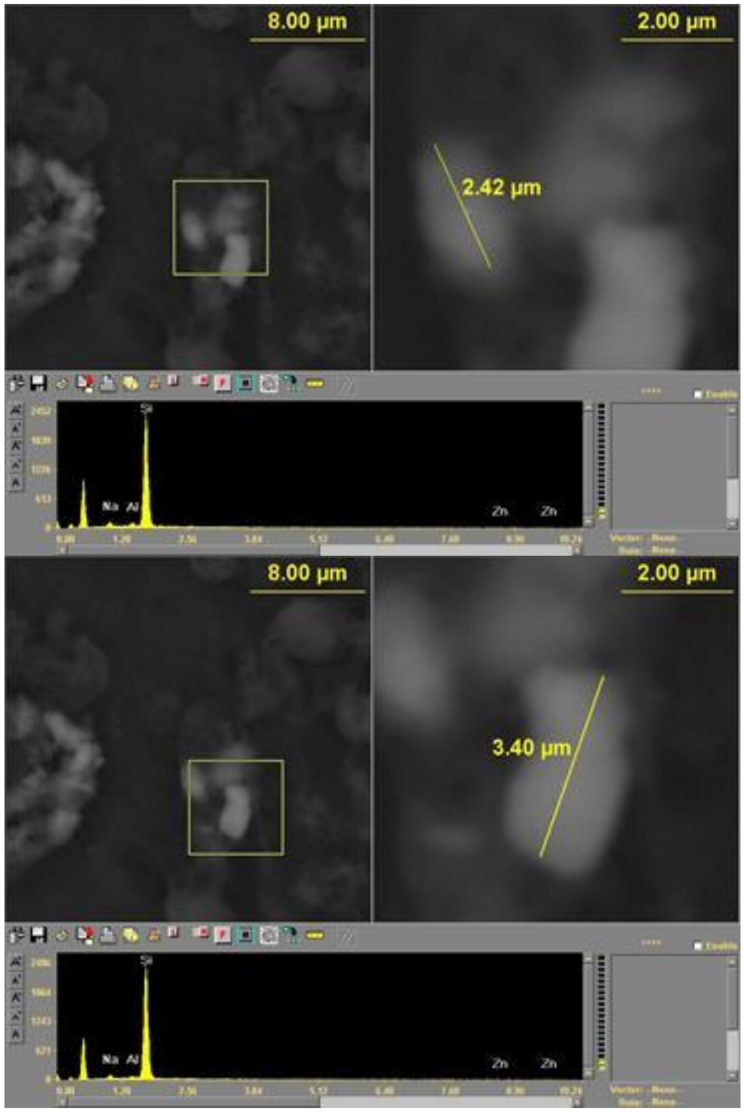

8.00 µm
2.00 µm
1.12 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
3.38 µm
Si
Na
Al
Zn
Zn

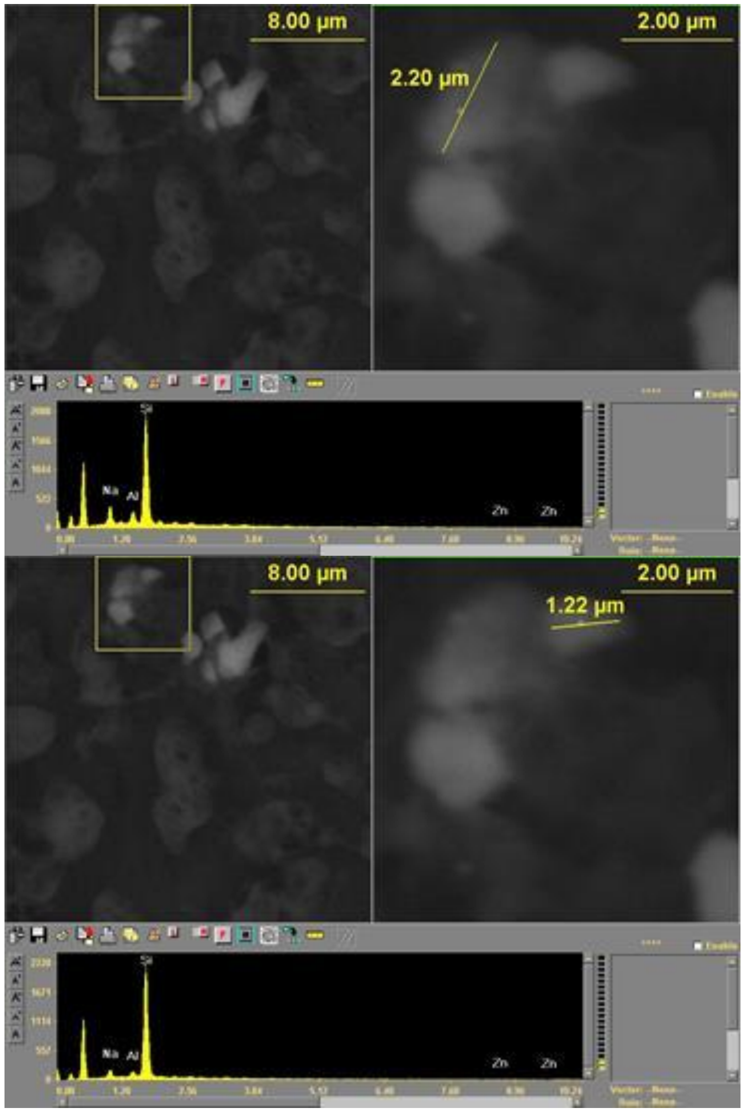

8.00 µm
2.00 µm
2.81 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
0.997
Si
Na
Al
K
Zn
Zn

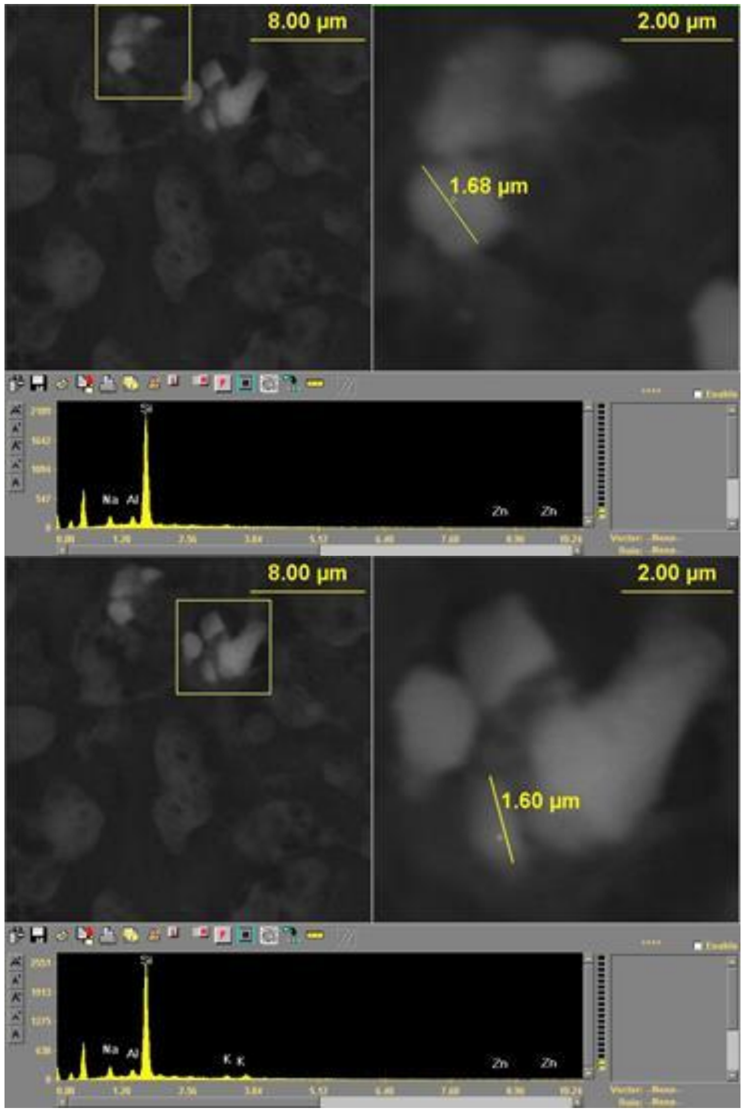

8.00 µm
2.00 µm
0.800 µm
Si
Al
Na
K
Zn
Zn
8.00 µm
2.00 µm
2.15 µm
Si
Na
Al
Zn
Zn


8.00 µm
2.00 µm
0.784 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
2.51 µm
Si
Na
Al
Zn
Zn

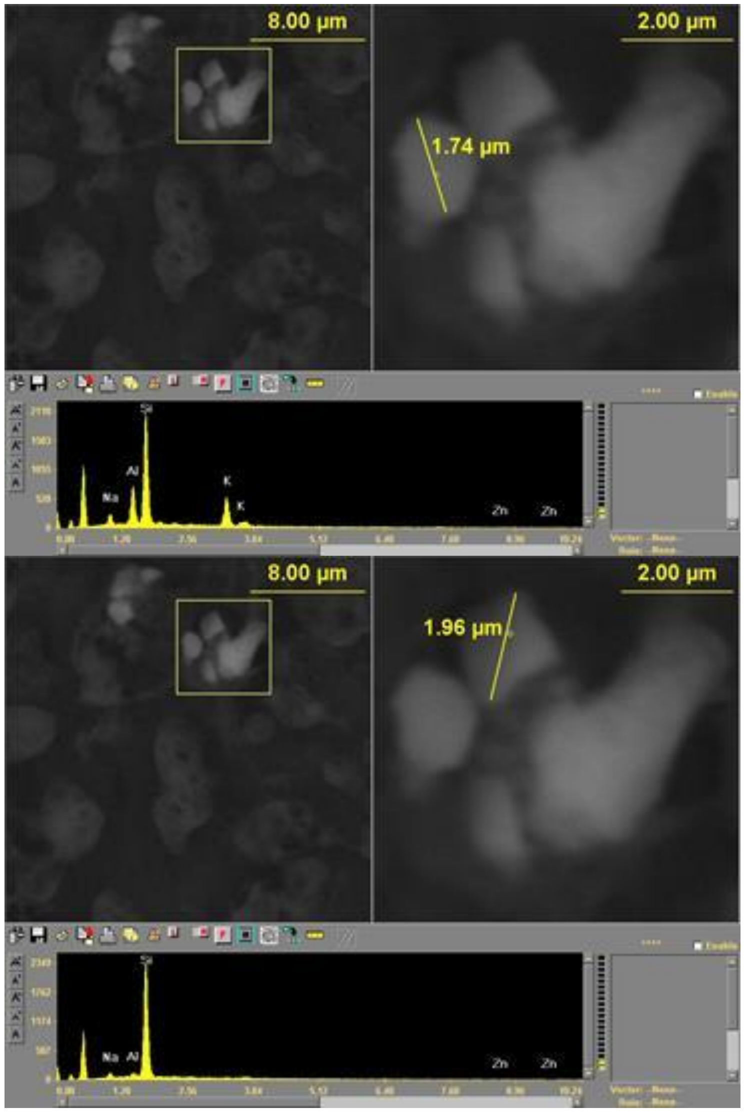

8.00 µm
2.00 µm
2.20 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
1.90 µm
Si
Na
Al
K
Zn
Zn

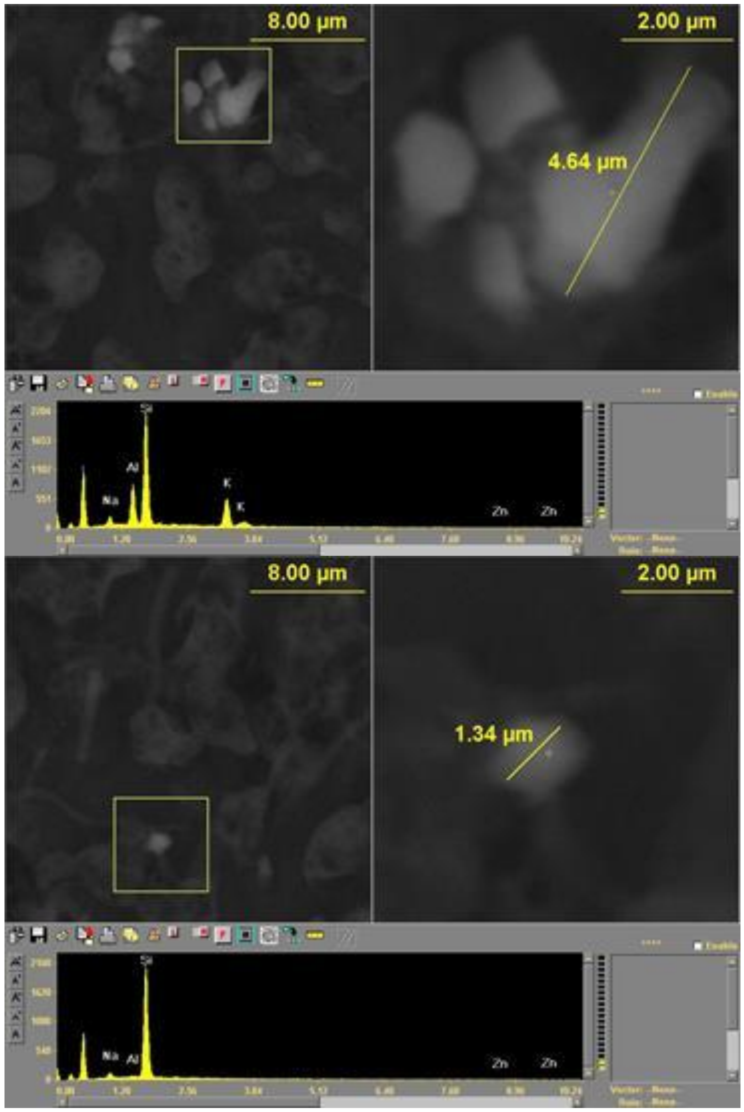

8.00 µm
2.00 µm
2.42 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
3.40 µm
Si
Na
Al
Zn
Zn


8.00 µm
2.00 µm
2.20 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
1.22 µm
Si
Na
Al
Zn
Zn

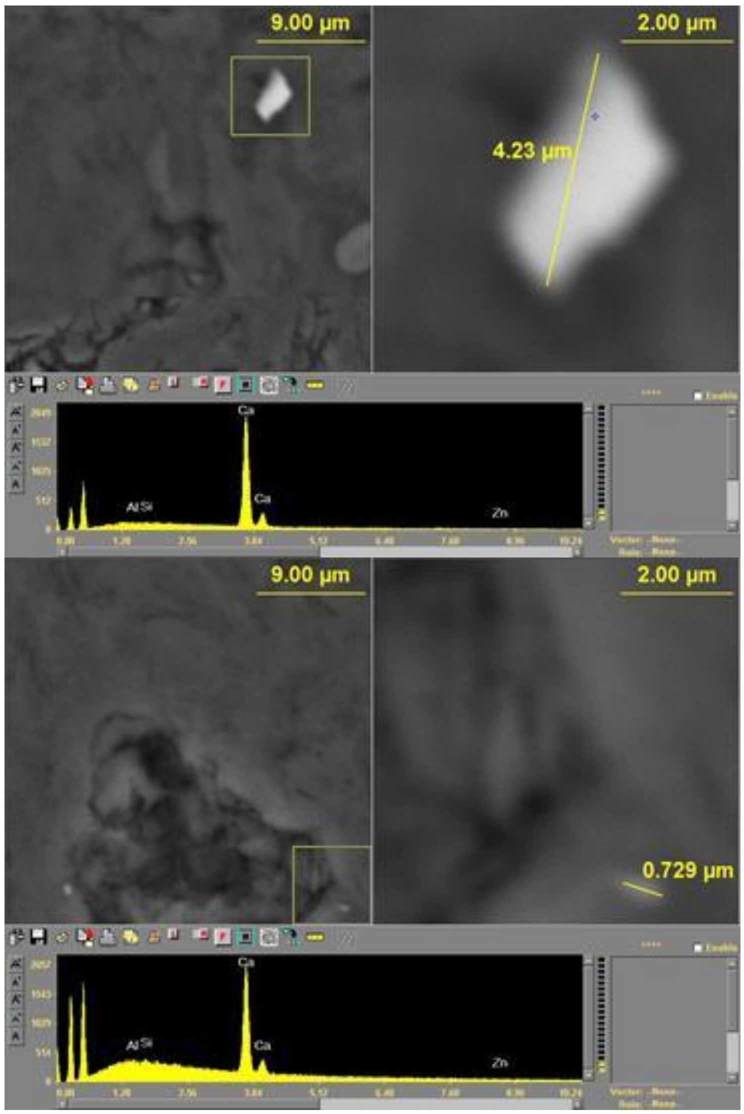


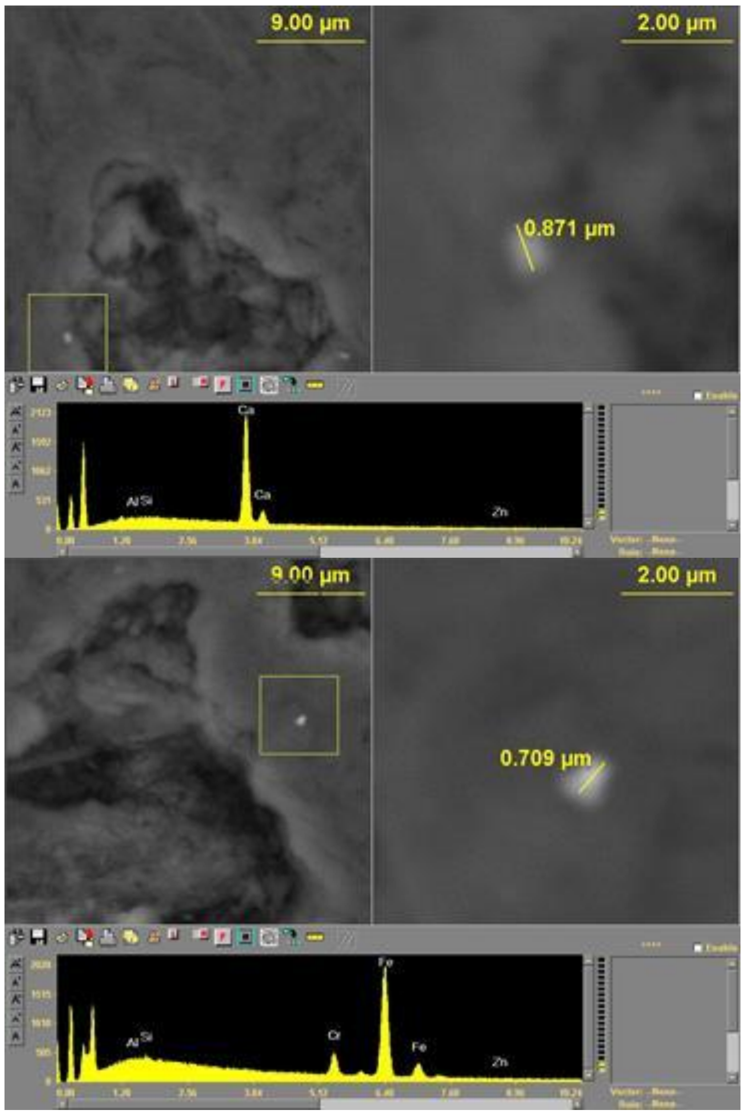
8.00 µm
2.00 µm
1.68 µm
Si
Na
Al
Zn
Zn
8.00 µm
2.00 µm
1.60 µm
Si
Na
Al
K
K
Zn
Zn

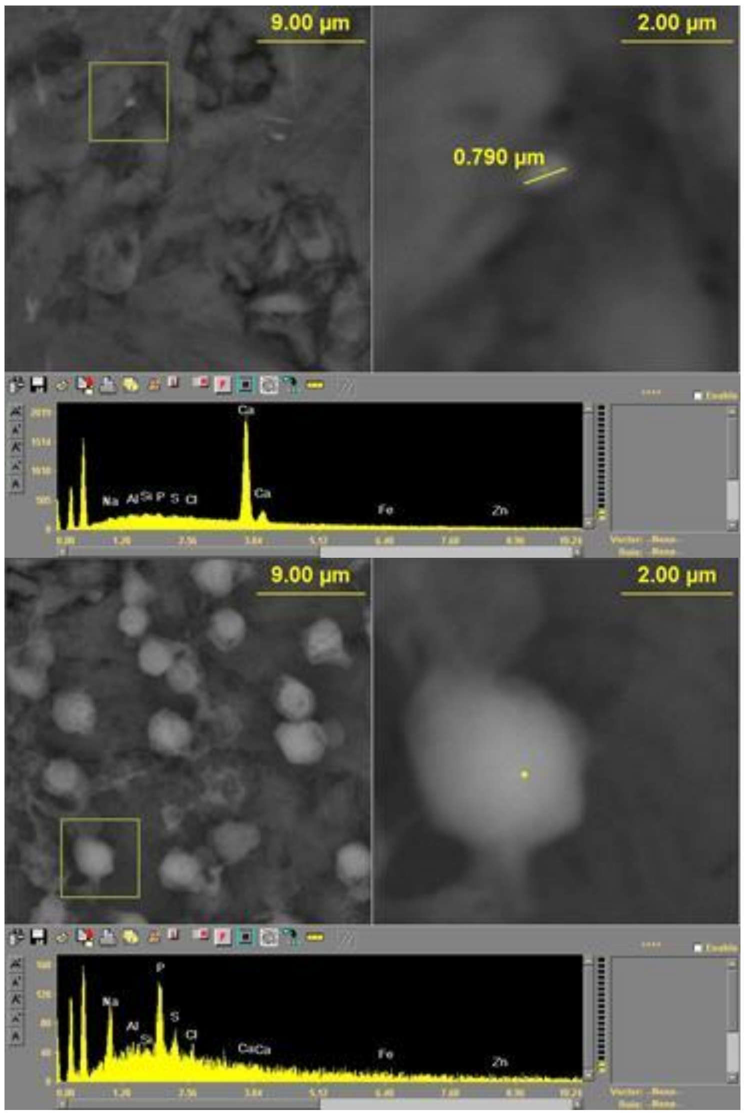

8.00 µm
2.00 µm
1.74 µm
Si
Al
Na
K
K
Zn
Zn
8.00 µm
2.00 µm
1.96 µm
Si
Na
Al
Zn
Zn

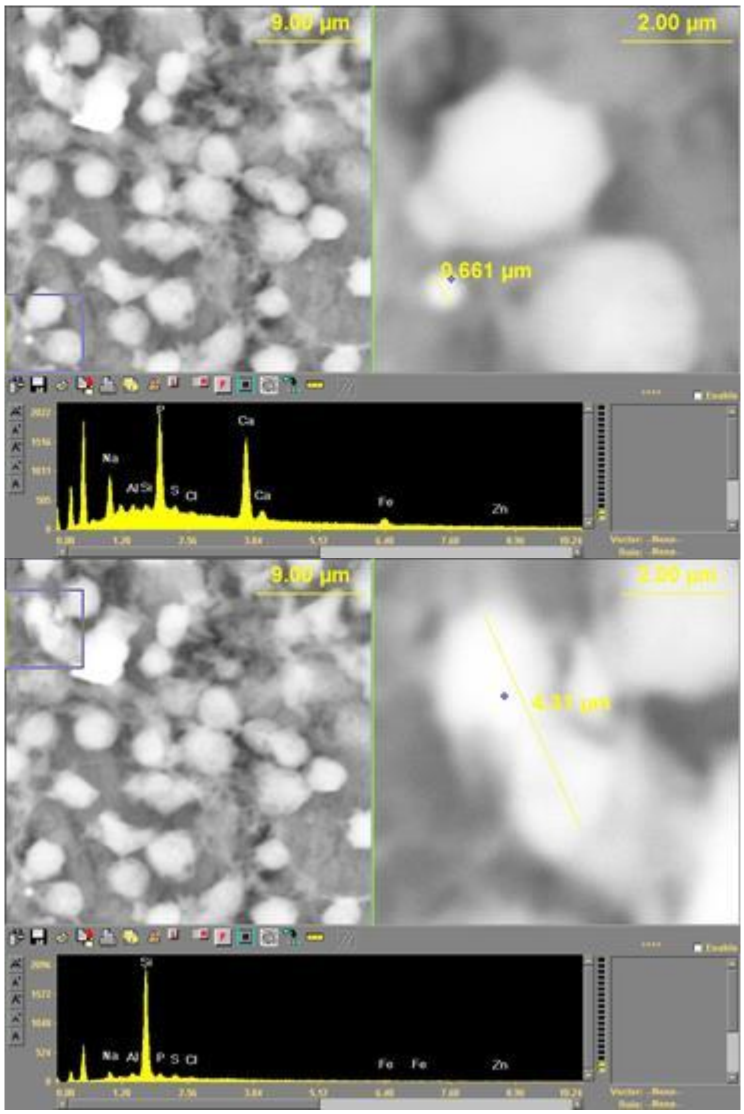

8.00 µm
2.00 µm
4.64 µm
Si
Al
Na
K
K
Zn
Zn
8.00 µm
2.00 µm
1.34 µm
Si
Na
Al
Zn
Zn

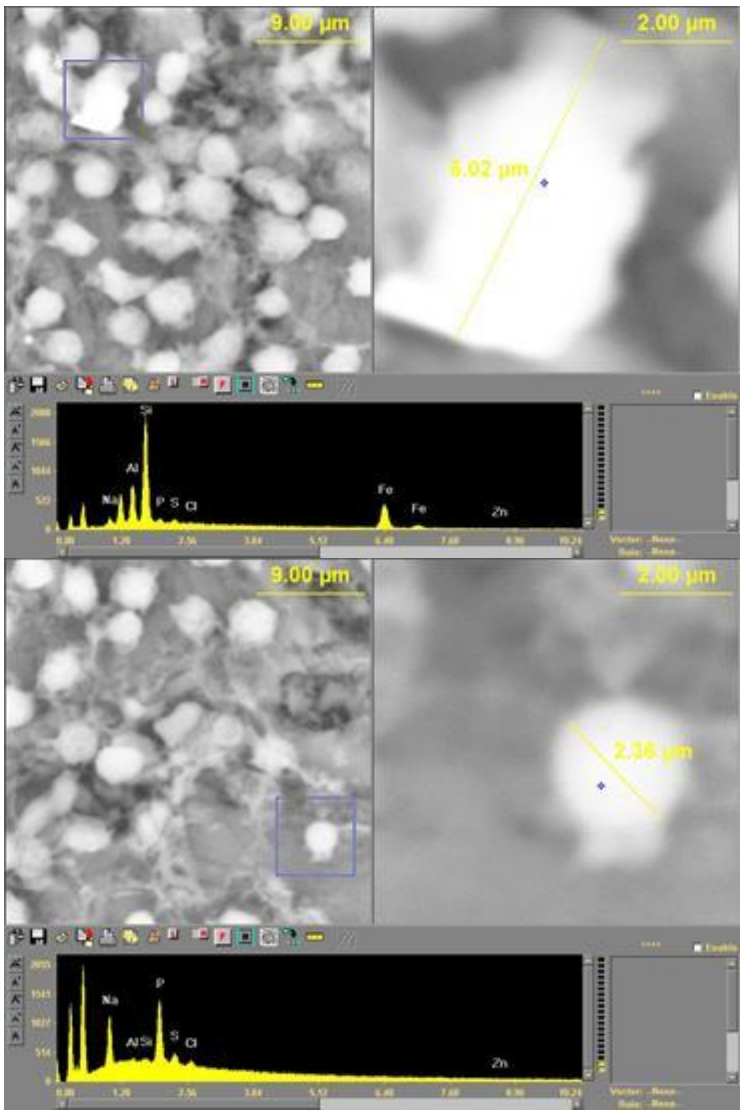
Sample 2A

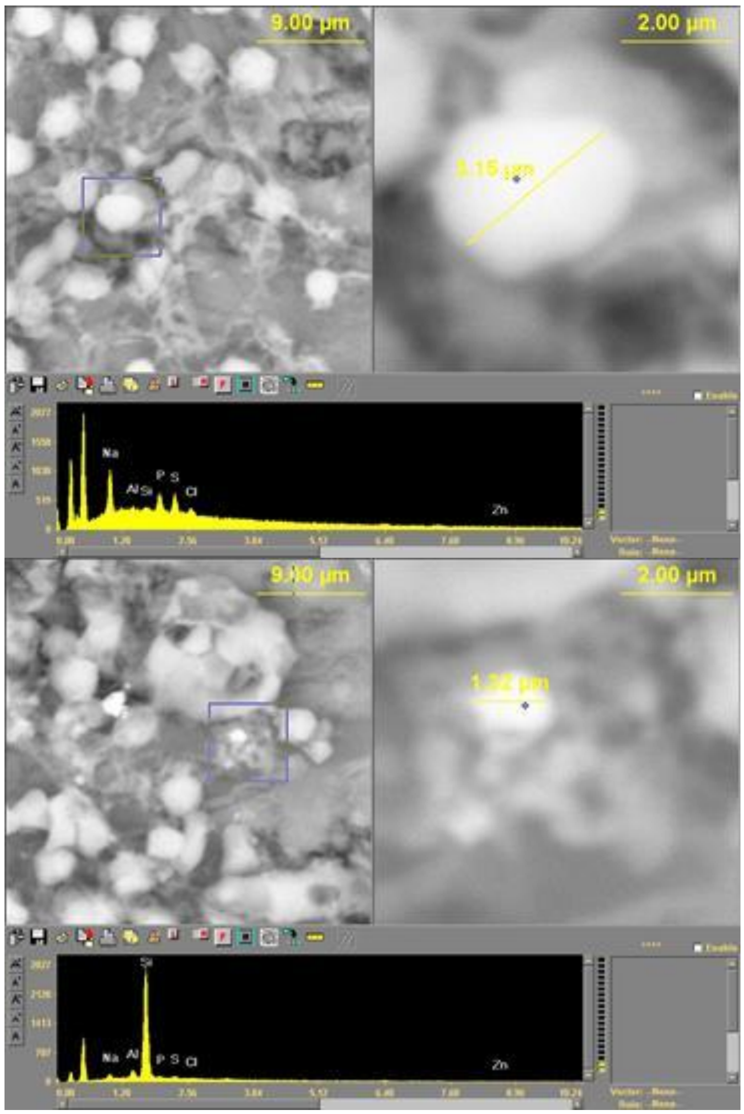

9.00 µm
2.00 µm
4.23 µm
Ca
Al Si
Ca
Zn
9.00 µm
2.00 µm
0.729 µm
Ca
Al Si
Ca
Zn

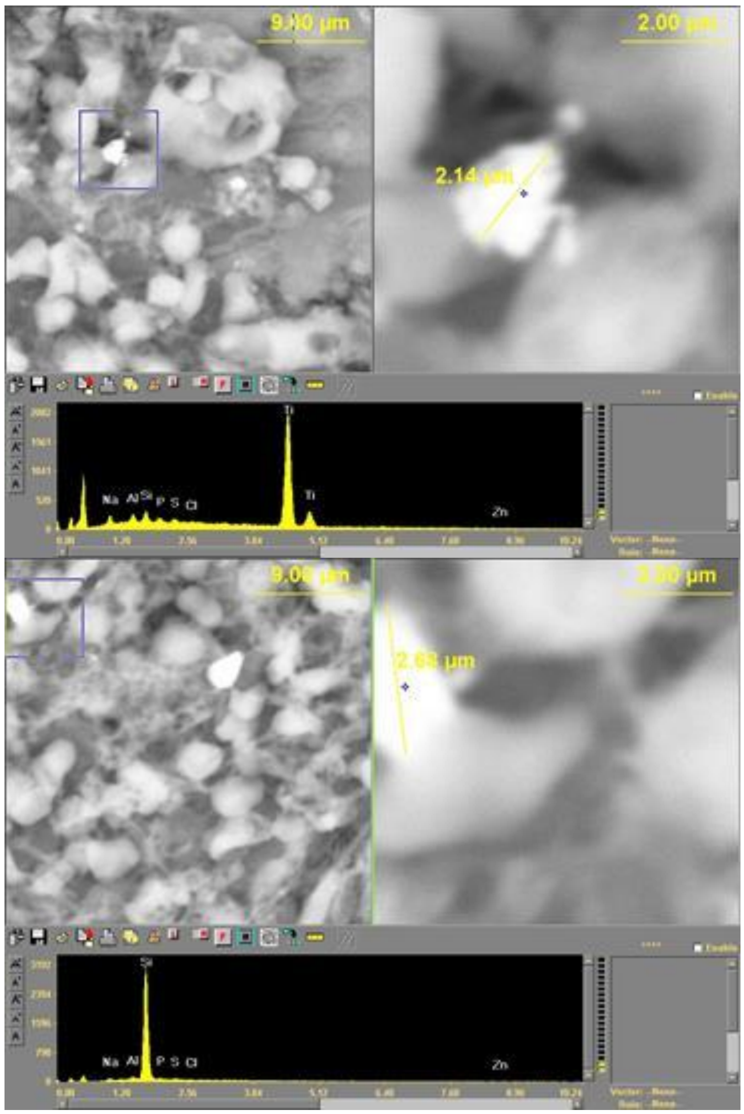

9.00 µm
2.00 µm
0.871 µm
Ca
Al Si
Ca
Zn
9.00 µm
2.00 µm
0.709 µm
Fe
Al Si
Cr
Fe
Zn

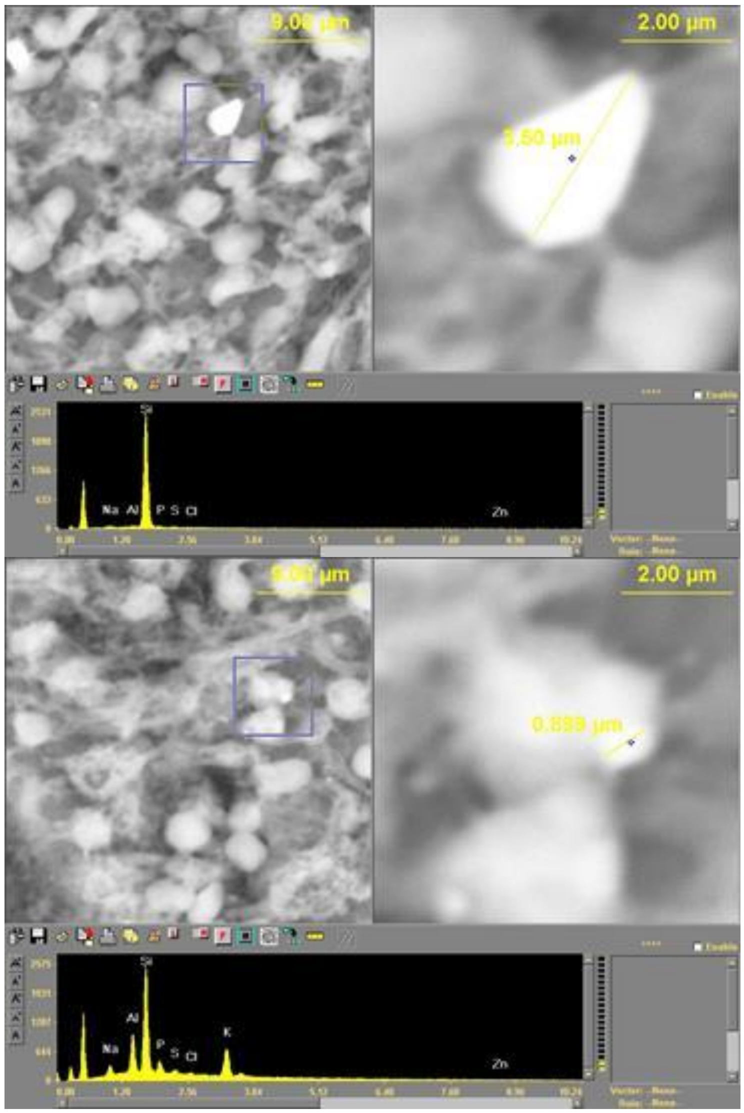

9.00 µm
2.00 µm
0.790 µm
Ca
Na Al Si P S Cl
Ca
Fe
Zn
9.00 µm
2.00 µm
P
Na
Al
Si
S
Cl
CaCa
Fe
Zn

