Technical Appendix S5

Ichthys Gas Field Development Project: the effect of simulated dredge material from Darwin Harbour on eggs and larvae of barramundi (*Lates calcarifer*)

Ichthys Gas Field Development Project: The Effect of Simulated Dredge Material from Darwin Harbour on Eggs and Larvae of Barramundi (*Lates calcarifer*)

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1. Introduction

INPEX is proposing to construct a shipping channel, turning basin and berthing pockets in Darwin Harbour to support the development of a major gas processing facility. The dredge spoil will be disposed of at an offshore location north east of Darwin Harbour. These dredging activities and offshore spoil disposal has the potential to cause elevated suspended sediment concentrations in Darwin Harbour, from sediment released during dredging and at Howard River and Hope Inlet, from sediment that may be transported away from the dredge spoil disposal ground by the prevailing currents (the INPEX Draft EIS provides details of the location and characteristics of these areas).

The Howard River estuary and, to a lesser extent, Darwin Harbour are thought to be spawning and nursery areas for barramundi. During consultation for the Project, concerns were expressed as to the potential impact of elevated suspended sediment concentrations on fish spawning success and larval survival. The species of most concern was barramundi, which is a popular target for recreational fishermen and an important commercial fisheries resource. The potential for elevated suspended sediment concentration, caused by sediments released during dredging and dredge spoil disposal, to effect barramundi eggs and larvae have been investigated.

The ill effects of suspended solids and associated turbidity on fish are extremely varied. Effects can be broadly categorized into direct effects, such as physical damage and abrasion (Buermann et al., 1997), and indirect effects such as feeding impairment

caused by reduced light penetration (Kerr, 1995). The effects of suspended solids on fish are determined primarily by the concentration, size and shape of the particles and the duration of exposure (Newcombe and MacDonald, 1991; Newcombe and Jensen, 1996).

A significant body of literature exists on the effects of turbidity and suspended sediments on fish, however, few studies have dealt with fish eggs and larvae. For example, Newcombe and Jensen (1996) reviewed over 80 publications on the effects of turbidity on fish and of these, over 85% were focused on juvenile or adult fish. Estuarine fish species have been shown to be more tolerant of turbidity than marine species (O'Connor et al., 1976; Bristow et al., 1996) and larvae are the most sensitive of all of the life stages (Appleby and Scarratt, 1989). Unlike juveniles and adults, fish larvae cannot avoid high levels of turbidity; they are very fragile and more prone to physical damage, they have a greater metabolic demand (per unit body weight) and are therefore more prone to oxygen depletion (O'Connor et al., 1976; Appleby and Scarratt, 1989; Isono et al., 1998). Larval fish are visual feeders and prone to the impacts of reduced light penetration associated with turbidity (Blaxter, 1968; 1969; Utne-Palm, 2004).

The aim of this study was to determine the direct and indirect effects of suspended solids on eggs and larvae of barramundi. The direct effects on eggs were assessed by determining the effects of concentration and exposure time on egg viability, sediment

adherence to eggs and egg hatch rate. The same factors were investigated on the survival of pre-feeding larvae.

2. Preparation and characterisation of phyllite dredge material

2.1. Introduction

It has as been demonstrated that due to the wide range of physical characteristics of different sediment particles, ecological studies investigating the effects of these sediments should utilise the sediment in question. Dredge material used in this study was therefore sourced from East Arm.

2.2. Methods

Phyllite material was taken from the site in the form of core samples and sent to Microanalysis Australia where it was ground to a particle size distribution reflective of the sediment plume predicted to occasionally be transported into the Howard River estuary (refer to INPEX Draft EIS, Technical Appendix 13 for details of sediment transport modelling)(http://www.inpex.com.au/media/20949/appendix_13-dredging_and_spoi_disposal_modelling.pdf). Phyllite was firstly homogenised into a slurry (10% maximum w/w) then placed into the reservoir of a Mozley hydrocyclone size abstractor. The slurry was maintained in an agitate state within this reservoir by continuous mixing and pumping via a by-pass valve allowing the slurry to dispense back into the reservoir. Once the correct pressure and flow rates were achieved the slurry

was re-directed through the hydrocyclone and the resultant overflow and underflow collected separately for particle size distribution analysis and further homogenisation and separation if required. The desired cut size was obtained by varying the flow rate and pressure, cyclone configuration (internally variable components) and slurry concentration. Particle sizes were measured using laser diffraction (Malvern Instruments, Mastersizer MS2000). A number of blends of various particle size distributions were combined in the ratios required to achieve the desired particle size distribution.

Scanning electron microscopy (SEM) and sphericity analysis were conducted on a representative sub-sample from the final slurry. This sample was placed on top of a double sided carbon tab before being carbon coated. The sample was analysed using a JEOL 5800LV scanning electron microscope (SEM) fitted with an Oxford INCA energy dispersive spectrometer (EDS).

Energy dispersive spectrometery is a semi-quantitative technique used to identify elemental composition of the particles from well prepared, optically flat samples.

The scale used to classify roundness and sphericity is shown in Figure 1.

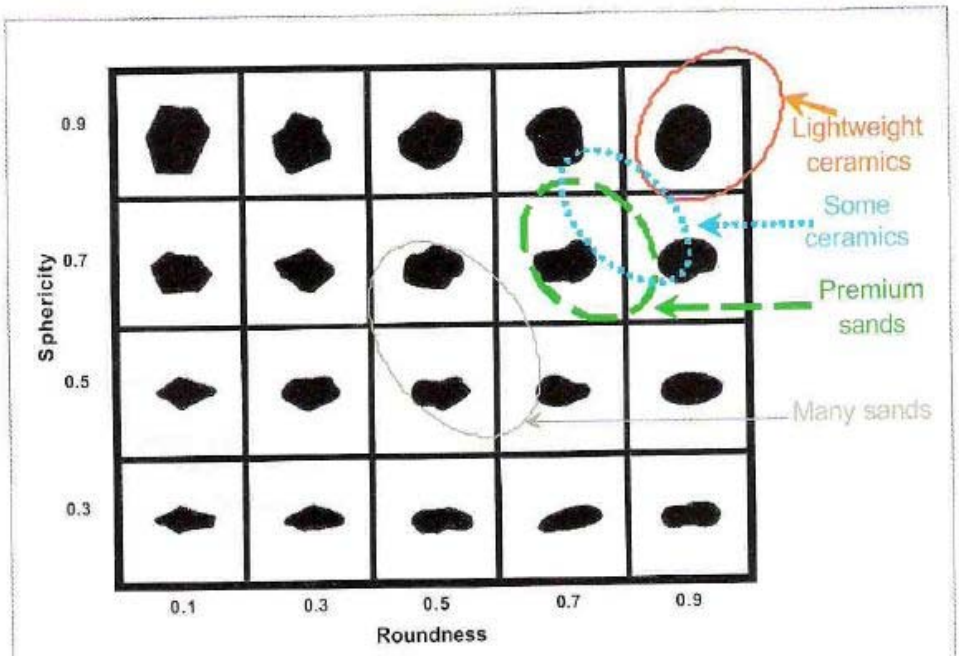


Figure 1: API RP60 Sphericity and roundness index.

The final ground material was supplied to Challenger Institute by Microanalysis Australia as slurry in freshwater. Here, the suspended solids concentration of this slurry was determined by oven drying triplicate 5 mL samples in pre-weighed glass vials.

2.3. Results and Discussion

The particle size distribution of the final slurry is shown in Figure 2.

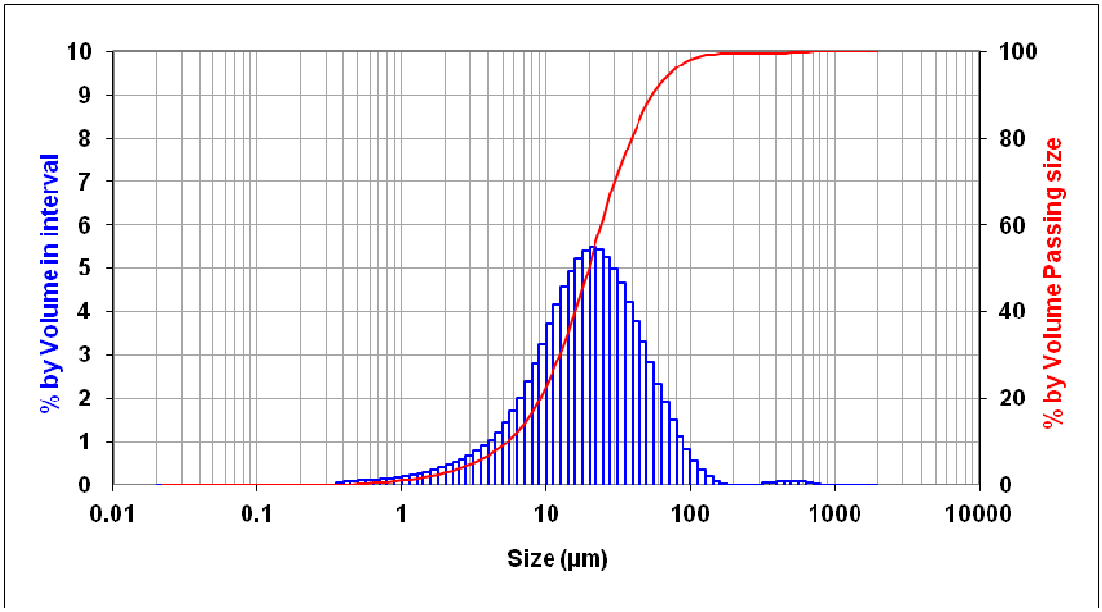


Figure 2: Particle size distribution of the phyllite-based simulated dredge material used in the current study.

Scanning electron microscopy revealed a range of particle sizes from ~ 1 µm to ~ 400 µm, with the following distinct compositions:

- Dominant quantities of alumino silicate (possible mica) containing minor potassium, titanium and iron, displaying lamellar cleavage morphologies with roundness values typically at 0.5 and sphericity values of less than 0.5 (refer to

Figure 1 for details on roundness and sphericity values), indicating that although not very spherical, the particles were not extremely sharp and angular.

- Trace quantities of discrete particles containing copper, zinc, zirconium and higher concentrations of titanium were observed.

Further details of the SEM analysis can be found in Appendix 1.

3. The effect of sediment concentration and exposure duration on the viability and hatch rate of barramundi eggs.

3.1. Introduction

The shipping channel, turning basin and berthing pockets proposed for the INPEX project are located in East Arm of the Darwin Harbour, adjacent to Balydin Point, while the dredge spoil disposal ground is outside of Darwin Harbour, as shown by Figure 4

Neither the dredging operations nor spoil disposal operations are likely to interfere directly with the fish's spawning events, however the released eggs may be exposed to suspended sediments released during dredge operations or transported from the spoil disposal ground. The aim of this trial was to determine the potential negative effects of dredge-induced suspended sediment on fertilised barramundi eggs.

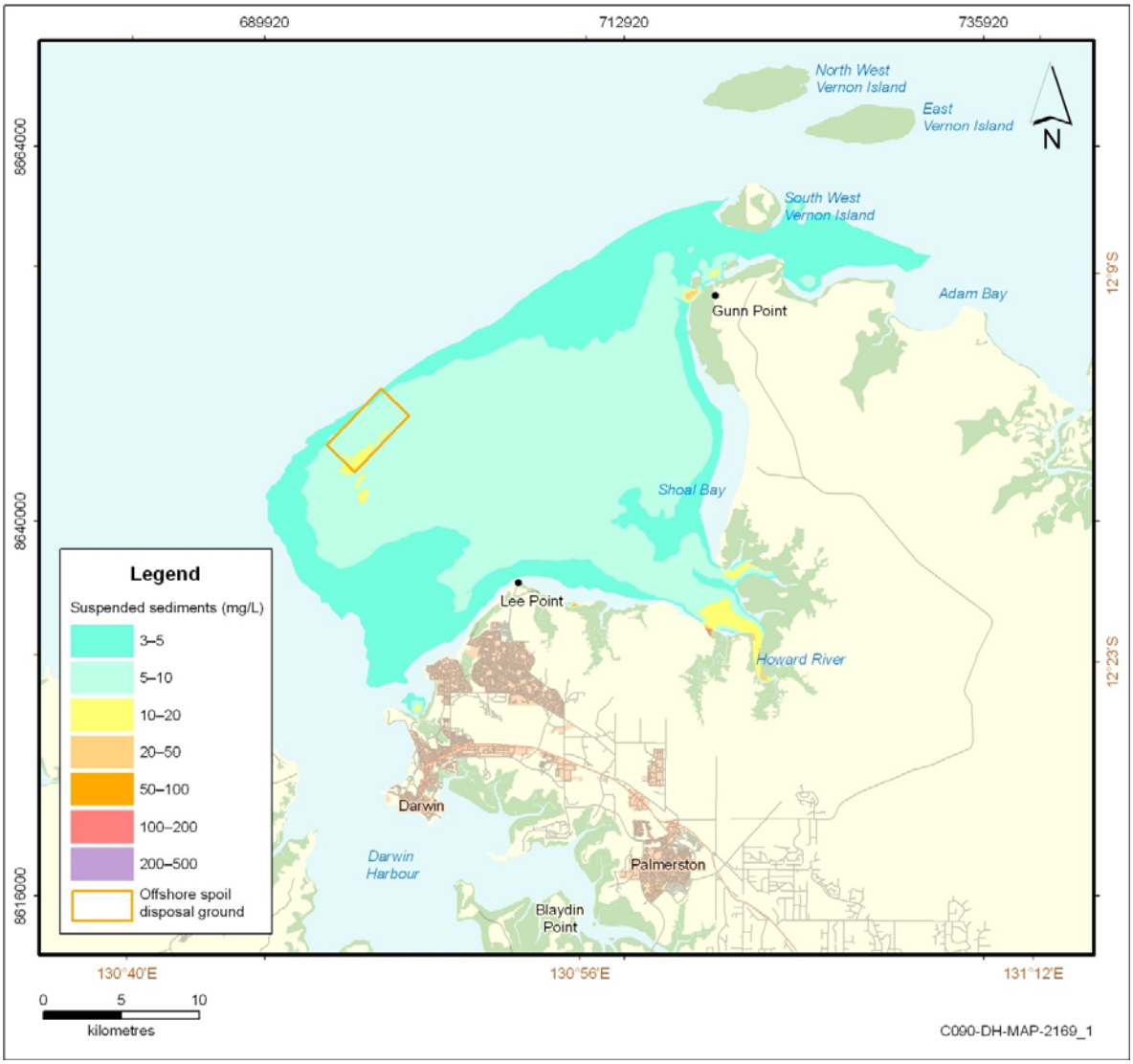


Figure 3: **Location of proposed dredge spoil disposal ground and contours showing the predicted 95th percentile concentration of suspended sediments transported from the spoil ground by prevailing currents during Phase 5 dredge operations**

3.2. Materials and Methods

The methods used to investigate the effects of suspended solids exposure on barramundi eggs were based on those described by Isono et al. (1998) and Partridge and Michael (2009).

Barramundi eggs were collected within 3 hours of being naturally spawned by broodstock held at Good Fortune Bay's hatchery in Bowen, Queensland. Barramundi broodstock are held in a 40m^3 tank with partially re-circulated seawater at 32 ppt. The temperature during spawning events ranged from 28-29°C.

During the trial, eggs were exposed to eight concentrations of simulated dredge material in seawater (20, 50, 100, 250, 500, 750, 1000, 2000 mg/L). Two control treatments were also included. These comprised a 0 mg/L TSS treatment (i.e. seawater) and filtrate from the 2,000 mg/L solution of simulated dredge material (to determine if any toxic residues are leached from the sediment). Two sets of triplicate 50 mL suspension vials for each of the above treatments were prepared. Approximately twenty fertilised eggs were stocked volumetrically into each vial. Vials were continuously rotated along their horizontal axis at 6 rpm for the duration of exposure to ensure the sediment remained in suspension (Figure 4). Water temperature within the vials was maintained at 29.0 ± 0.5°C by a reverse cycle air conditioner which maintained the air within the laboratory at this temperature.

Figure 4: Experimental setup showing the rotating mechanism and 50 mL exposure vials.

