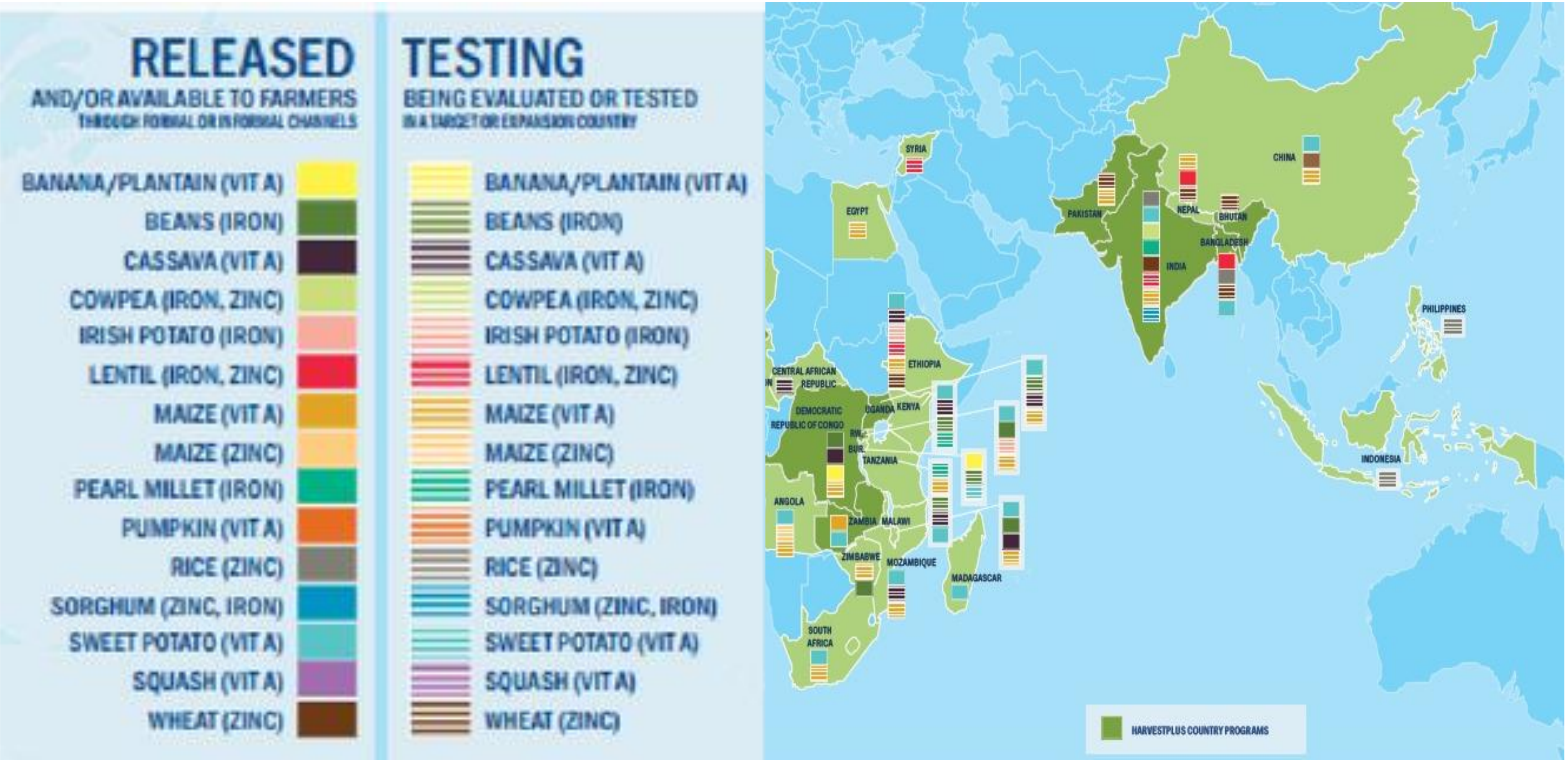
Evaluation of Synchrotron Based X-ray Fluorescence Spectroscopy for Quantification of Minerals in Pea Seeds

Ramandeep Kaur Bamrah

M.Sc. Candidate

Supervisor: Dr. Tom Warkentin

Biofortification



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Pulse Growing Regions of Canada

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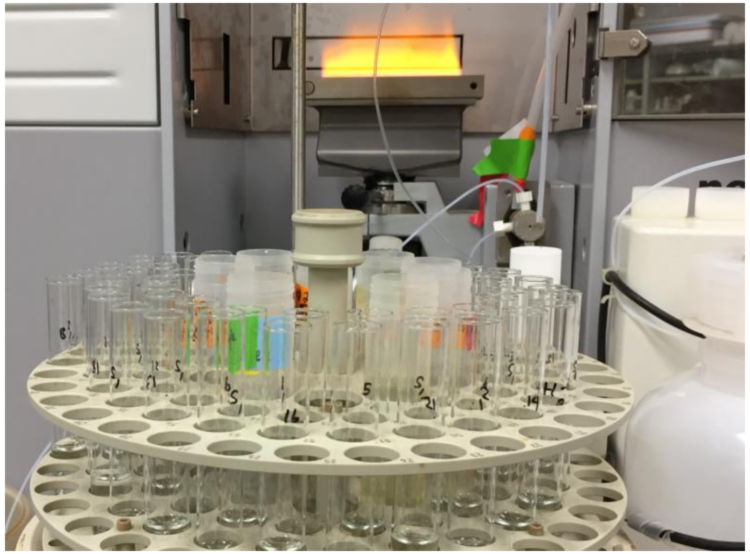
Mineral Analysis

- Fast, accurate and inexpensive methods for screening large numbers of breeding lines and germplasm
- Common methods: digestion based methods
  - Atomic Absorption Spectroscopy (AAS)
  - Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Problems with Digestion Based Methods