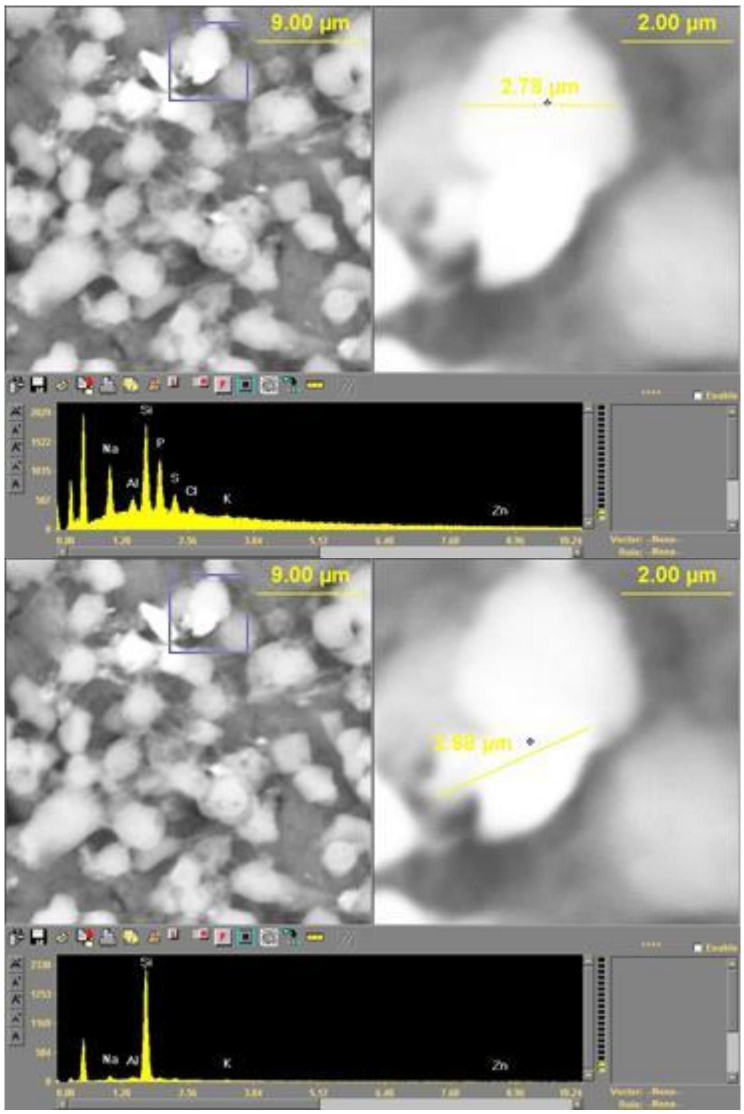

9.00 µm
2.00 µm
0.661 µm
P
Ca
Na
Al Si
S
Cl
Ca
Fe
Zn
9.00 µm
2.00 µm
Si
Na
Al
P
S
Cl
Fe
Fe
Zn


9.00 µm
2.00 µm
6.02 µm
Si
Al
Na
P S Cl
Fe
Fe
Zn
9.00 µm
2.00 µm
2.36 µm
P
Na
Al Si
S Cl
Zn

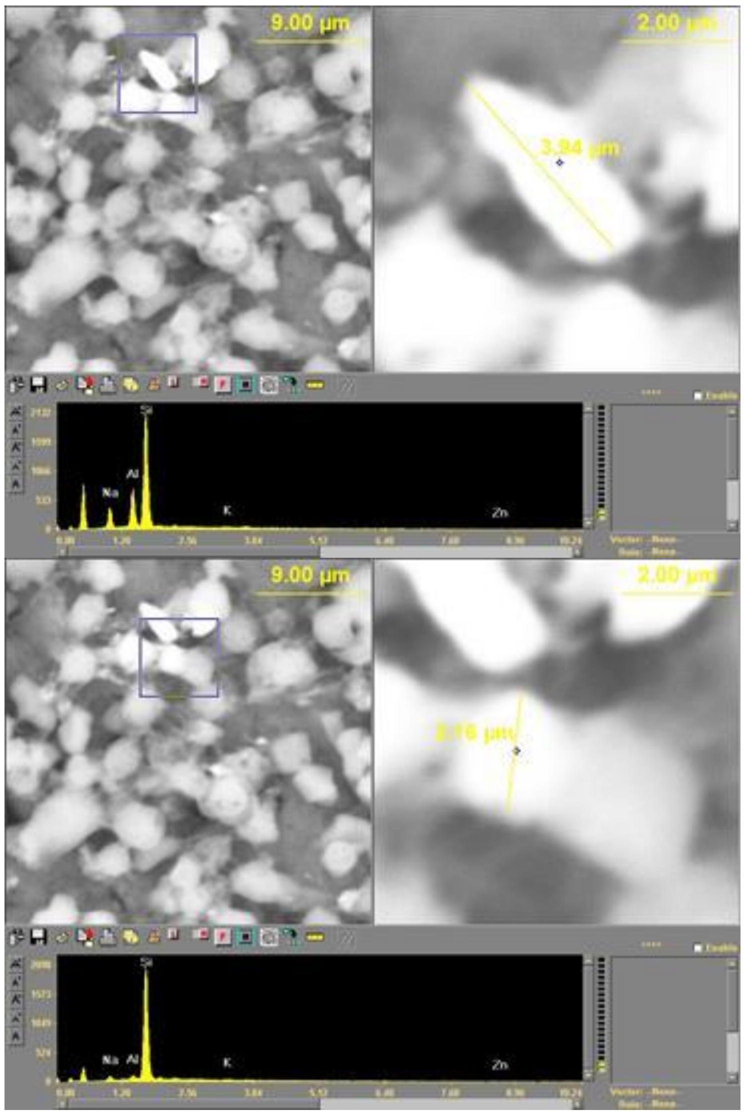

9.00 µm
2.00 µm
Na
Al Si
P S
Cl
Zn
9.00 µm
2.00 µm
1.32 µm
Si
Na Al
P S Cl
Zn


9.00 µm
2.00 µm
2.14 µm
Ti
Na Al Si P S Cl
Ti
Zn
9.00 µm
2.00 µm
2.68 µm
Si
Na Al P S Cl
Zn

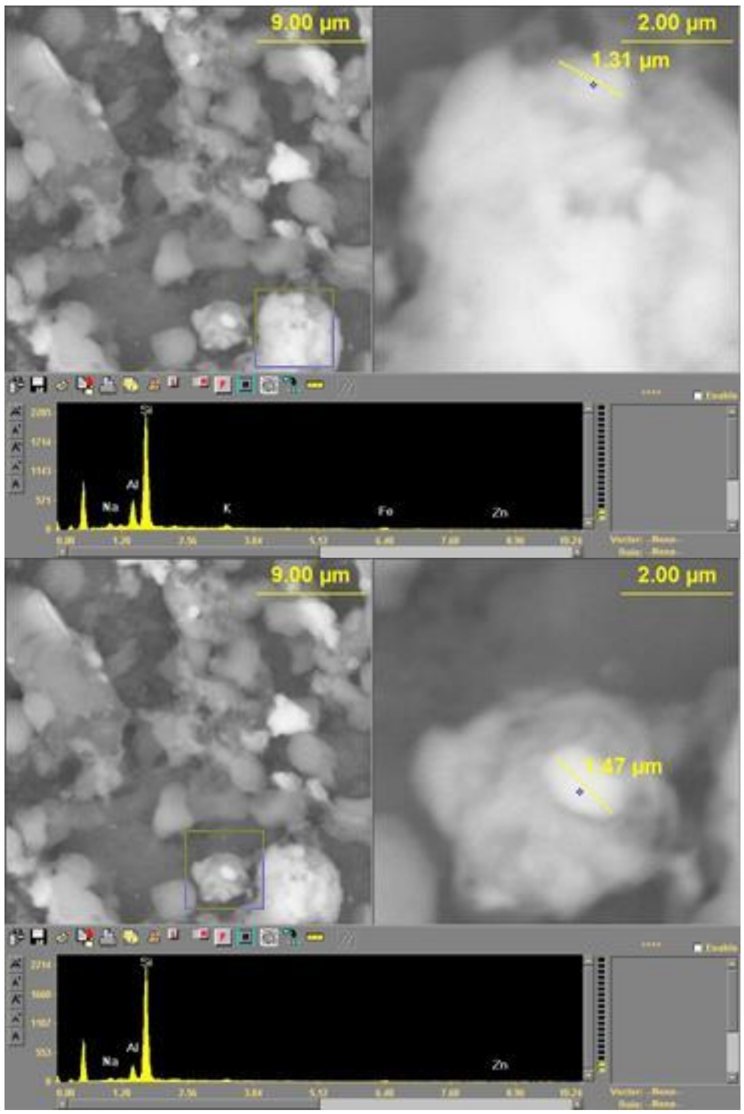

9.00 µm
2.00 µm
3.50 µm
Si
Na
Al
P
S
Cl
Zn
9.00 µm
2.00 µm
0.889 µm
Si
Al
Na
P
S
Cl
K
Zn

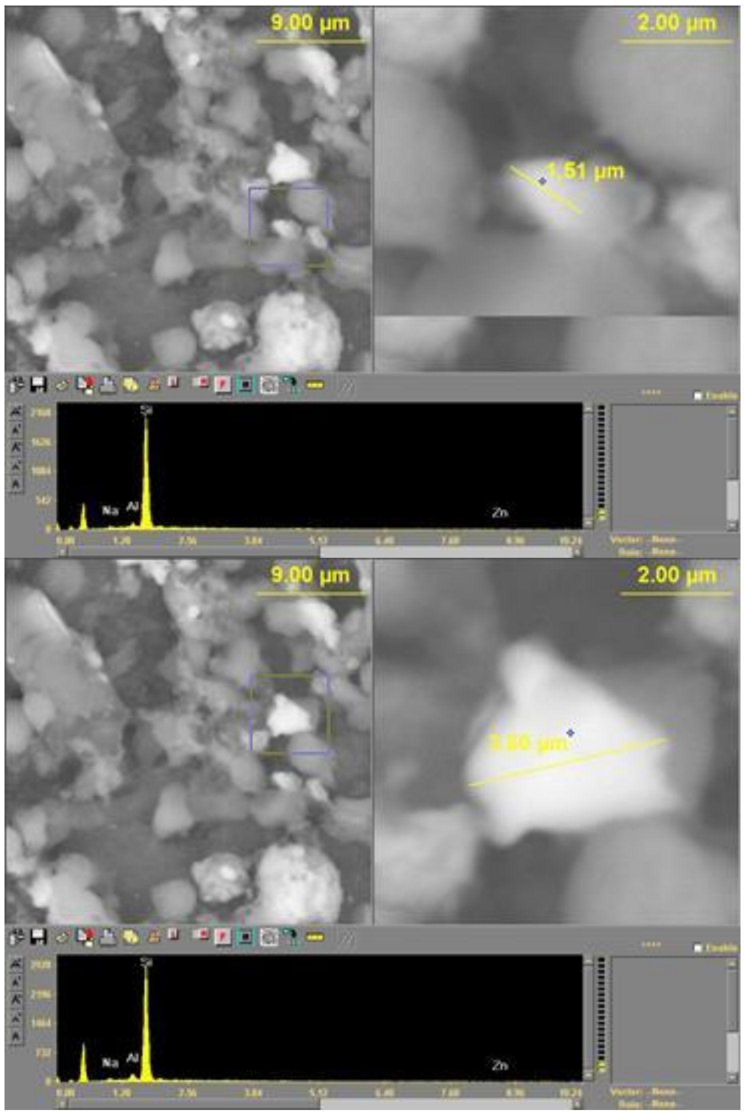


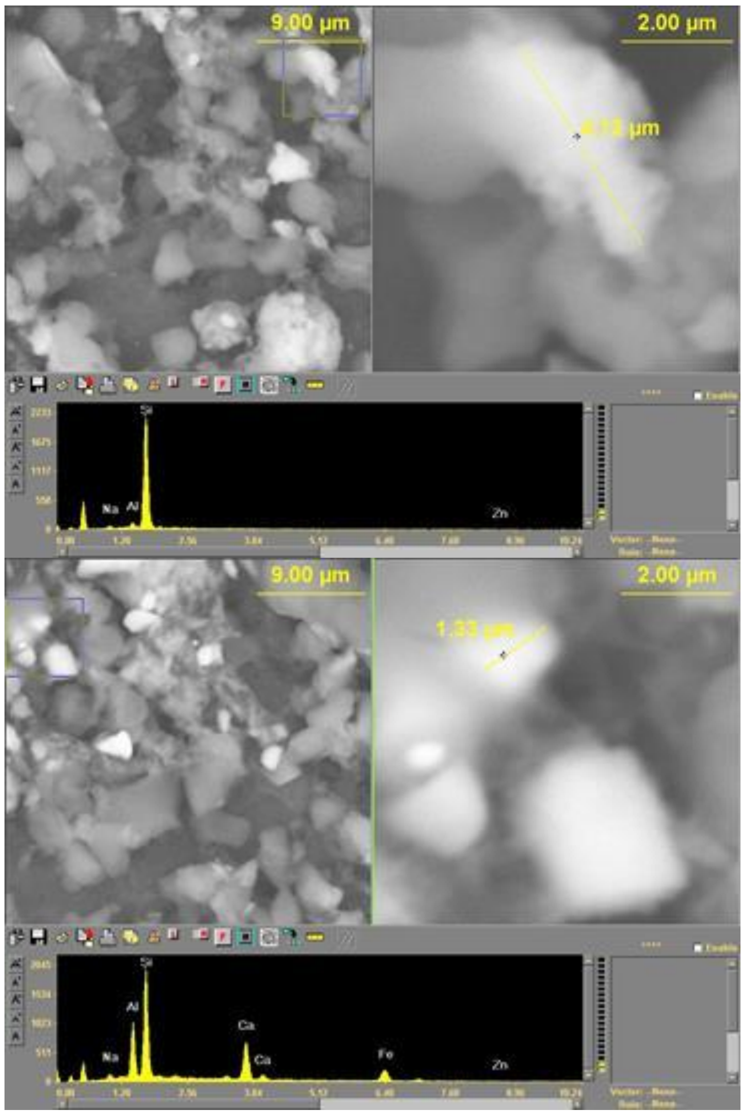
9.00 µm
2.00 µm
2.78 µm
Si
Na
P
Al
S
Cl
K
Zn
9.00 µm
2.00 µm
Si
Na
Al
K
Zn

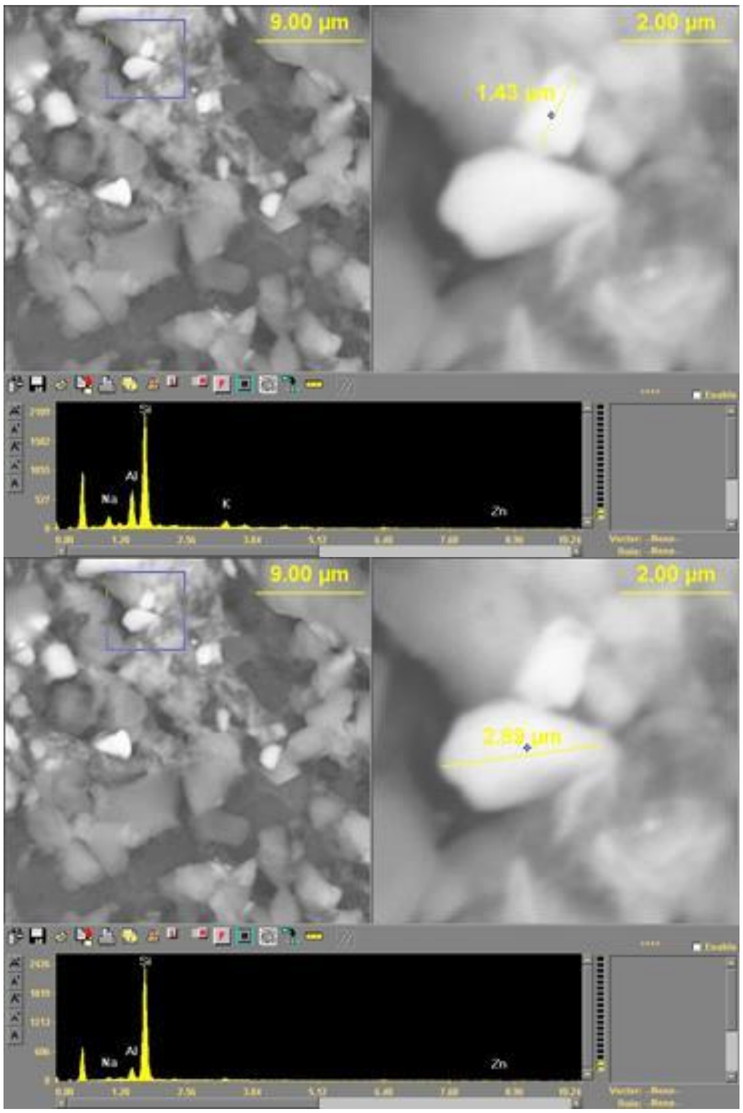

9.00 µm
2.00 µm
3.94 µm
Si
Al
Na
K
Zn
9.00 µm
2.00 µm
Si
Na Al
K
Zn

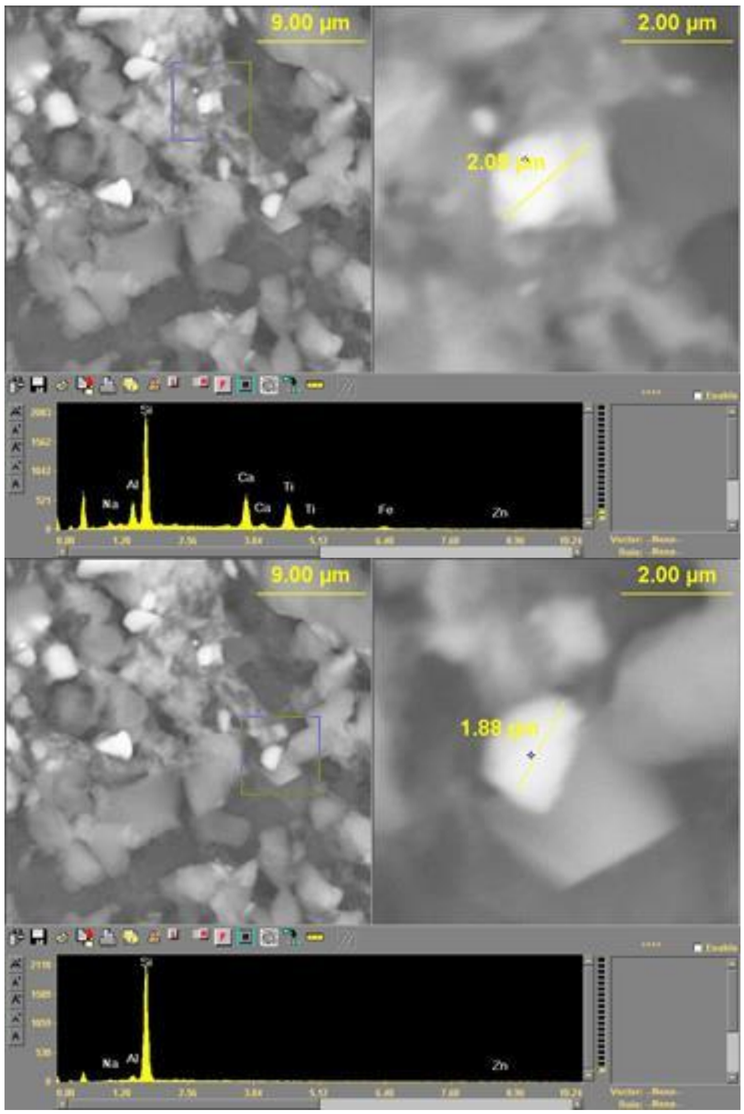
Sample 2B

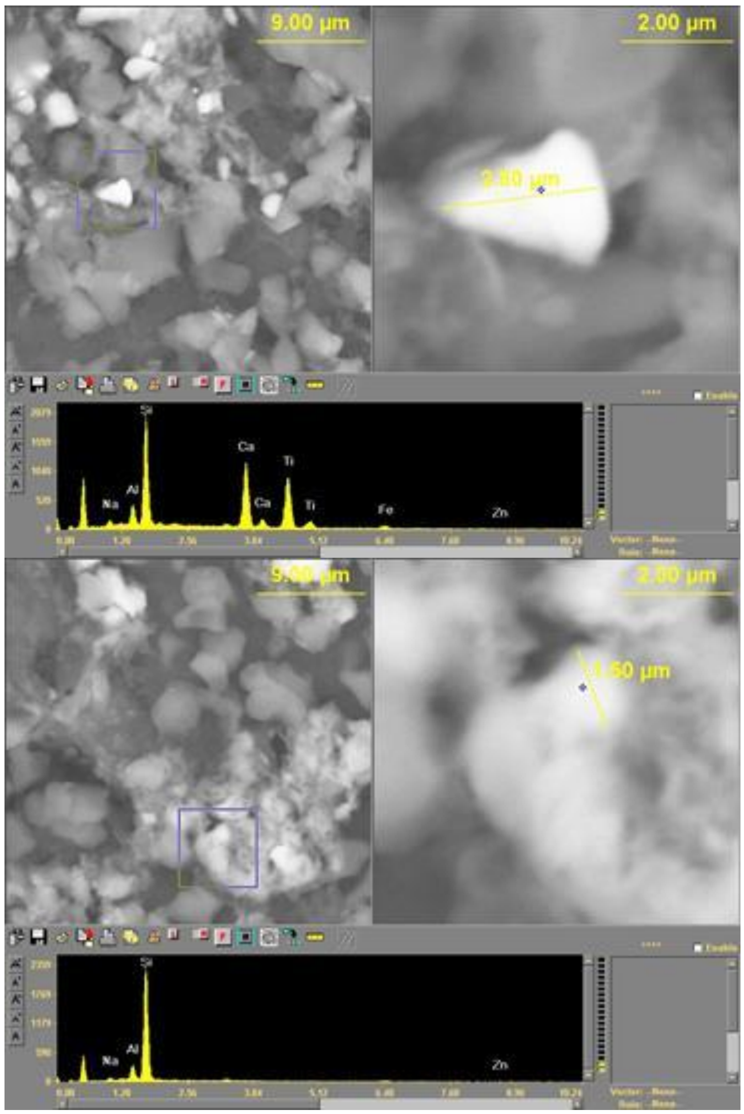


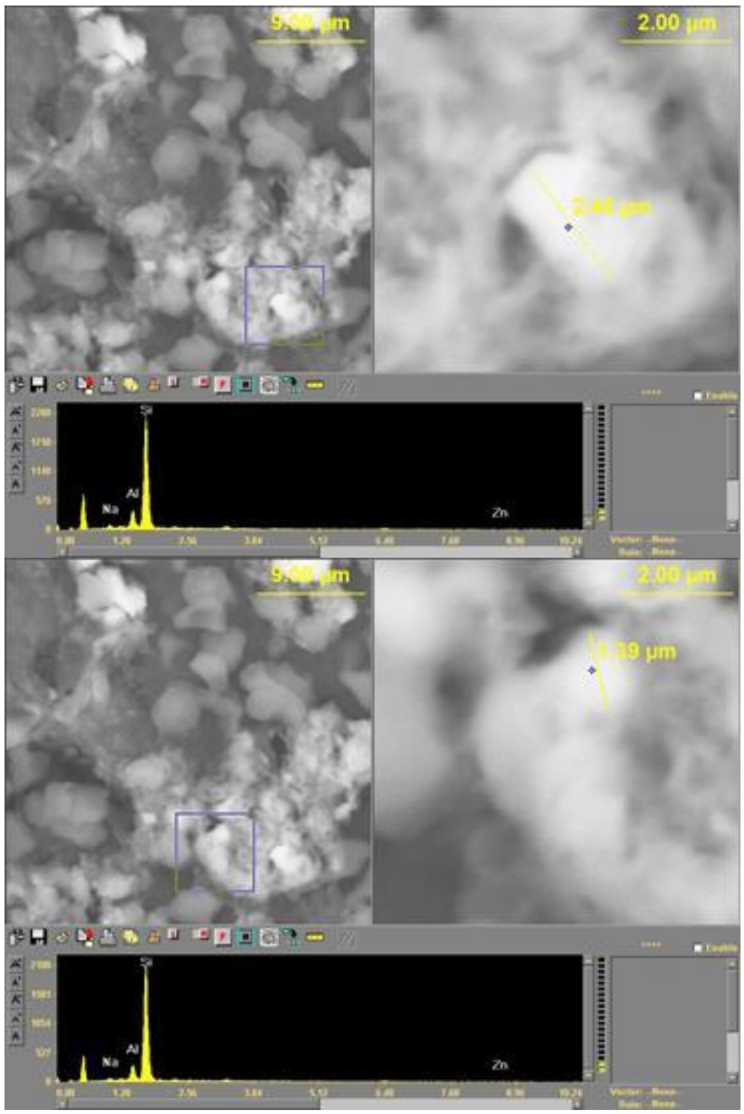

9.00 µm
2.00 µm
1.51 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
Si
Na
Al
Zn