After 4.5 hours of exposure, one set of triplicate vials was removed and the sediment separated from the eggs by pouring through a 200 µm screen. Seawater was gently rinsed through this screen then the eggs transferred to beakers containing 50 mL of clean seawater. After 11.5 hours of exposure, the second set of vials was processed in the same manner. After 15 hours (and just prior to hatch) each beaker was observed under a dissecting microscope. The total number of eggs, viable number of eggs (i.e. those containing a live embryo) and number of eggs with sediment adherence was counted. Percentage viability was calculated as follows:

$$\%\,\text{viability} = \left(\frac{\text{number of viable eggs}}{\text{total number of eggs}} \right) \text{x}100$$

After 17 hours all beakers were again observed and the number of hatched larvae recorded. The percentage hatch rate was calculated as:

$$\%\,\text{hatch} = \left(\frac{\text{number of eggs hatched}}{\text{number of viable eggs}} \right) \text{x}\, 100 .$$

A two-way analysis of variance investigated the effects of control type (0 mg/L and filtrate) and exposure duration (4.5 or 11.5 hours) on egg viability and hatch rate to determine if any substances toxic to the developing embryos were being leached from the simulated dredge material. A second two-way analysis of variance compared the effects of sediment concentration and exposure duration on egg viability and hatch rate.

3.3. Results and Discussion

No adherence of phyllite particles to eggs was observed at any concentration or for any exposure duration.

A comparison between the 0 mg/L control and the filtrate-exposed treatment revealed no significant differences in egg viability or hatch rate (regardless of exposure time) (two-way ANOVA $F = 0.76$, $P = 0.72$ for hatch rate and $F = 1.06$, $P = 0.42$ for viability), demonstrating that no toxic substances were leaching from the simulated dredge material.

Egg viabilities in the various treatments are shown in Figure 5. Viability ranged from 58 ± 10% to 90 ± 10%, with a two-way analysis of variance revealing there to be no effect of sediment concentration (F= 0.49, P = 0.85) or exposure duration (F= 2.29, P = 0.14) on viability.

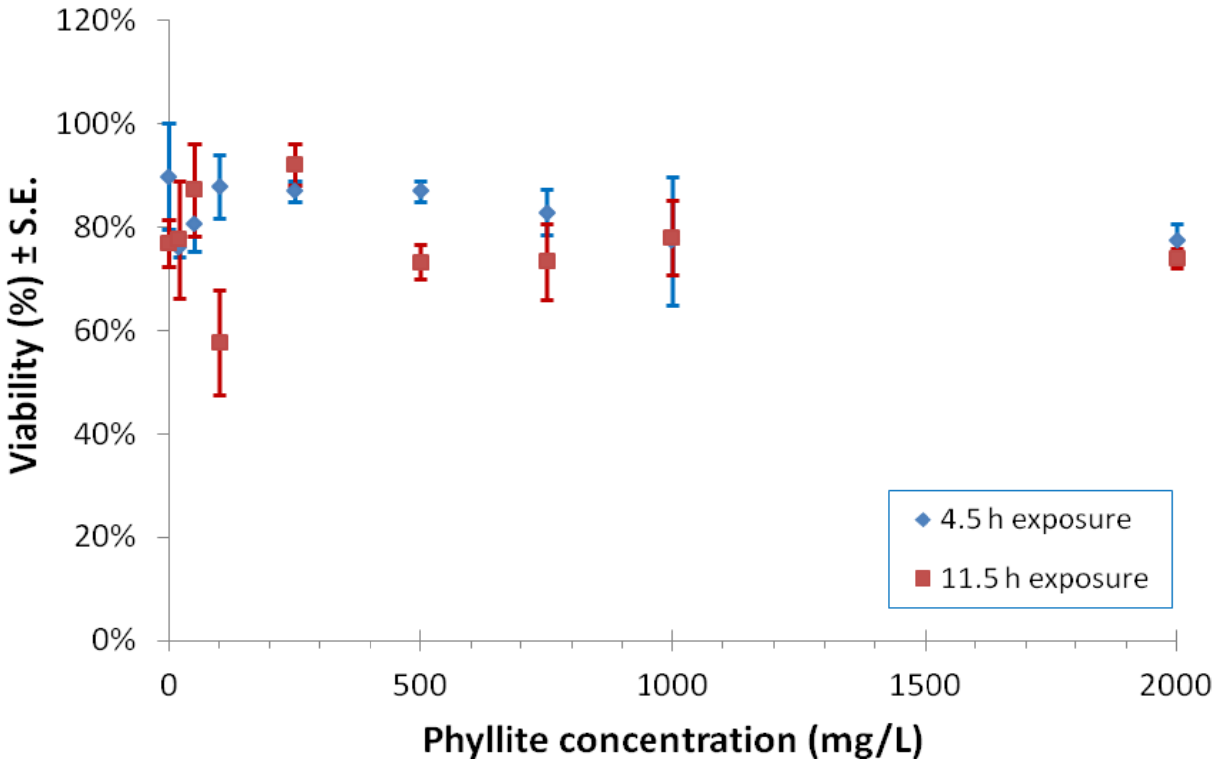


Figure 5: **Viability of barramundi eggs exposed to simulated dredge material at various concentrations and times.**

Hatch rates in the various treatments are shown in Figure 6. Hatch rate ranged from 89 ± 1% to 100 ± 0%, with a two-way analysis of variance revealing no effect of sediment concentration (F= 1.45, P = 0.21) or exposure duration (F = 0.13, P = 0.72). These data

together effectively highlight that barramundi eggs are extremely tolerant of suspended solids.

Although it has been demonstrated that high concentrations of suspended solids can interfere with oxygen uptake, developmental rates and hatching success of fish eggs (Kiorboe et al., 1981), the tolerance of eggs to suspended solids is generally high and has been described for a number of species (Auld and Schubel, 1978; Kiorboe et al., 1981; Morgan et al., 1983; Boehlert and Morgan, 1985; Isono et al., 1998; Partridge and Michael, 2009).

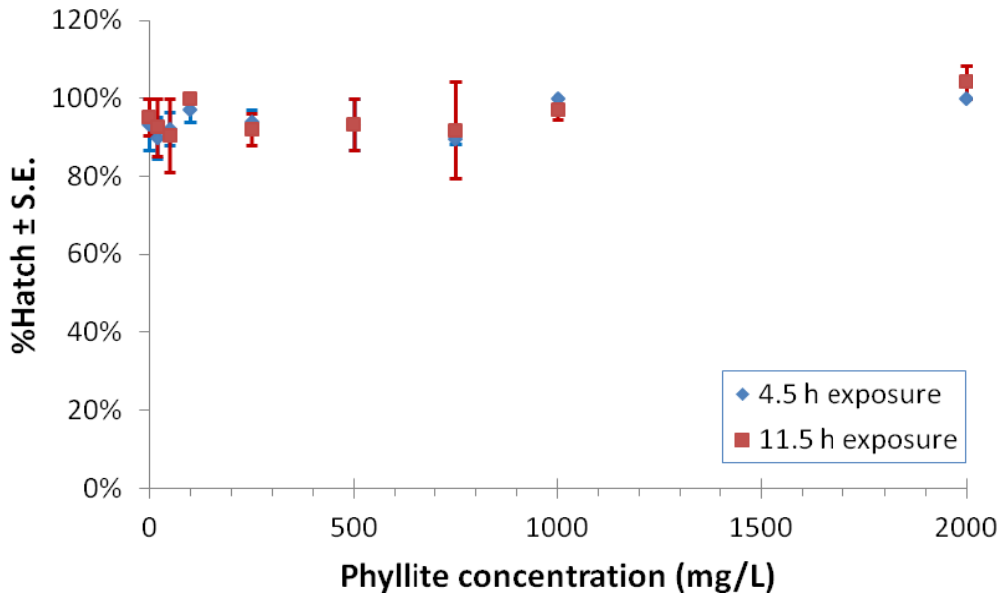


Figure 6: **Hatch rate of barramundi eggs exposed to simulated dredge material at various concentrations and times.**

Morgan et al (1983) found that the percent hatch rates for white perch and striped bass eggs were unaffected by the highest sediment concentrations tested (5,280 and 2,300 mg/L, for each species, respectively). Although hatch rate was unaffected, the rate of embryonic development of both species was negatively affected by concentrations in excess of approximately 1,300 mg/L. White perch eggs are demersal and completely covering them with 2 mm of sediment resulted in 100% mortality, but a layer of 0.45 mm had no effect on hatch. Barramundi eggs are not demersal and no sediment adhered to them, even at the highest concentration tested. Our data demonstrating no effect of sediment concentrations up to 2,000 mg/L on hatch rate also suggests that sediment exposure was not sufficient to interfere with oxygen uptake and subsequent development. All viable eggs hatched within 2 hours of each other, regardless of the sediment concentration they were exposed to, demonstrating that the rate of egg development (i.e. the time taken to hatch) was similar between the controls and those exposed to sediment.

4. Acute toxicity of phyllite sediment to barramundi larvae at two stages of development.

4.1. Introduction

The aim of this trial was to provide baseline data on the toxicity of Darwin Harbour dredge spoil to barramundi larvae at two stages of development and for various exposure times. Data from Isono et al. (1998) showed that the 12 hour LC_{50} of kaolinite to newly hatched snapper larvae was approximately 1,000 mg/L. Although this concentration is well in excess of that predicted for the spoil disposal plumes in the Howard River estuary, these authors only exposed larvae to sediment prior to their mouths or opercula opening. With the gills of larval fish being highly susceptible to physical abrasion and clogging (Auld and Schubel, 1978), it is important to also assess the toxicity of the dredge spoil to larvae whose gills are exposed to the suspened sediment.

In 1991, Newcombe and MacDonald demonstrated that the concentration of suspended solids alone is a poor indicator of the effects of sediment on fish and that a combination of concentration and exposure time provides much greater predictive power into the effects of suspended sediment.

The effects of exposure to nine concentrations of phyllite dredge spoil were measured on mouth-closed larvae for 12 hours (Experiment 1) and on mouth-open larvae for durations of 6, 12 and 18 hours (Experiment 2).

4.2. Materials and Methods

The experimental protocol and equipment used in these two trials was similar to that described for the egg exposure trials above and by Isono et al. (1998) and Partridge and Michael (2009). In both trials larvae were exposed to the same sediment concentrations described above (0 to 2,000 mg/L) and all treatments were again tested in triplicate. Control treatments of seawater and filtrate of the 2,000 mg/L simulated dredge material were also included in Experiment 1 to test the possibility of toxic substances being leached from the sediment. Water temperature in both trials was again maintained at 29 ± 0.5°C. Experiment 1 tested the effects of simulated dredge material on the survival of 'mouth-closed' larvae exposed to sediments for 12 hours. Experiment 2 exposed mouth-open larvae to simulated dredge material for various exposure times (6, 12 and 18hrs).

Although the ANZECC/ARMCANZ Water Quality Guidelines (2000) use 48 – 96 hr EC_{50} values for the derivation of chemical toxicity guidelines, it was not possible to use these time frames in the testing of TSS toxicity for early stage larvae. Once the larvae's mouth opens they need to start feeding within one day. If trial durations of 48 - 96 hours were employed, then the larvae would begin starving to death, thereby confounding the

results. In addition, this was not considered to be a limitation given that elevations in suspended sediment concentrations from spoil disposal are only expected to occur in pulses coinciding with spring flood tides; the duration of which is likely to be in the order of several hours.

Twenty larvae were hand counted into each 50 mL exposure vial containing the required concentration of TSS. Mouth-closed larvae (Experiment 1) were stocked within 3 hours of hatching, whilst mouth-open larvae (Experiment 2) were stocked at 36 hours post-hatch (within 4 hours of mouth opening). The vials were continuously rotated along their horizontal axis at 6 rpm for the duration of exposure. After the allocated exposure time, sediment was removed from the vials by gentle rinsing through a 200 µm screen as previously described. The numbers of live and dead larvae were immediately counted under a dissecting microscope and survival assessed as follows:

$$\%\,\text{survival} = \left(\frac{\text{number of live larvae}}{20} \right) \text{x} 100$$

A one-way analysis of variance was used to compare survival data between the two control treatments of Experiment 1 to determine if any substances toxic to newly hatched larvae were present in the sediment. A one-way analysis of variance was also used to compare the effects of sediment concentration on survival of mouth-closed larvae. The effects of exposure duration and sediment concentration on the survival of mouth-open larvae (Experiment 2) were compared with two-way analysis of variance.

Lowest observable effect concentrations (LOEC) were calculated using ToxCalc™ (Environmental Toxicity Data Analysis System v5.0.32).

4.3. Results and Discussion

4.3.1. Experiment 1 – Mouth-closed larvae

There was no significant difference in survival of mouth-closed larvae between the 0 mg/L control (56 ± 5%) and the filtrate control (40 ± 14%), ($F = 1.26$, $P = 0.32$) again demonstrating that no toxic substances are leached from the phyllite dredge material.

Figure 7 shows the effect of sediment concentration on survival of mouth closed larvae exposed for 12 hours. One-way analysis of variance revealed a significant effect of suspended sediment concentration on larval mortality ($F = 5.77$, $P = 0.001$). Tukey's HSD post-hoc comparison of treatment means showed there to be no differences in mortality at concentrations from 0 to 500 mg/L. Although there were no significant differences within this range of concentrations, survival at 20 mg/L (68%), 50 mg/L (79%) and 100 mg/L (67%) were all higher than obtained in the seawater control (56%). These data may suggest a beneficial effect of sediment to early barramundi, which is not unexpected given the natural habitat in which this species spawns (Moore, 1982; Schipp et al., 2007).

Those larvae exposed to a concentration of 750 mg/L or higher had significantly lower survival than those exposed to a sediment concentration of 50 mg/L. Similarly, ToxCalc determined the lowest observable effect concentration for 12 hour exposed, mouth closed larvae to be 750 mg/L.

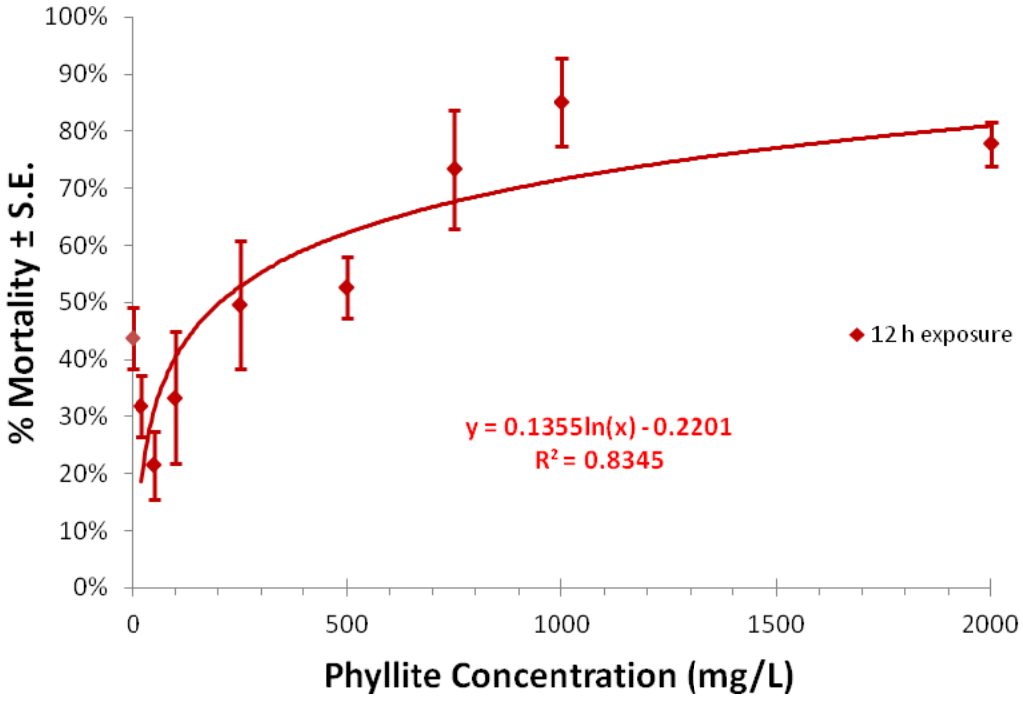


Figure 7: **Mortality of barramundi larvae of different life stages exposed for 12hrs to varying concentrations of simulated dredge material.**

4.3.2. Experiment 2 – Mouth-open larvae

Figure 8 shows the survival of larvae exposed to the various concentrations of phyllite for three exposure durations.

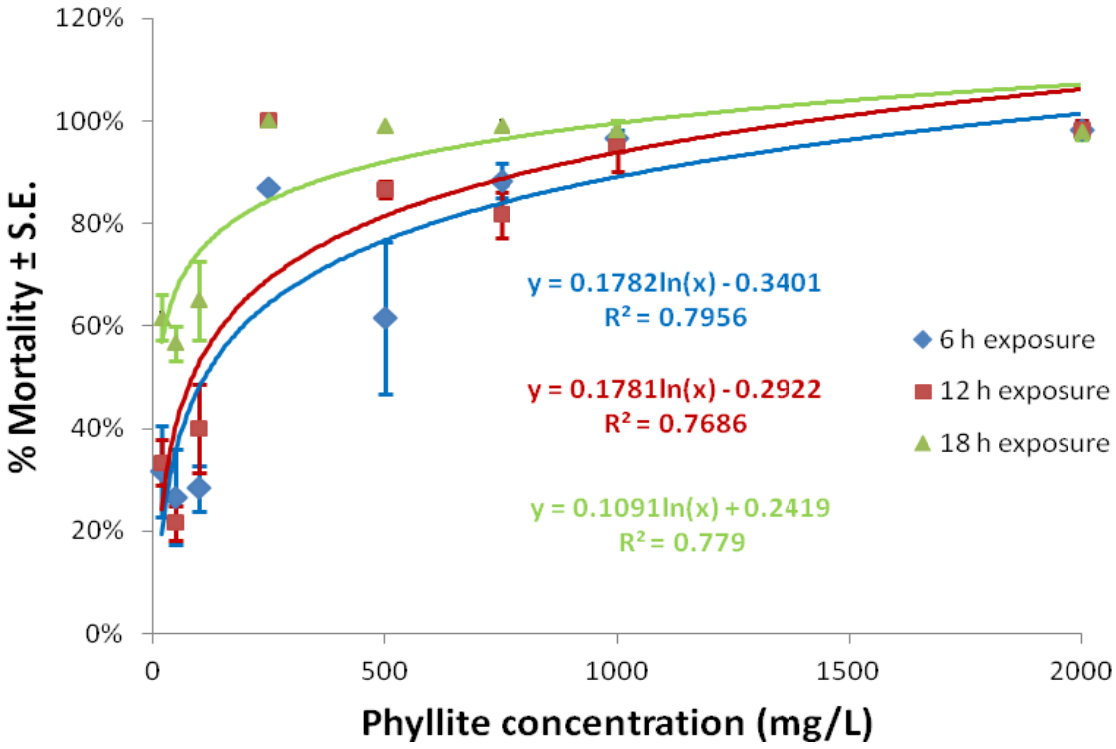


Figure 8: Mortality of mouth-open barramundi larvae exposed to varying concentrations of simulated dredge material for 6, 12 & 18 hours.

Two way analysis of variance revealed a significant effect of both sediment concentration ($F = 29.3$, $P<0.0001$) and exposure time ($F = 5.78$, $P = 0.005$) on the survival of mouth-open larvae, with no significant interaction between these terms ($F = 0.55$, $P = 0.91$). Tukey's HSD test on least square means demonstrated that survival of larvae exposed to concentrations greater than and equal to 250 mg/L were significantly

higher than concentrations of 0 – 100 mg/L. For each exposure time, the lowest observable effect concentration was 250 mg/L i.e. no effects were seen on larvae at concentrations below this concentration.

The mortality of larvae exposed to sediment for 6 and 12 hours was significantly lower than those exposed for 18 hours, which Tukey's HSD analysis of Least Squares Means (LSM) showed were not different from each other. A positive correlation between exposure time and sediment toxicity has been described for a number of species and life stages (Morgan et al., 1983; Newcombe and MacDonald, 1991; Newcombe and Jensen, 1996) and the significant differences we obtained between 6 and the 12 hour and 18 hour are consistent with these findings. That we obtained no significant difference in toxicity between 12 and 18 hours exposure suggests that the majority of harm is done between the 12^{th} and 18^{th} hours of exposure. These findings are consistent with those described for sediment exposed snapper larvae by Partridge and Michael (2009).

A plot comparing survival of mouth-closed (Experiment 1) and mouth-open (Experiment 2) larvae each exposed to sediment for 12 hours is shown in Figure 9.

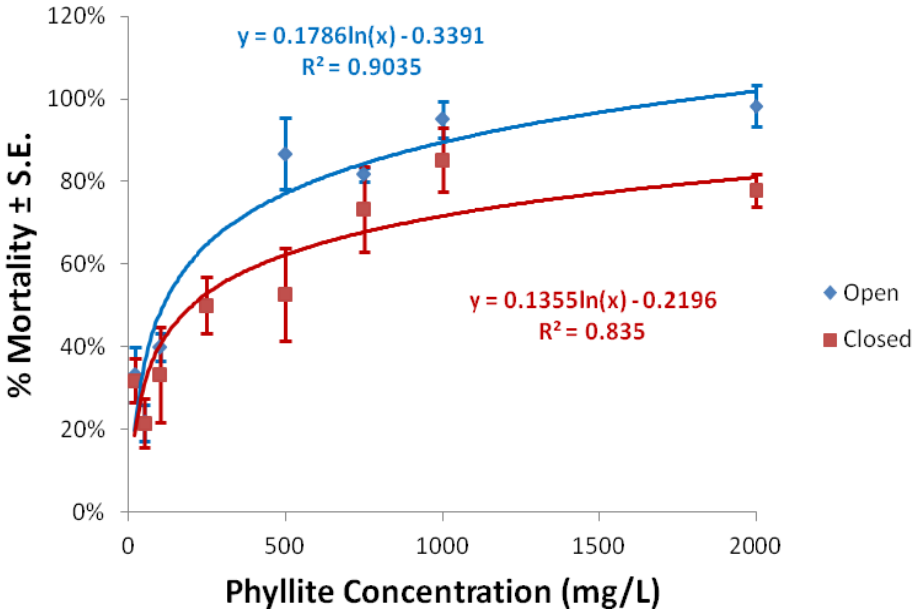


Figure 9 **A comparison of survival between mouth-closed and mouth-open larvae exposed to various sediment concentrations for 12 hours.**

The data in this figure demonstrate that once the larvaes' mouths open they become less tolerant of suspended solids, a feature previously reported for snapper larvae by Partridge and Michael (2009). Two factors likely contribute to this lower tolerance. Once the larvae's mouth opens, they begin to drink seawater as a mechanism to offset the diffusional loss of water to the surrounding, hyperosmotic medium (Varsamos et al., 2004). As such, those larvae in the sediment treatments will have ingested sediment which may have contributed to their death. Secondly, once the larvae's mouth opens, so too does their operculum; exposing the gills to sediment. With gill abrasion and damage having been previously described for juvenile and adult fish (Appleby and Scarratt, 1989; Buermann et al., 1997), this is a likely mechanism for the higher mortality seen in larvae with open mouths.

5. References

Appleby, J.P., Scarratt, D.J., 1989. Physical effects of suspended solids on marine and estuarine fish and shellfish with special reference to ocean dumping: A literature review. Canadian Fisheries and Aquatic Sciences, Nova Scotia, pp. 33.

Auld, A.H., Schubel, J.R., 1978. Effects of suspended sediment on fish eggs and larvae: a laboratory assessment. Estuarine and Coastal Marine Science 6, 153-164.

Blaxter, J.H.S., 1968. Visual Thresholds and Spectral Sensitivity of Herring Larvae. Journal of Experimental Biology 48, 39-53.

Blaxter, J.H.S., 1969. Visual Thresholds and Spectral Sensitivity of Flatfish Larvae. Journal of Experimental Biology 51, 221-230.

Boehlert, G.W., Morgan, J.B., 1985. Turbidity enhances feeding abilities of larval pacific herring, *Clupea harengus pallasi*. Hydrobiologia 123, 161-170.

Bristow, B.T., Summerfelt, R.C., Clayton, R.D., 1996. Comparative performance of intensively cultured larval walleye in clear, turbid and coloured water. The Progressive Fish Culturist 58, 1-10.

Buermann, Y., Du Preez, H.H., Steyn, G.J., Smit, L., 1997. Tolerance levels of redbreast tilapia, *Tilapia rendalli* (Boulenger, 1896) to natural suspended silt. Hydrobiologia 344, 11-18.

Isono, R.S., Kita, J., Setoguma, T., 1998. Acute effects of Kaolinite suspension on eggs and larvae of some marine teleosts. Comparative Biochemistry and Physiolgy 120, 449-455.

Kerr, S.J., 1995. Silt, Turbidity and Suspended Sediments in the Aquatic Environment. Ministry of Natural Resources, Ontario, pp. 277.

Kiorboe, T., Frantsen, E., Jensen, C., Sorensen, G., 1981. Effects of suspended sediment on development and hatching of Herring (*Clupea harengus)* eggs. Estuarine Coastal and Shelf Science 13, 107-111.

Moore, R., 1982. Spawning and early life history of barramundi, *Lates calcarifer*, (Bloch), in Papua New Guinea. Aust. J. Mar. Freshw. Res. 33, 647-661.

Morgan, R.P., Rasin, V.J., Noe, L.A., 1983. Sediment effects on eggs and larvae of striped bass and white perch. Transactions of the American Fisheries Society 112, 220-224.

Newcombe, C.P., MacDonald, D.D., 1991. Effects of suspended sediments on aquatic ecosystems. North American Journal of Fisheries Management 11, 72-82.

Newcombe, C.P., Jensen, J.O.T., 1996. Channel Suspended Sediment and Fisheries: A synthesis for quantitative assesment of risk and impact. North American Journal of Fisheries Management 16, 693-272.

O'Connor, J.M., Neumann, D.A., Shark, J.A.J., 1976. Lethal effects of suspended sediments on estuarine fish. US Coastal Engineering Research Technical Paper.

Partridge, G.J., Michael, R.J., 2009. Direct and indirect effects of simulated calcareous dredge material on eggs and larvae of pink snapper *Pagrus auratus* (Bloch and Schneider). Journal of Fish Biology 75, 1518-1523.

Schipp, G.R., Bosmans, J.M.P., Humphrey, J., 2007. Barramundi Farming Handbook. Northern Territory Government, Darwin, 80 pp.

Utne-Palm, A.C., 2004. Effects of larvae ontogeny, turbidity, and turbulence on prey attack rate and swimming activity of Atlantic herring larvae. Journal of Experimental Marine Biology and Ecology 310, 147-161.

Varsamos, S., Wendelaar Bonga, S.E., Charmantier, G., Flik, G., 2004. Drinking and Na^+/K^+ ATPase activity during early development of European sea bass, *Dicentrarchus labrax*: Ontogeny and short-term regulation following acute salinity changes. J. Exp. Mar. Biol. Ecol. 311, 189-200.

6. Appendices

6.1. Appendix 1

Suite 6
642 Albany Hwy
Victoria Park
WA 6100

Client: Challenger Institute of Technology

Job number: 10_536

Sample: 10_536_01

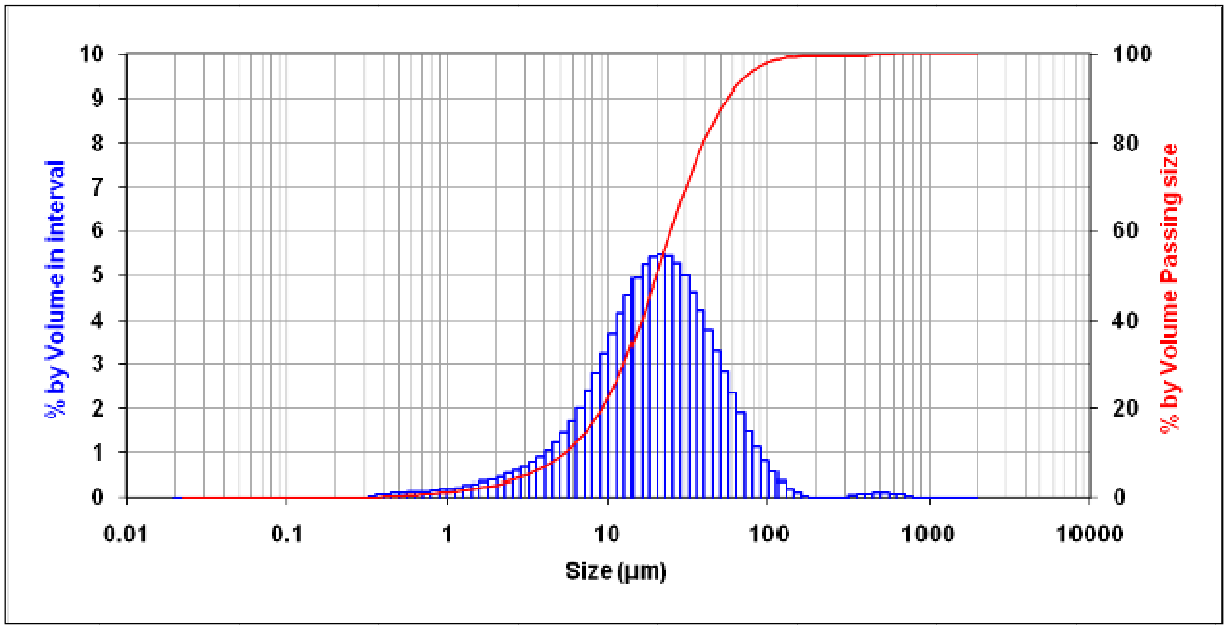
Client ID: Dredge Core -53µm

Date: 24/11/2010

Analysis: Scanning electron microscopy (SEM) with elemental analysis by energy dispersive spectroscopy (EDS)

Sample preparation

The sample was supplied to Microanalysis Australia as a coarse rock fragment. The fragment was crushed by a range of techniques including coarse compression (hammer), ring mill and microniser to produce a target particle size distribution (as shown in graph 1) with a D50 of approximately 20 µm.



A representative sub-sample was removed and placed on top of a double sided carbon tab before being carbon coated. Non-conducting samples require coating prior to SEM analysis to prevent charging whilst being analysed by the electron beam.

Analysis

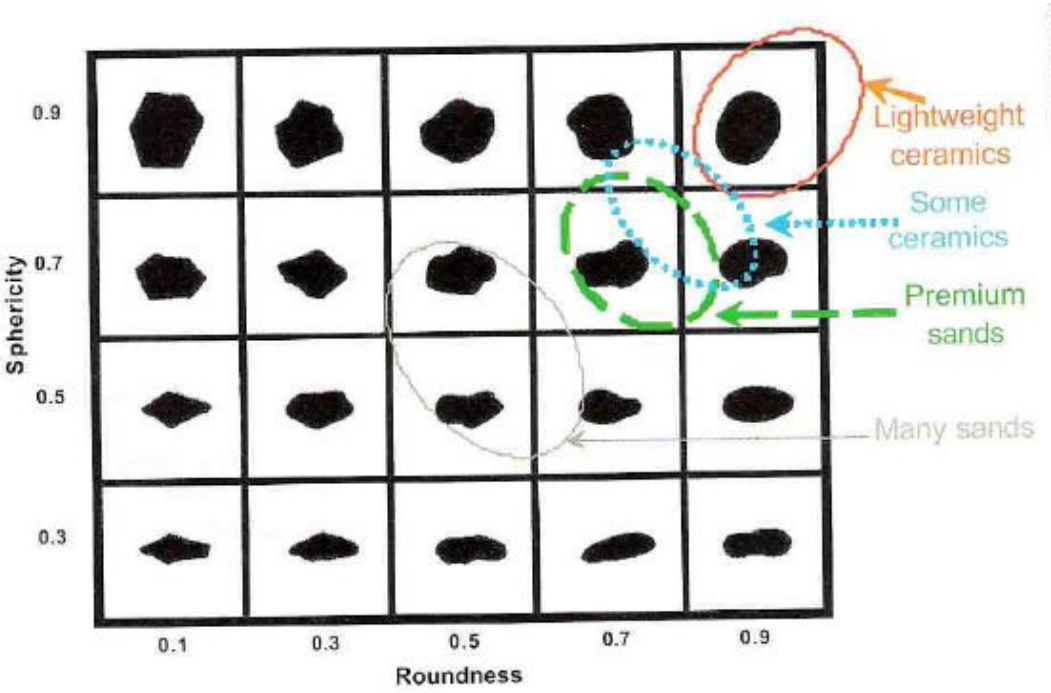
The sample was analysed using a JEOL 5800LV scanning electron microscope (SEM) fitted with an Oxford INCA energy dispersive spectrometer (EDS).