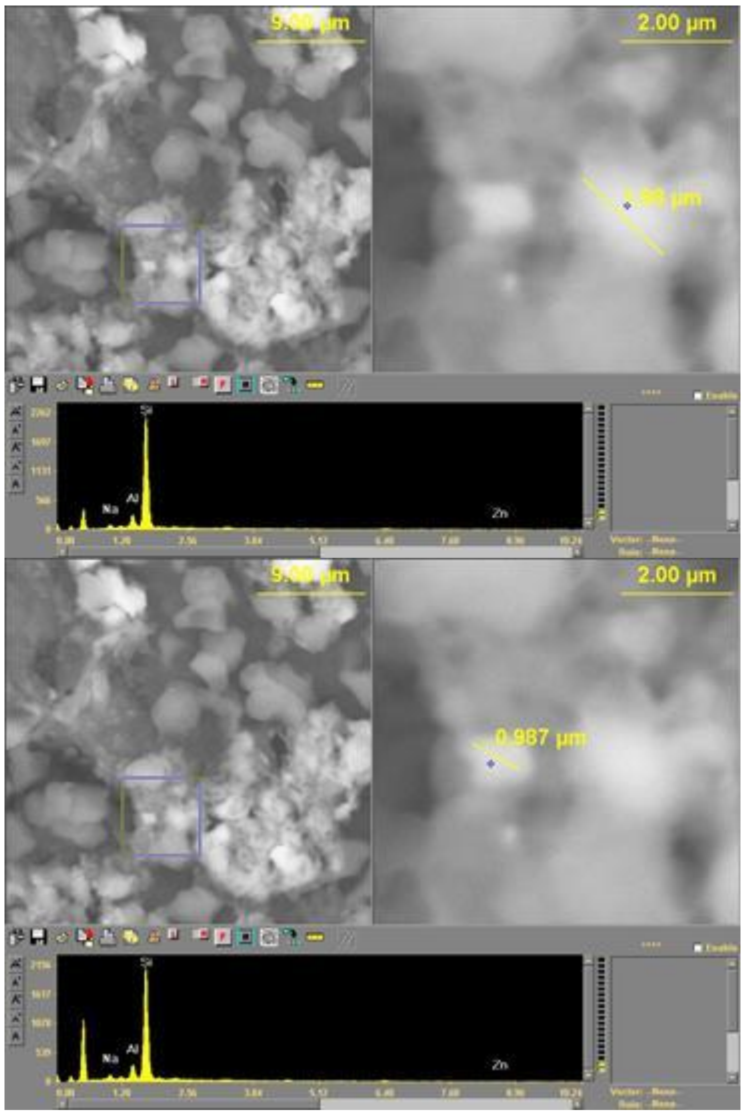

9.00 µm
2.00 µm
Si
Na
Al
Zn
9.00 µm
2.00 µm
1.33 µm
Si
Al
Ca
Na
Ca
Fe
Zn

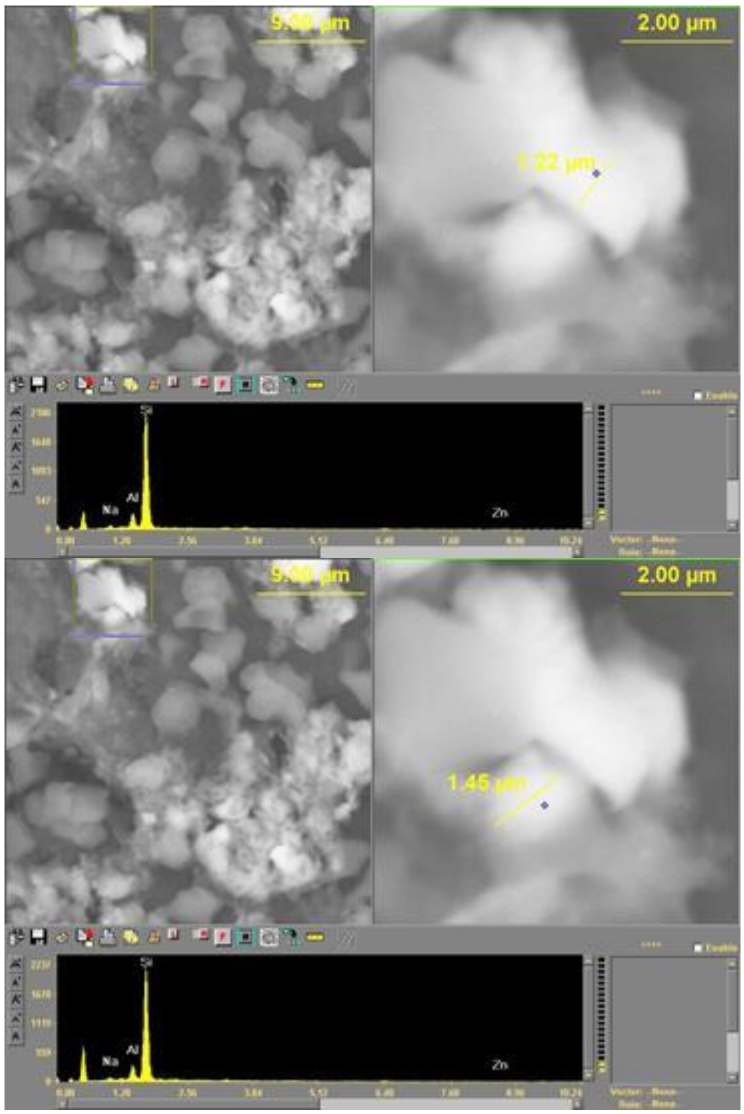

9.00 µm
2.00 µm
1.43 µm
Si
Al
Na
K
Zn
9.00 µm
2.00 µm
Si
Al
Na
Zn

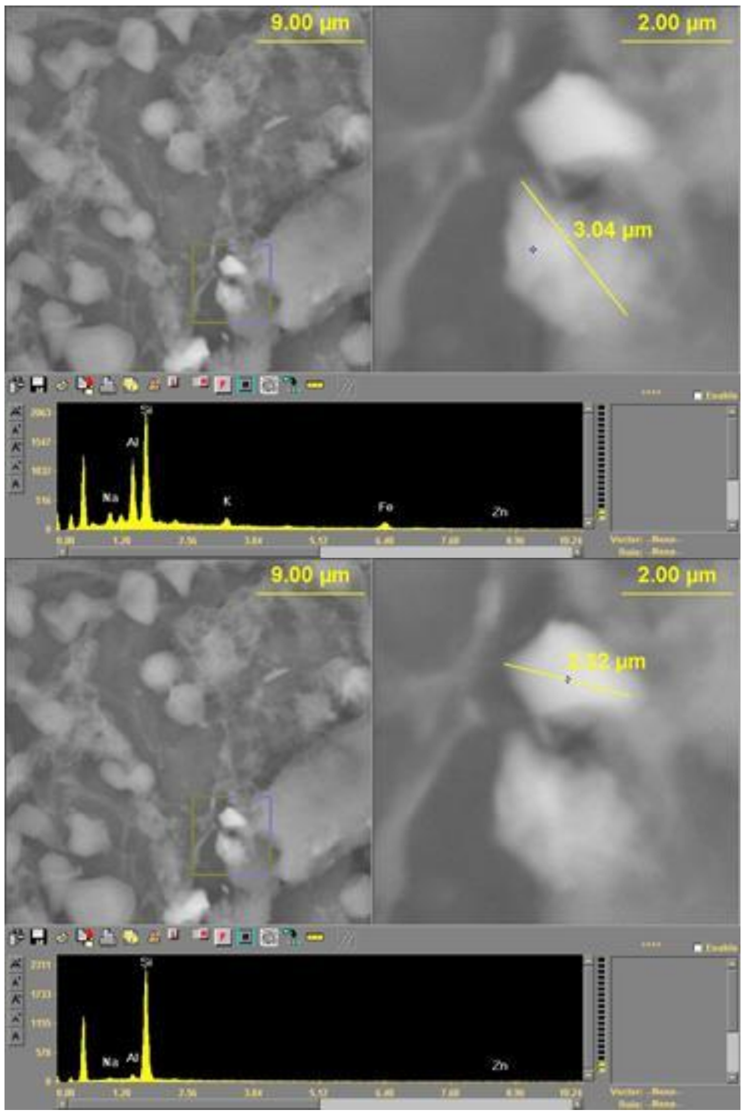

9.00 µm
2.00 µm
Si
Al
Na
Ca
Ca
Ti
Ti
Fe
Zn
9.00 µm
2.00 µm
1.88
Si
Na
Al
Zn


9.00 µm
2.00 µm
2.80 µm
Si
Ca
Ti
Na
Al
Fe
Zn
1.60 µm

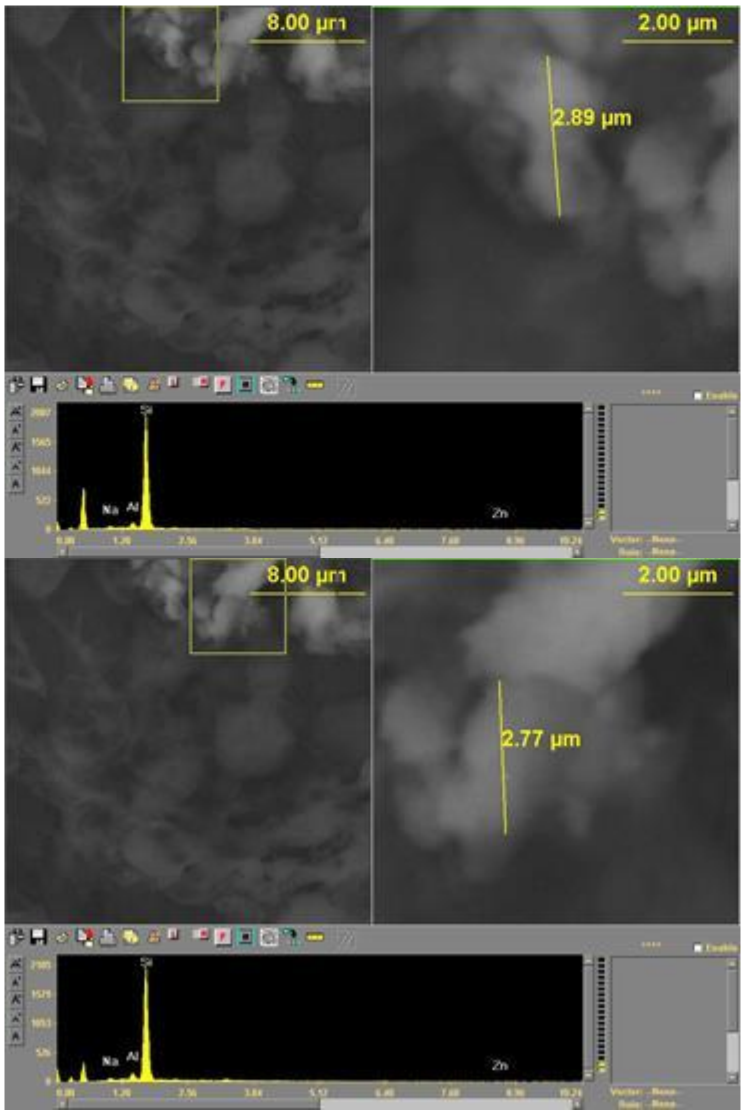

2.00 µm
Si
Al
Na
Zn
2.00 µm
Si
Al
Na
Zn

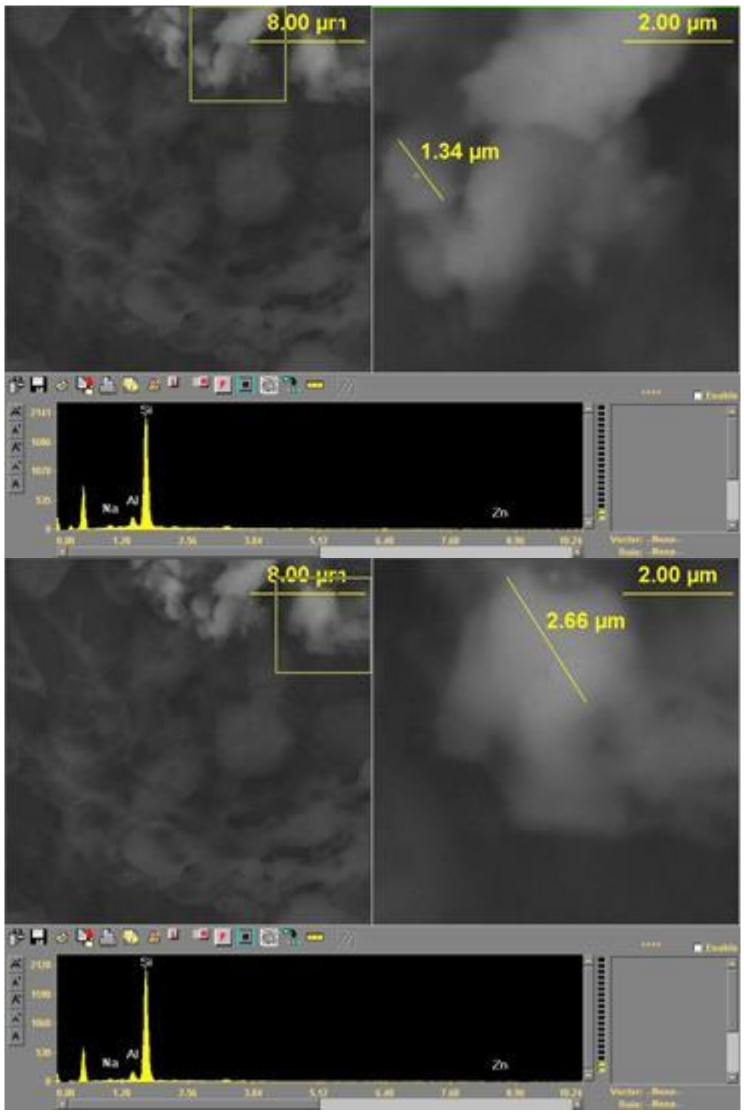

2.00 µm
Si
Na
Al
Zn
0.987 µm

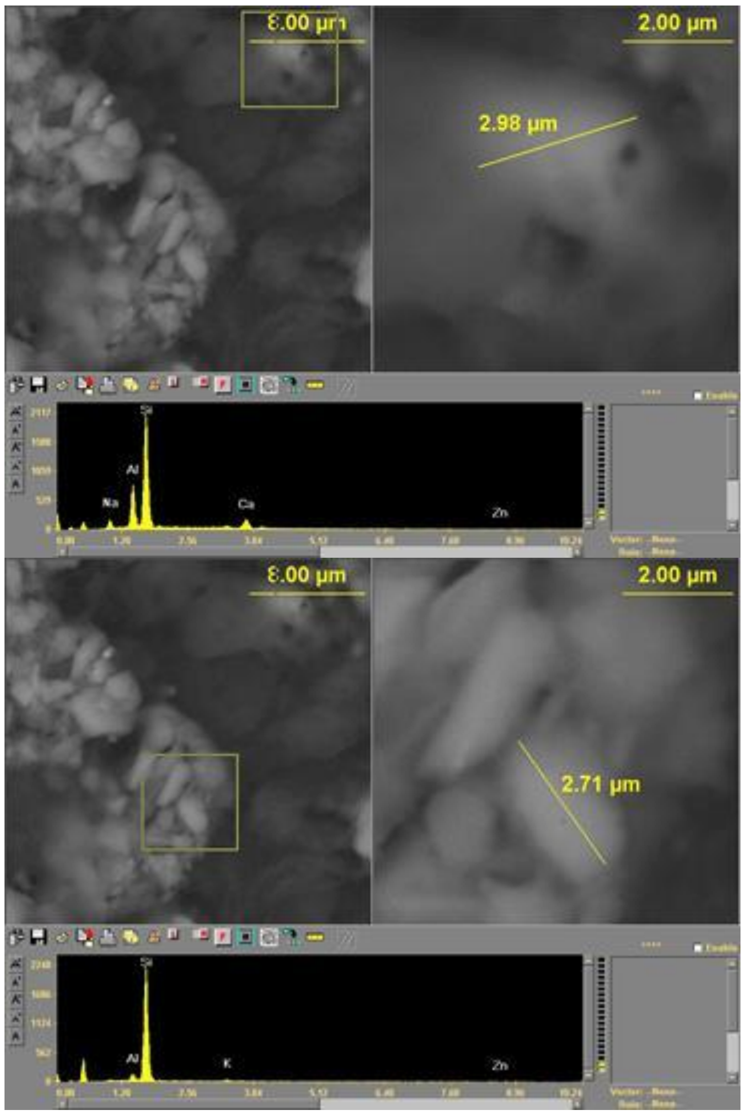

2.00 µm
1.22 µm
Si
Na
Al
Zn
2.00 µm
1.45 µm
Si
Na
Al
Zn

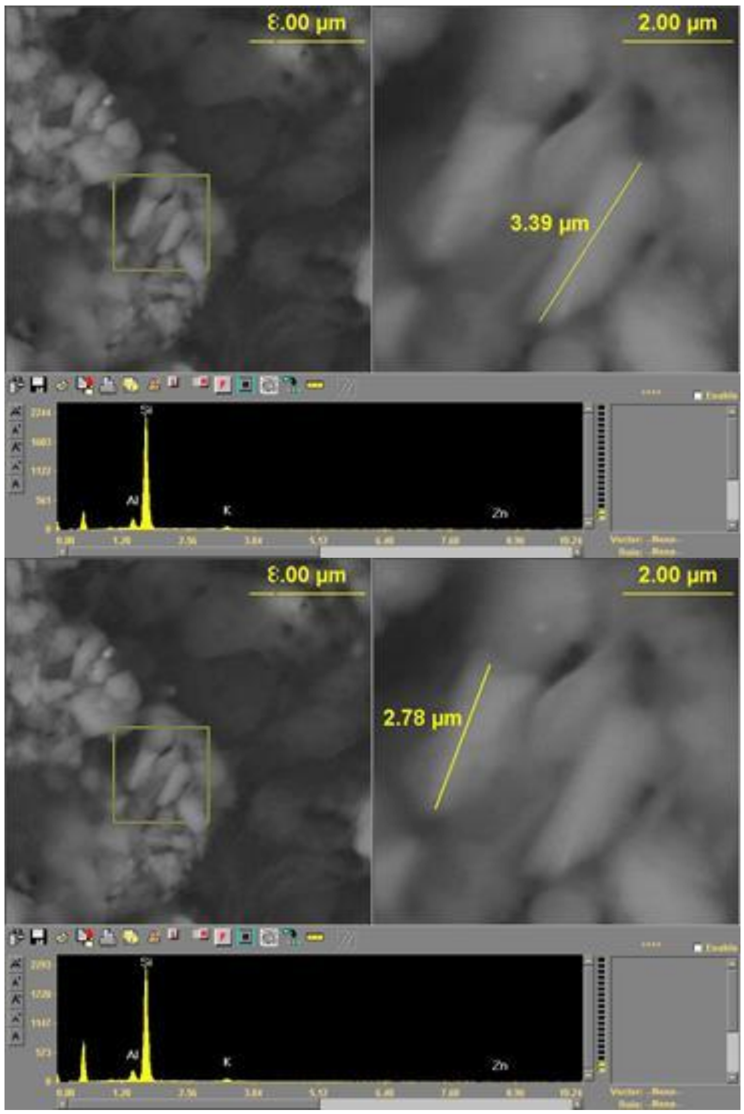

9.00 µm
2.00 µm
3.04 µm
Si
Al
Na
K
Fe
Zn
9.00 µm
2.00 µm
Si
Na
Al
Zn

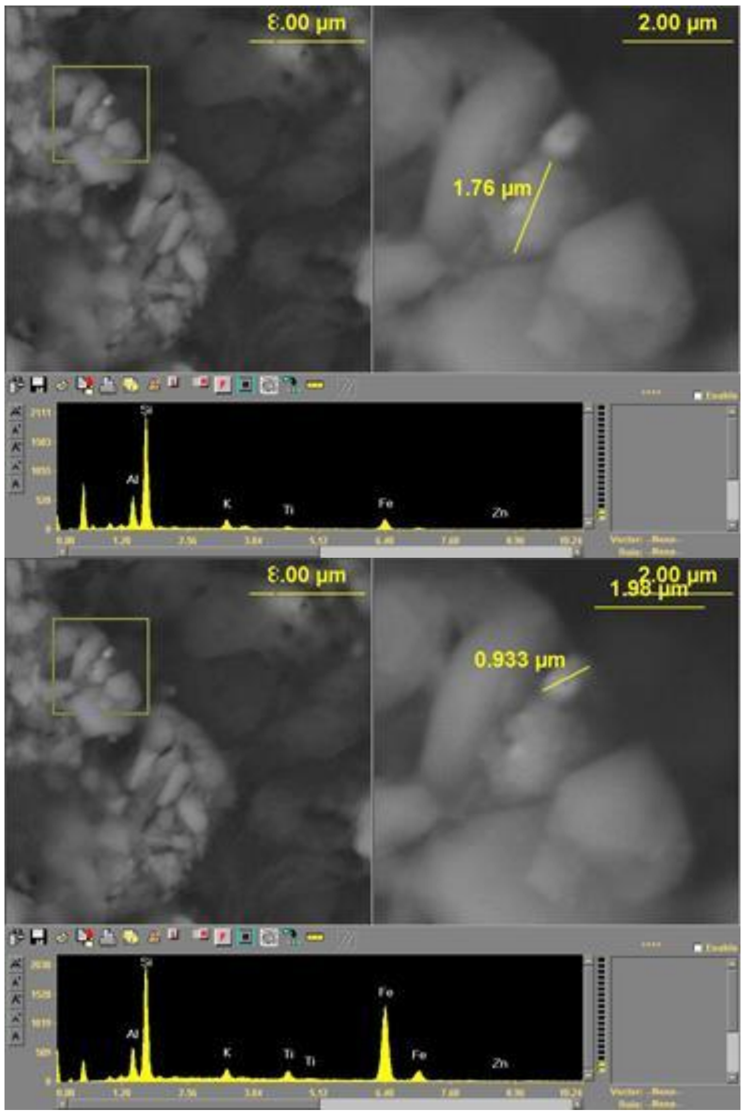
Sample 3A

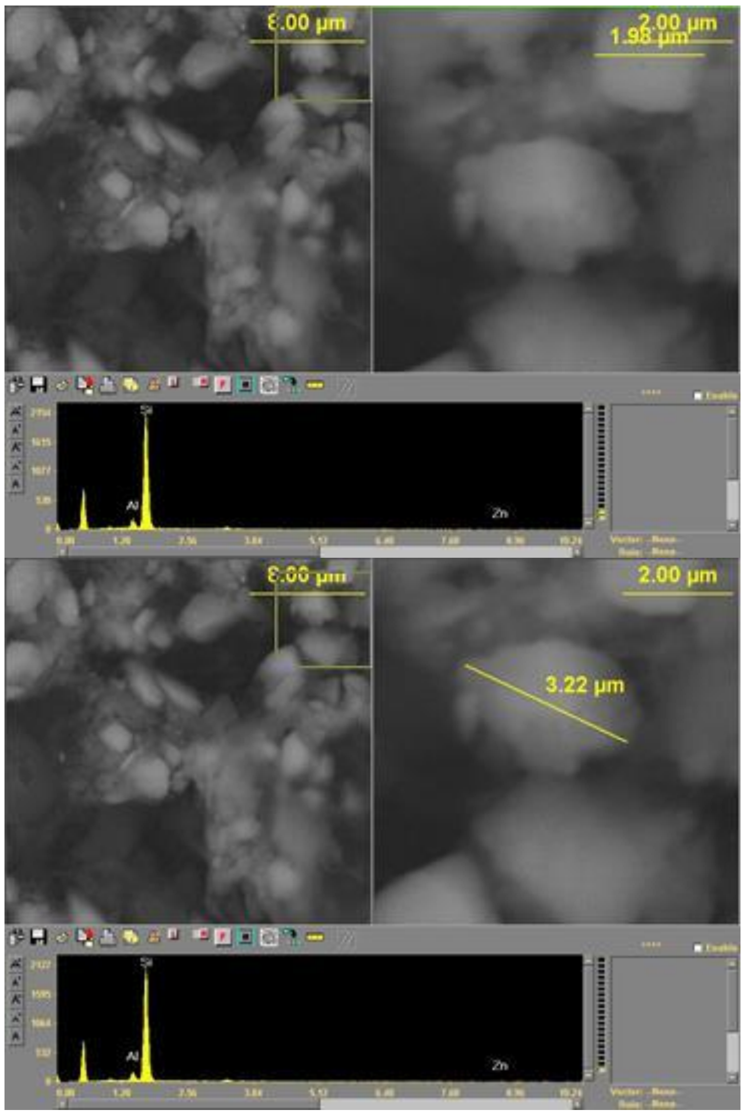


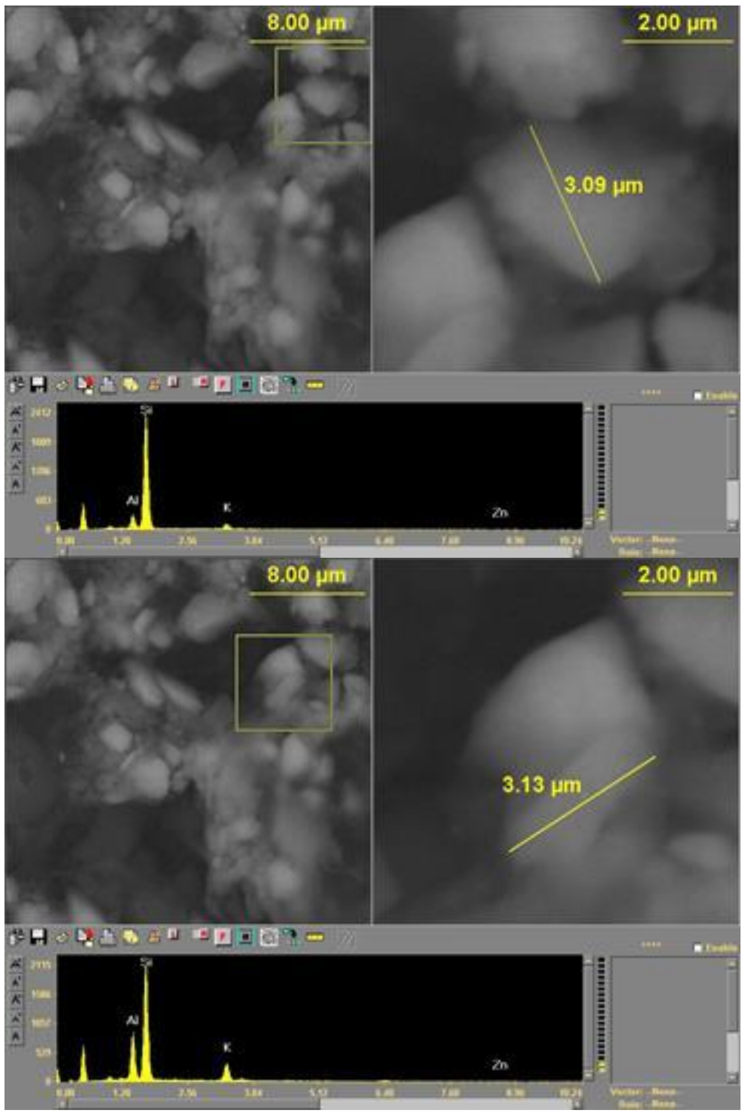

8.00 µm
2.00 µm
1.34 µm
Si
Na
Al
Zn
8.00 µm
2.00 µm
2.66 µm
Si
Na
Al
Zn

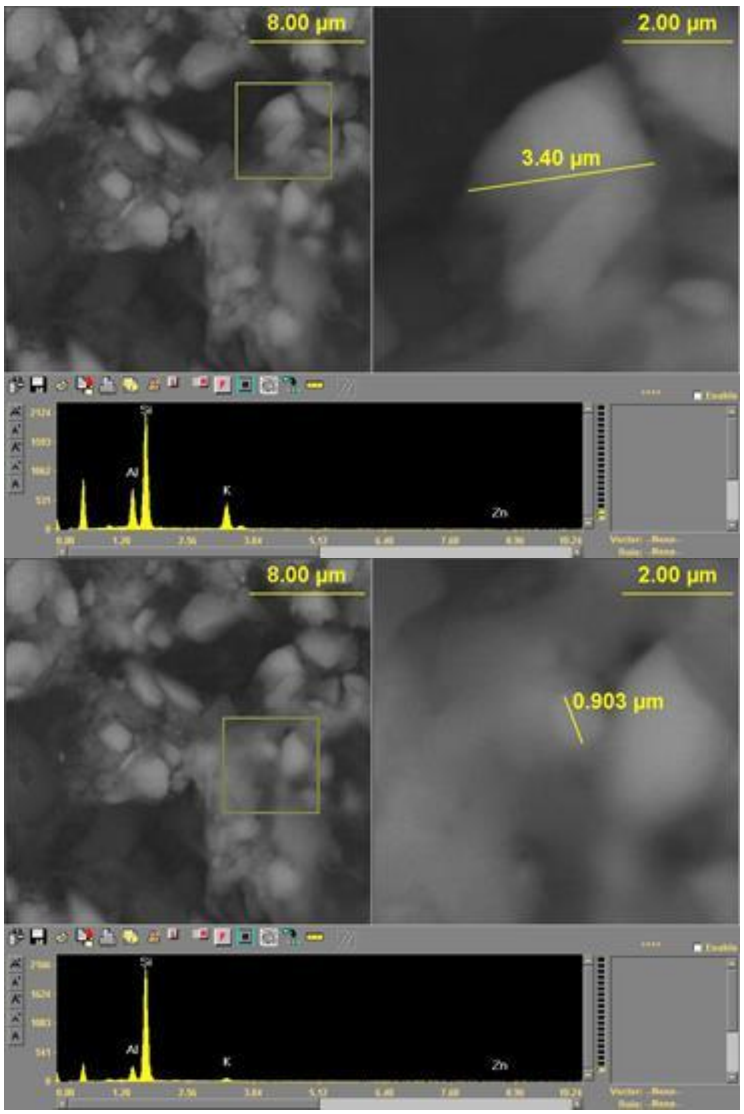

2.00 µm
2.98 µm
Si
Al
Na
Ca
Zn
2.00 µm
2.71 µm
Si
Al
K
Zn

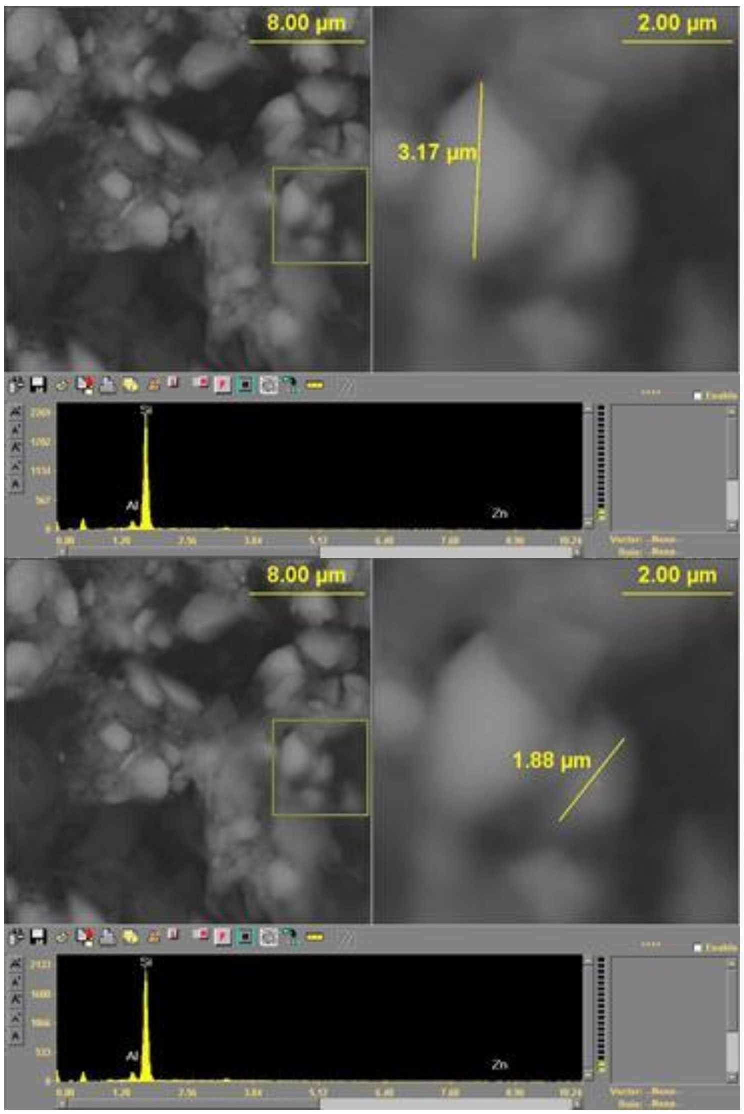

8.00 µm
2.00 µm
3.39 µm
Si
Al
K
Zn
8.00 µm
2.00 µm
2.78 µm
Si
Al
K
Zn

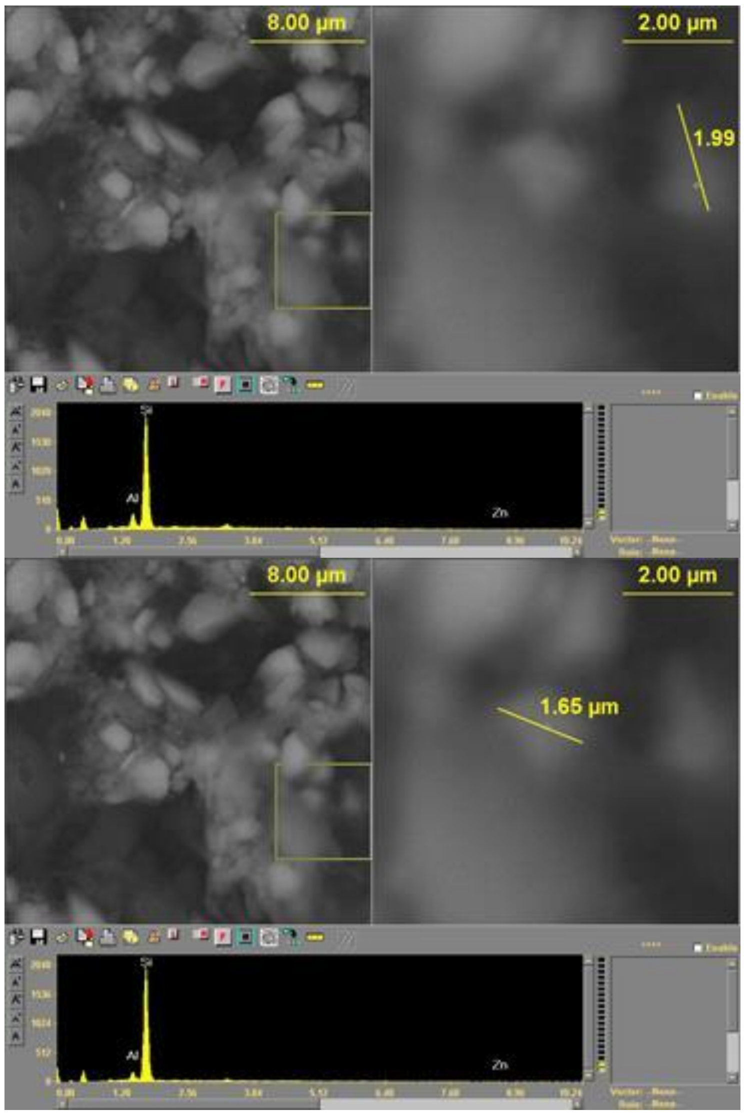

8.00 µm
2.00 µm
1.76 µm
Si
Al
K
Ti
Fe
Zn
8.00 µm
2.00 µm
1.98 µm
0.933 µm
Si
Al
K
Ti
Ti
Fe
Fe
Zn

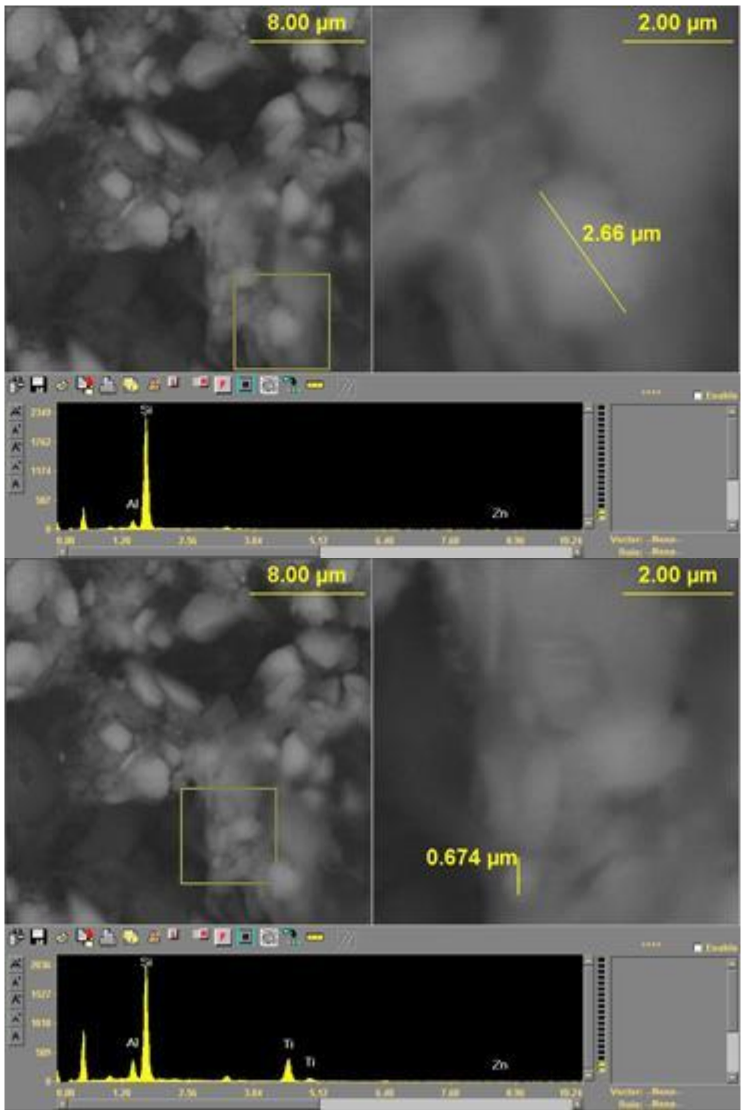

8.00 µm
2.00 µm
1.98 µm
Si
Al
Zn
8.00 µm
2.00 µm
3.22 µm
Si
Al
Zn

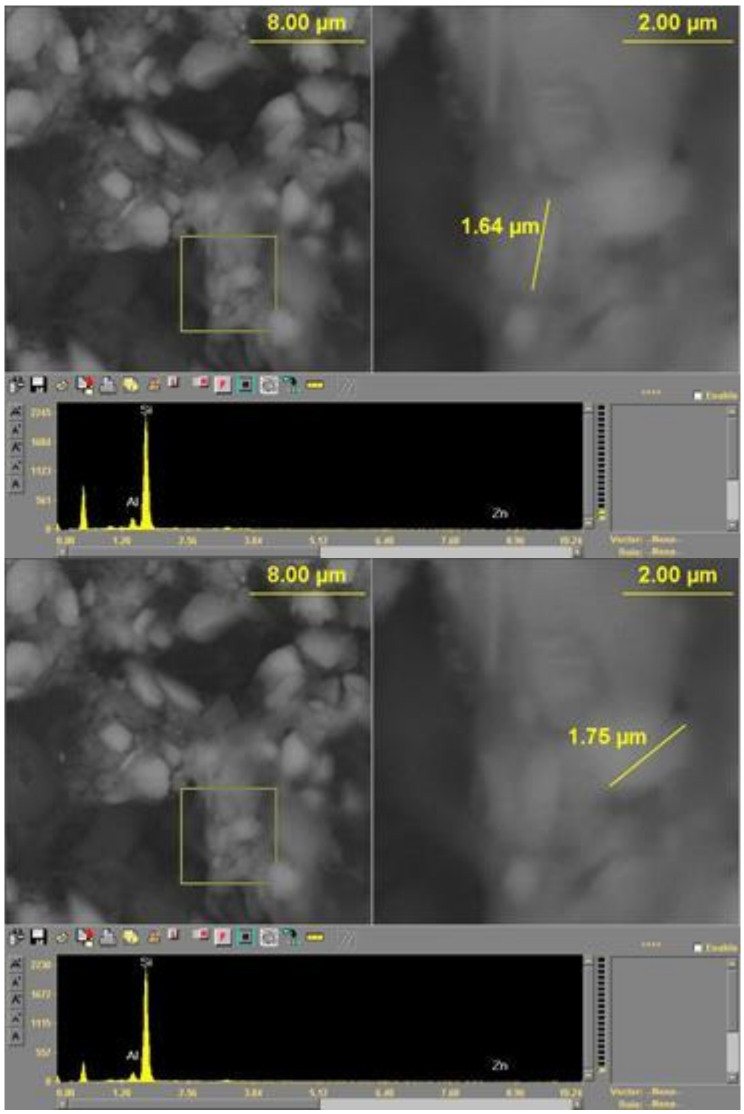

8.00 µm
2.00 µm
3.09 µm
Si
Al
K
Zn
8.00 µm
2.00 µm
3.13 µm
Si
Al
K
Zn


8.00 µm
2.00 µm
3.40 µm
Si
Al
K
Zn
8.00 µm
2.00 µm
0.903 µm
Si
Al
K
Zn

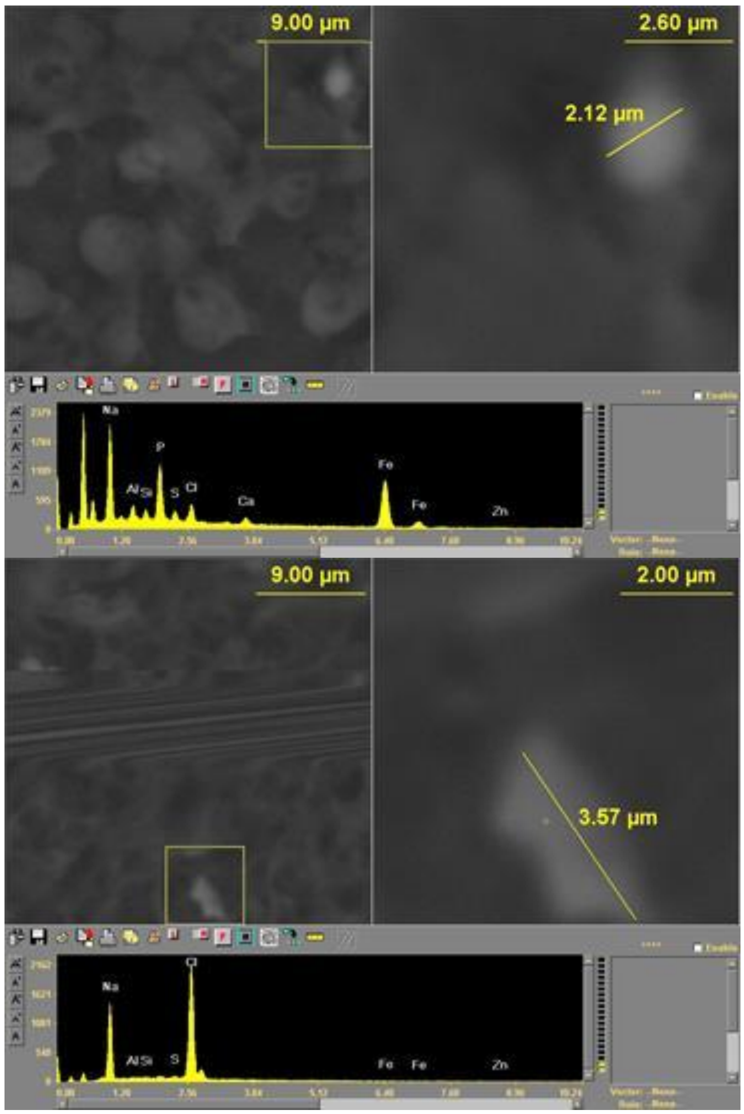

8.00 µm
2.00 µm
3.17 µm
Si
Al
Zn
8.00 µm
2.00 µm
1.88 µm
Si
Al
Zn

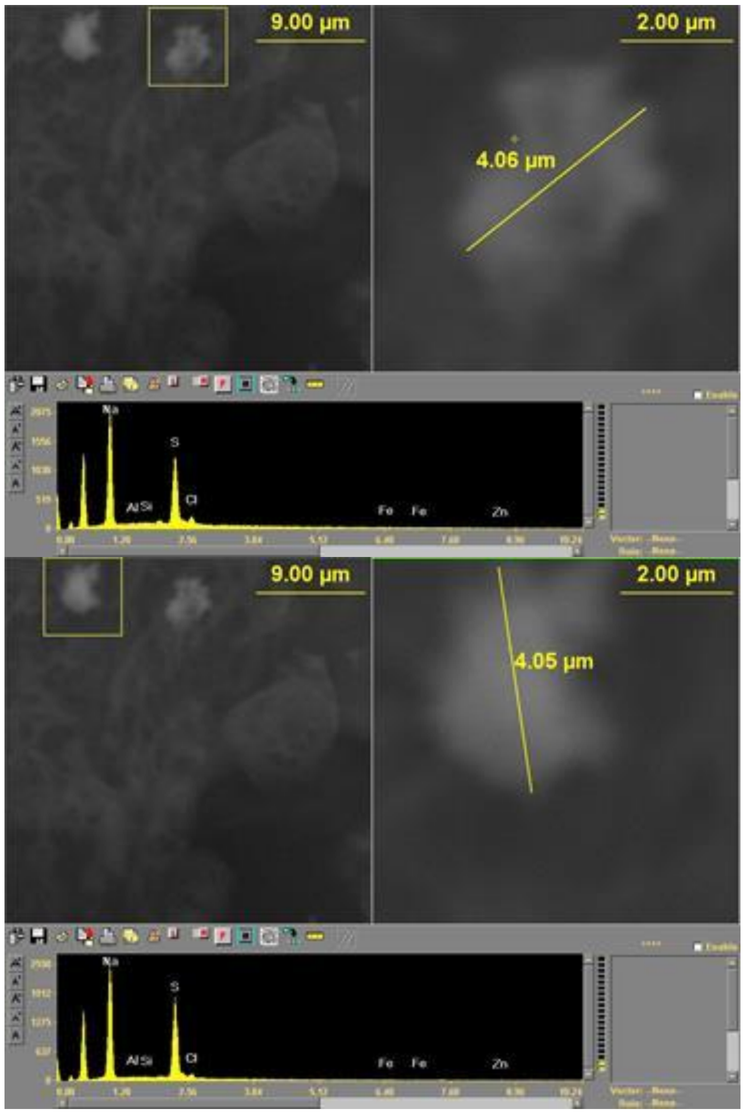

8.00 µm
2.00 µm
1.99
Si
Al
Zn
8.00 µm
2.00 µm
1.65 µm
Si
Al
Zn


8.00 µm
2.00 µm
2.66 µm
Si
Al
Zn
8.00 µm
2.00 µm
0.674 µm
Si
Al
Ti
Ti
Zn

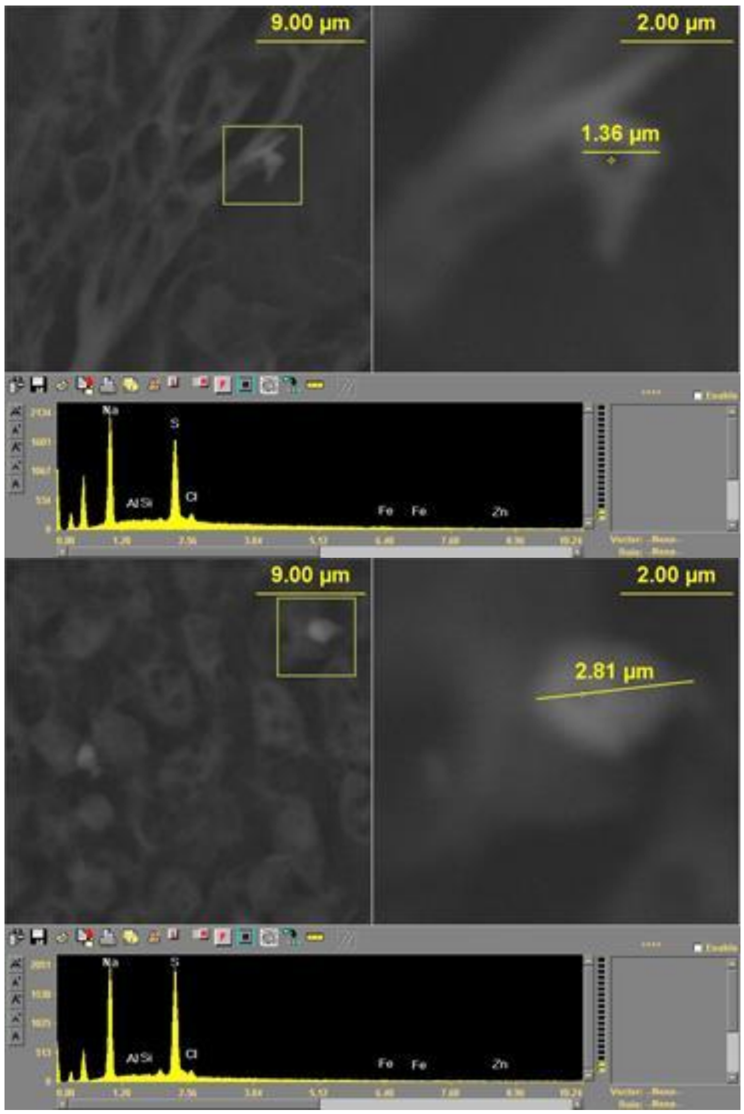

8.00 µm
2.00 µm
1.64 µm
Si
Al
Zn
8.00 µm
2.00 µm
1.75 µm
Si
Al
Zn

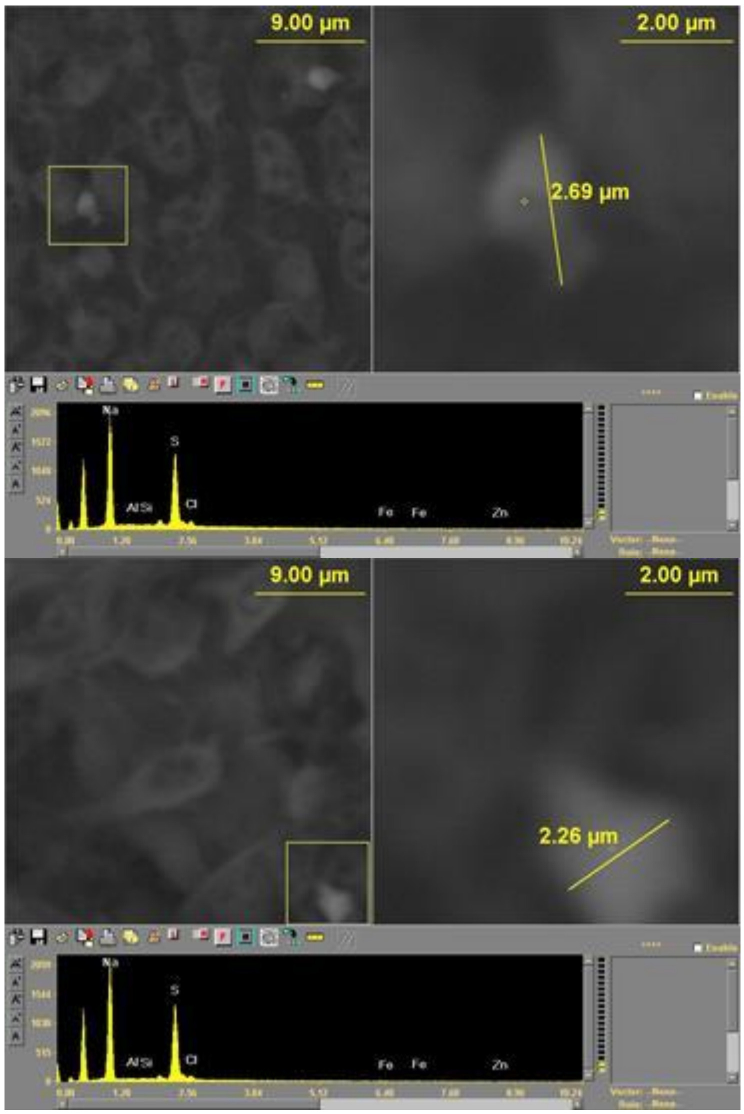
Sample 3B

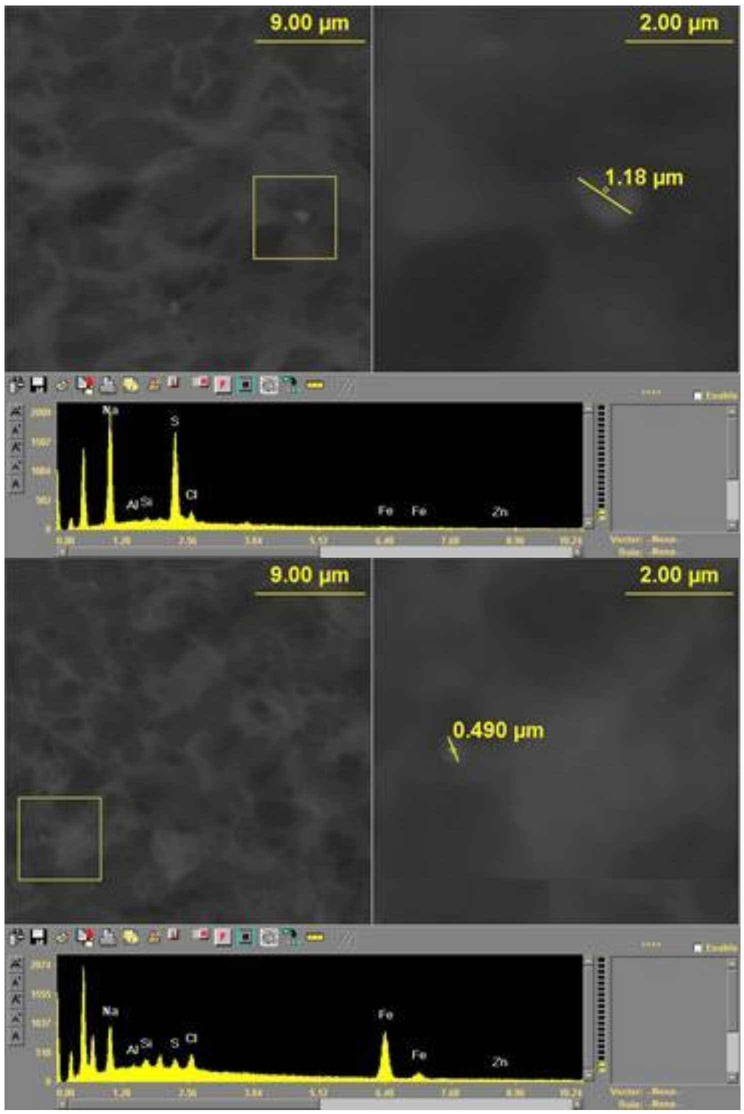

9.00 µm
2.60 µm
2.12 µm
Na
P
Al Si
S Cl
Ca
Fe
Fe
Zn
9.00 µm
2.00 µm
3.57 µm
Cl
Na
Al Si
S
Fe Fe
Zn

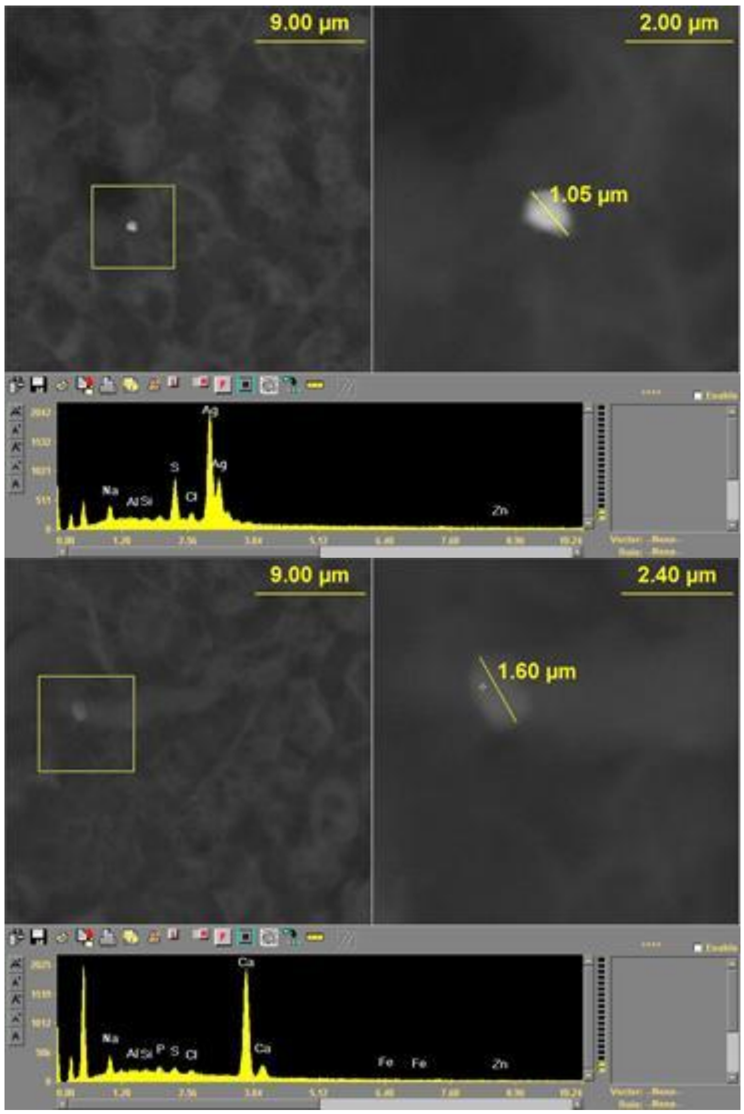


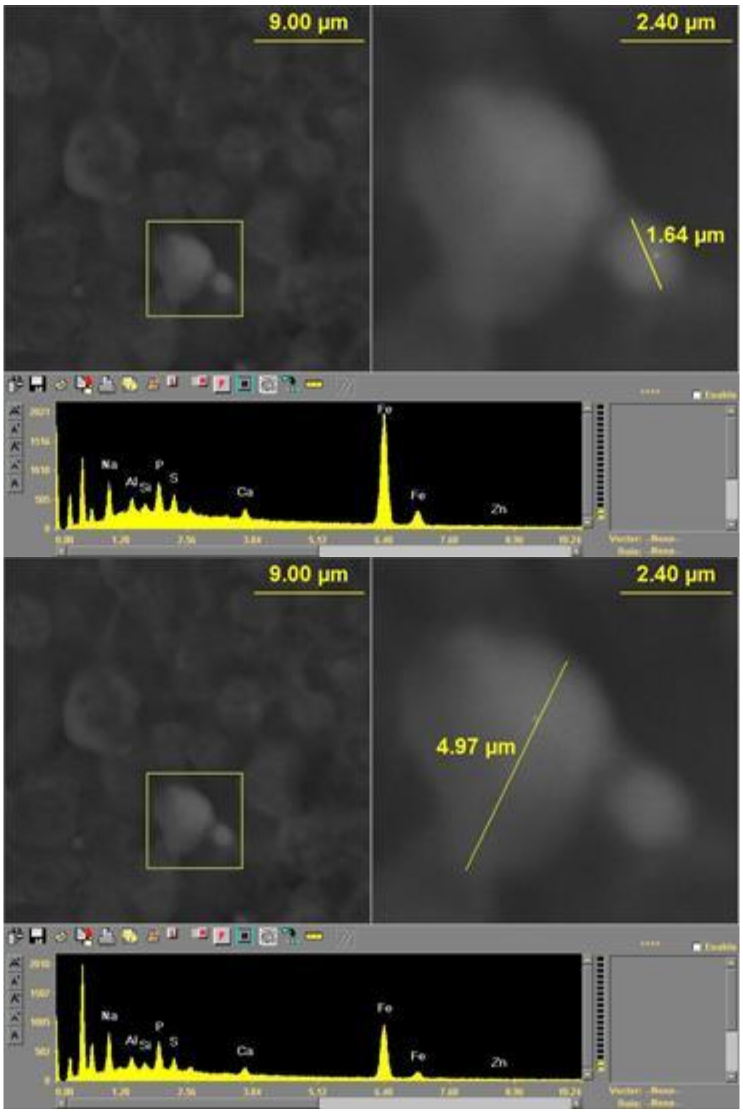
9.00 µm
2.00 µm
4.06 µm
Na
S
Al Si
Cl
Fe Fe
Zn
9.00 µm
2.00 µm
4.05 µm
Na
S
Al Si
Cl
Fe Fe
Zn


9.00 µm
2.00 µm
1.36 µm
Na
S
Al Si
Cl
Fe Fe
Zn
9.00 µm
2.00 µm
2.81 µm
Na
S
Al Si
Cl
Fe Fe
Zn

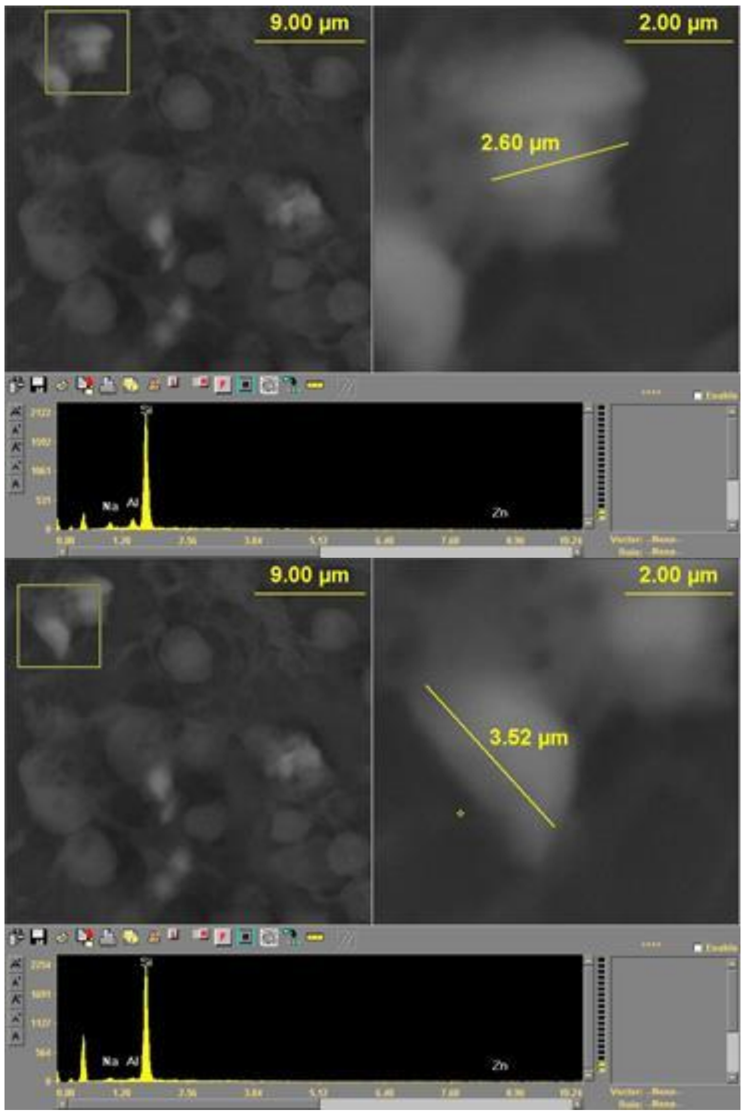

9.00 µm
2.00 µm
2.69 µm
Na
S
Al Si
Cl
Fe
Fe
Zn
9.00 µm
2.00 µm
2.26 µm
Na
S
Al Si
Cl
Fe
Fe
Zn

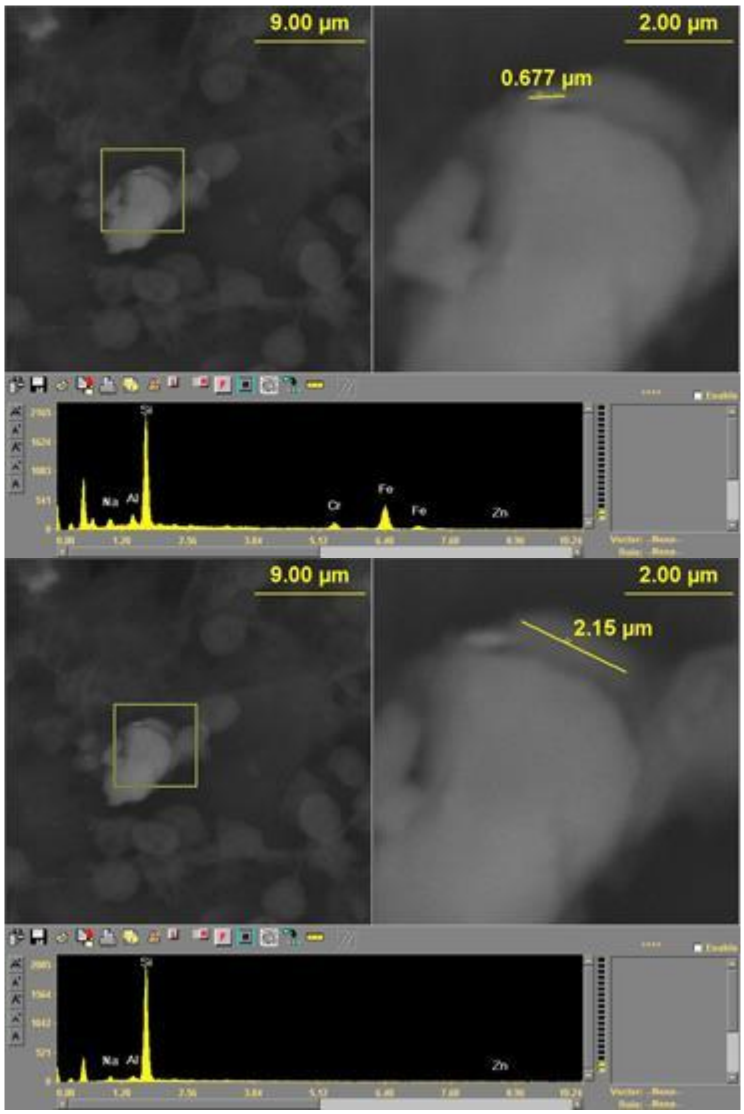

9.00 µm
2.00 µm
1.18 µm
Na
S
Al
Si
Cl
Fe
Fe
Zn
9.00 µm
2.00 µm
0.490 µm
Na
Al
Si
S
Cl
Fe
Fe
Zn

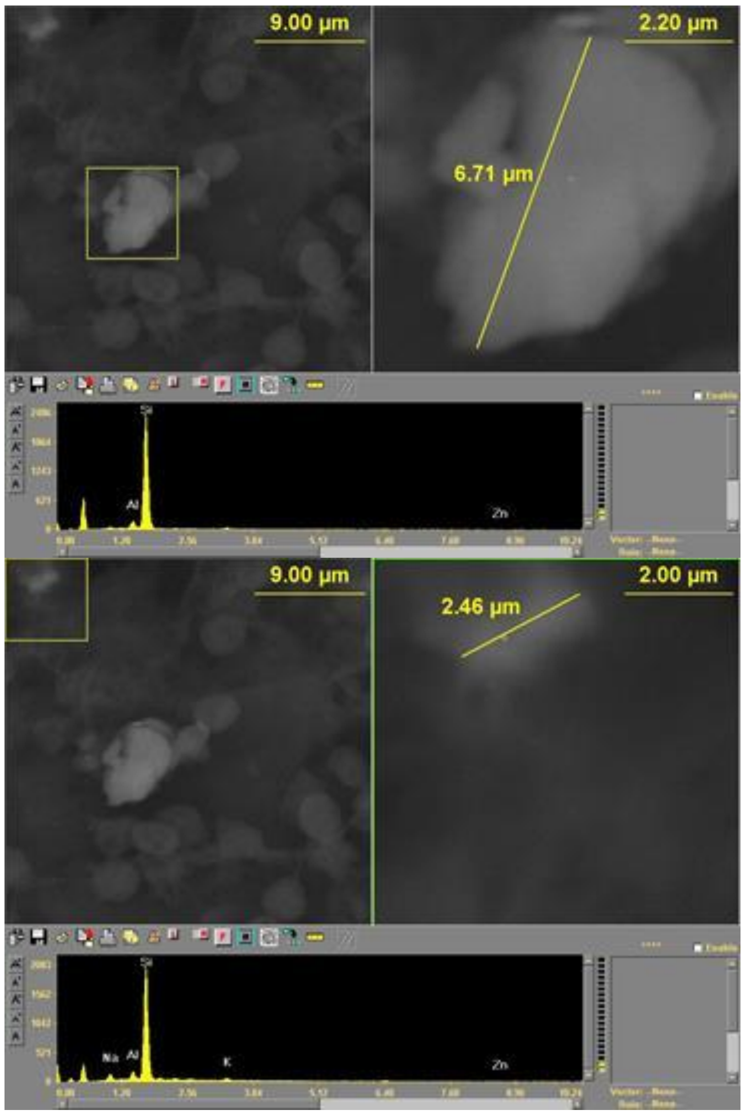

9.00 µm
2.00 µm
1.05 µm
Ag
S
Ag
Na
Al Si
Cl
Zn
9.00 µm
2.40 µm
1.60 µm
Ca
Na
Al Si P S Cl
Ca
Fe Fe
Zn

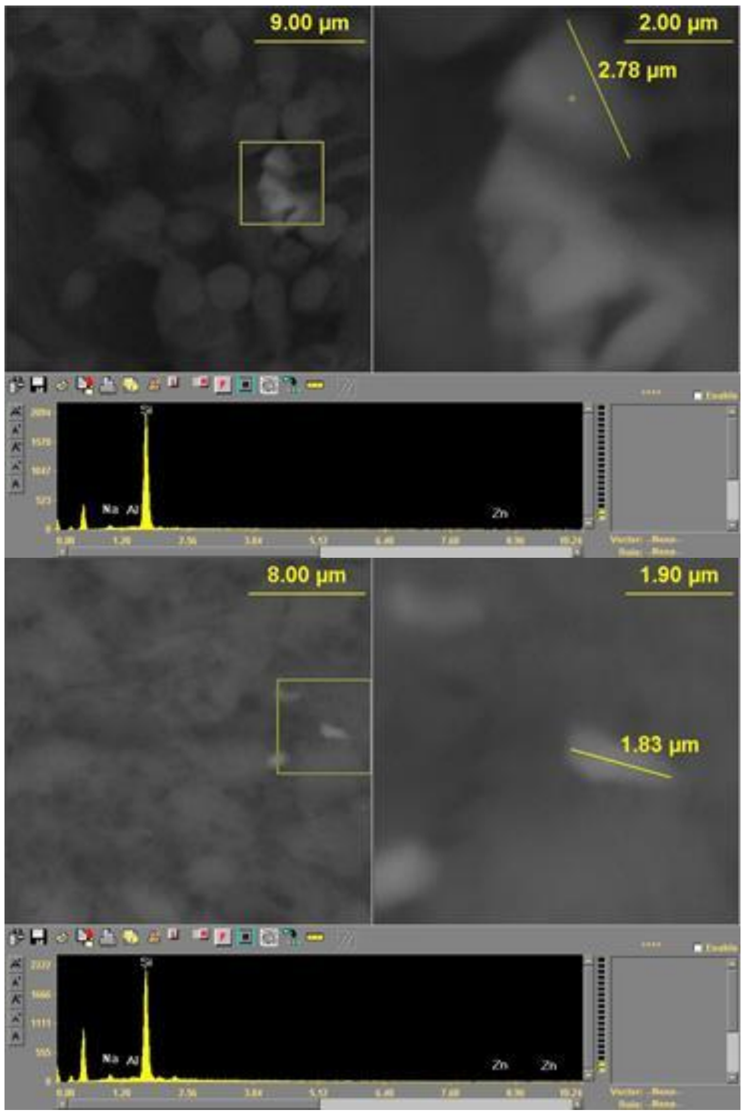

9.00 µm
2.40 µm
1.64 µm
Fe
Na
Al
Si
P
S
Ca
Fe
Zn
9.00 µm
2.40 µm
4.97 µm
Na
Al
Si
P
S
Ca
Fe
Fe
Zn

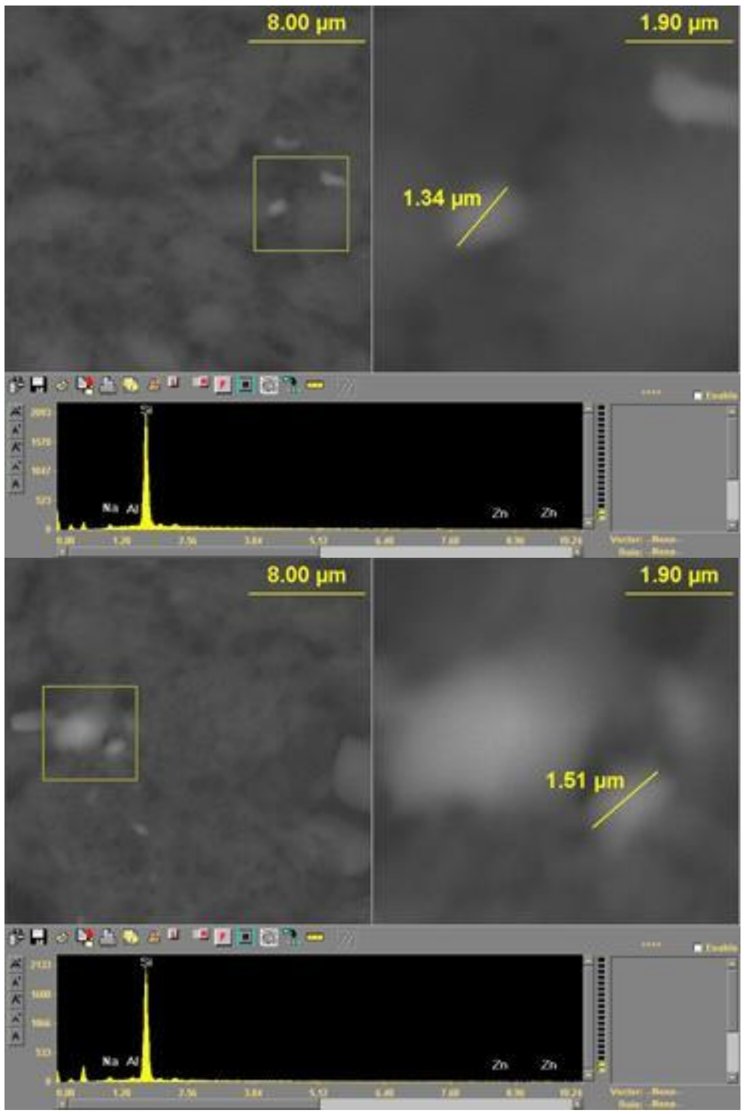
Sample 4A

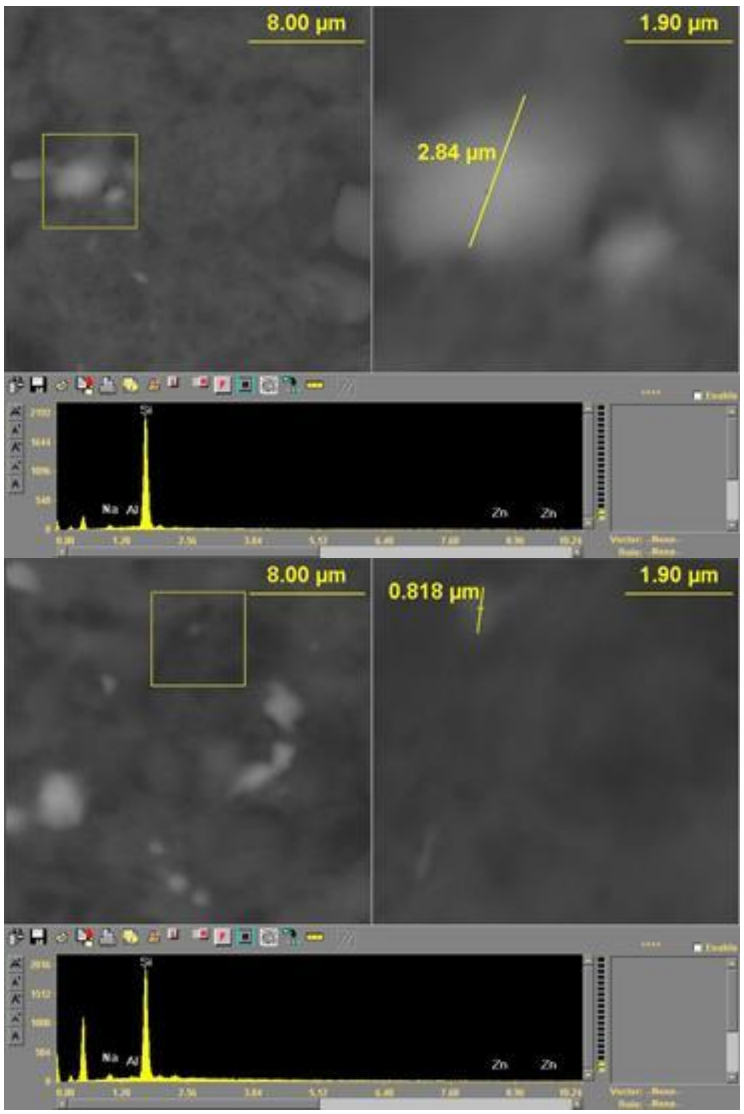


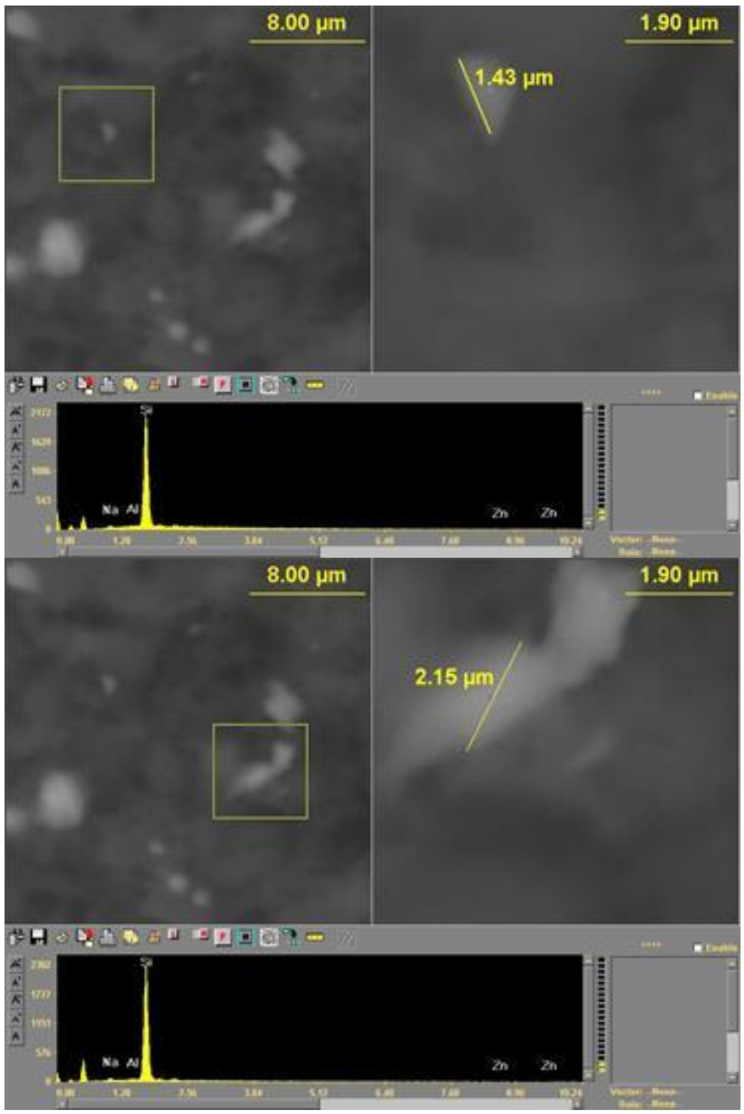

9.00 µm
2.00 µm
0.677 µm
Si
Na
Al
Cr
Fe
Fe
Zn
9.00 µm
2.00 µm
2.15 µm
Si
Na
Al
Zn