EDS is a semi-quantitative technique (at best) on well prepared, optically flat samples. Factors such as sample unevenness may adversely bias elemental concentration interpretation. EDS has a spatial resolution of ~5 µm meaning spectra from particles less than this size may contain elemental concentrations biased by their surroundings.

Summary

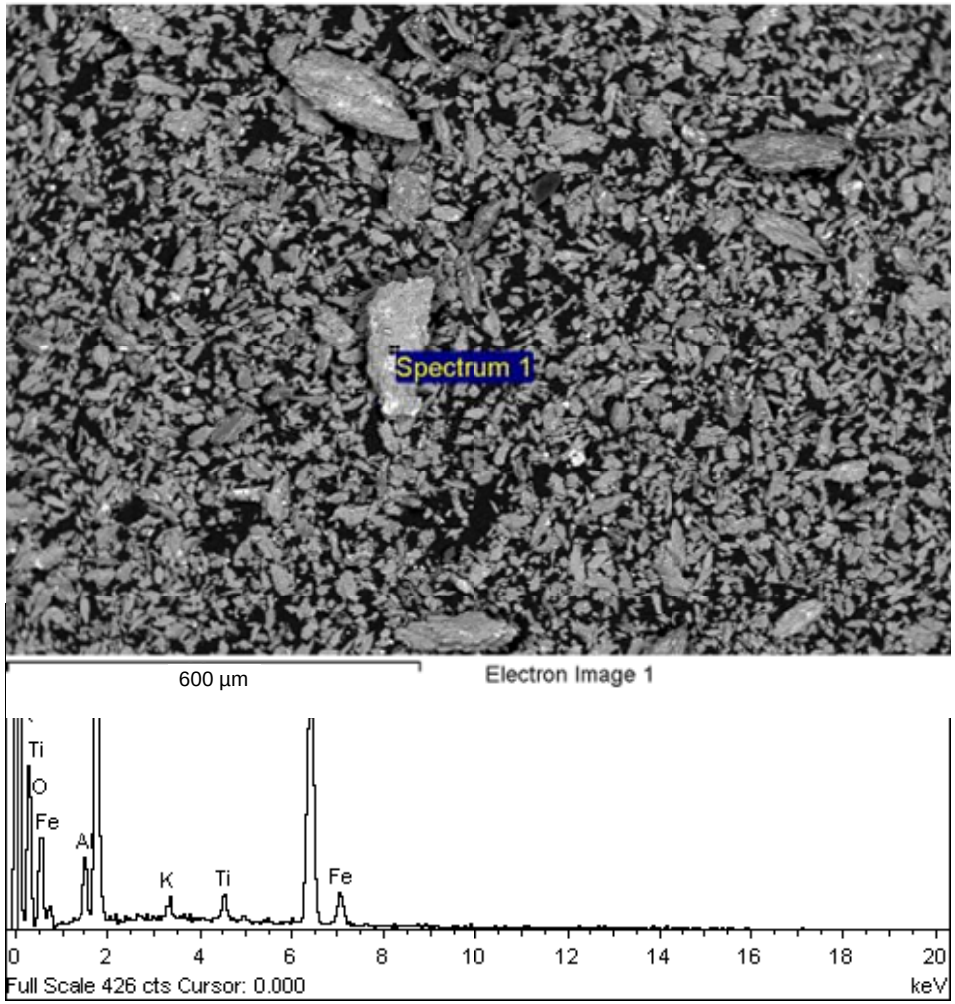
All images were acquired using backscatter electrons. Image contrast is directly proportional to average atomic number i.e. the brighter the area, the higher the atomic number.

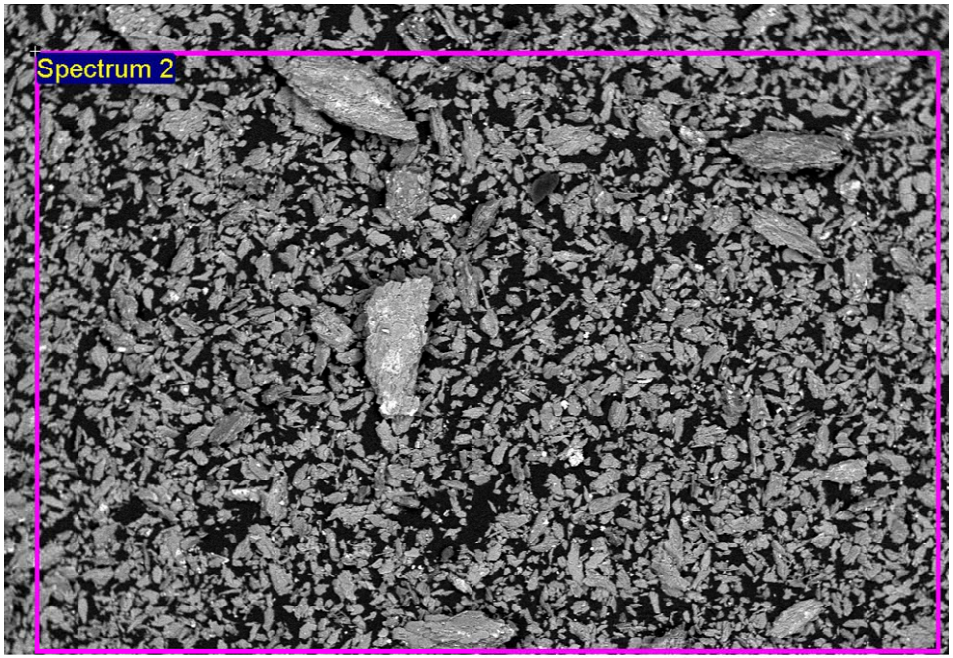
The scale used to classify roundness and sphericity is as below, from the API RP60 standard classification of rock materials.



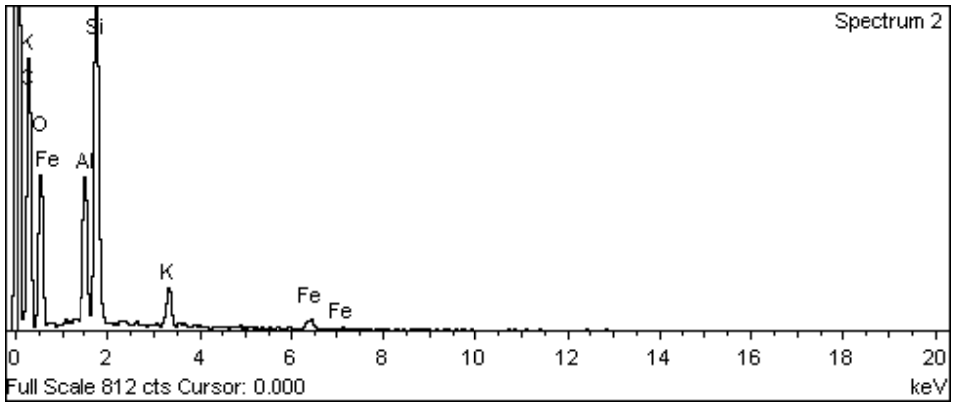
A range of particle sizes from ~ 1 µm to ~ 400 µm were observed in the following distinct compositions –

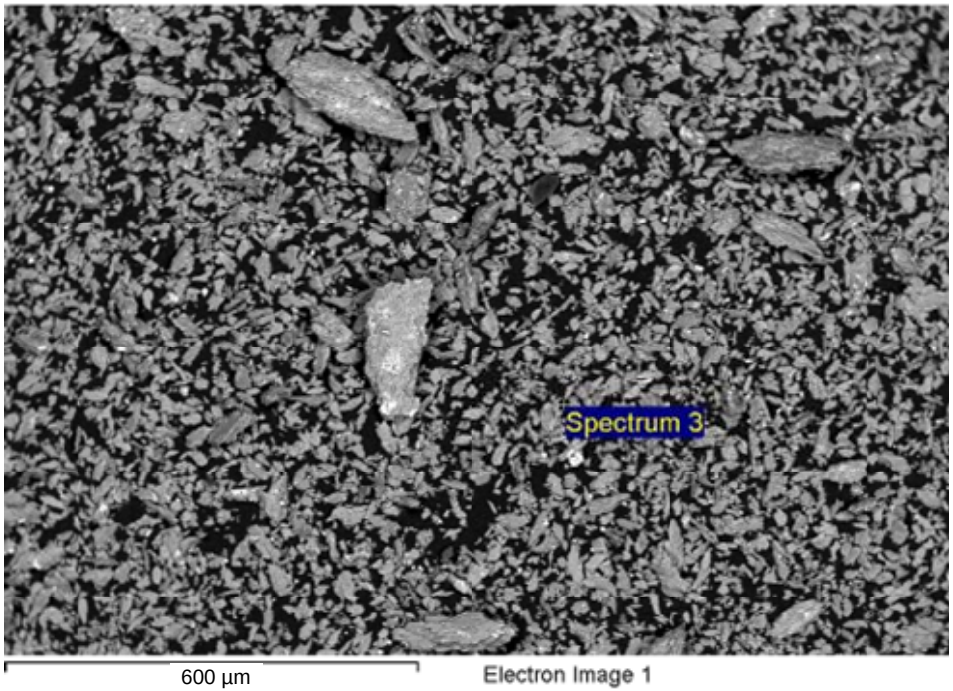
- Dominant quantities of alumino silicate (possible mica) containing minor potassium, titanium and iron, displaying lamellar cleavage morphologies with roundness values typically at 0.5 and sphericity values of less than 0.5;
- Trace quantities of discrete particles containing copper, zinc, zirconium and higher concentrations of titanium were observed.

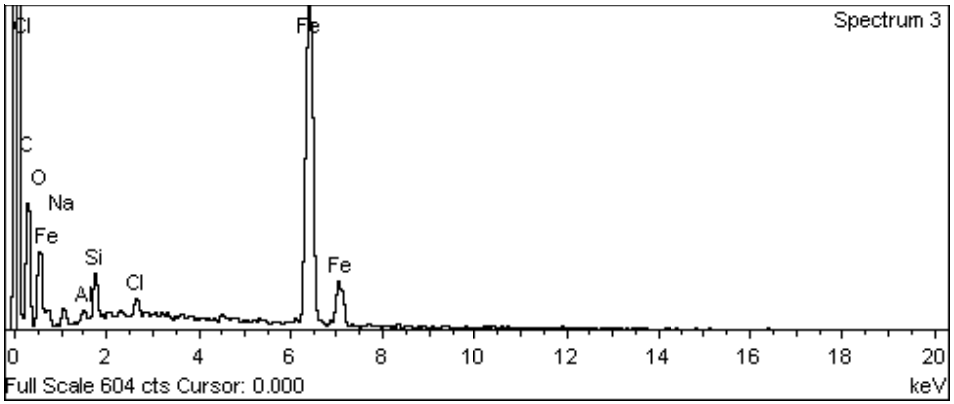

Spectrum 1
600 µm
Electron Image 1
Ti
O
Fe
Al
K
Ti
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 426 cts Cursor: 0.000
keV

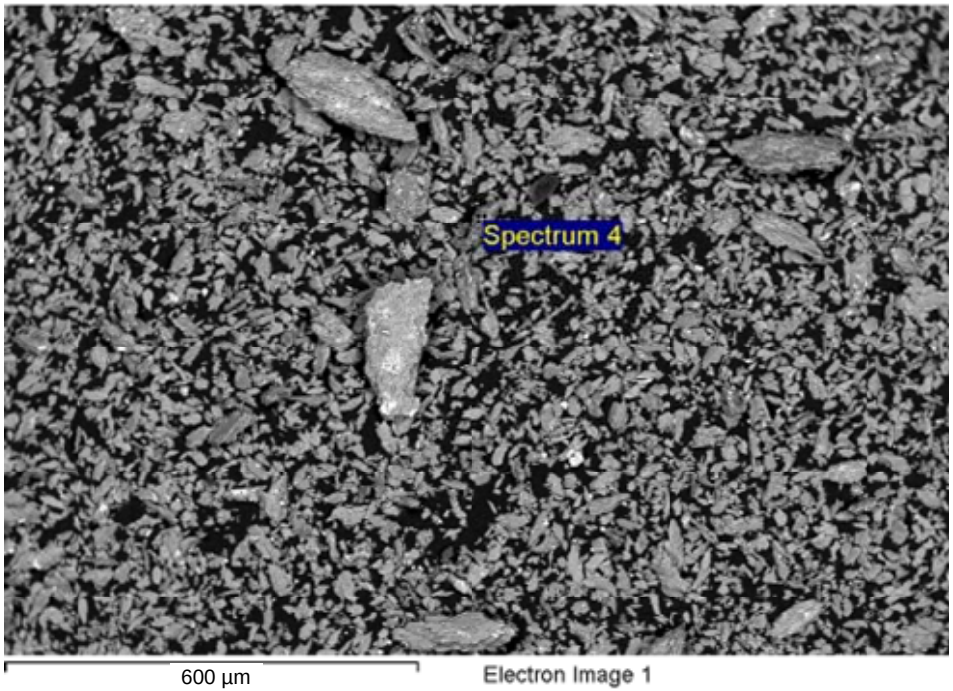


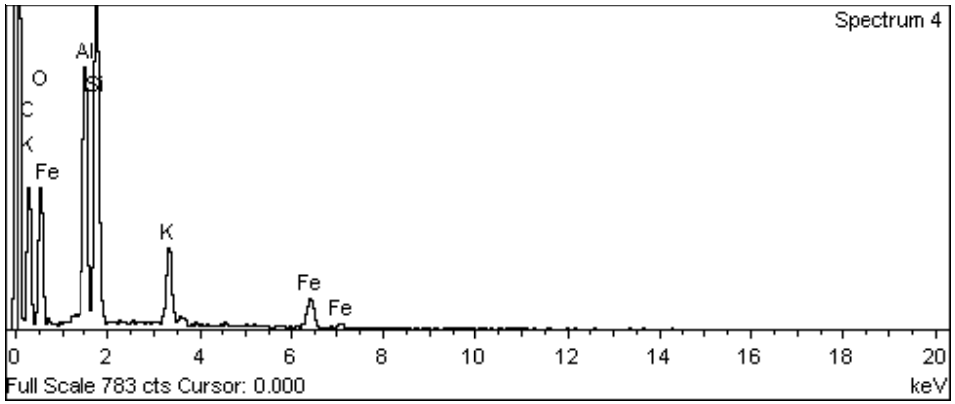
Electron Image 1

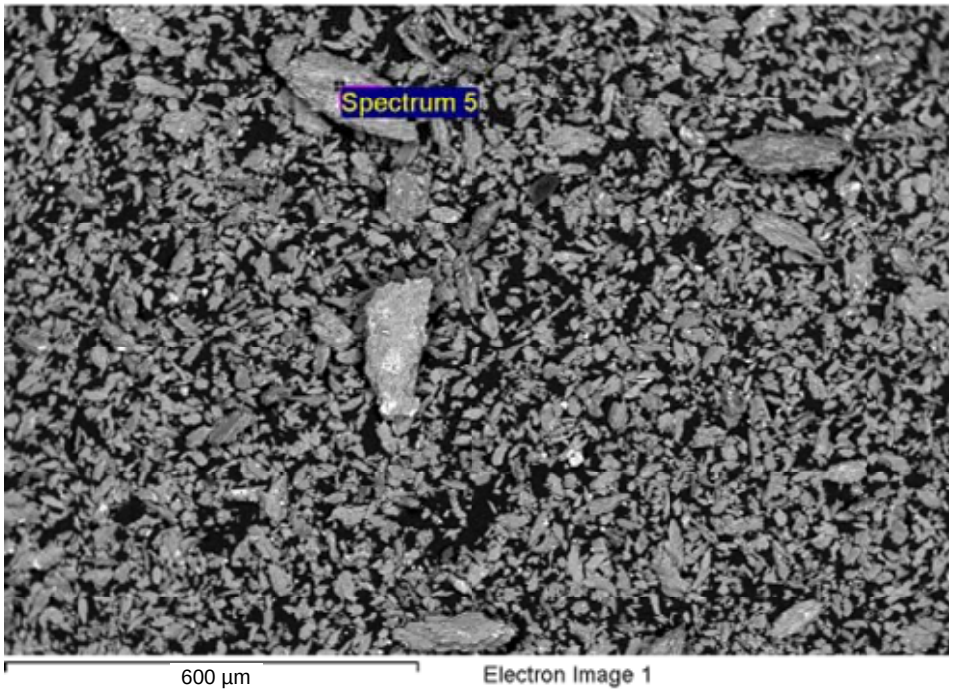


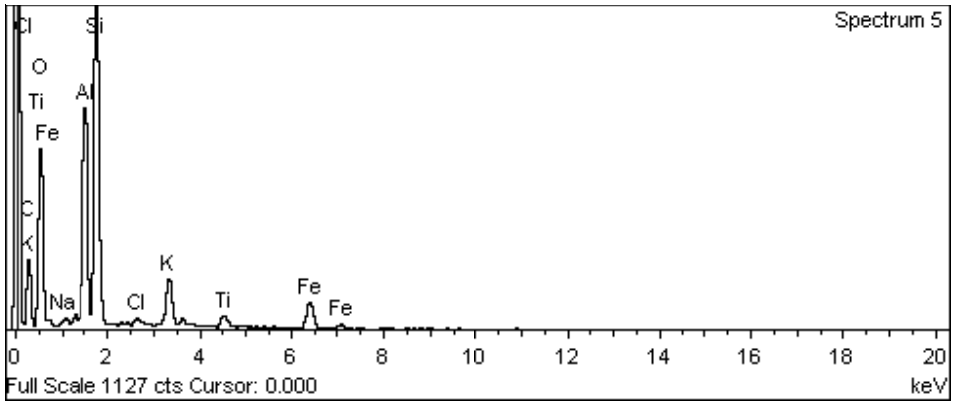

Spectrum 3
600 µm
Electron Image 1

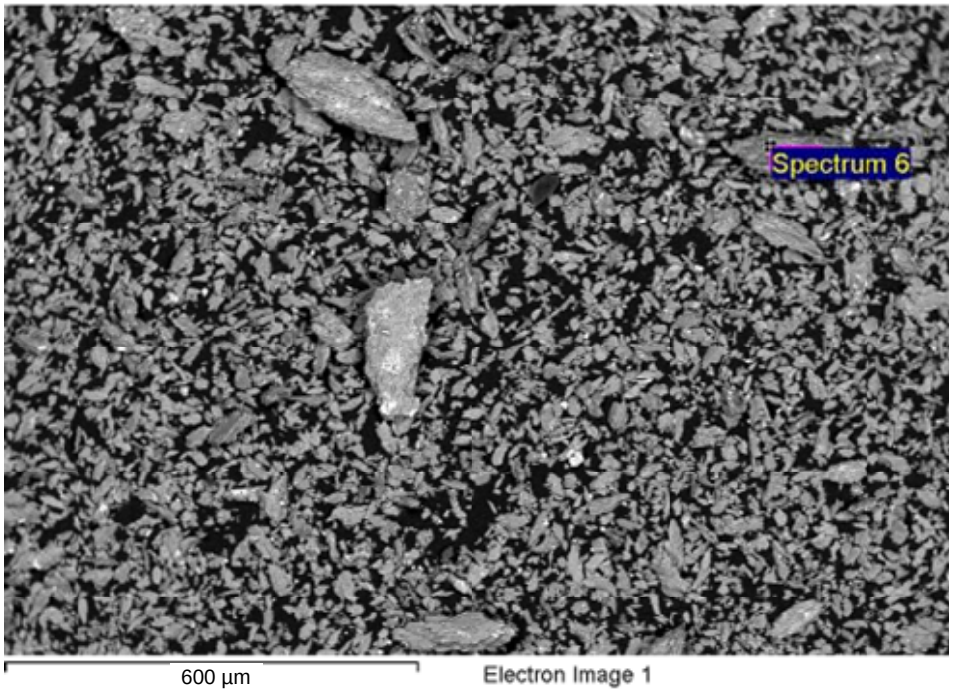

Spectrum 3
Cl
Fe
C
O
Na
Fe
Si
Al
Cl
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 604 cts Cursor: 0.000
keV

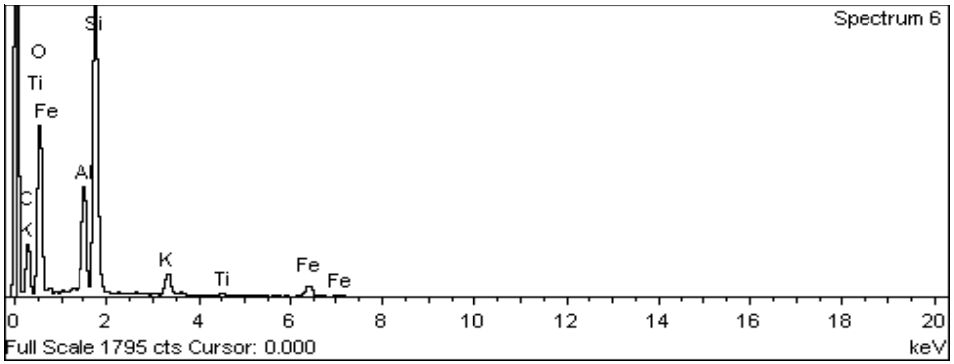

Spectrum 4
600 µm
Electron Image 1

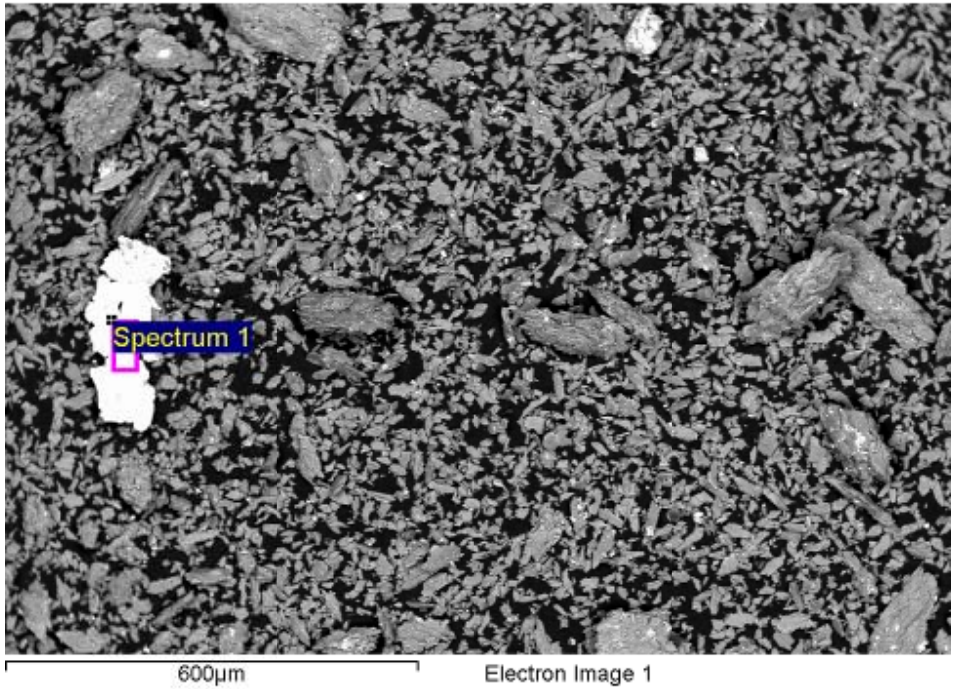

Spectrum 4
O
C
K
Fe
Al
Si
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 783 cts Cursor: 0.000
keV

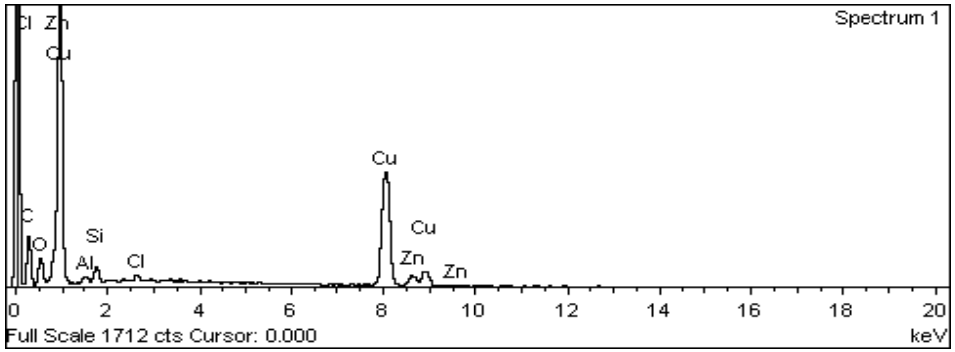

Spectrum 5
600 µm
Electron Image 1

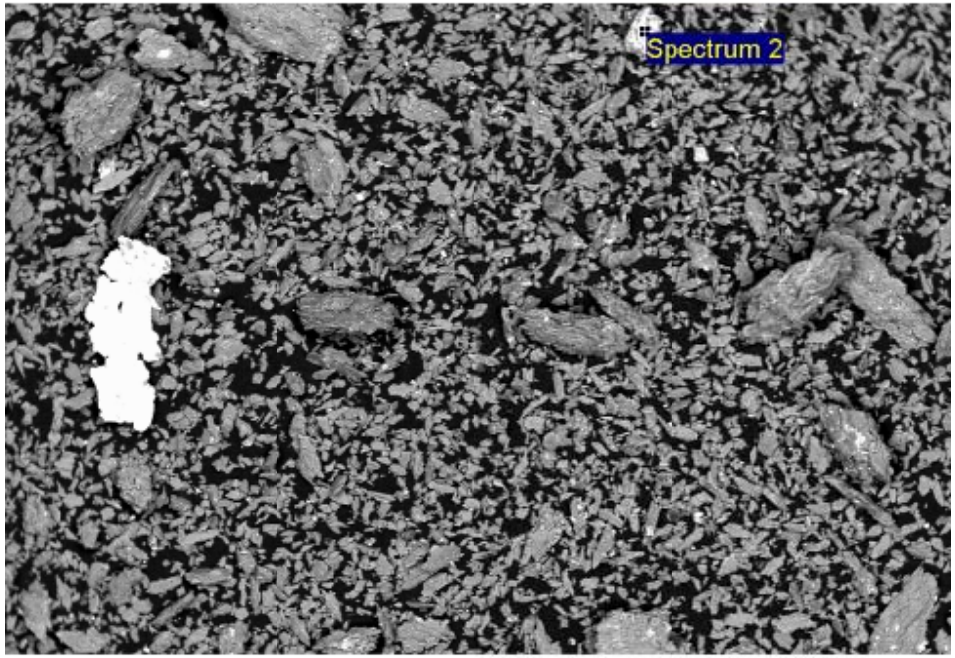

Spectrum 5
Cl
Si
O
Ti
Al
Fe
C
K
Na
Cl
K
Ti
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1127 cts Cursor: 0.000
keV

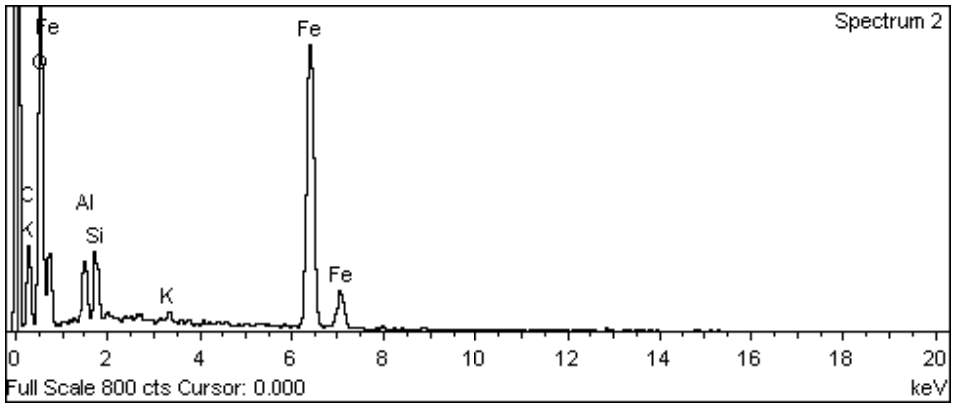

Spectrum 6
600 µm
Electron Image 1

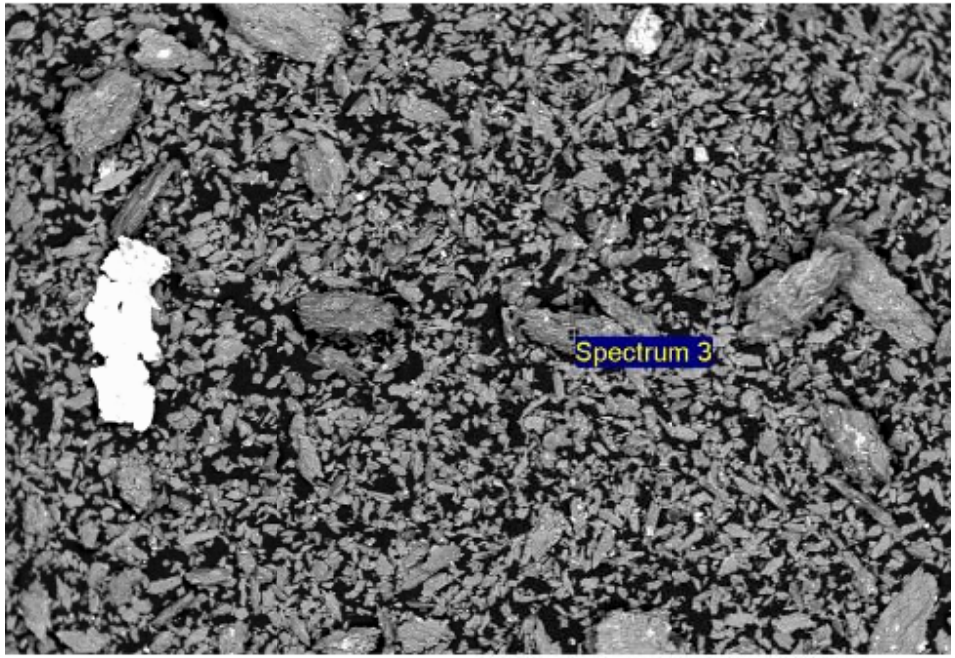

Spectrum 6
Si
O
Ti
Fe
Al
C
K
K
Ti
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1795 cts Cursor: 0.000
keV

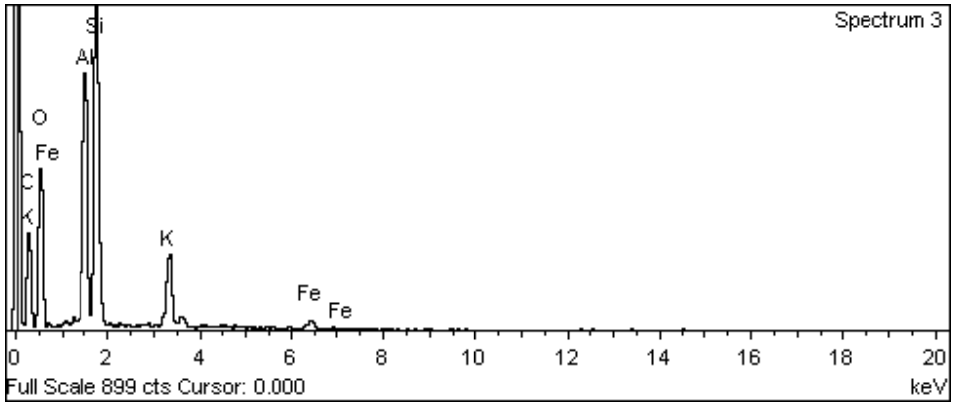

Spectrum 1
600µm
Electron Image 1

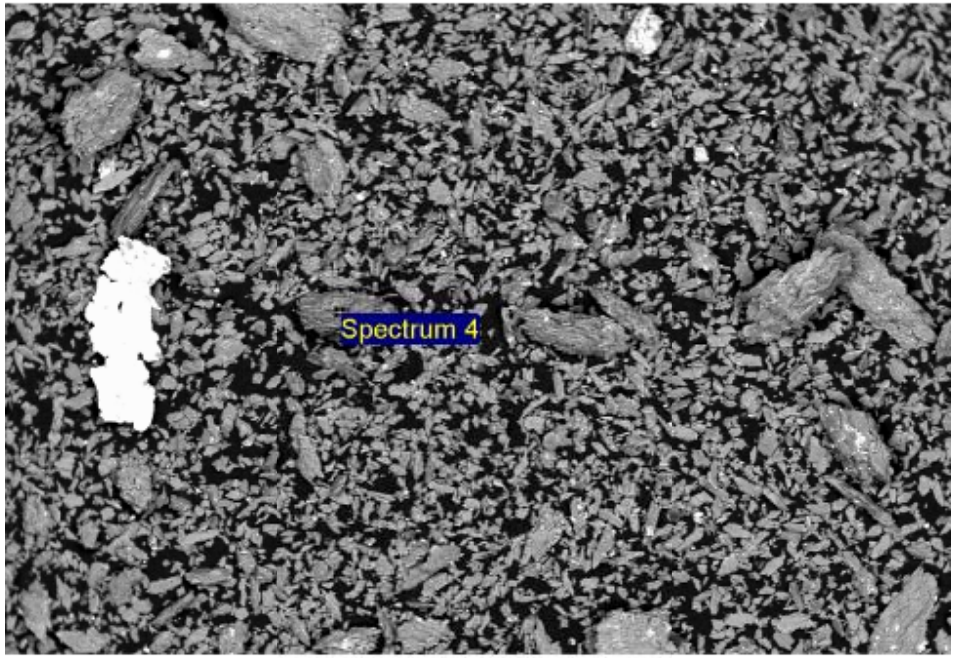

Spectrum 1
Cl
Zn
Cu
C
O
Si
Al
Cl
Cu
Cu
Zn
Zn
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1712 cts Cursor: 0.000
keV