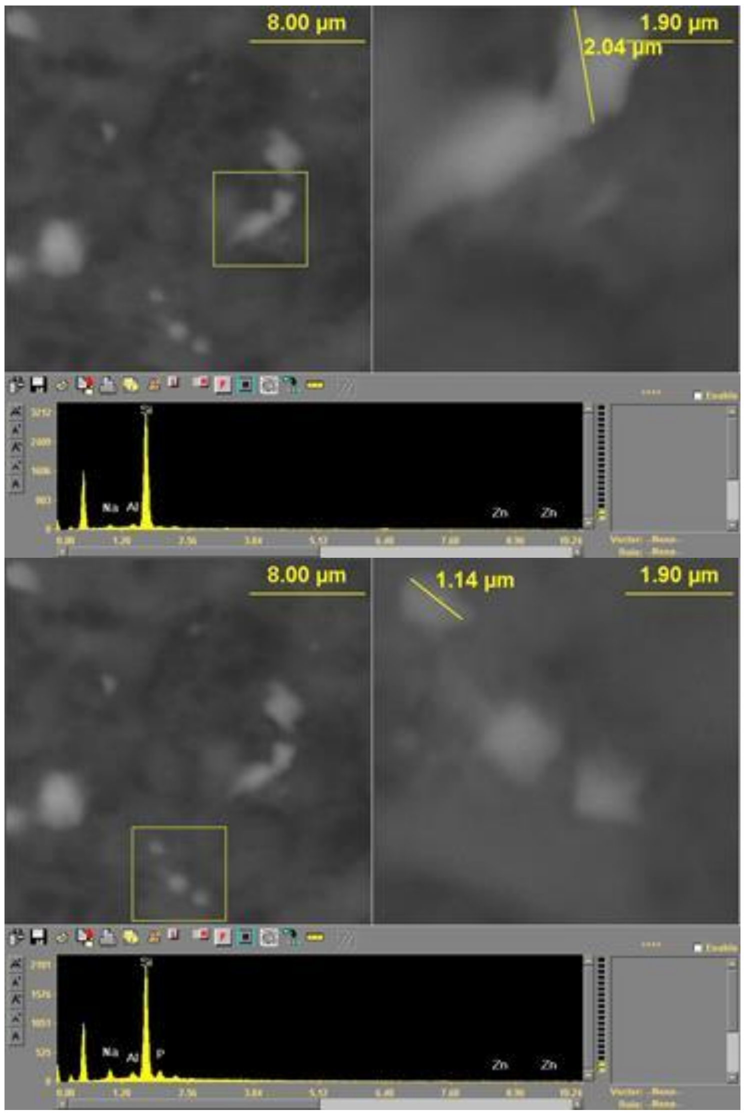

9.00 µm
2.20 µm
6.71 µm
Si
Al
Zn
9.00 µm
2.00 µm
2.46 µm
Si
Na
Al
K
Zn

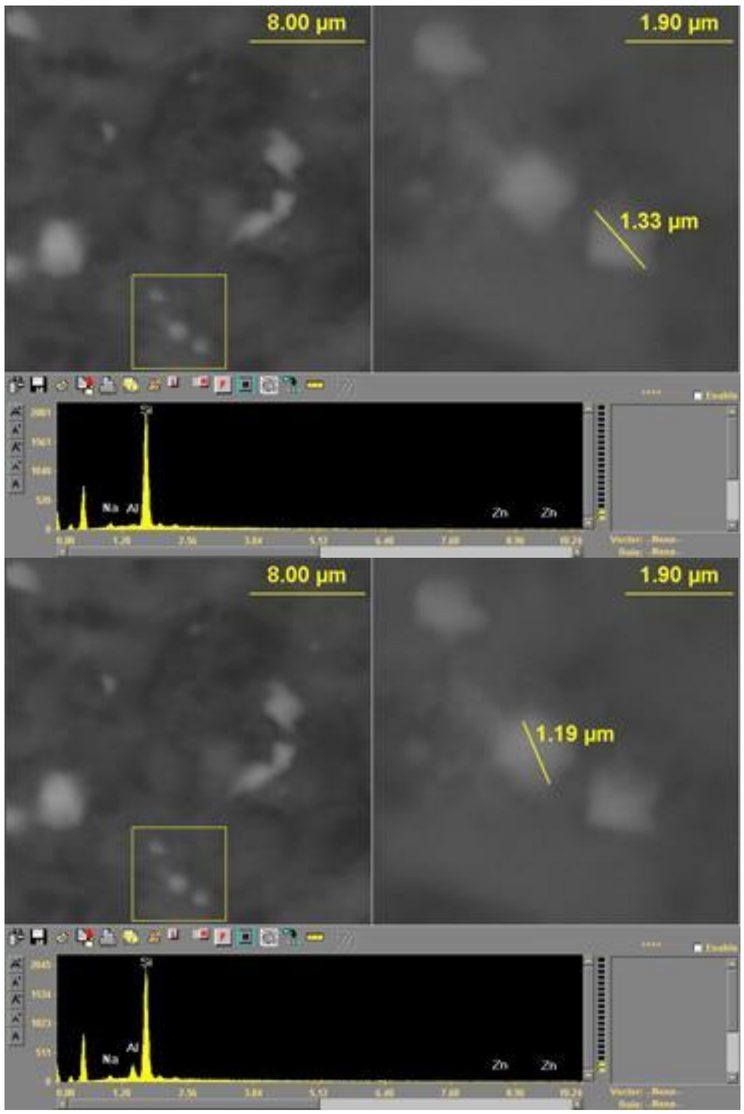

9.00 µm
2.00 µm
2.78 µm
Si
Na
Al
Zn
8.00 µm
1.90 µm
1.83 µm
Si
Na
Al
Zn
Zn

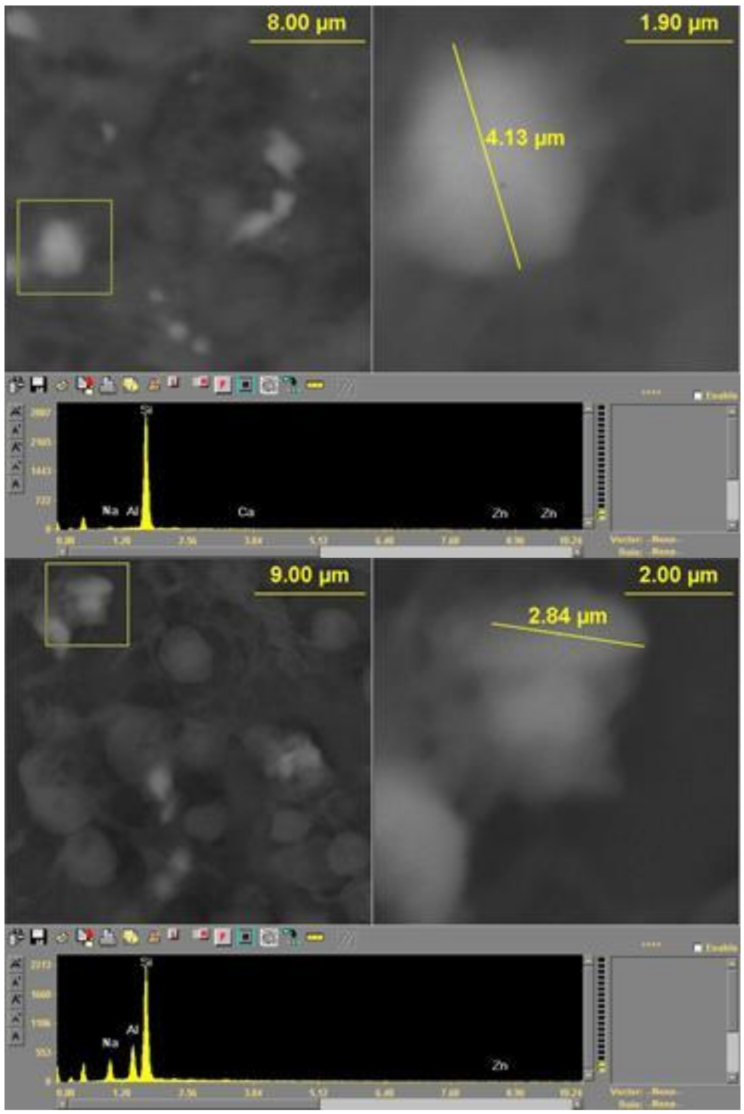

8.00 µm
1.90 µm
1.34 µm
Si
Na
Al
Zn
Zn
8.00 µm
1.90 µm
1.51 µm
Si
Na
Al
Zn
Zn


8.00 µm
1.90 µm
2.84 µm
Si
Na
Al
Zn
Zn
8.00 µm
0.818 µm
1.90 µm
Si
Na
Al
Zn
Zn

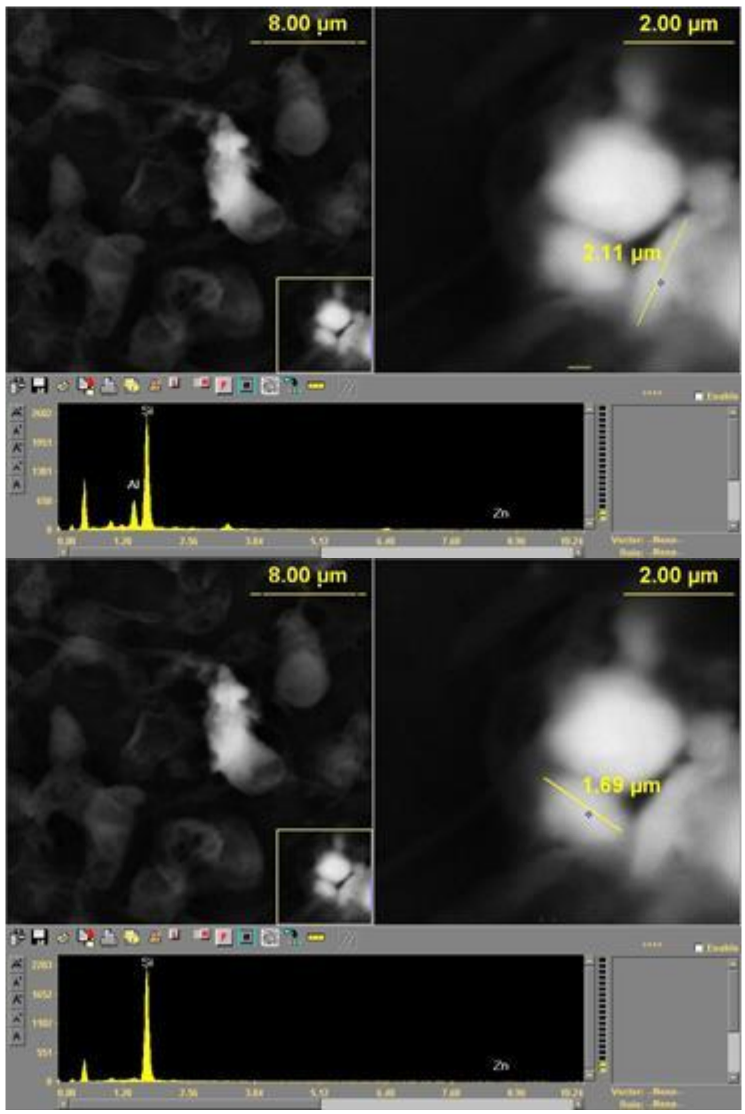

8.00 µm
1.90 µm
1.43 µm
Si
Na
Al
Zn
Zn
8.00 µm
1.90 µm
2.15 µm
Si
Na
Al
Zn
Zn

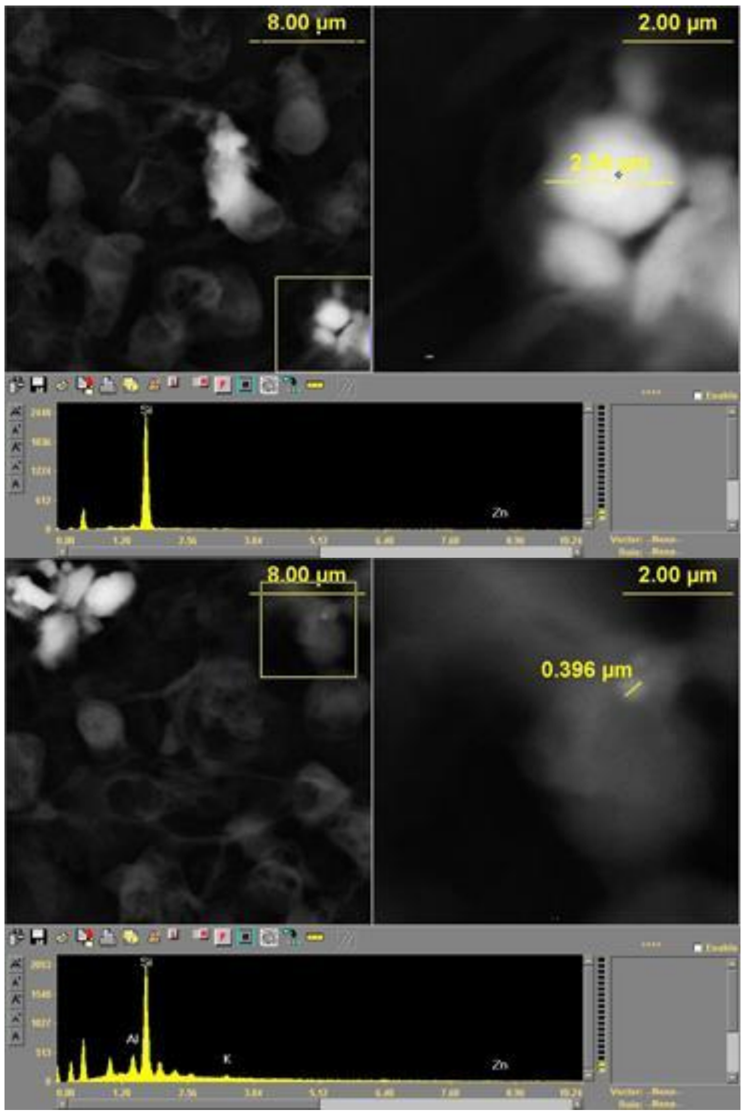

8.00 µm
1.90 µm
2.04 µm
Si
Na
Al
Zn
Zn
8.00 µm
1.14 µm
1.90 µm
Si
Na
Al
P
Zn
Zn

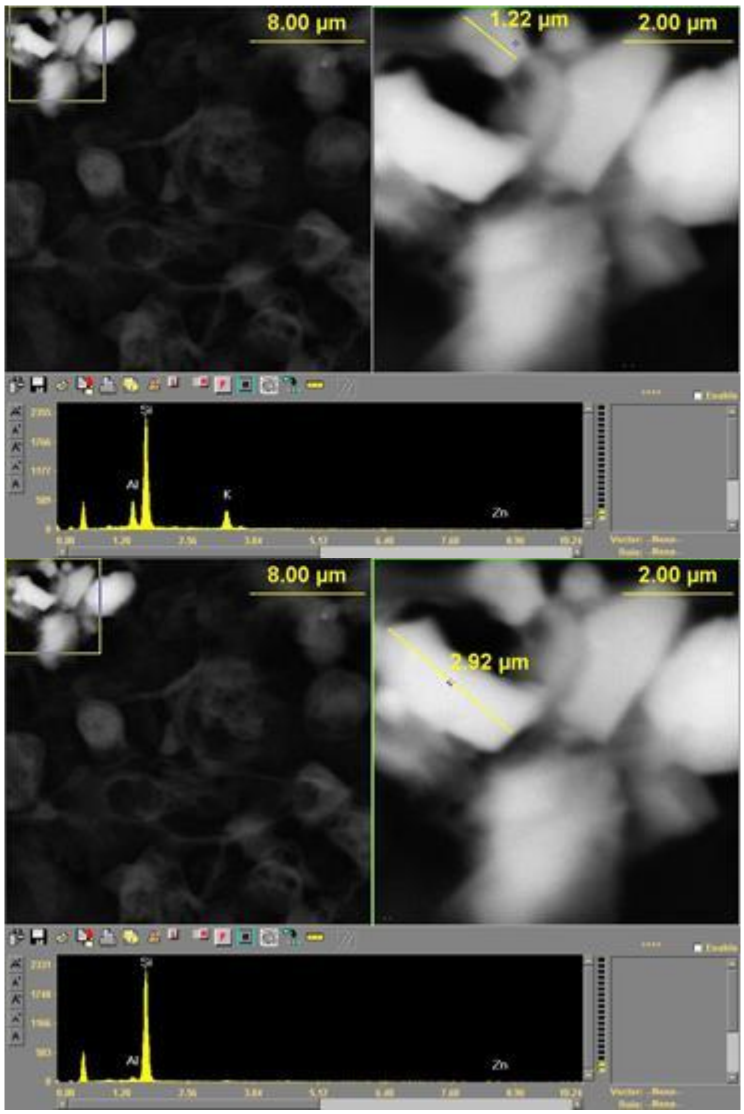

8.00 µm
1.90 µm
1.33 µm
Si
Na
Al
Zn
Zn
8.00 µm
1.90 µm
1.19 µm
Si
Na
Al
Zn
Zn

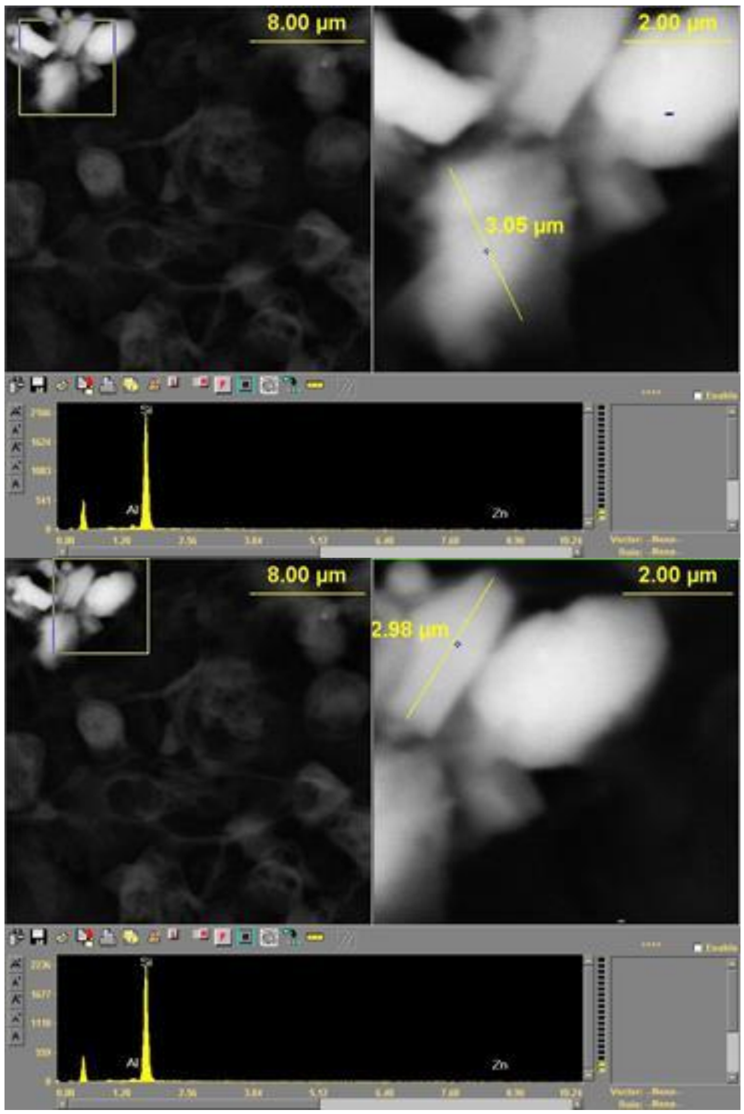

8.00 µm
1.90 µm
4.13 µm
Si
Na
Al
Ca
Zn
Zn
9.00 µm
2.00 µm
2.84 µm
Si
Al
Na
Zn

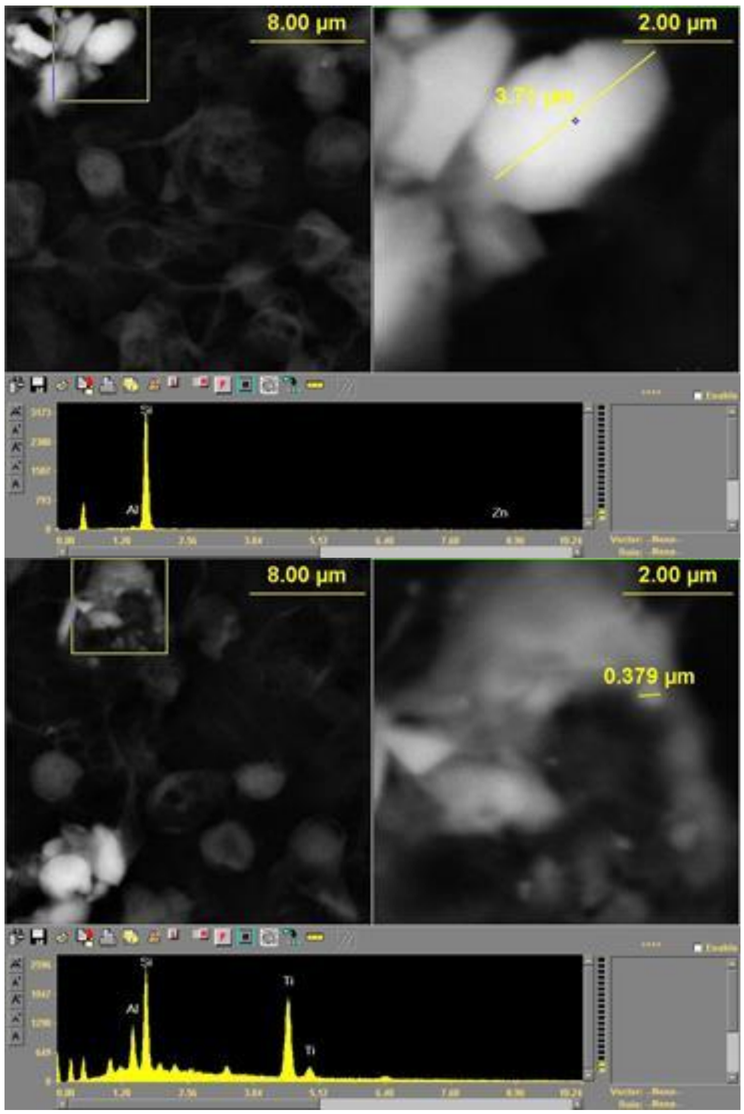
Sample 4B

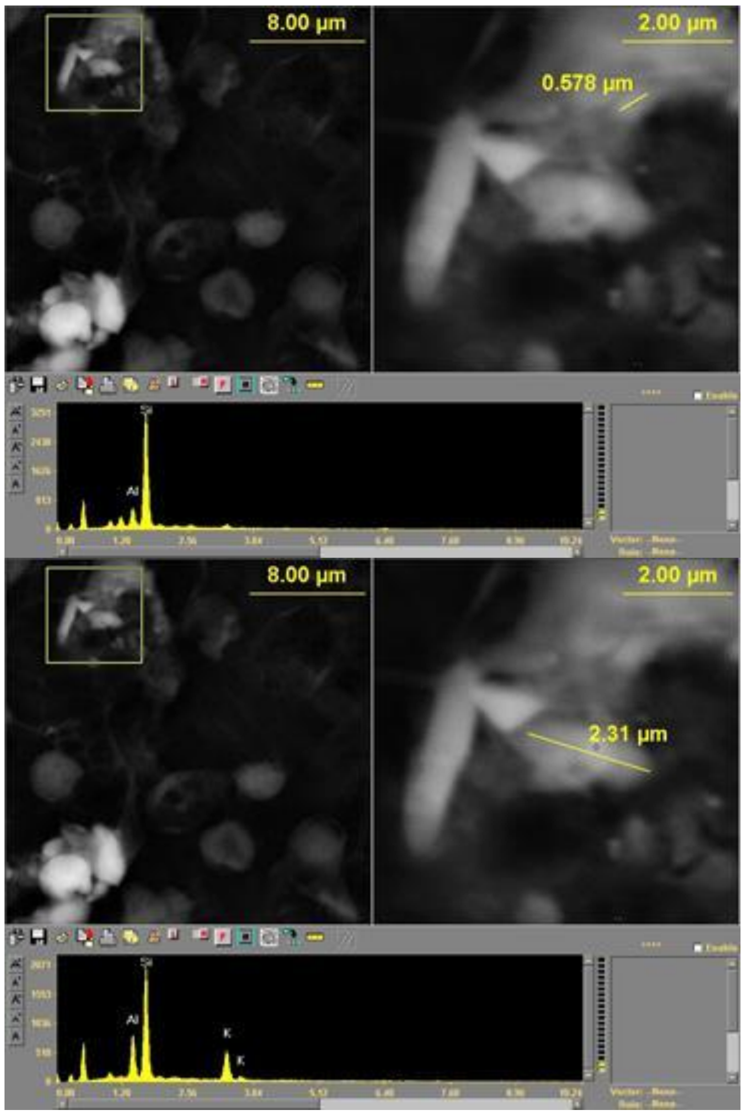


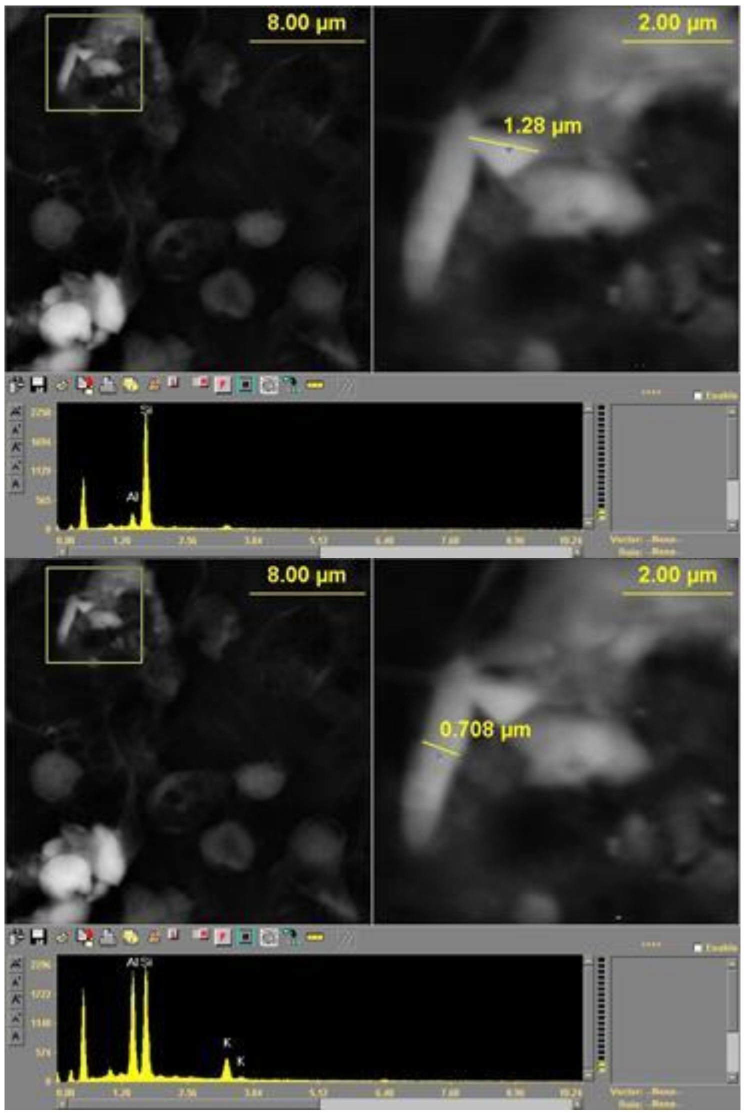

8.00 µm
2.00 µm
Si
Zn
8.00 µm
2.00 µm
0.396 µm
Si
Al
K
Zn

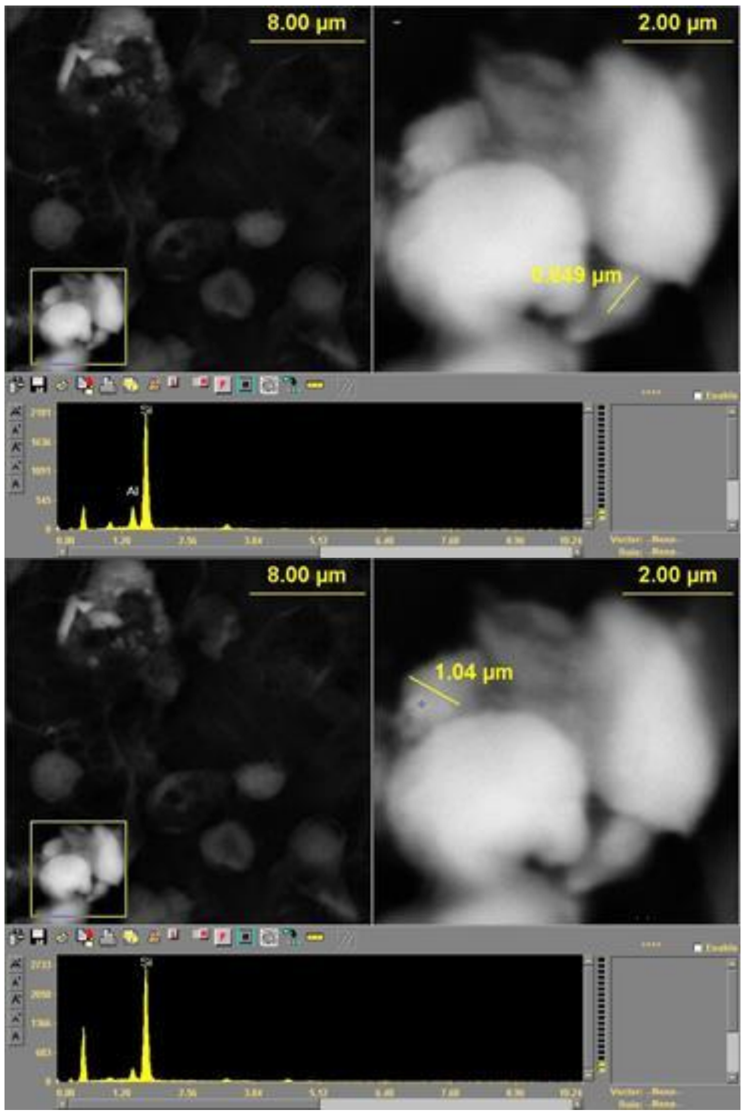

8.00 µm
1.22 µm
2.00 µm
Si
Al
K
Zn
8.00 µm
2.00 µm
2.92 µm
Si
Al
Zn

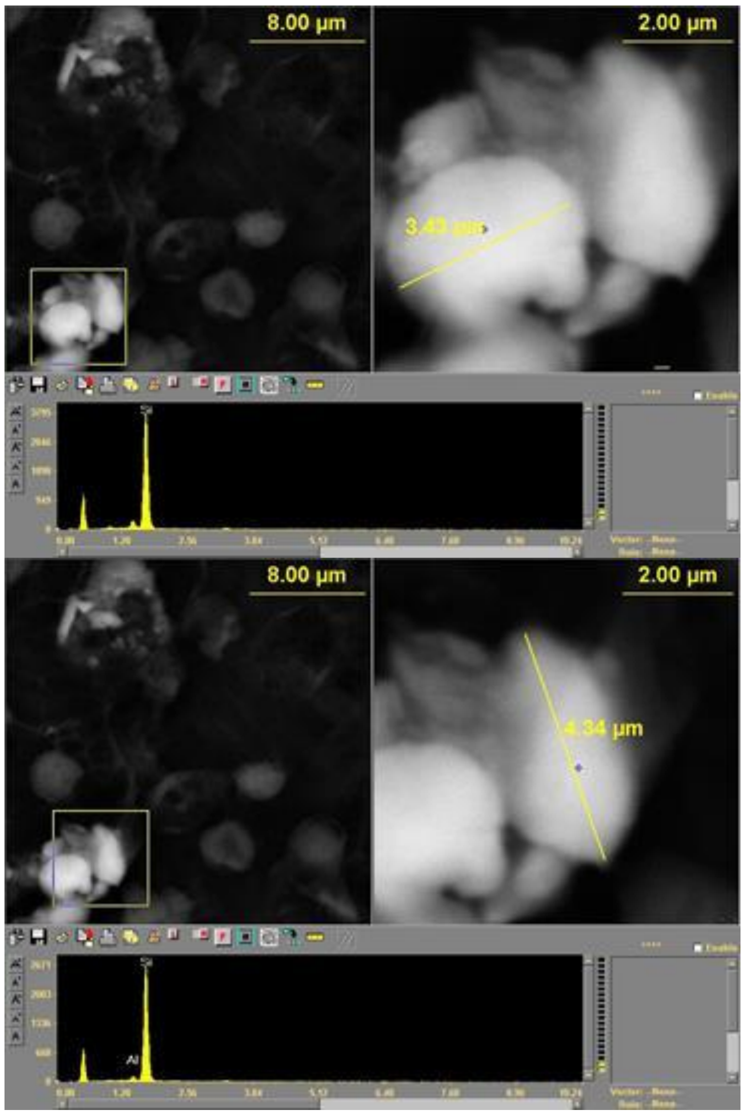

8.00 µm
2.00 µm
3.05 µm
Si
Al
Zn
8.00 µm
2.00 µm
2.98 µm
Si
Al
Zn

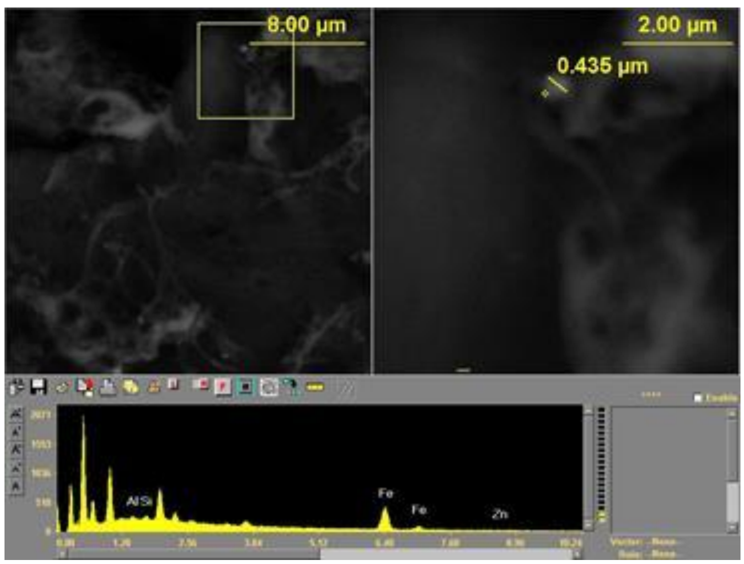

8.00 µm
2.00 µm
Si
Al
Zn
8.00 µm
2.00 µm
0.379 µm
Si
Al
Ti
Ti


8.00 µm
2.00 µm
0.578 µm
Si
Al
8.00 µm
2.00 µm
2.31 µm
Si
Al
K
K

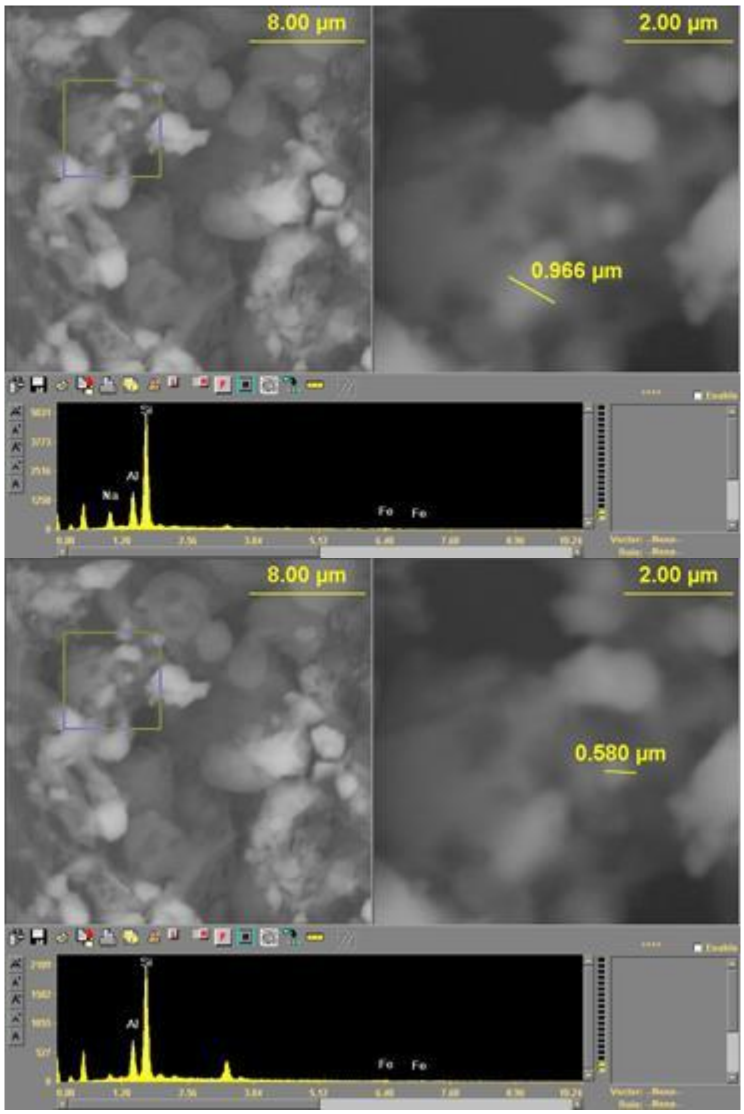

8.00 µm
2.00 µm
1.28 µm
Si
Al
8.00 µm
2.00 µm
0.708 µm
Al Si
K
K

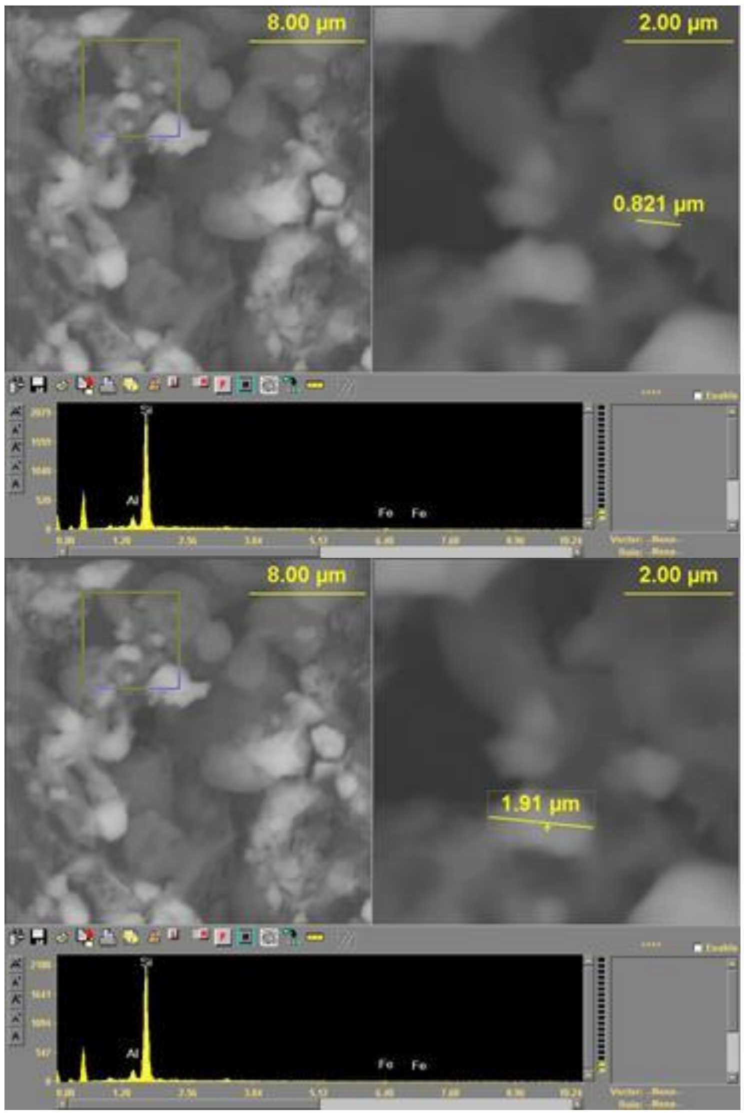

8.00 µm
2.00 µm
0.849 µm
Si
Al
8.00 µm
2.00 µm
1.04 µm
Si

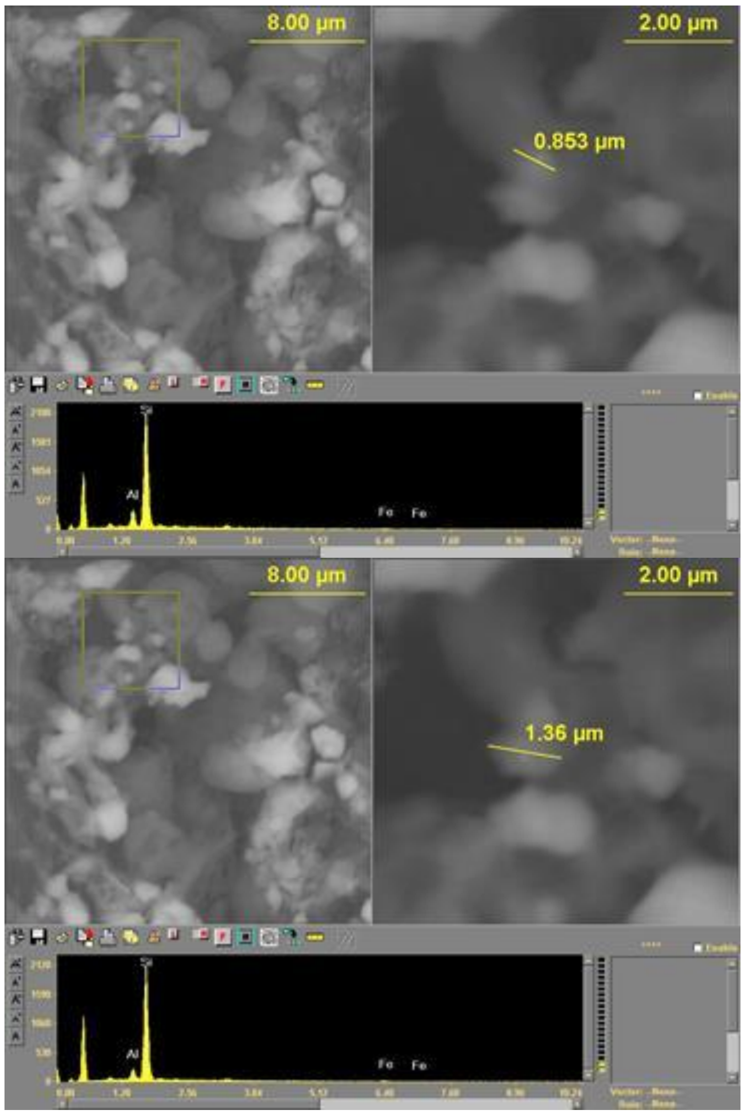

8.00 µm
2.00 µm
3.43 µm
Si
8.00 µm
2.00 µm
4.34 µm
Si
Al

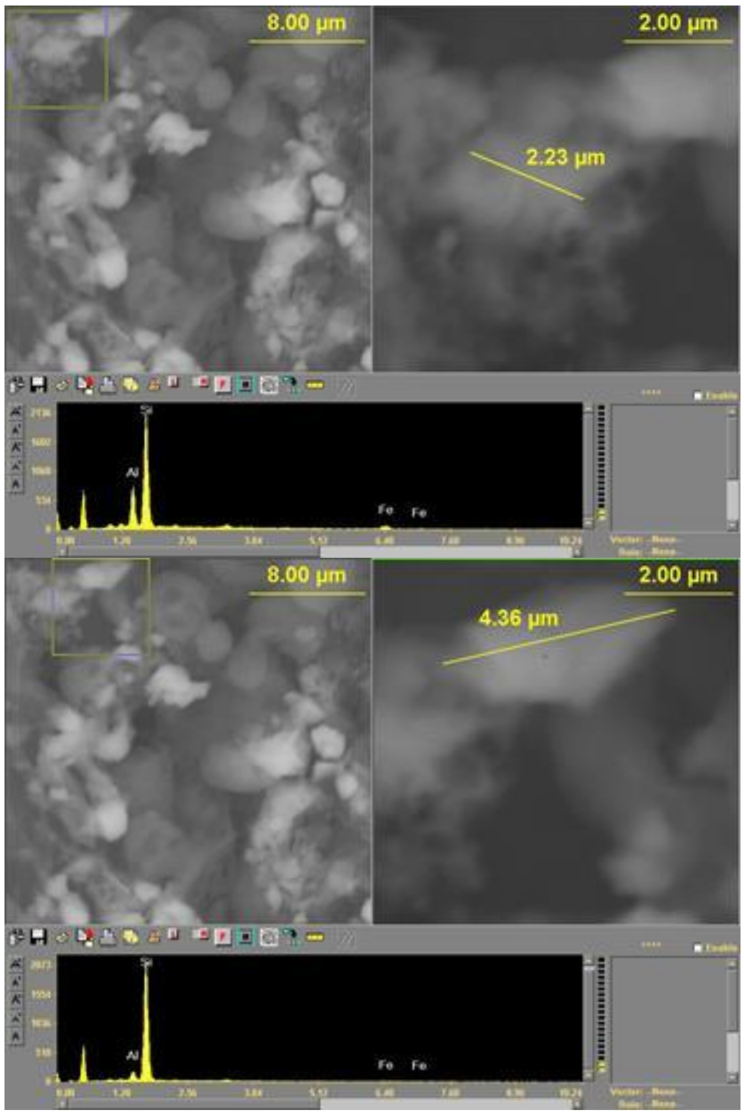

8.00 µm
2.00 µm
0.435 µm
Al Si
Fe
Fe
Zn

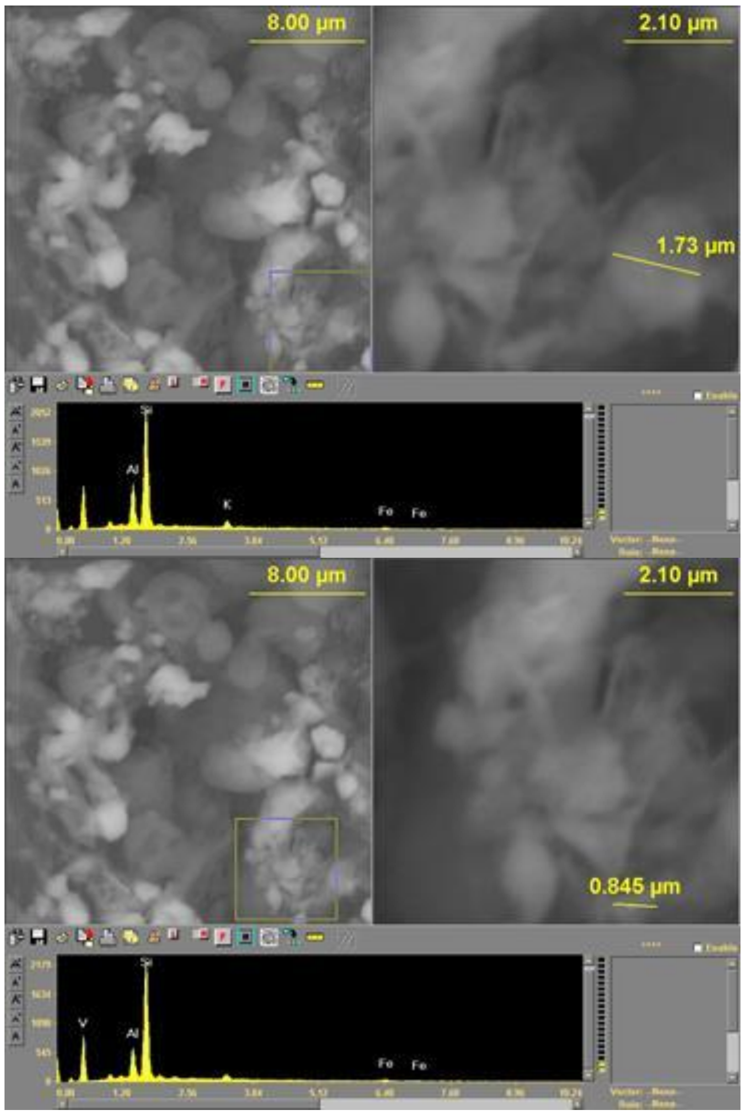
Sample 6A

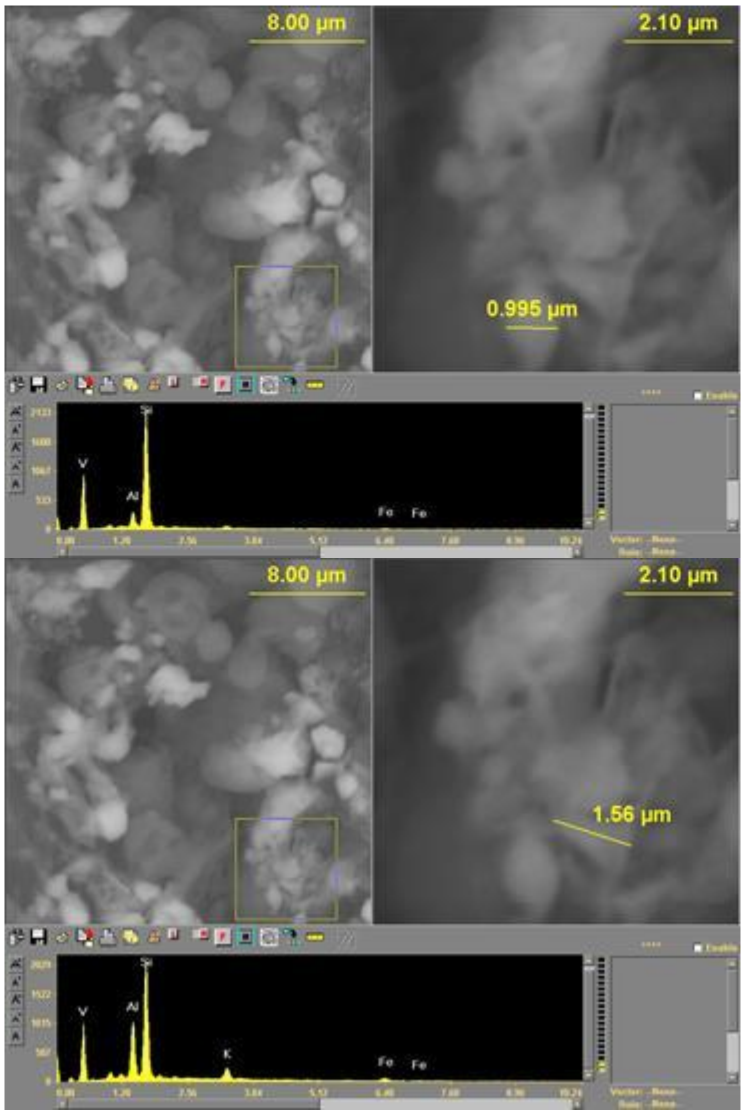

8.00 µm
2.00 µm
0.966 µm
Si
Al
Na
Fe
Fe
8.00 µm
2.00 µm
0.580 µm
Si
Al
Fe
Fe

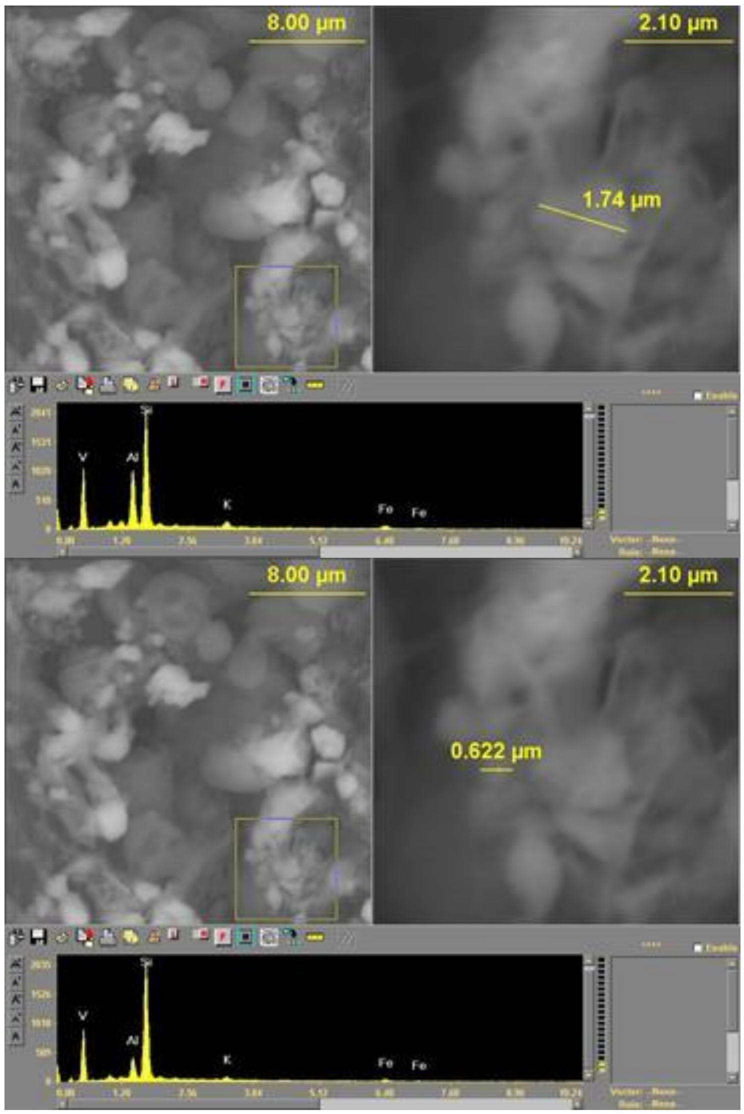

8.00 µm
2.00 µm
0.821 µm
Si
Al
Fe Fe
8.00 µm
2.00 µm
1.91 µm
Si
Al
Fe Fe

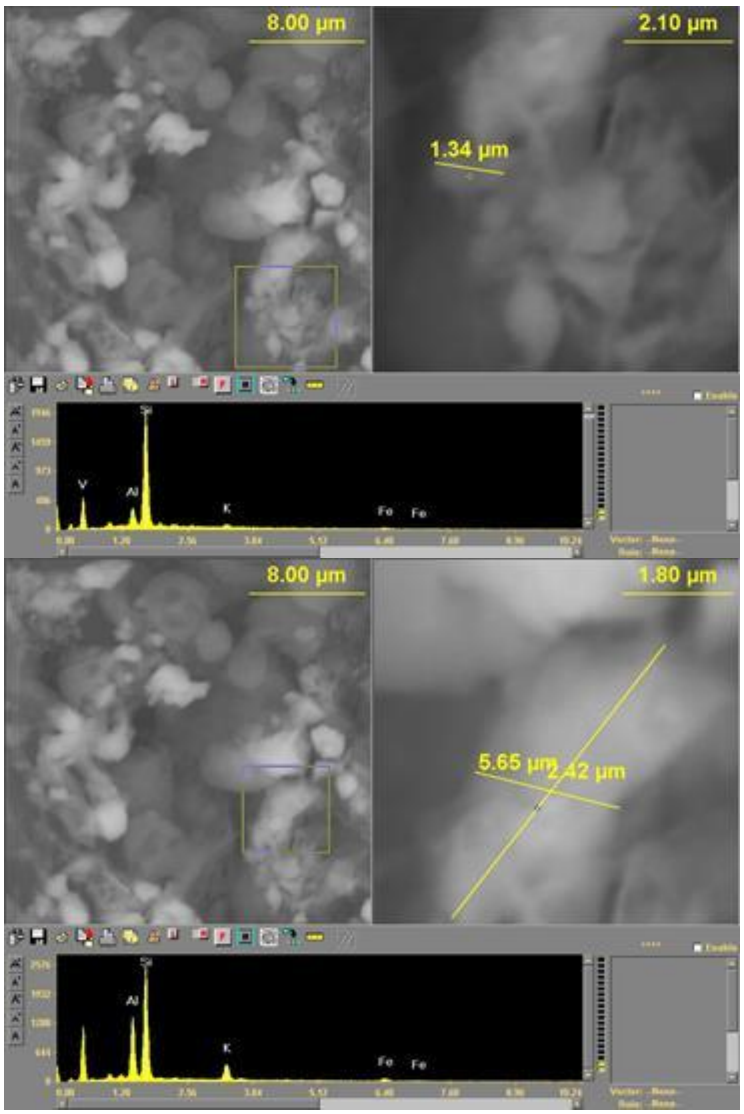

8.00 µm
2.00 µm
0.853 µm
Si
Al
Fe Fe
8.00 µm
2.00 µm
1.36 µm
Si
Al
Fe Fe

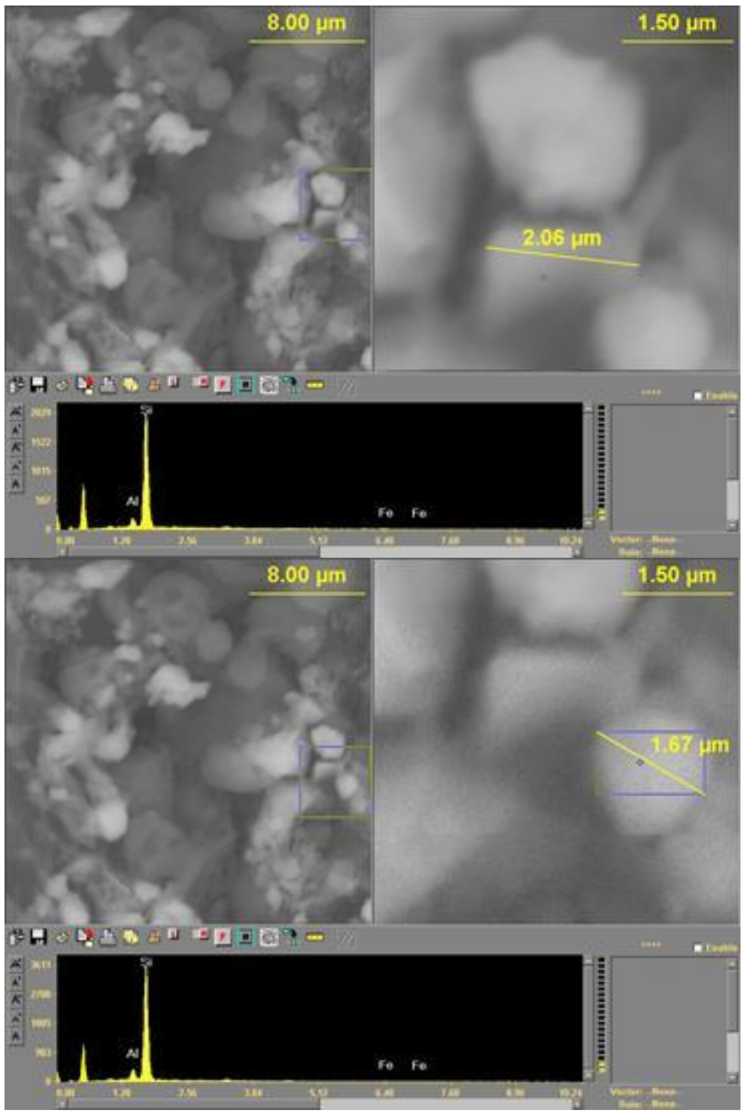

8.00 µm
2.00 µm
2.23 µm
Si
Al
Fe
Fe
8.00 µm
2.00 µm
4.36 µm
Si
Al
Fe
Fe

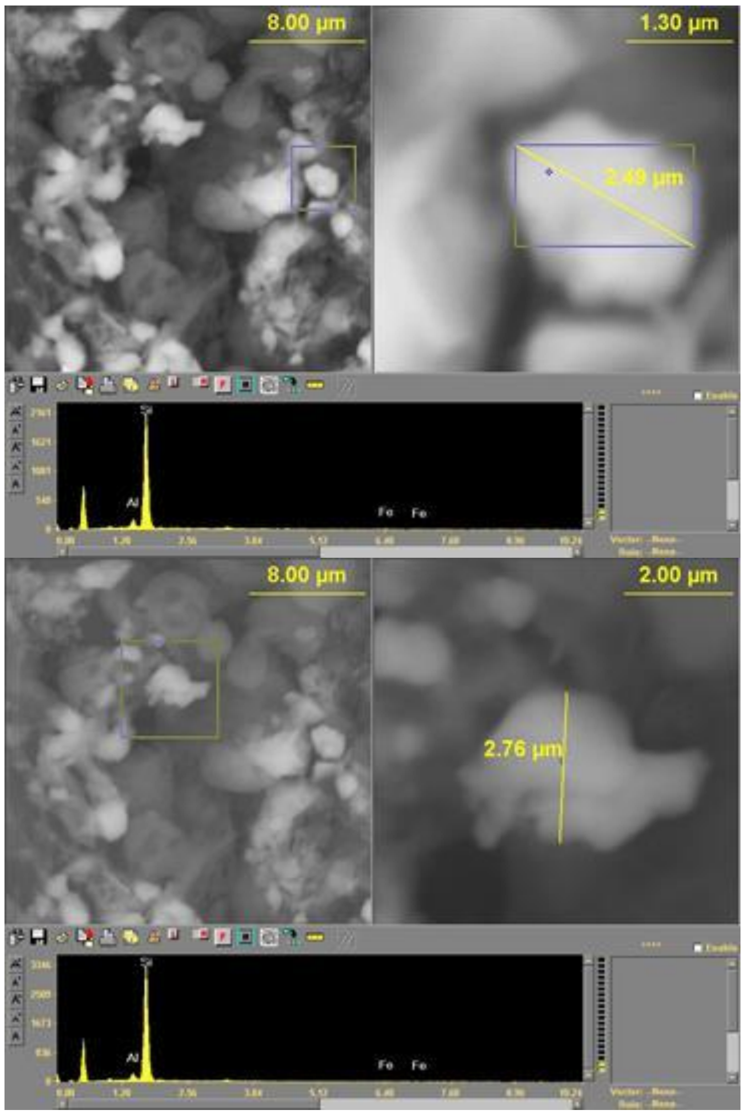

8.00 µm
2.10 µm
1.73 µm
Si
Al
K
Fe
Fe
8.00 µm
2.10 µm
0.845 µm
Si
V
Al
Fe
Fe


8.00 µm
2.10 µm
0.995 µm
Si
V
Al
Fe
Fe
8.00 µm
2.10 µm
1.56 µm
Si
V
Al
K
Fe
Fe

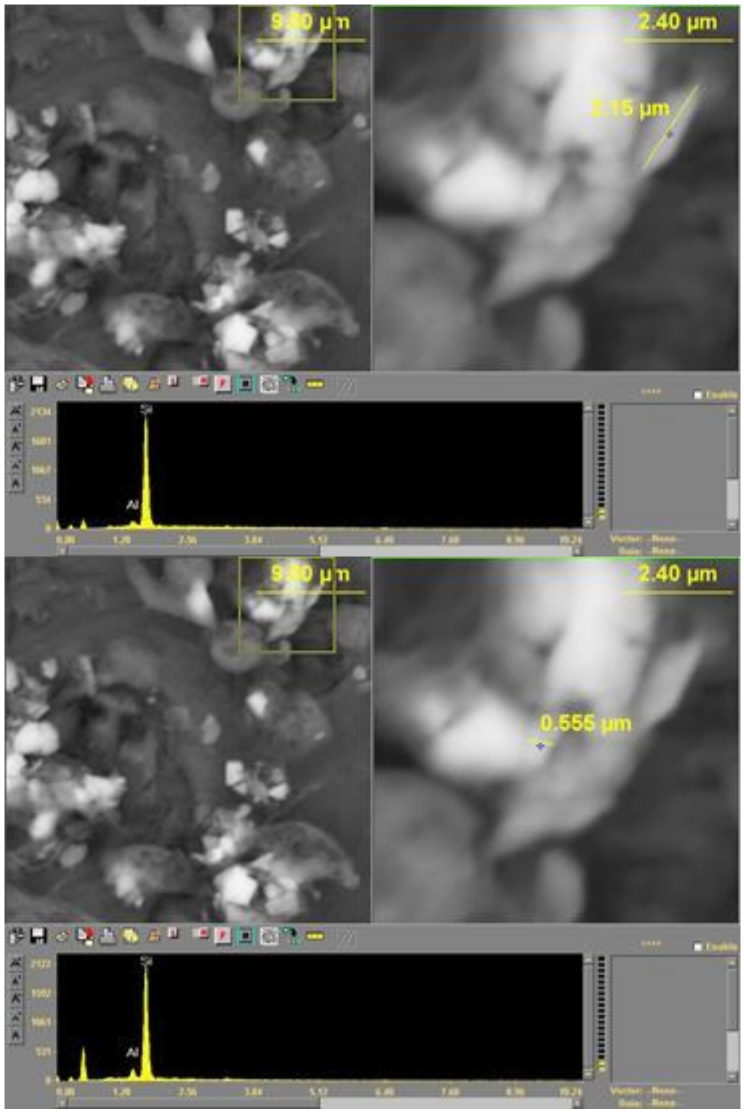

8.00 µm
2.10 µm
1.74 µm
V
Al
Si
K
Fe
Fe
8.00 µm
2.10 µm
0.622 µm
V
Al
Si
K
Fe
Fe

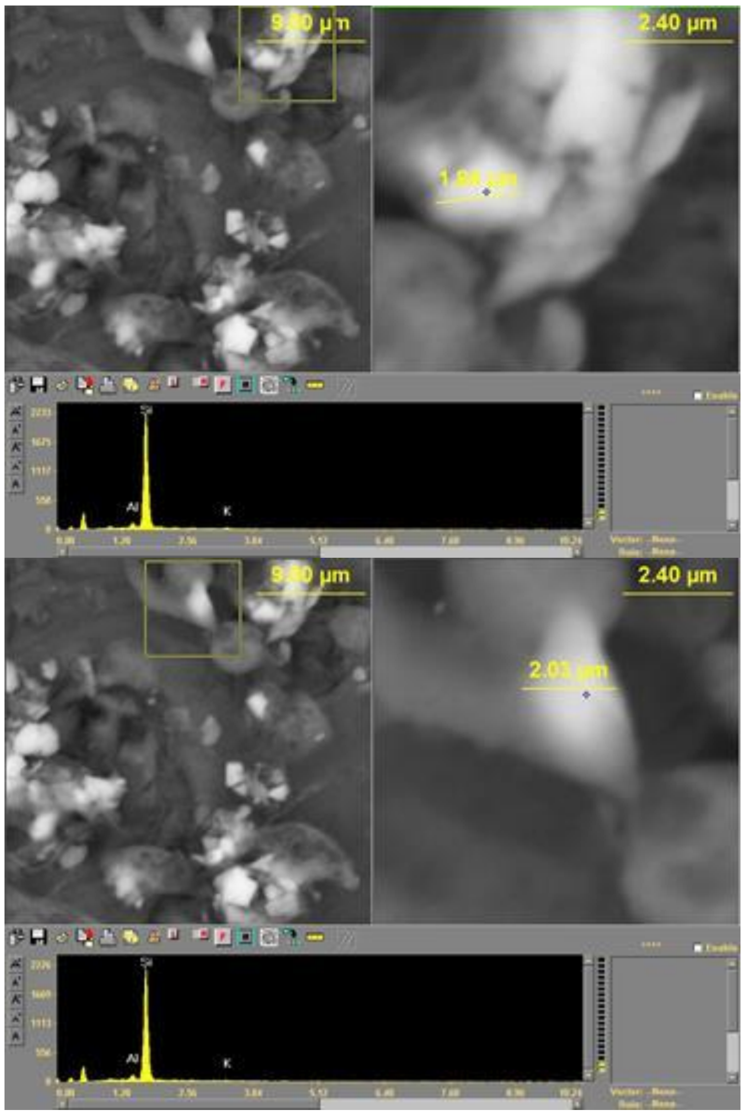

8.00 µm
2.10 µm
1.34 µm
Si
V
Al
K
Fe Fe
8.00 µm
1.80 µm
5.65 µm
2.42 µm
Si
Al
K
Fe Fe