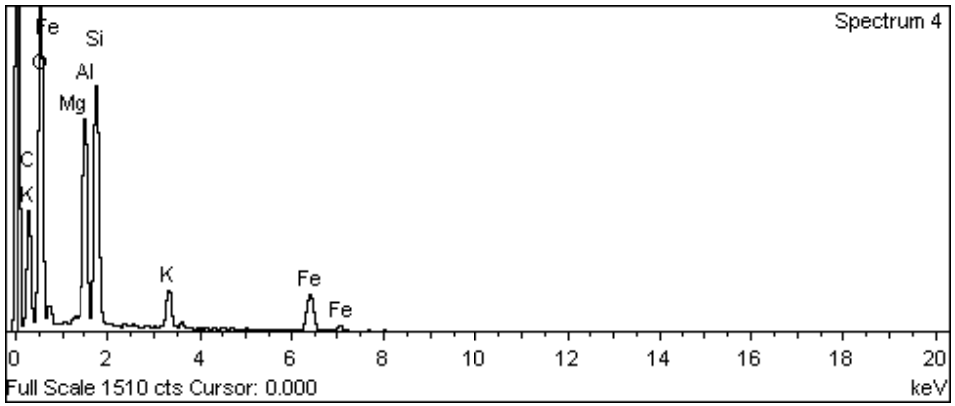

Spectrum 2
600µm
Electron Image 1

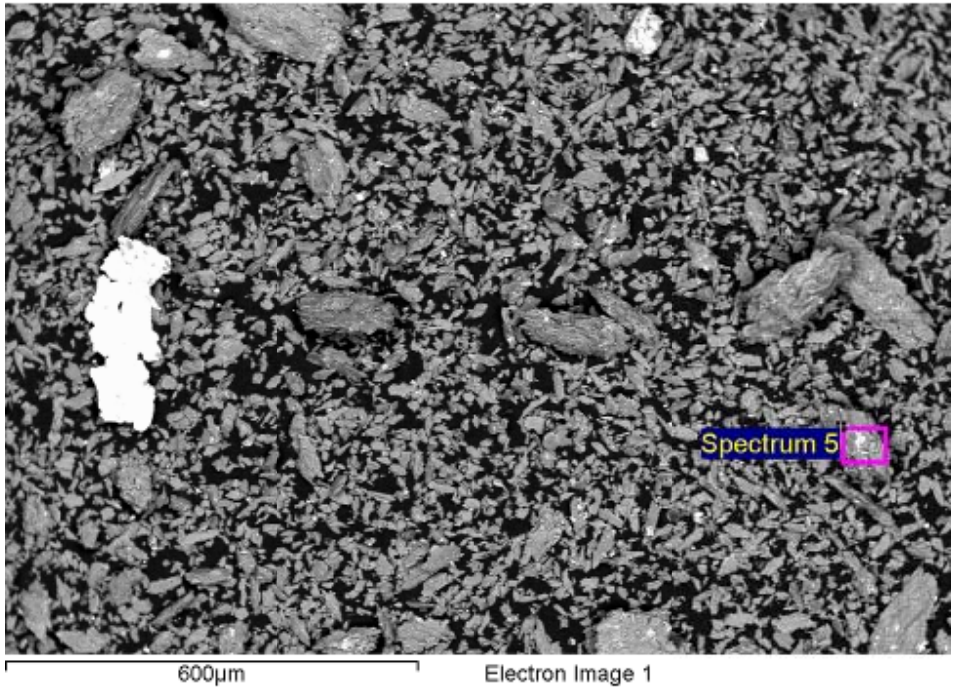

Spectrum 2
Fe
O
C
K
Al
Si
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 800 cts Cursor: 0.000
keV

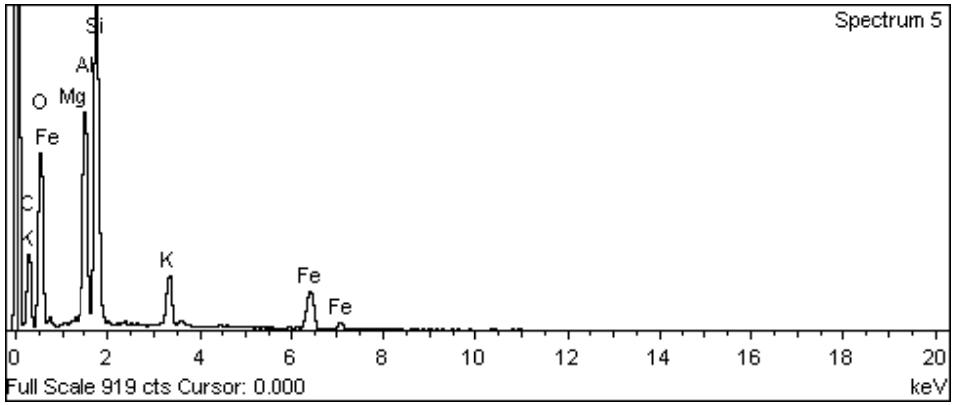

Spectrum 3
600µm
Electron Image 1

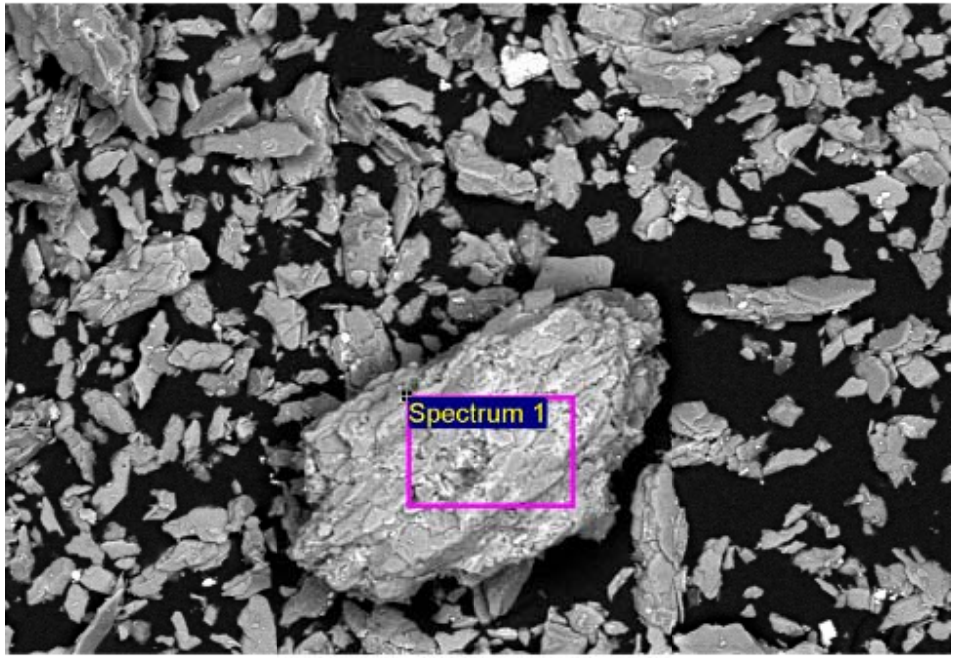

Spectrum 3
Si
Al
O
Fe
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 899 cts Cursor: 0.000
keV

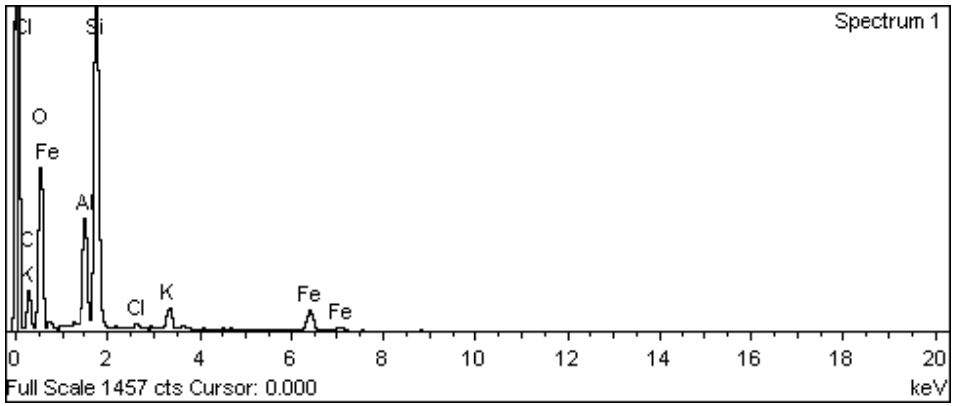

Spectrum 4
600µm
Electron Image 1

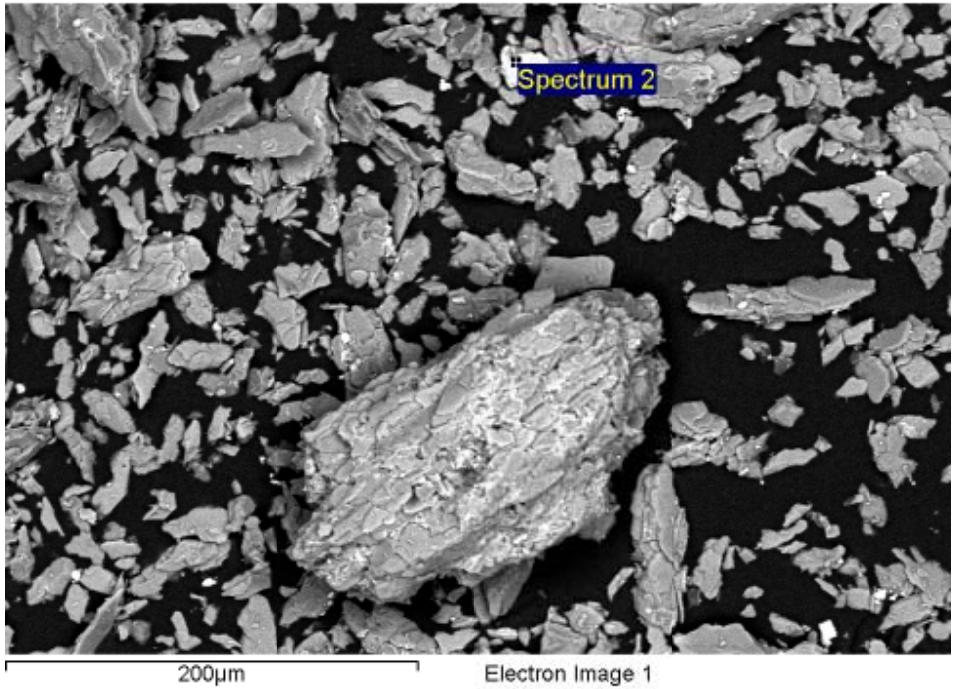

Spectrum 4
Fe
Si
Al
Mg
O
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1510 cts Cursor: 0.000
keV

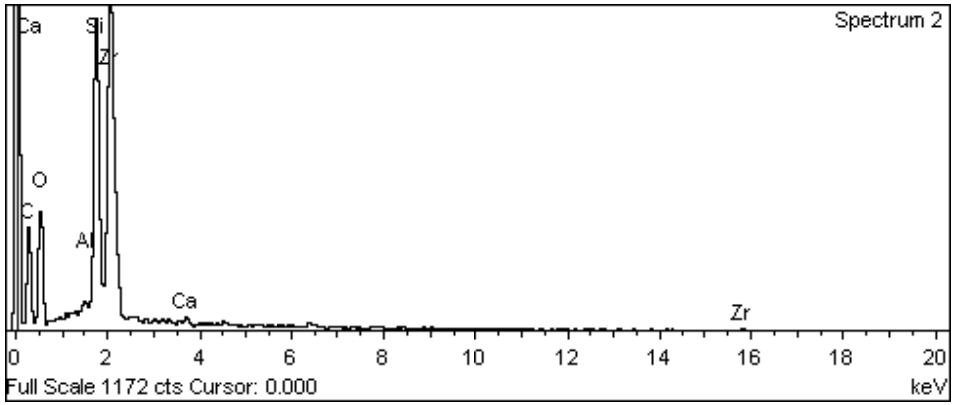

Spectrum 5
600µm
Electron Image 1

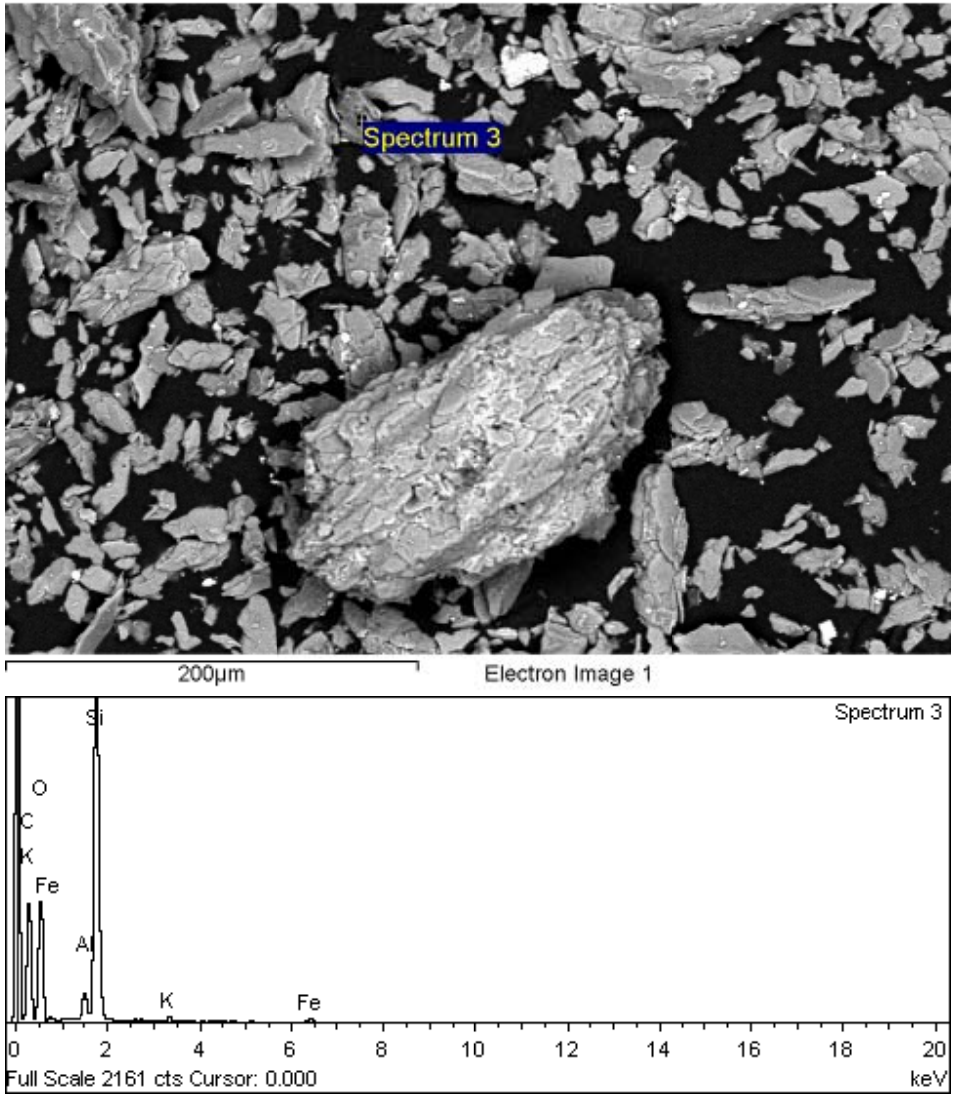

Spectrum 5
Si
Al
O
Mg
Fe
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 919 cts Cursor: 0.000
keV

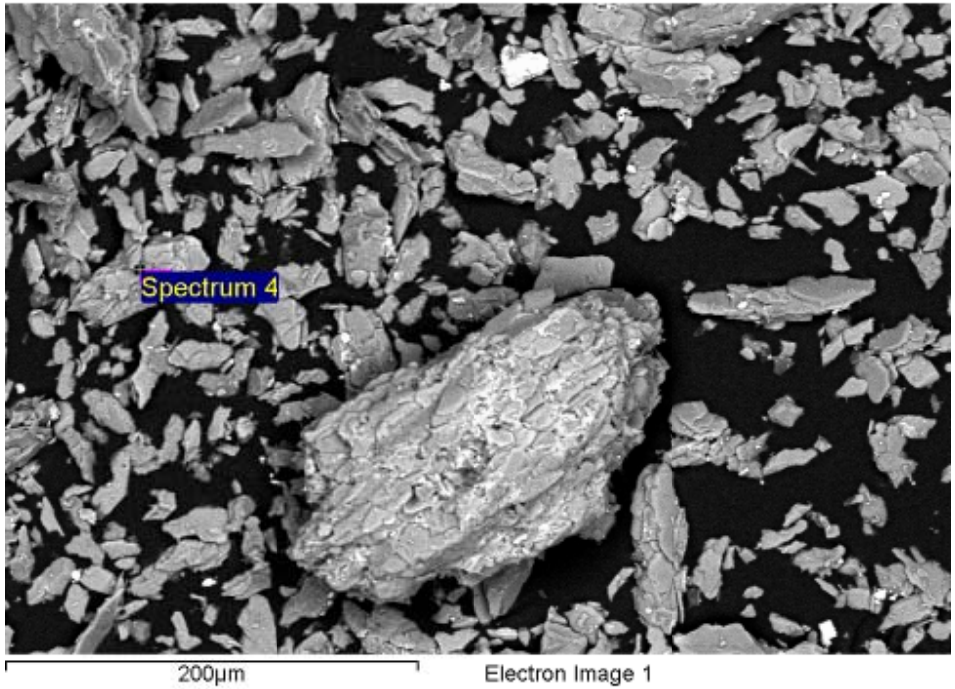

Spectrum 1
200µm
Electron Image 1

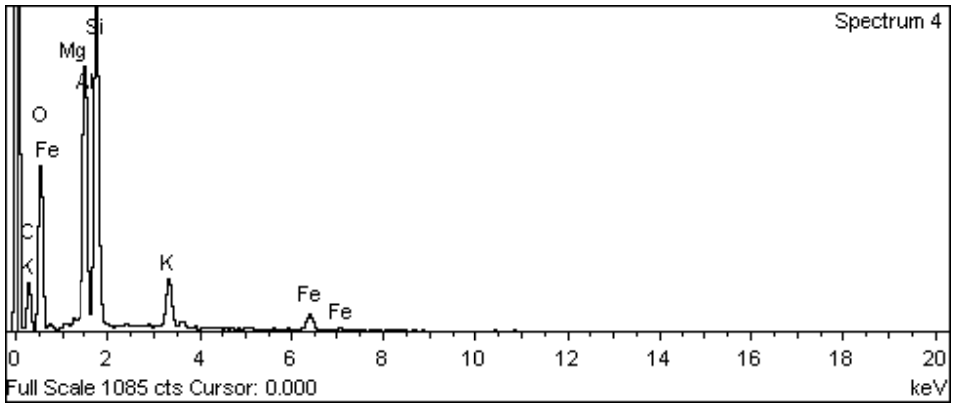

Spectrum 1
Cl
Si
O
Fe
Al
C
K
Cl
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1457 cts Cursor: 0.000
keV

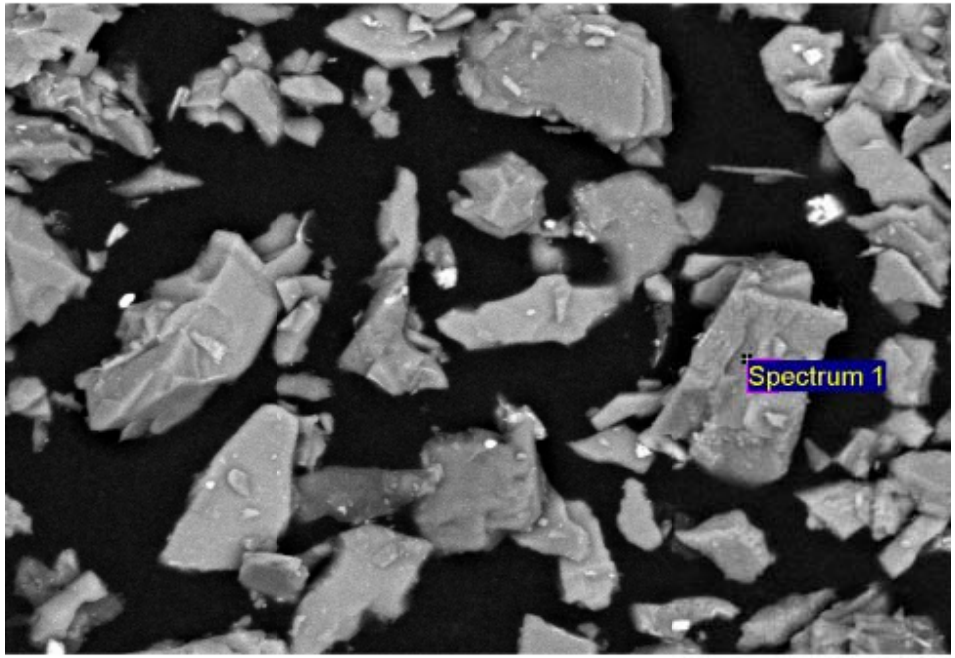

Spectrum 2
200µm
Electron Image 1

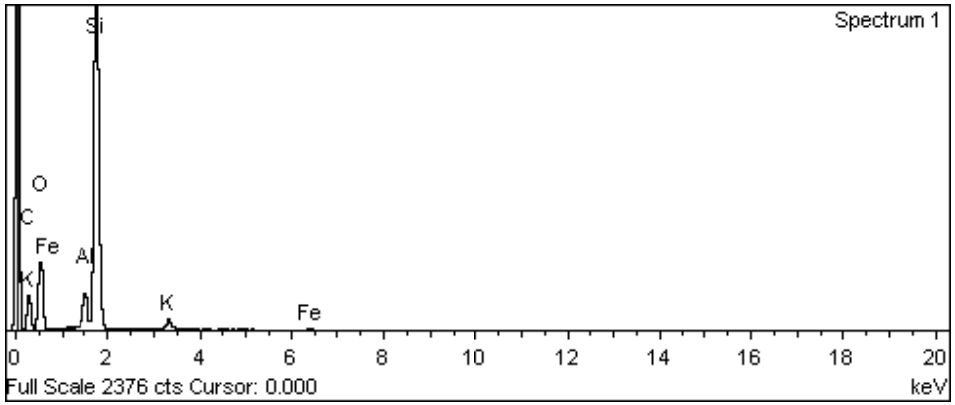

Spectrum 2
Ca
Si
Zr
O
C
Al
Ca
Zr
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1172 cts Cursor: 0.000
keV

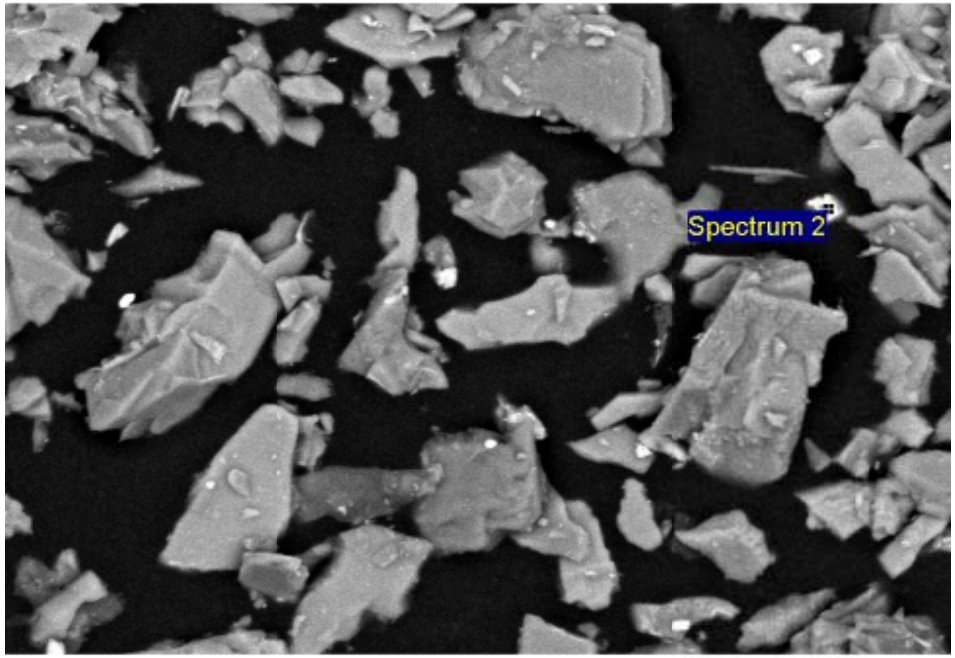

Spectrum 3
200µm
Electron Image 1
Spectrum 3
Si
O
C
K
Fe
Al
K
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 2161 cts Cursor: 0.000
keV

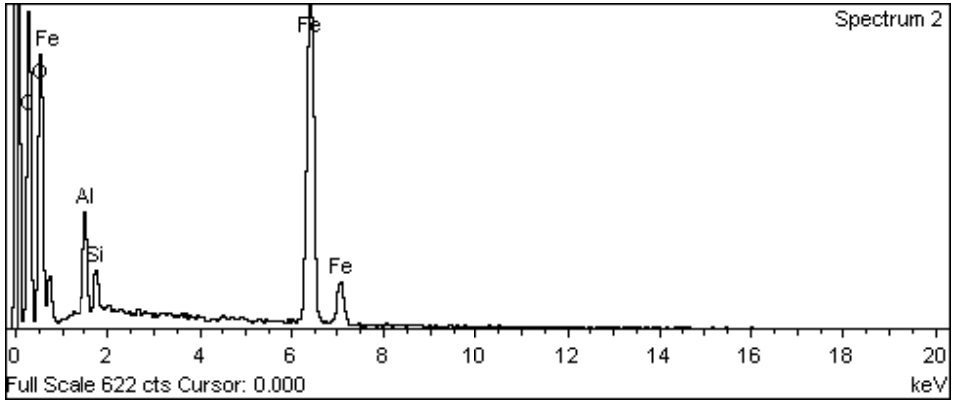

Spectrum 4
200µm
Electron Image 1

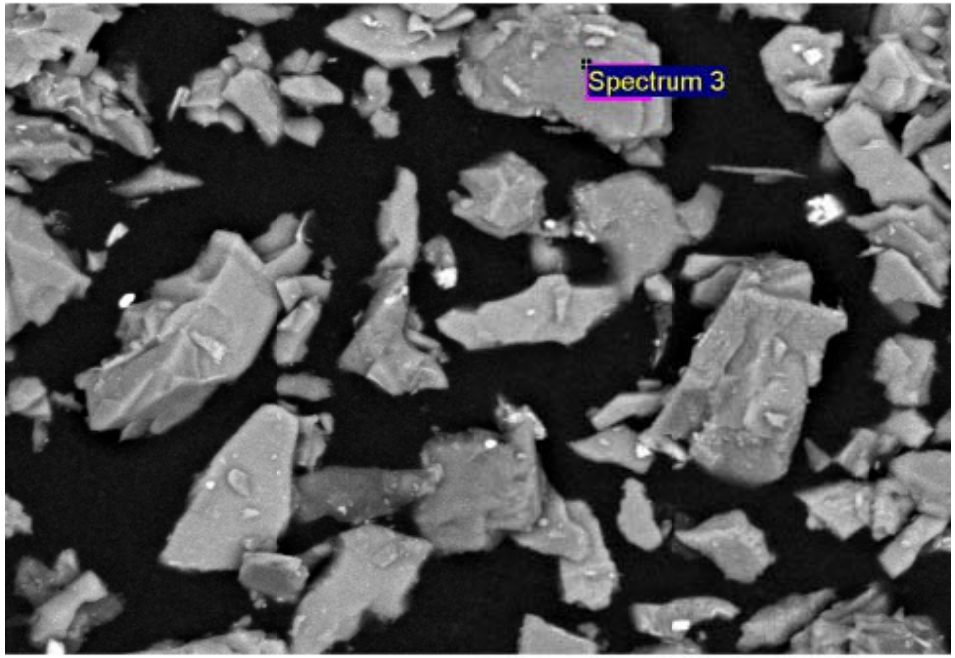

Spectrum 4
Si
Mg
Al
O
Fe
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 1085 cts Cursor: 0.000
keV

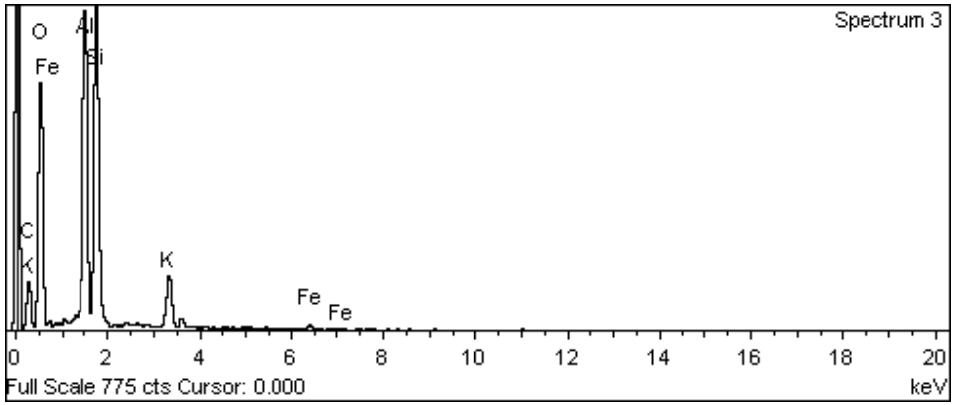

Spectrum 1
60µm
Electron Image 1

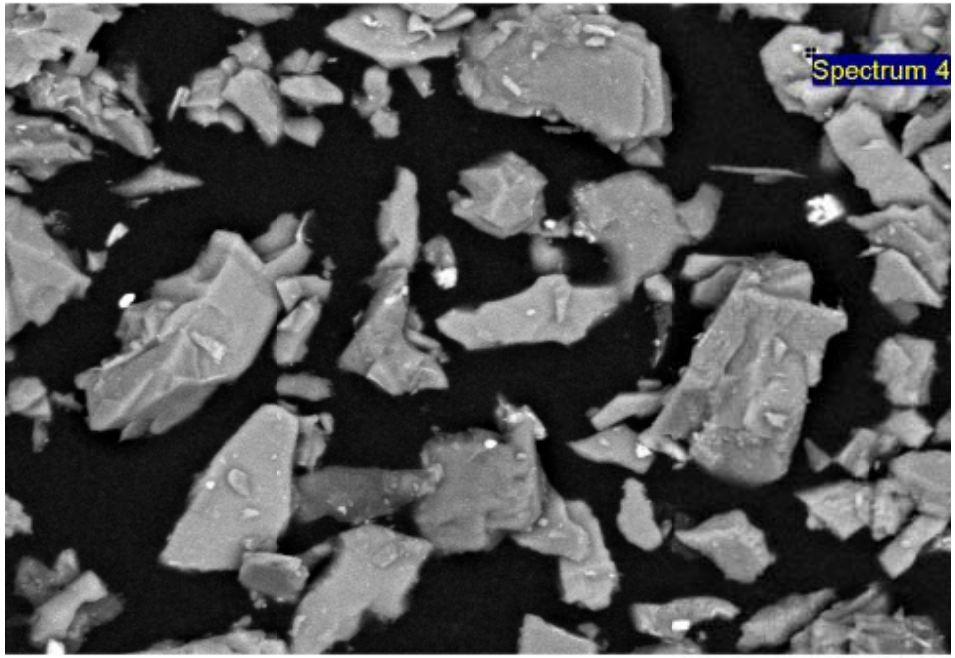

Spectrum 1
Si
O
C
Fe
Al
K
K
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 2376 cts Cursor: 0.000
keV