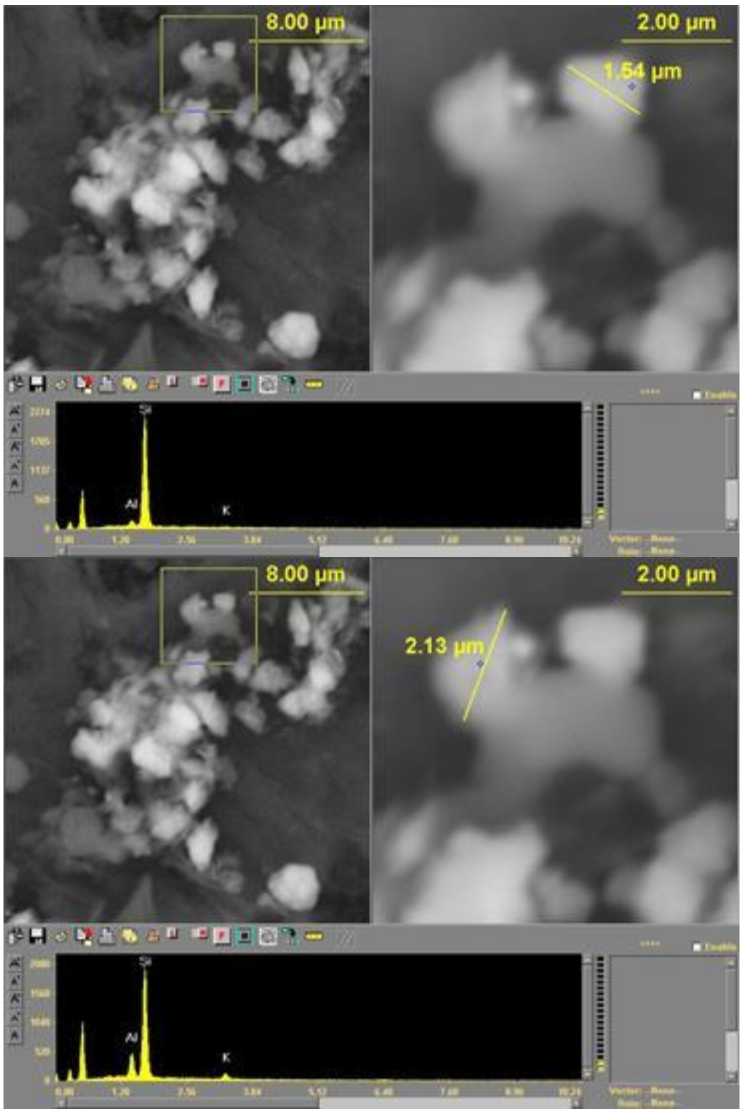

8.00 µm
1.50 µm
2.06 µm
Si
Al
Fe
Fe
8.00 µm
1.50 µm
1.67 µm
Si
Al
Fe
Fe

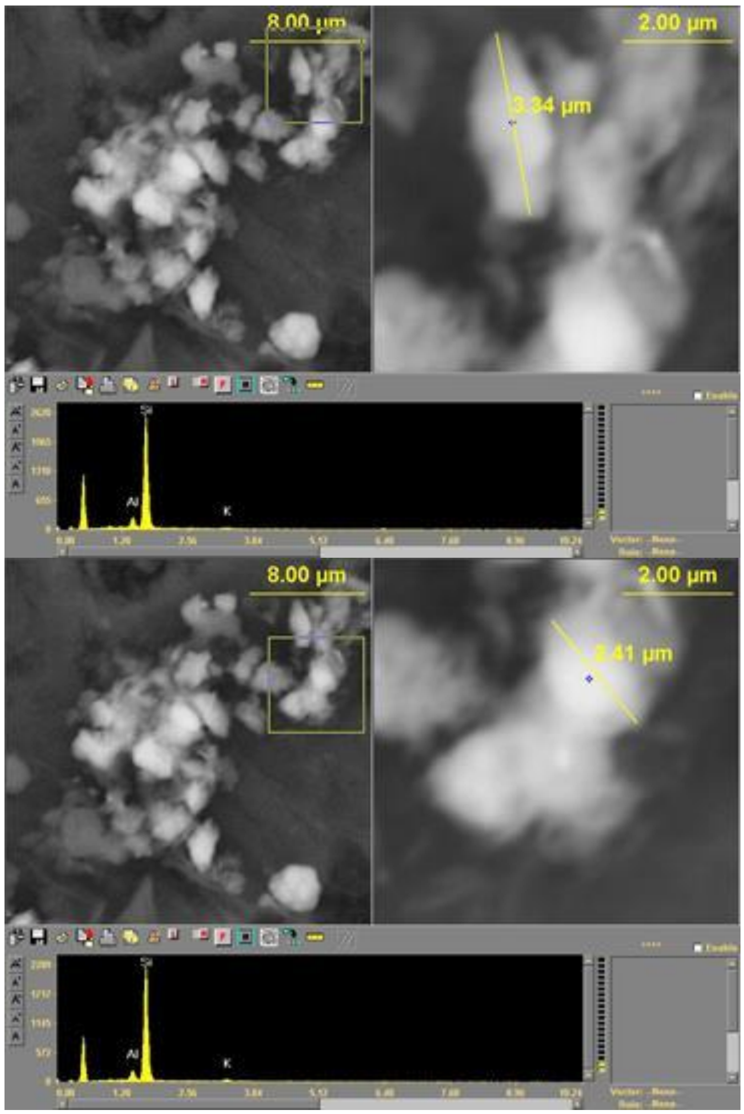

8.00 µm
1.30 µm
2.49 µm
Si
Al
Fe Fe
8.00 µm
2.00 µm
2.76 µm
Si
Al
Fe Fe

Sample 6B

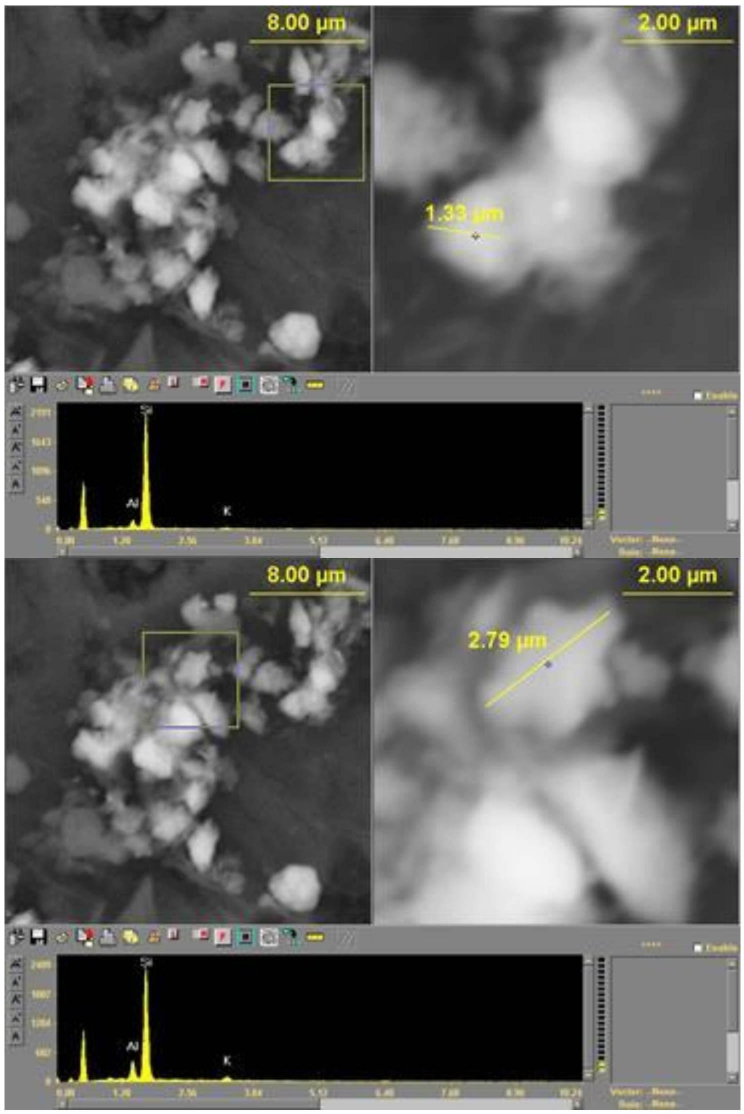


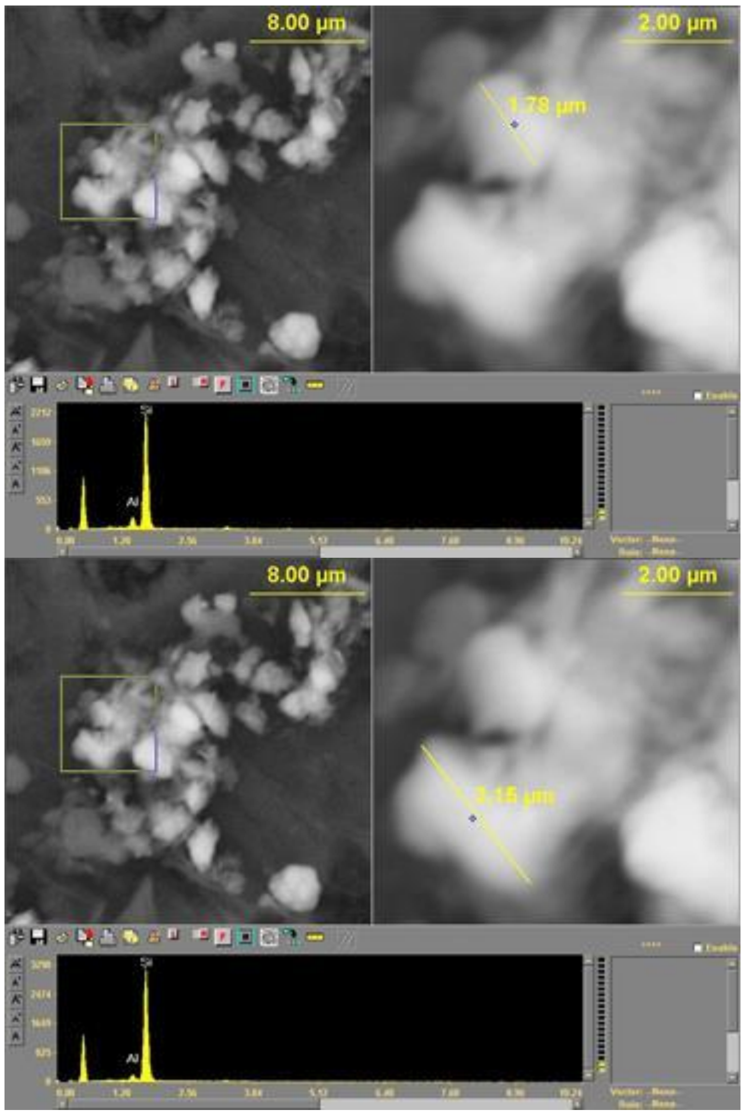

2.40 µm
Si
Al
K
2.40 µm
Si
Al
K

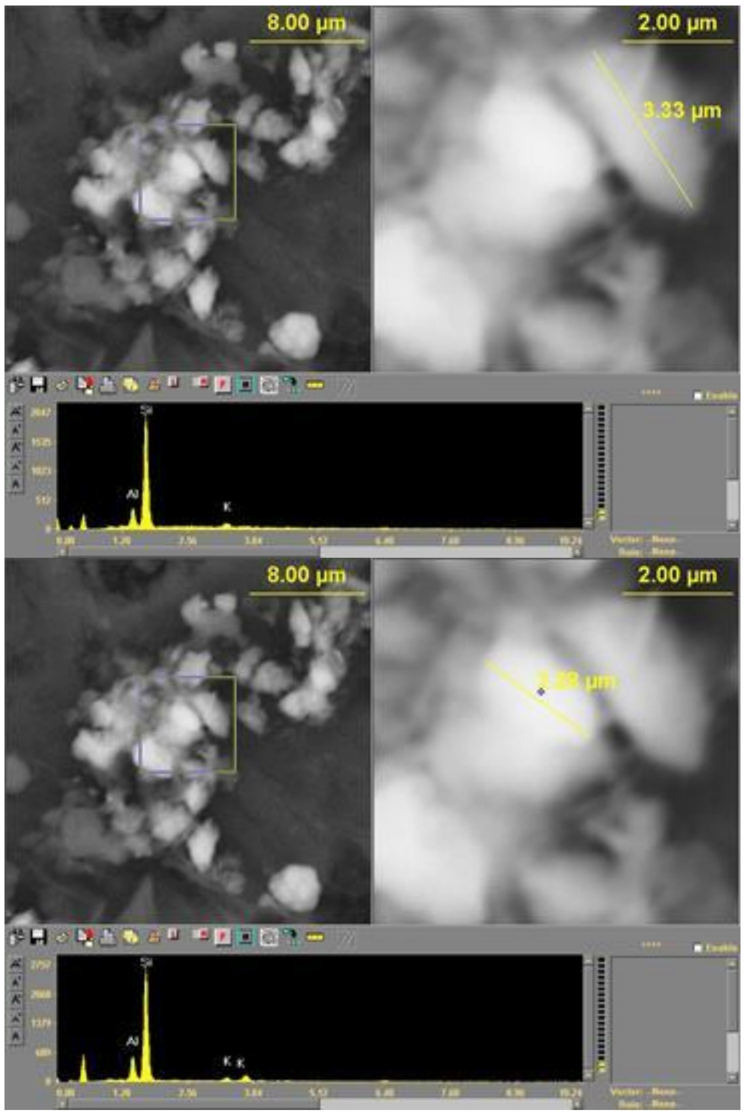

8.00 µm
2.00 µm
1.54 µm
Si
Al
K
8.00 µm
2.00 µm
2.13 µm
Si
Al
K

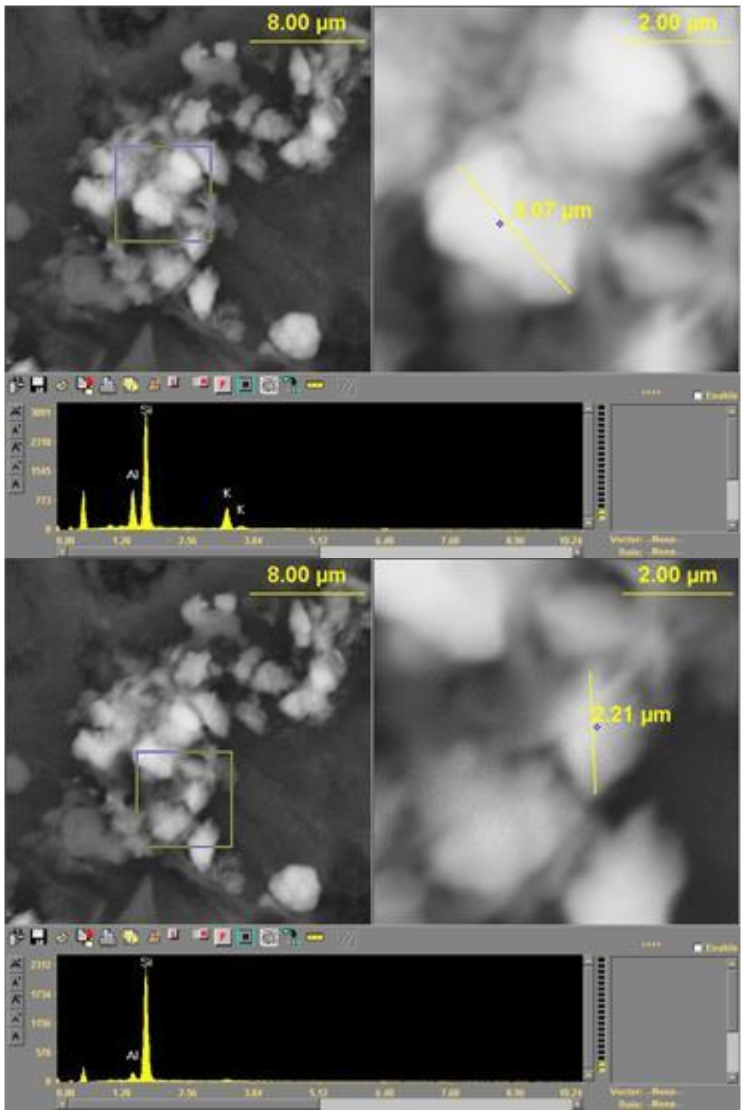


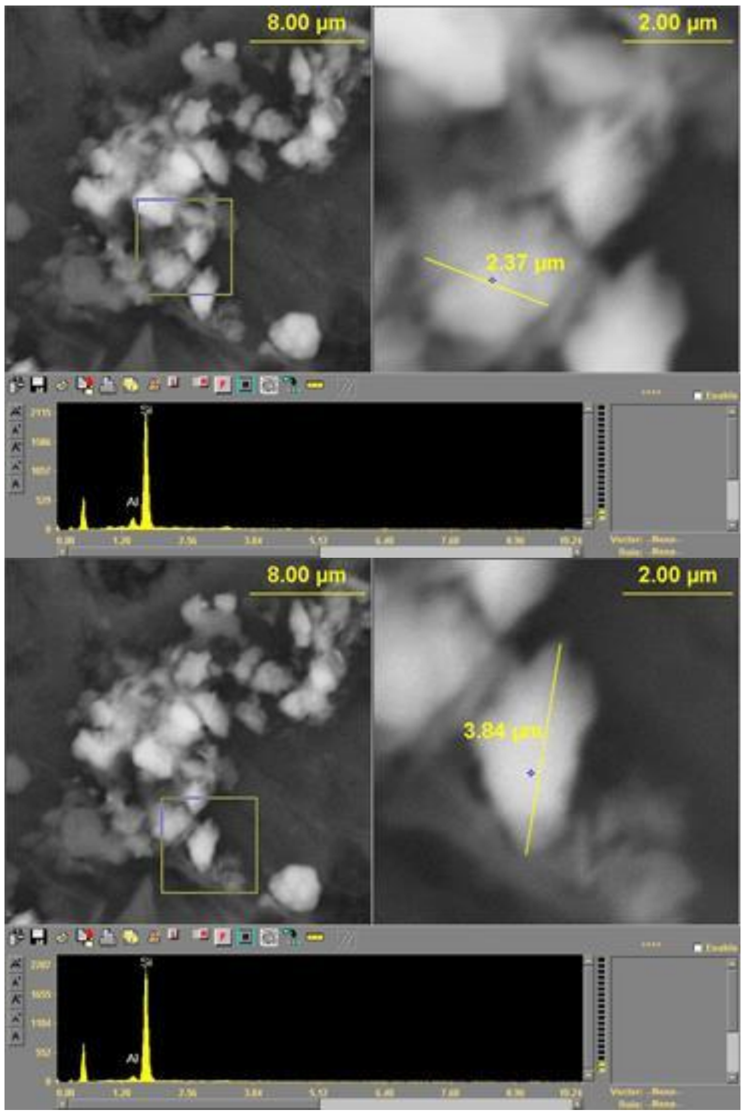
8.00 µm
2.00 µm
3.34 µm
Si
Al
K
8.00 µm
2.00 µm
2.41 µm
Si
Al
K

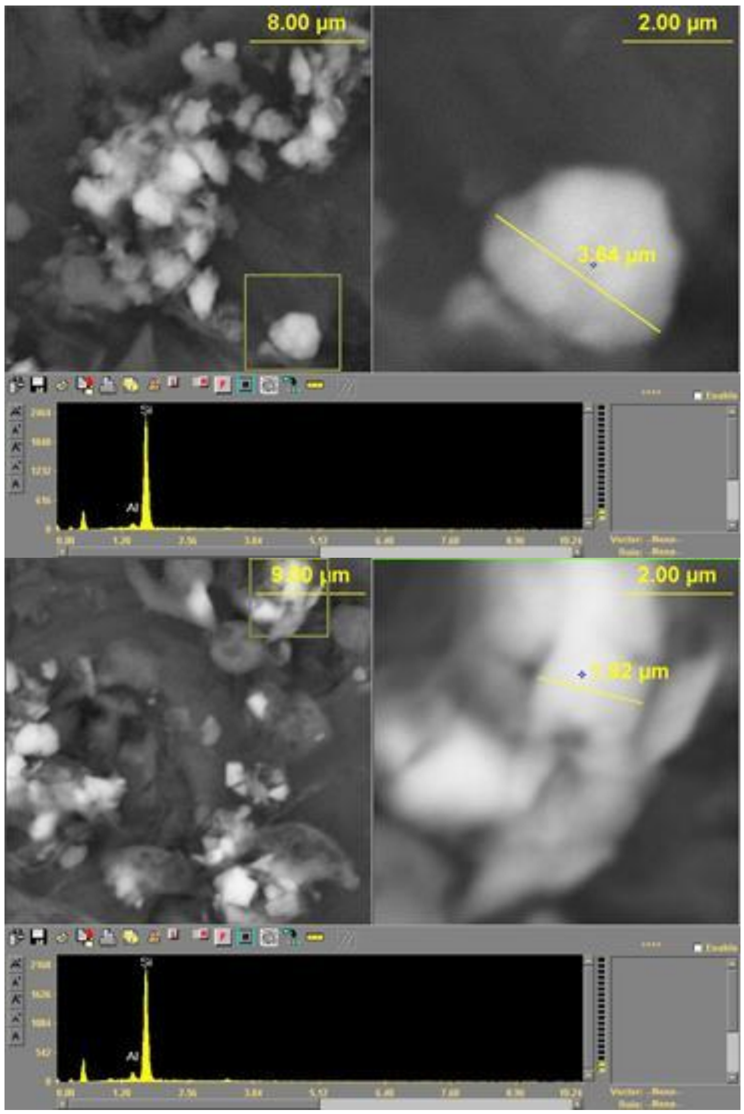

8.00 µm
2.00 µm
1.33 µm
Si
Al
K
8.00 µm
2.00 µm
2.79 µm
Si
Al
K


8.00 µm
2.00 µm
1.78 µm
Si
Al
8.00 µm
2.00 µm
3.16 µm
Si
Al

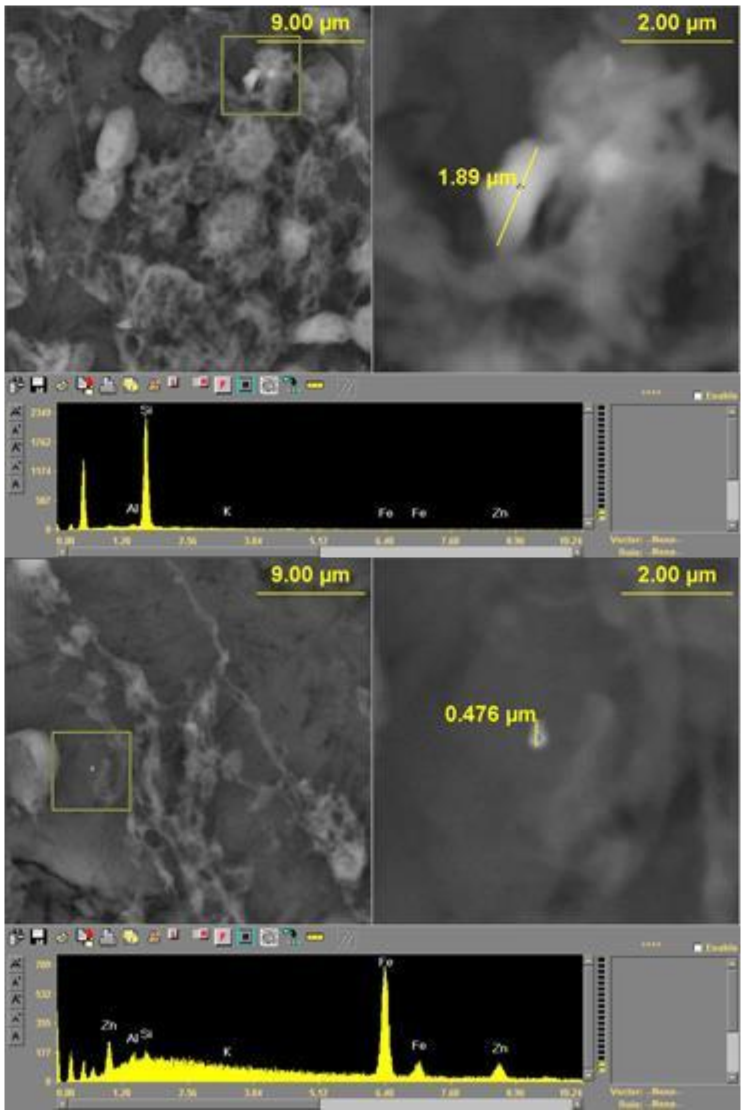

8.00 µm
2.00 µm
3.33 µm
Si
Al
K
8.00 µm
2.00 µm
Si
Al
K K

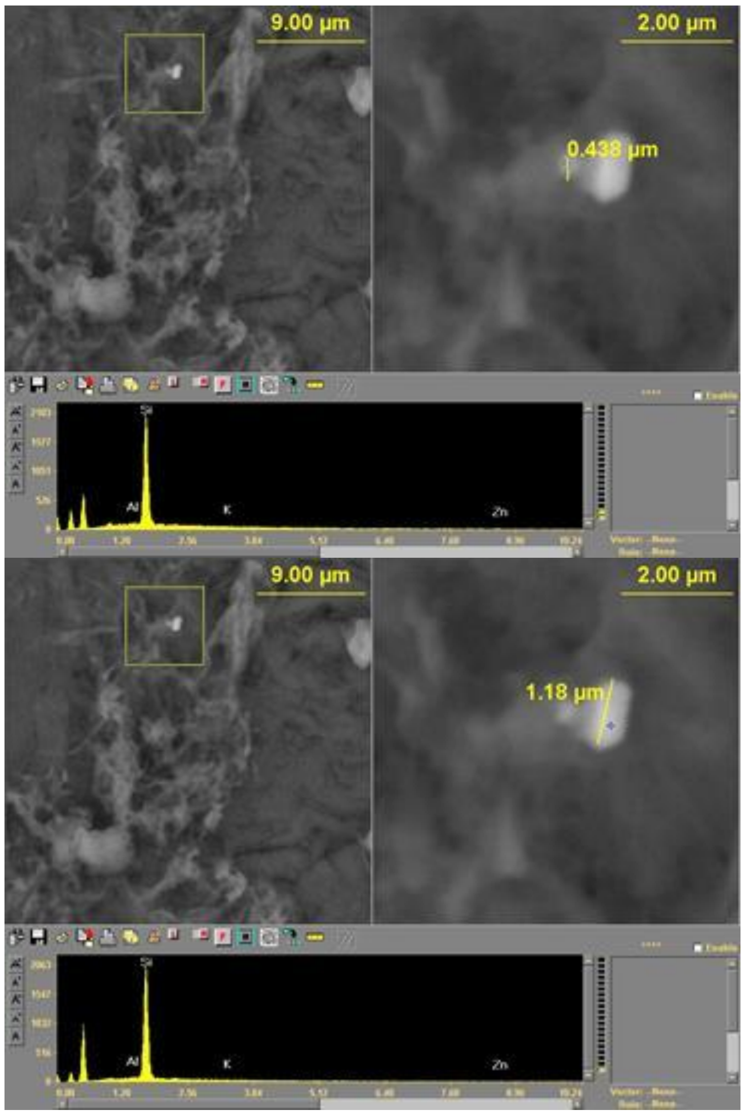

8.00 µm
2.00 µm
Al
K
K
8.00 µm
2.00 µm
2.21 µm
Al

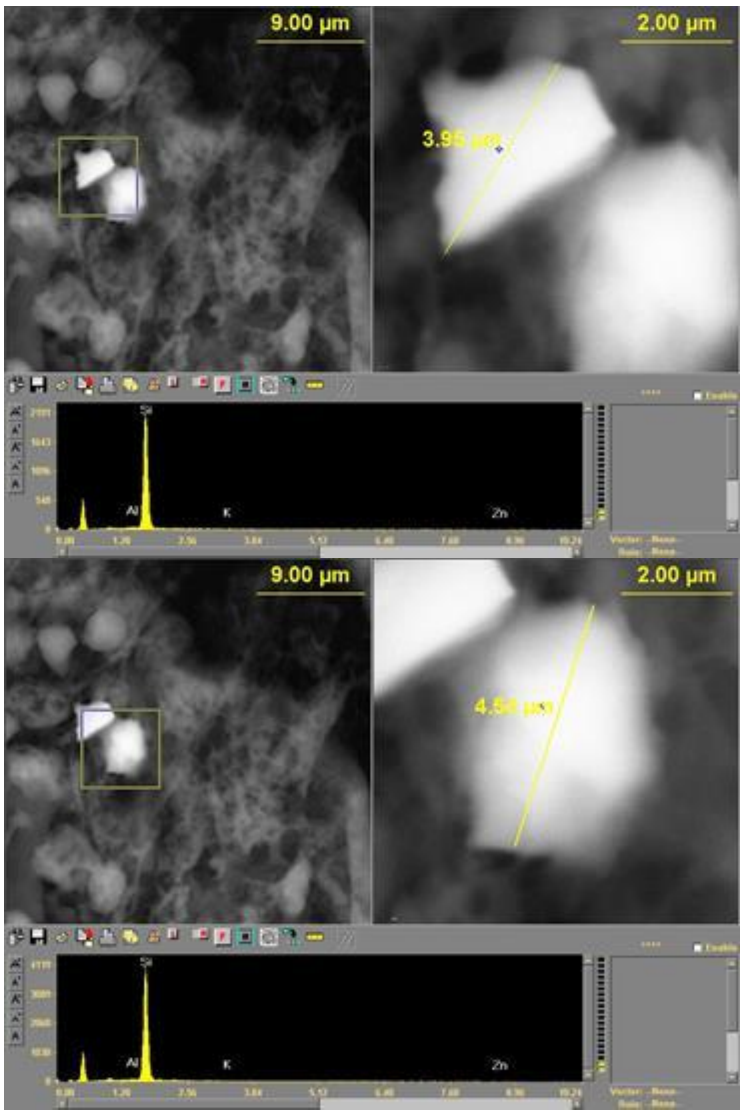

8.00 µm
2.00 µm
2.37 µm
Si
Al
8.00 µm
2.00 µm
3.84 µm
Si
Al

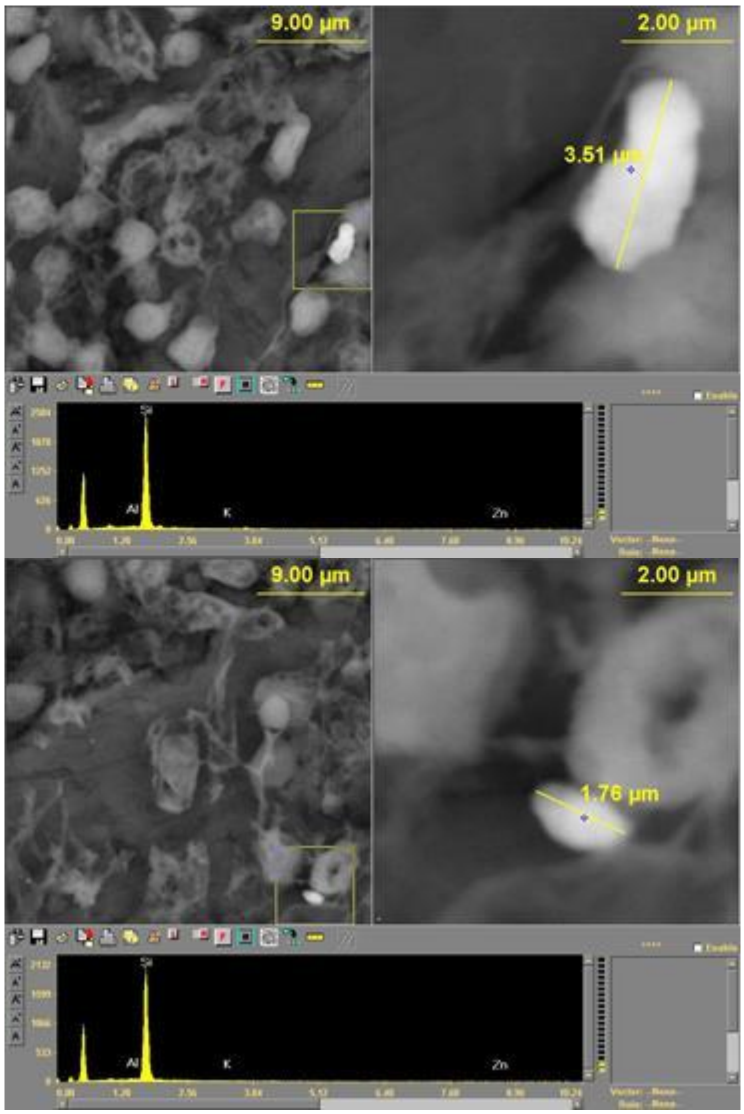

8.00 µm
2.00 µm
3.64 µm
Si
Al
9.80 µm
2.00 µm
Si
Al

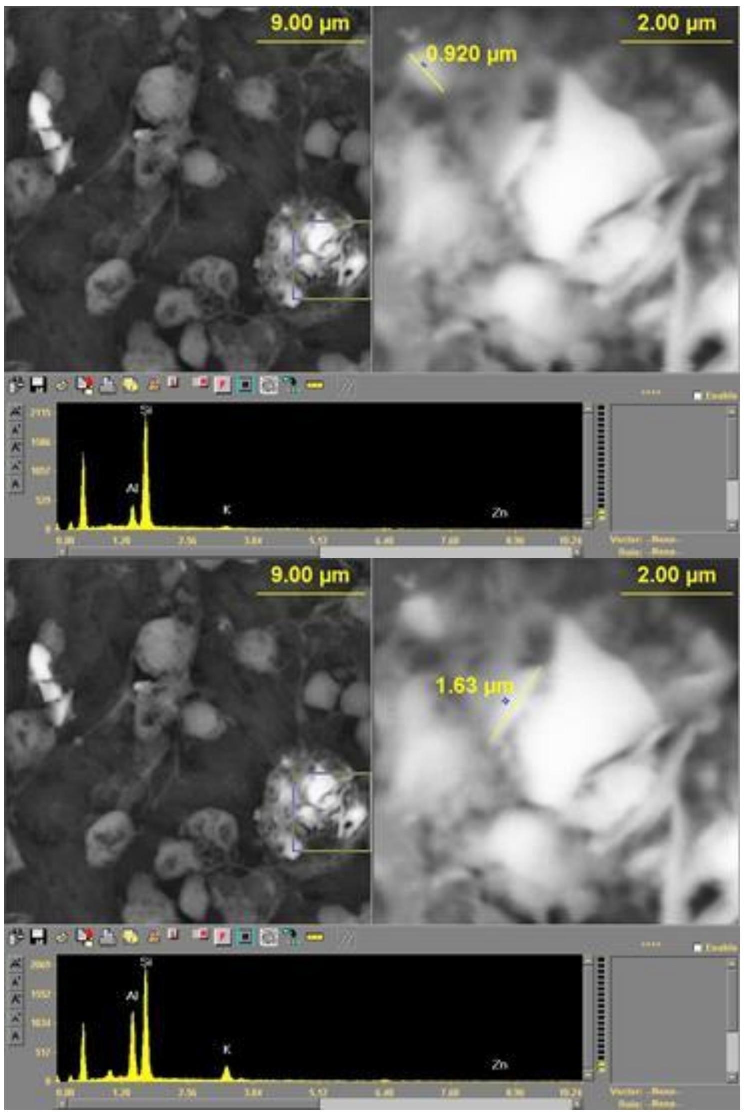
Sample 7A

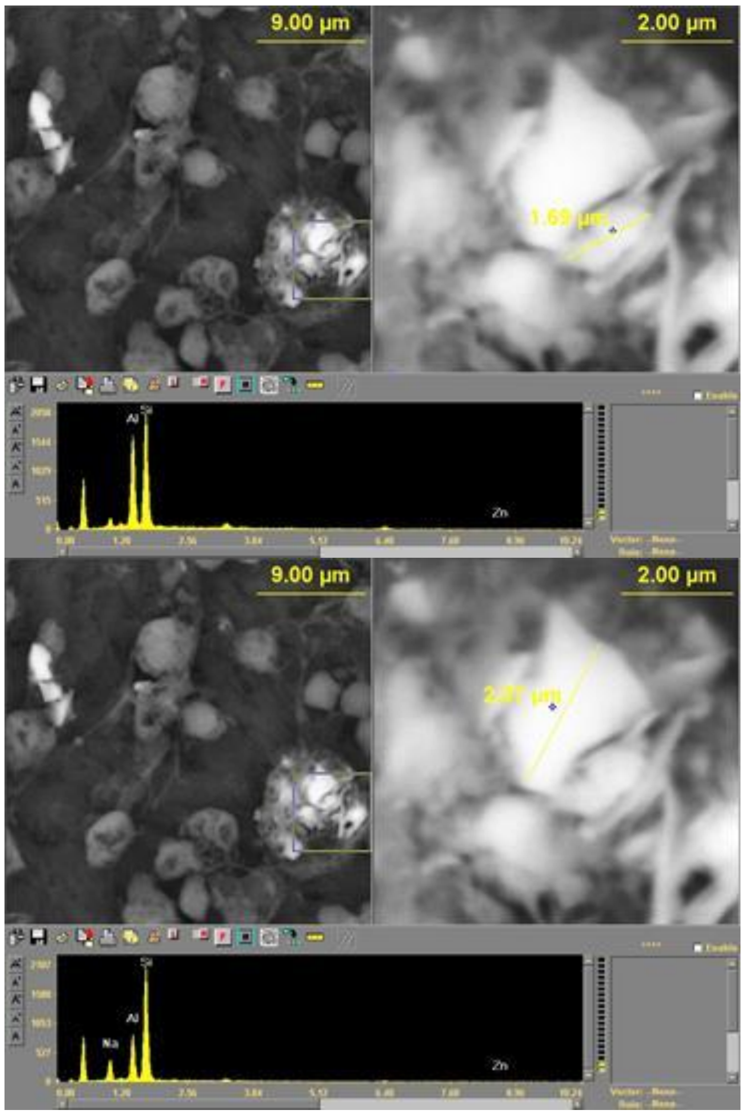

9.00 µm
2.00 µm
1.89 µm
Si
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
0.476 µm
Fe
Zn
Al
Si
K
Fe
Zn