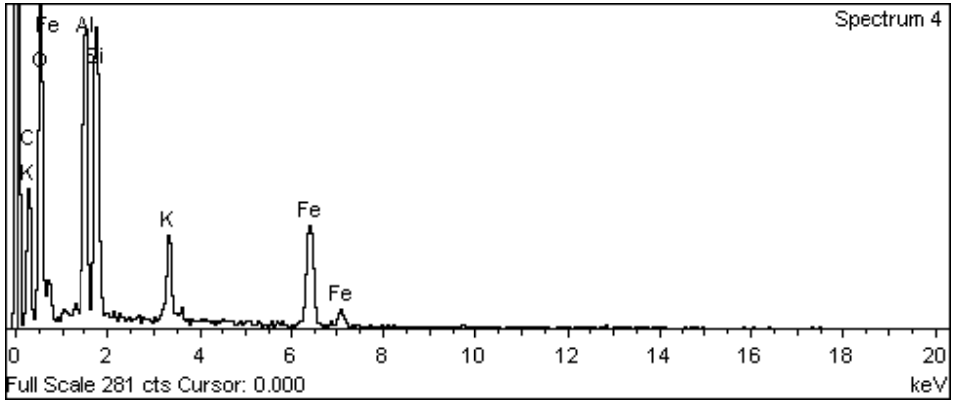

Spectrum 2
60µm
Electron Image 1

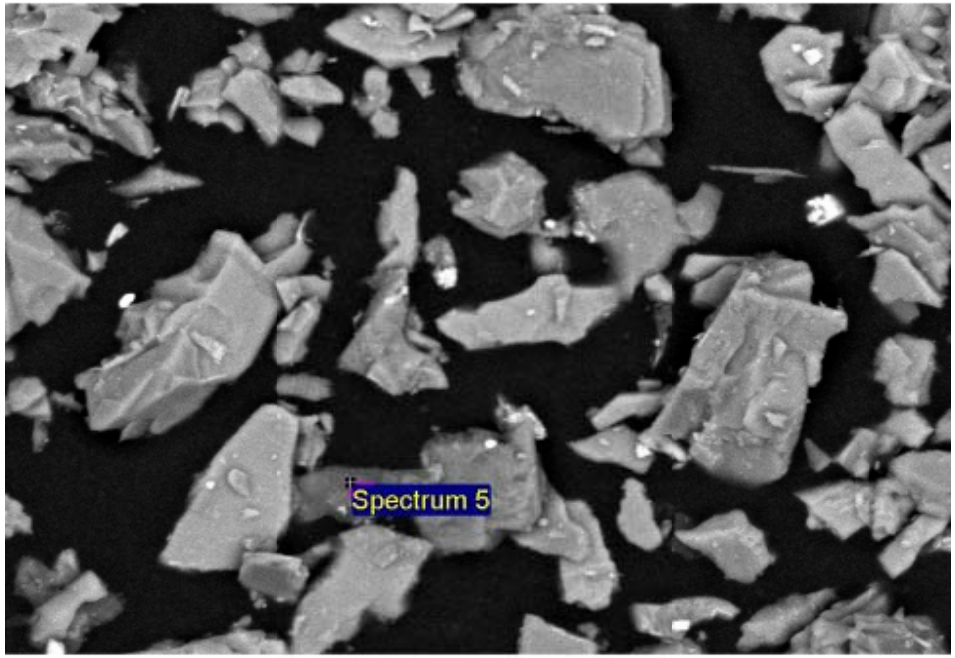

Spectrum 2
Fe
O
Al
Si
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 622 cts Cursor: 0.000
keV

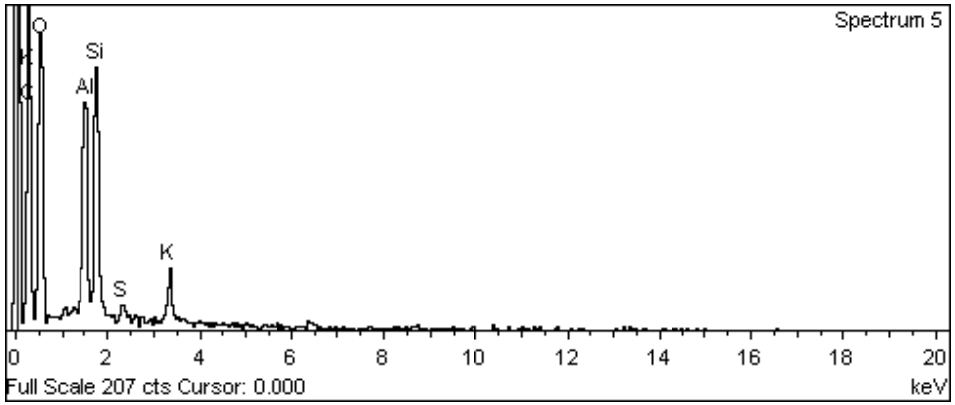

Spectrum 3
60µm
Electron Image 1

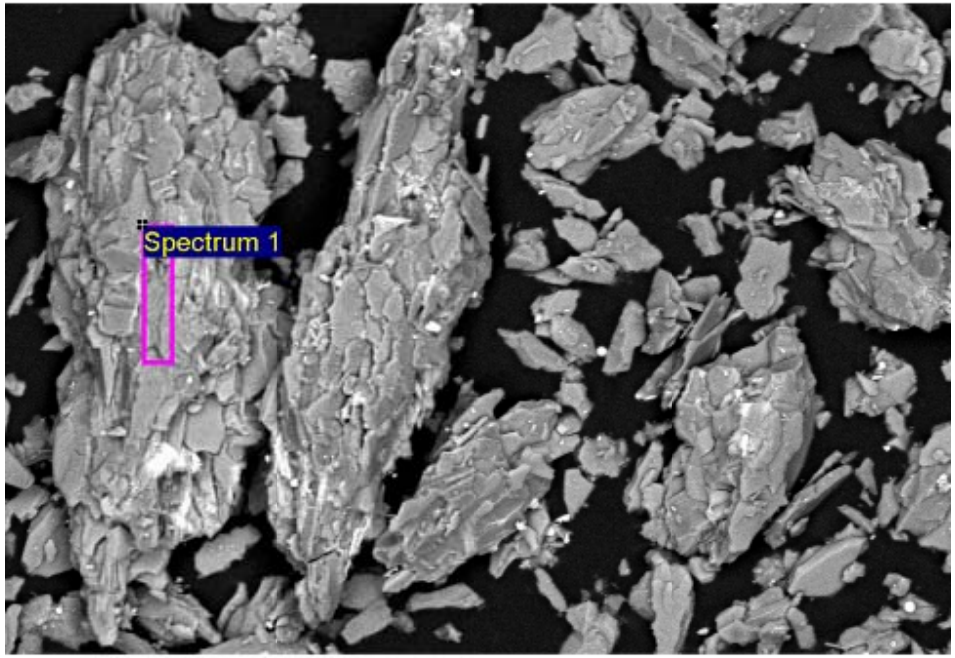

Spectrum 3
O
Al
Fe
Si
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 775 cts Cursor: 0.000
keV

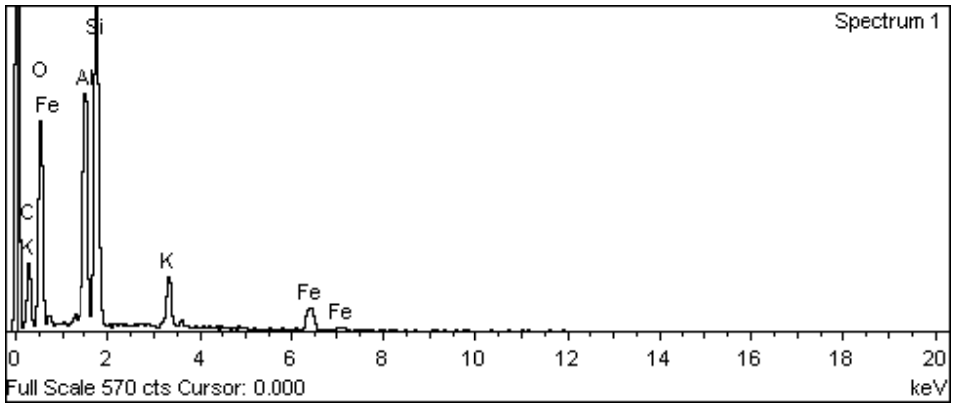

Spectrum 4
60µm
Electron Image 1

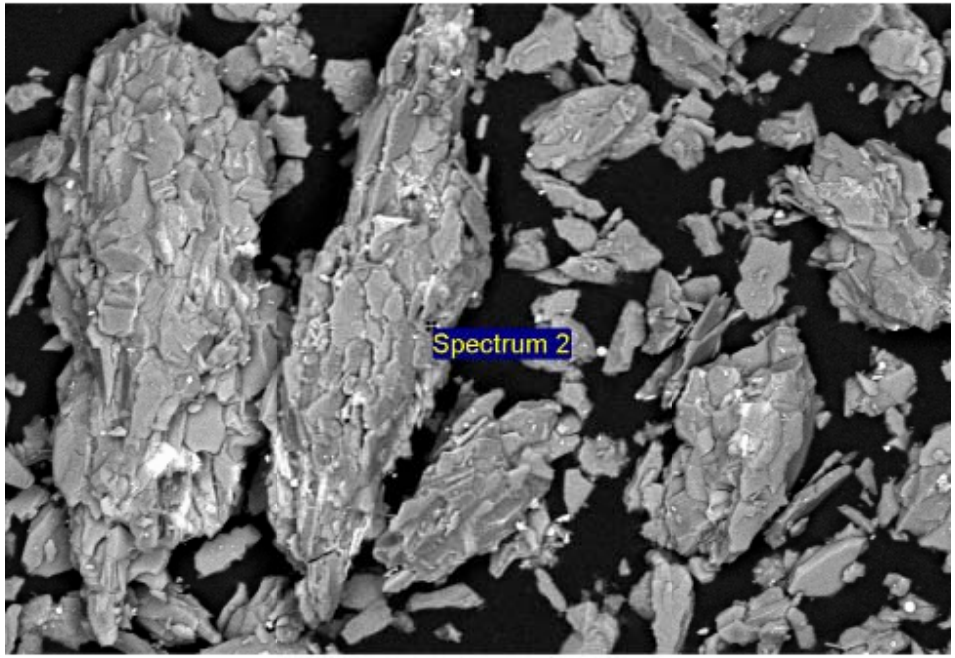

Spectrum 4
Fe
Al
Si
O
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 281 cts Cursor: 0.000
keV

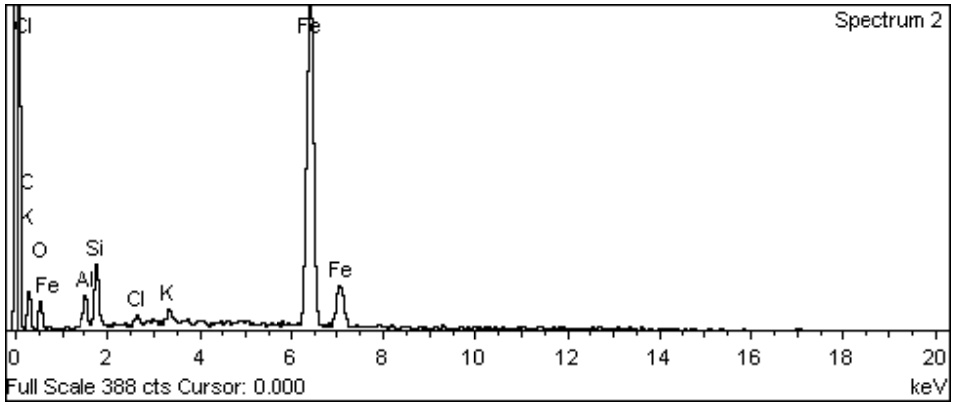

Spectrum 5
60µm
Electron Image 1

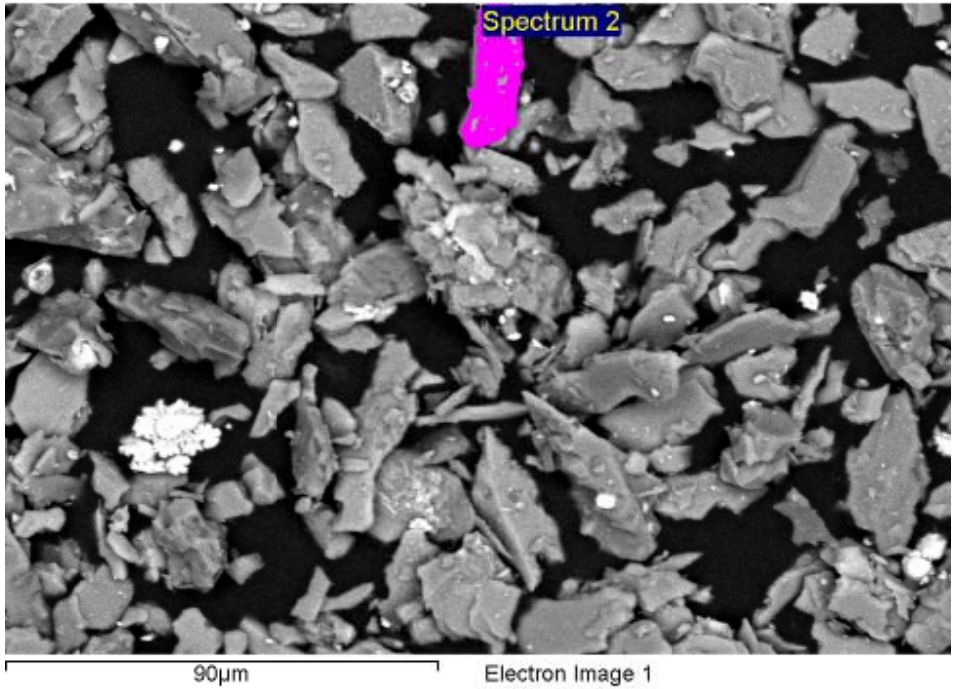

Spectrum 5
Si
Al
K
S
0
2
4
6
8
10
12
14
16
18
20
Full Scale 207 cts Cursor: 0.000
keV

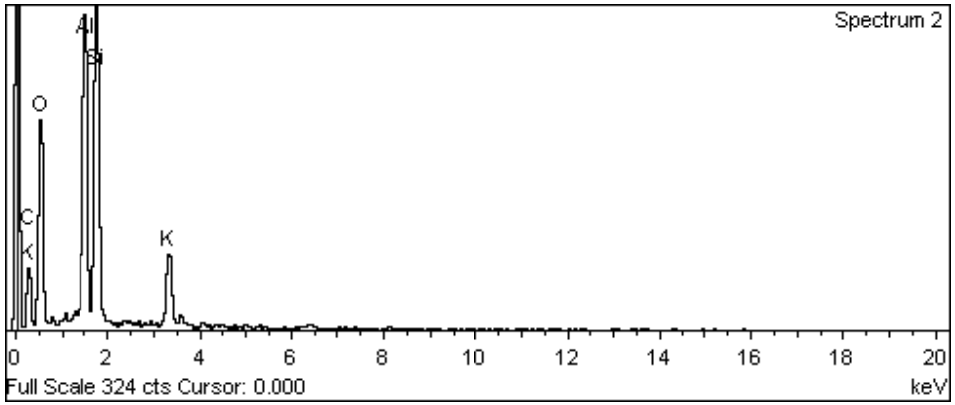

Spectrum 1
100µm
Electron Image 1


Spectrum 1
Si
O
Al
Fe
C
K
K
Fe
Fe
0
2
4
6
8
10
12
14
16
18
20
Full Scale 570 cts Cursor: 0.000
keV


Spectrum 2
100µm
Electron Image 1


Spectrum 2
Cl
Fe
C
K
O
Si
Fe
Al
Cl
K
0
2
4
6
8
10
12
14
16
18
20
Full Scale 388 cts Cursor: 0.000
keV


Spectrum 2
90µm
Electron Image 1


Spectrum 2
Al
O
C
K
K
0
2
4
6
8
10
12
14
16
18
20
Full Scale 324 cts Cursor: 0.000
keV