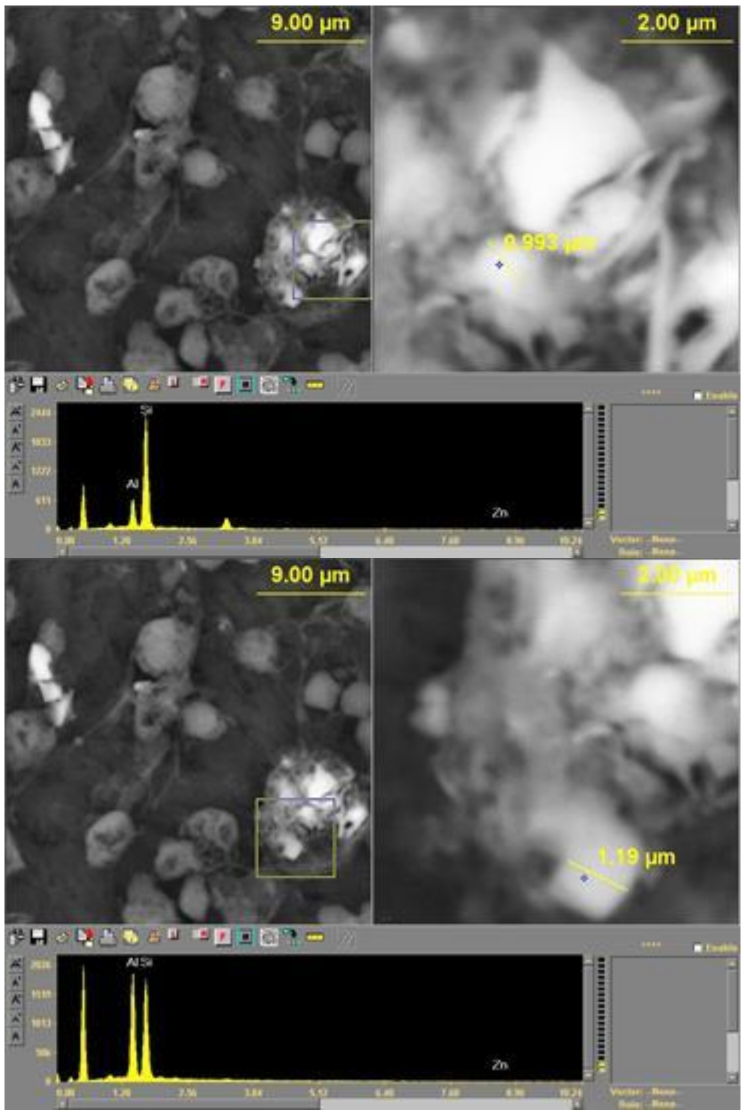

9.00 µm
2.00 µm
0.438 µm
Si
Al
K
Zn
9.00 µm
2.00 µm
1.18 µm
Si
Al
K
Zn

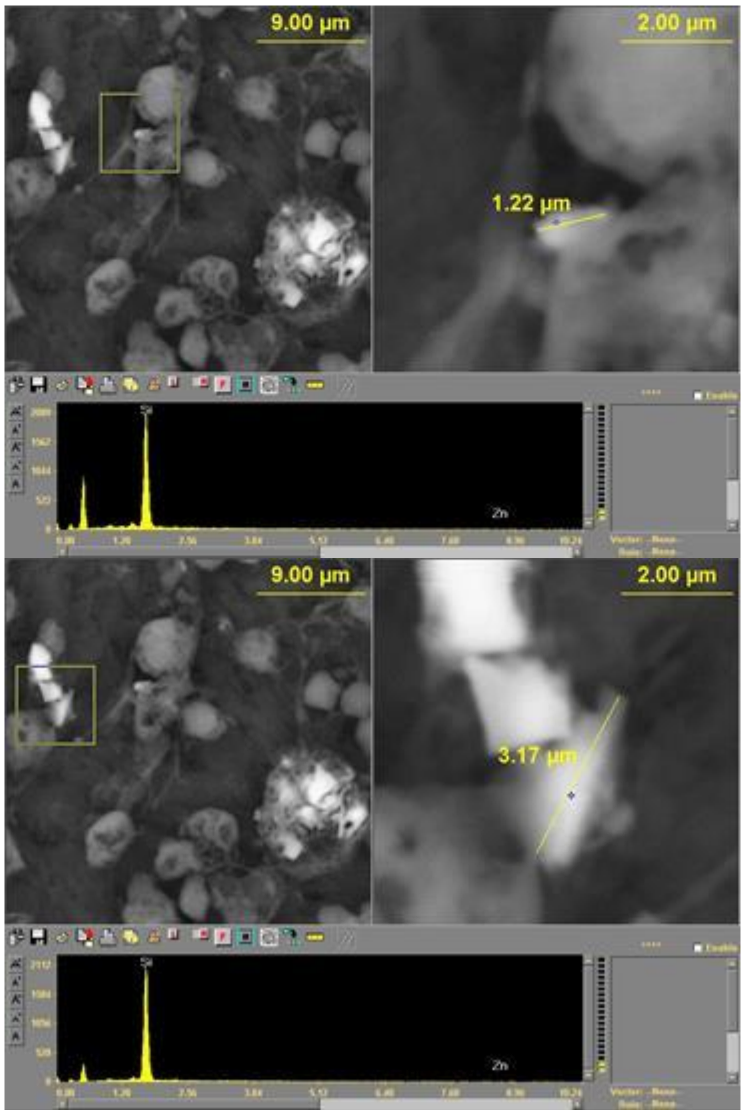

9.00 µm
2.00 µm
3.95 µm
Si
Al
K
Zn
9.00 µm
2.00 µm
4.58 µm
Si
Al
K
Zn


9.00 µm
2.00 µm
3.51 µm
Si
Al
K
Zn
9.00 µm
2.00 µm
1.76 µm
Si
Al
K
Zn

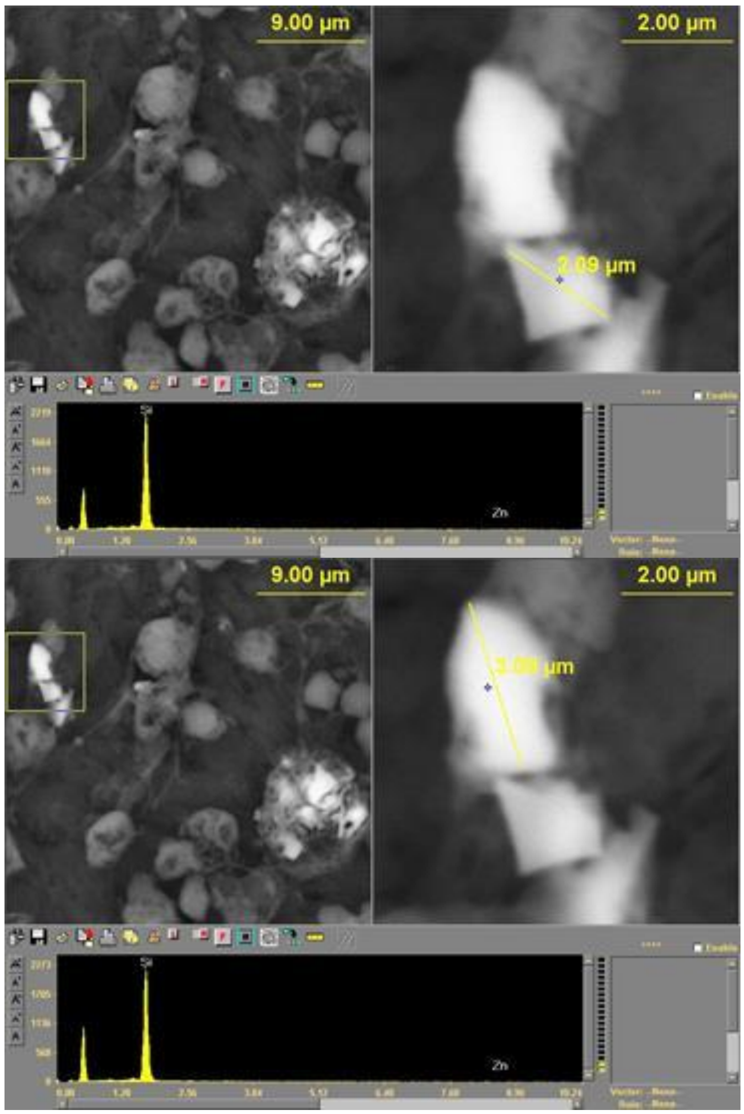

9.00 µm
2.00 µm
0.920 µm
Si
Al
K
Zn
9.00 µm
2.00 µm
1.63 µm
Si
Al
K
Zn

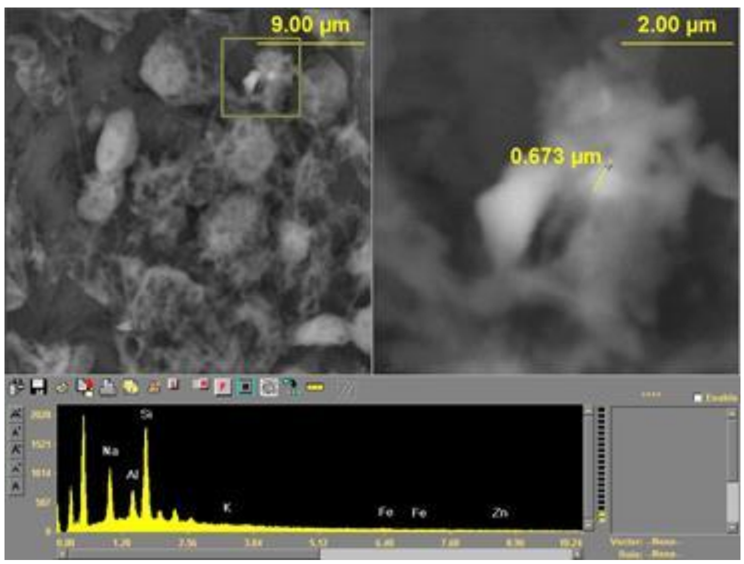

9.00 µm
2.00 µm
Al
Si
Zn
9.00 µm
2.00 µm
Si
Al
Na
Zn


9.00 µm
2.00 µm
Si
Al
Zn
9.00 µm
1.19 µm
Al Si
Zn

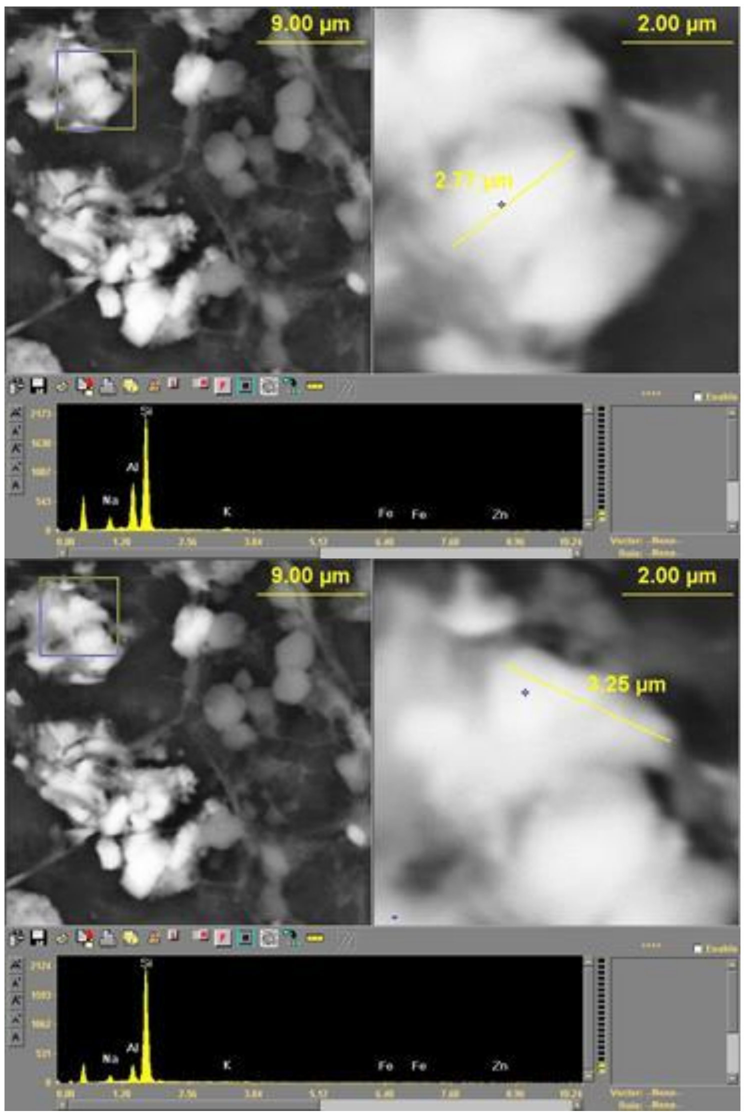


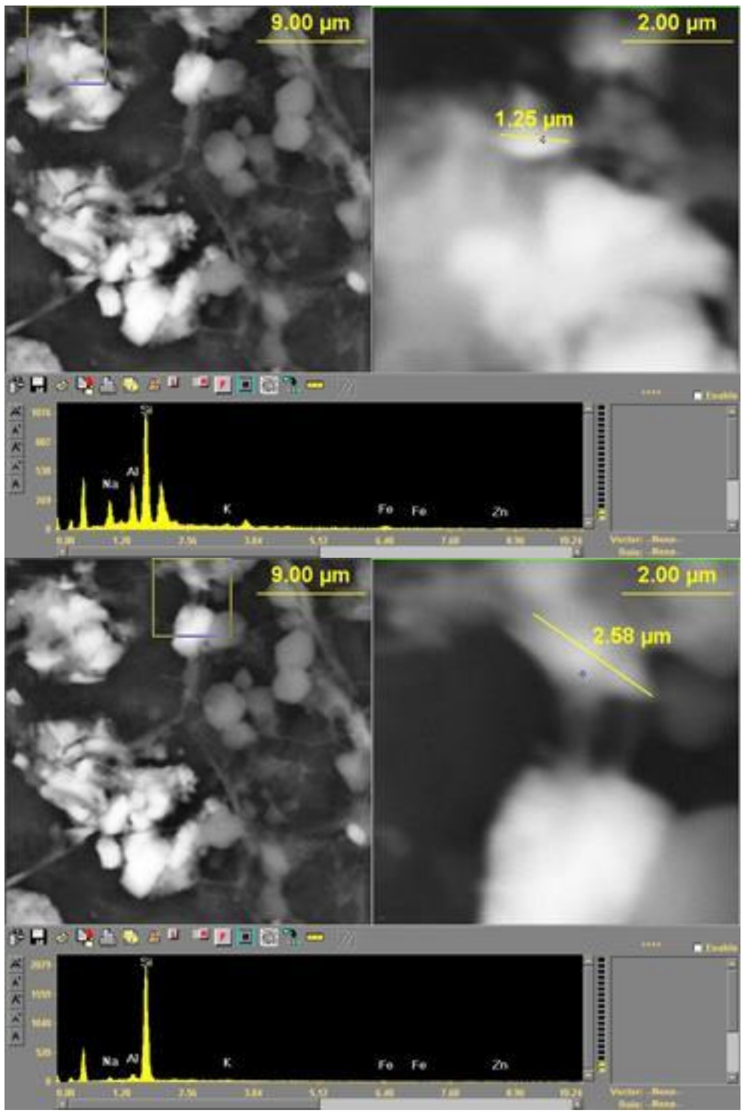
9.00 µm
2.00 µm
1.22 µm
Si
Zn
9.00 µm
2.00 µm
3.17 µm
Si
Zn

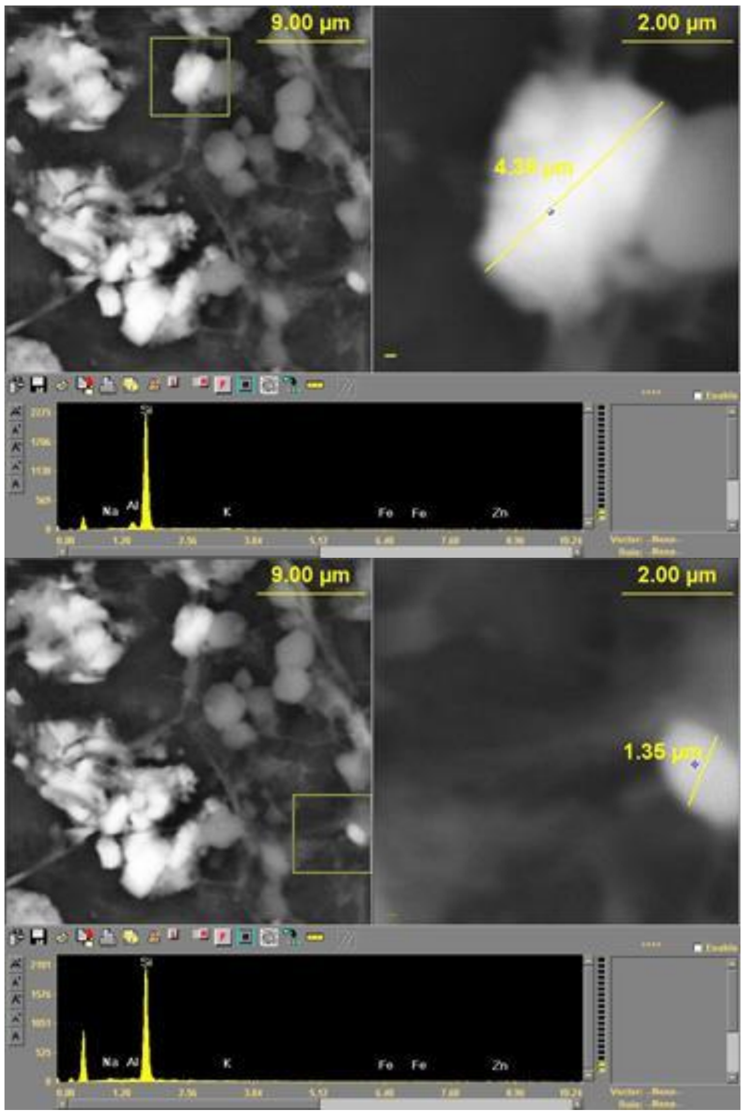

9.00 µm
2.00 µm
2.09 µm
Si
Zn
9.00 µm
2.00 µm
Si
Zn

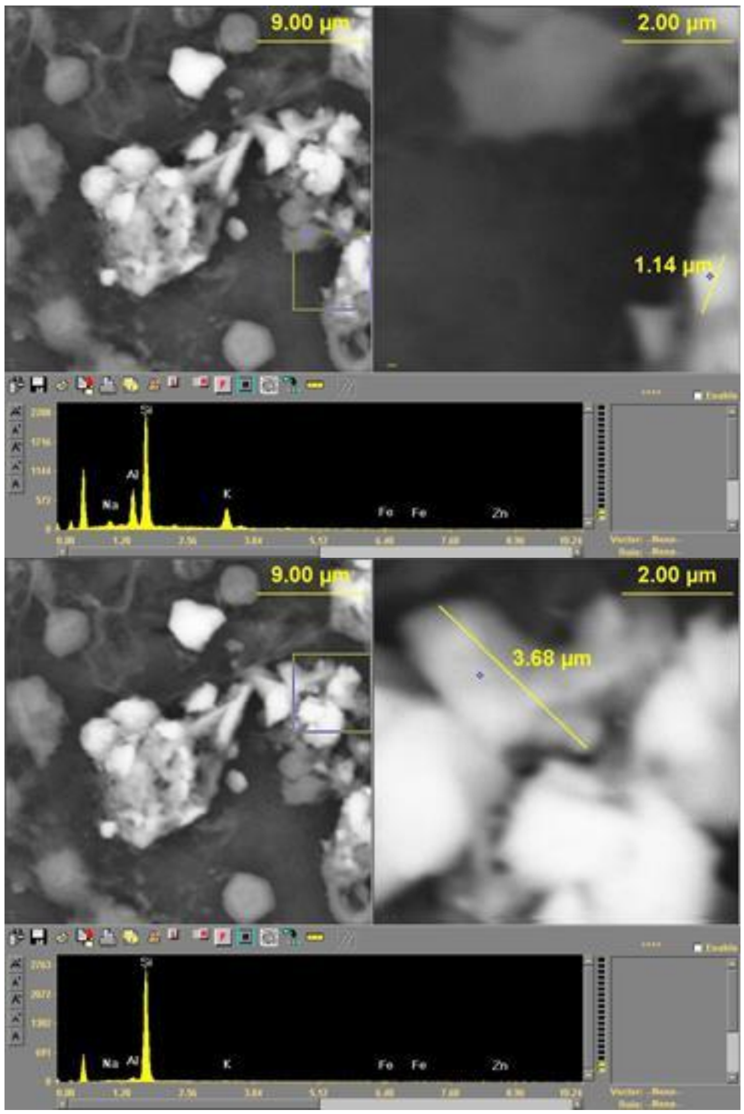

9.00 µm
2.00 µm
0.673 µm
Si
Na
Al
K
Fe
Fe
Zn
1.28
2.56
3.84
5.12
6.40
7.68
8.96

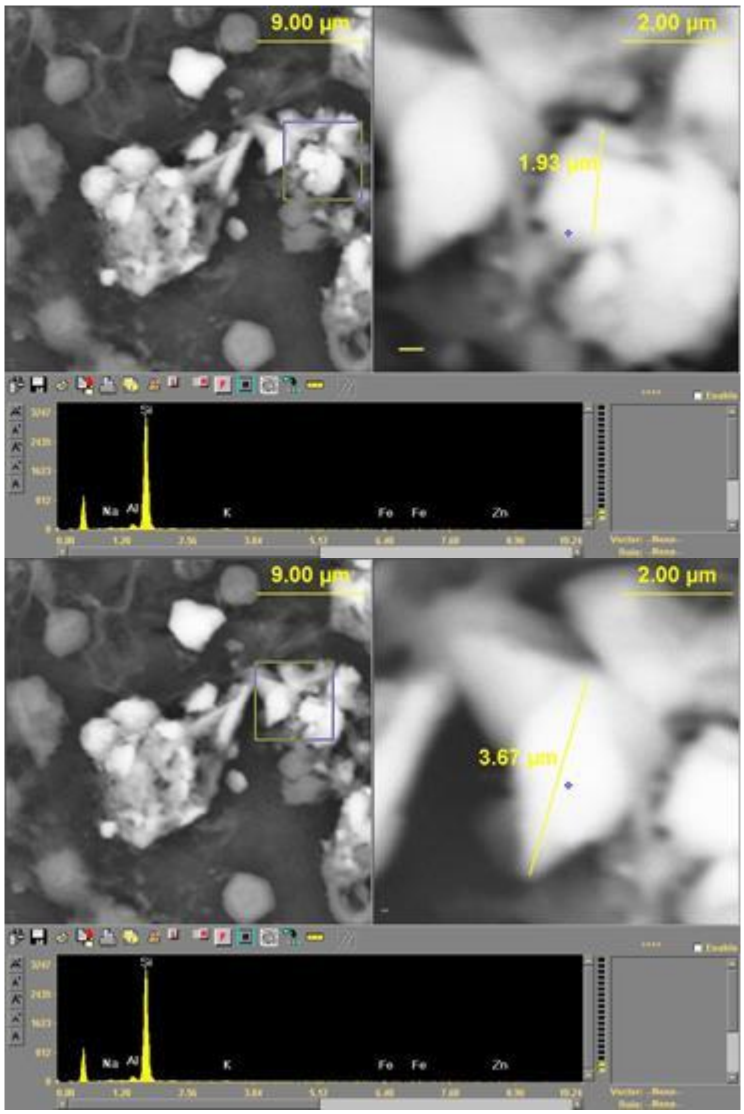
Samples 7B

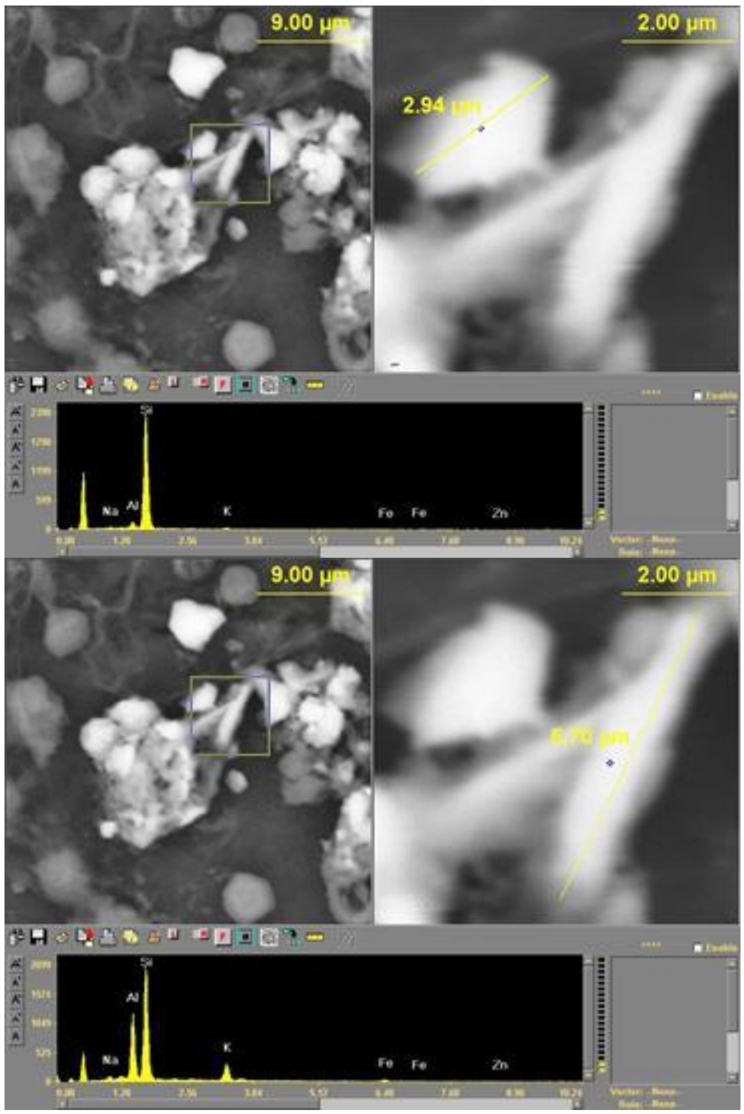

9.00 µm
2.00 µm
2.77 µm
Si
Al
Na
K
Fe
Fe
Zn
9.00 µm
2.00 µm
Si
Al
Na
K
Fe
Fe
Zn

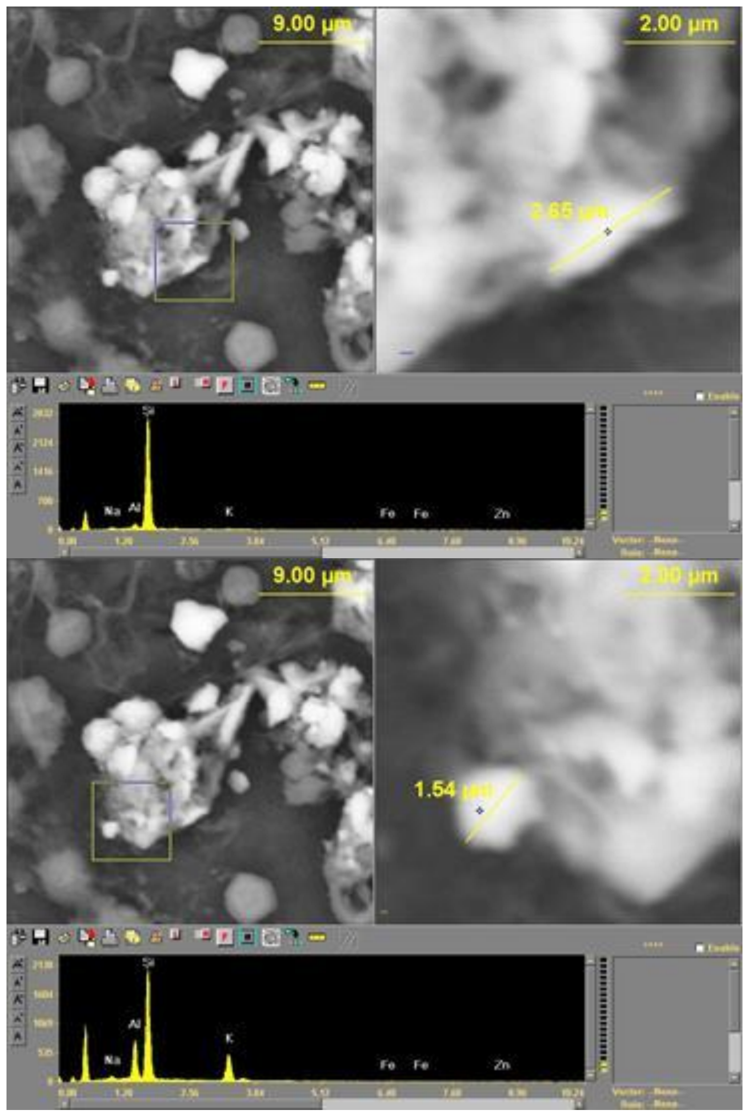

9.00 µm
2.00 µm
1.25 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
2.58 µm
Si
Na
Al
K
Fe
Fe
Zn

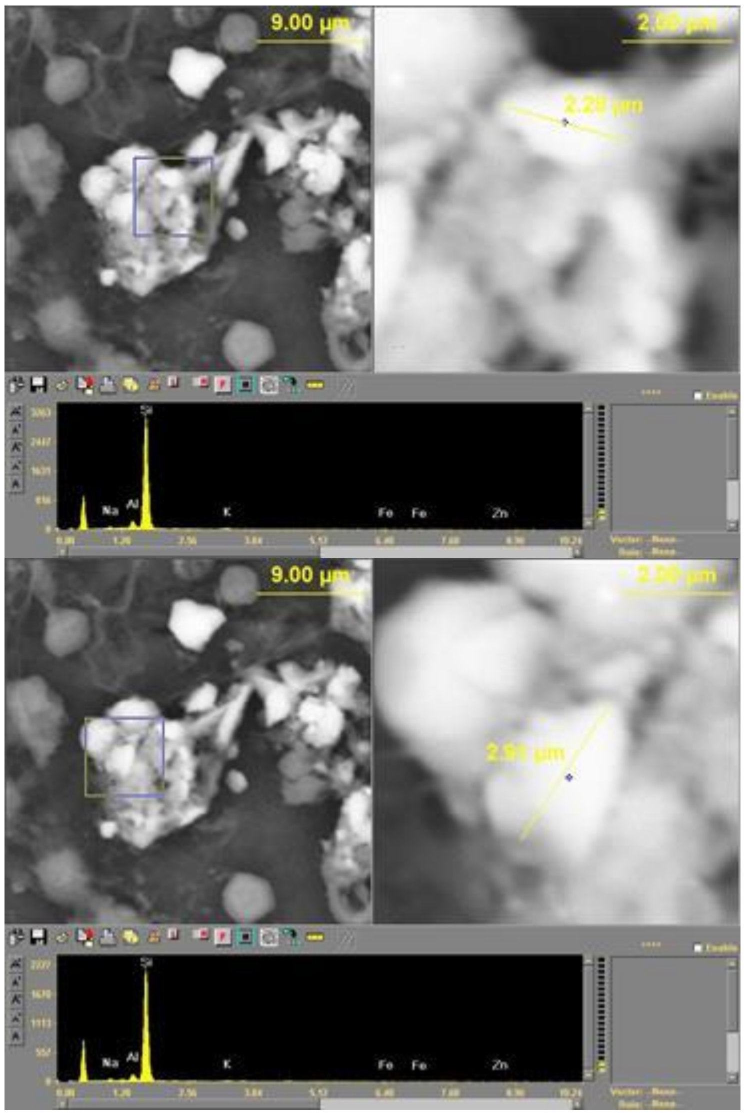

9.00 µm
2.00 µm
4.38 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
1.35 µm
Si
Na
Al
K
Fe
Fe
Zn

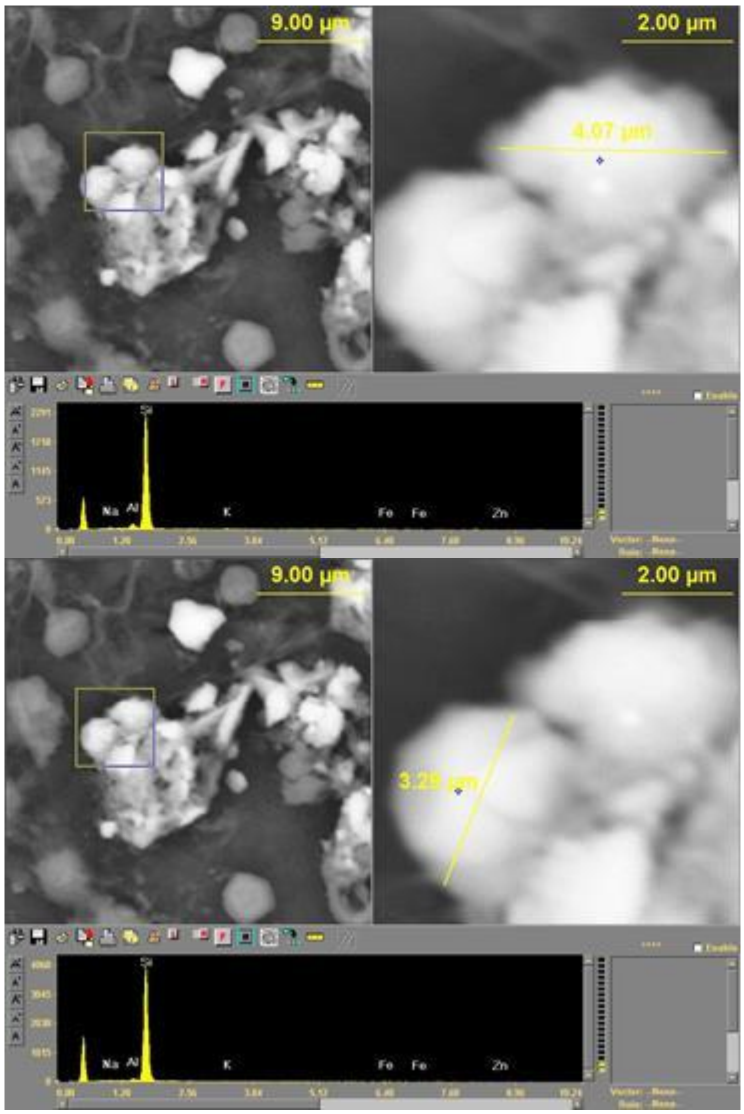

9.00 µm
2.00 µm
1.14 µm
Si
Al
Na
K
Fe
Fe
Zn
9.00 µm
2.00 µm
3.68 µm
Si
Na
Al
K
Fe
Fe
Zn

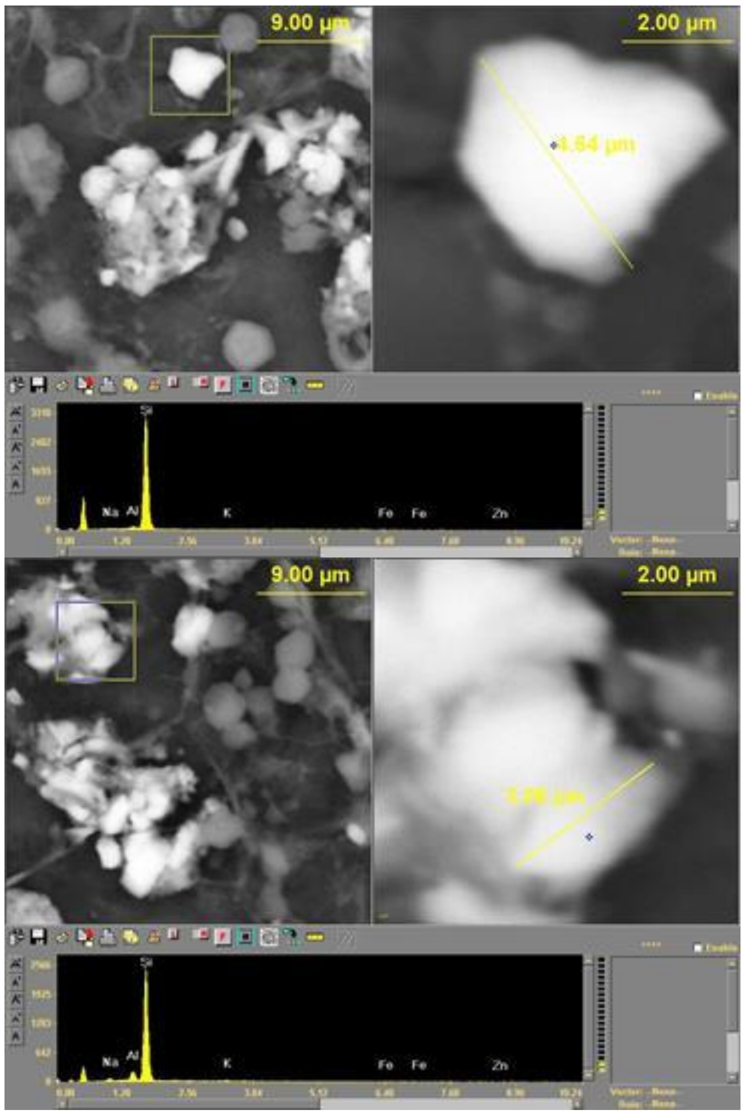

9.00 µm
2.00 µm
1.93 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
3.67 µm
Si
Na
Al
K
Fe
Fe
Zn


9.00 µm
2.00 µm
2.94 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
Si
Al
Na
K
Fe
Fe
Zn


9.00 µm
2.00 µm
2.65 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
1.54 µm
Si
Al
K
Na
Fe
Fe
Zn


9.00 µm
2.00 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
Si
Na
Al
K
Fe
Fe
Zn


9.00 µm
2.00 µm
4.07 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
3.29 µm
Si
Na
Al
K
Fe
Fe
Zn


9.00 µm
2.00 µm
Si
Na
Al
K
Fe
Fe
Zn
9.00 µm
2.00 µm
Si
Na
Al
K
Fe
Fe
Zn

References

Abraham, J. L. (1978). Analysis of Particulates in Tissues. La Jolia, California, United States of America.

Abraham, J. L., Burnett, B. R., & Hunt, A. (1990). Development and use of a Pneumoconiosis Database of Human Pulmonary Inorganic Particulate Burden in over 400 lungs. *Scanning Microscopy International*, 1-14- pg 95-108.

Adachi, K., & Tainosho, Y. (2004). Characterization of heavy metal particles emedded in tire dust. *Environment International*, 1009-1017.\

Adamson, I. Y. R., Prieditis, H., Hedgecock, C., and Vincent, R. (2000). Zinc is the toxic factor in the lung response to an atmospheric particulate sample. Toxicol. Appl. Pharmacol. 166, 111–119.

Balachandran, S., Meena, B. R., and Khillare, P. S. (2000). Particle size distribution and its elemental composition in the ambient air of Delhi. Environ. Int. 26, 49–54.

Councell, T. B., Duckenfield, K. U., Landa, E. R., & Callender, E. (2004). Tire-Wear Particles as a Source of Zince to the Environment. *Environmental Science and Technology*, 4206-4214.

Dye, J. A., Lehmann, J. R., McGee, J. K., Winsett, D. W., Ledbetter, A D., Everitt, J. I., Ghio, A. J., and Costa, D. L. (2001). Acute pulmonary toxicity of particulate matter filter extracts in rats: Coherence with epidemiological studies in Utah Valley residents. Environ. Health Perspect. 109(Suppl. 3), 395–403

EPA. (2010, November 17). *Environmental Protection Agency* . Retrieved from Particulate Matter: http://www.epa.gov/pm/

EPA, U. (2013, August 13). *Texas Commission on Environmental Quality* . Retrieved from PM2.5 Standars May Be Set Lower than Scientifically Justifiable : http://www.tceq.texas.gov/publications/pd/020/2013-NaturalOutlook/pm2.5-standards-may-be-set-lower-than-scientifically-justifiable

Harrison, R. M., and Yin, J. (2000). Particulate matter in the atmosphere: Which particle properties are important for its effects on health? Sci. Total Environ. 249, 85–101

Hunt, A., Abraham, J. L., Judson, B., & Berry, C. L. (2003). Toxicologic and Epidemiologic Clues from the Characterization of the 1952 London smog Fine Particulate matter in Archival Autopsy Lung Tissues. *Journal of the national Institue of Environmental Health sciences* , Vol 111-9.

Kodavanti, U., Schladweiler, M.C., Ledbetter, A.D., Hauser, R., Christiani, D.C., Samet, J.M., McGee, J., Richards, J.H., and Costa, D,L. (2002) Pulmonary and systemic effects of zinc-containing emission particles in three rat strains: multiple exposure scenarios. Toxicol Sci., 70(1):73-85.

Kodavanti, U. P., Mebane, R., Ledbetter, A. D., Krantz, T., McGee, J., Jackson, M. C., Walsh, L., Hilliard, H., Chen, B. Y., Richards, J., and Costa, D. L. (2000). Variable pulmonary responses from exposure to concentrated ambient air particles in a rat model of bronchitis. Toxicol. Sci. 54, 441–451.

Schoning, P., Abraham, J. L., & Burnett, B. R. (1996). Silicate and metal dust in lungs of Greyhounds. *Kansas Agricultural Experiment Station*, 1006-1008.

Soukup, J. M., Ghio, A. J., and Becker, S. (2000). Soluble components of Utah Valley particulate pollution alter alveolar macrophage function in vivo and in vitro. Inhal. Toxicol. 12, 401–414.

Statistcs, B. o. (2002, 2 14). *Estimated U.S. roadway Lane-Miles by Functional System*. Retrieved from Bureau of Transportation Statistics : http://222.bts.gov/publication/nts/Ch1_web/1-6.htm

Swinth, K.L. and Huth, G.C. (1974) Role Of Lymph-Nodes In Inhalation Exposures. Trans. Am. Nuc. Soc., 18: 385-386.

Biographical Information

Following this research Jamell Kennedy plans to apply for admission into medical school for 2015. The work done with this research will help formulate ideas and will prove useful when analyzing tissue under microscope. He plans to use this research to aid him in identifying exogenous and or xenobiotic substances that may find ways into human lung tissue. Use of the SEM-EDX and the morphometric point method will be a very valuable tool in the future. He interest is the cardiovascular system and how particulate matter effects the system. He has a bachelor's degree in Geology from SMU, following a Masters degree in Geoscience from The University Of Texas at Arlington.