- Time consuming and laborious
- Requires:
  - Highly trained analysts
  - Contamination free reagents and acids
- Large sample size (0.5-10 gm)
- Destructive method



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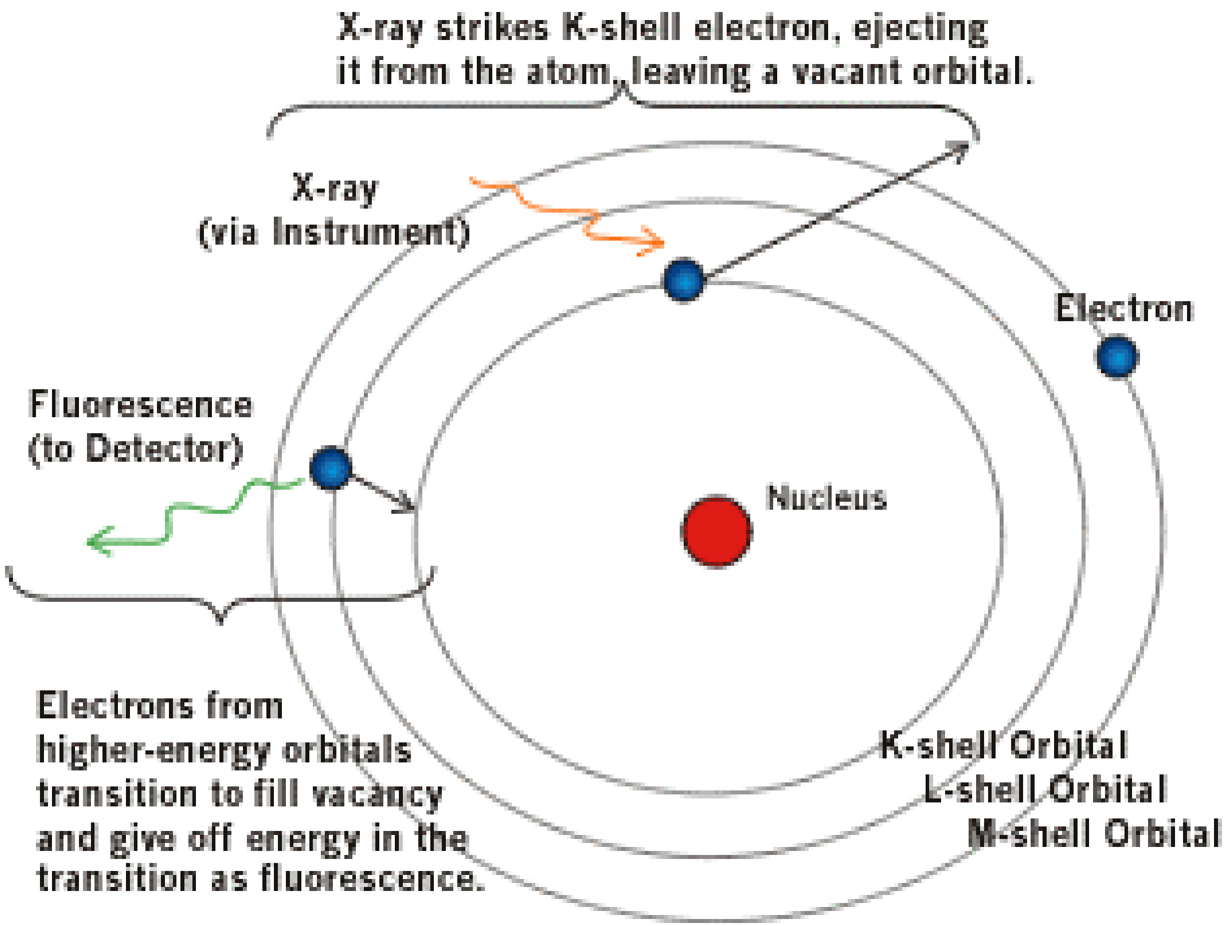
X-ray Fluorescence Spectroscopy (XRF)

Principle



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Hypothesis

XRF technique can be developed to quantify the concentration iron, zinc, potassium and selenium in pea seeds

Objectives

- Development of a semi-automated method for pea seed sample preparation for XRF analysis
- Evaluation of pea samples (test set) for Fe, Zn, K and Se concentration by atomic absorption spectroscopy and XRF
  - Correlation between these analyses
- Validate the X-ray fluorescence spectroscopy technique on new panel (validation set) of pea seeds

Material Selection

130 pea seed test samples:

- Three pea mapping populations- PAM, PR-07, GWAS
- Representing a wide range of minerals

Element	Min (ppm)	Max (ppm)
Se	0.08	6.94
K	8715	14313
Zn	14.39	53.78
Fe	25.71	93.68

Sample Preparation for XRF Analysis

Geno grinder

1st Grinding
(one steel ball)

→

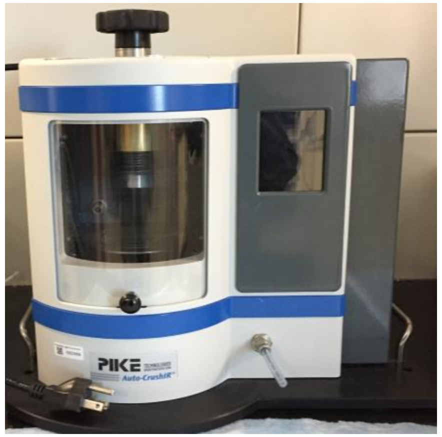
2nd Grinding
(30 Zirconia balls)

→

Fine powder
(<10µm dia)

Fine powder (120 mg)

→



Hydraulic Pellet press

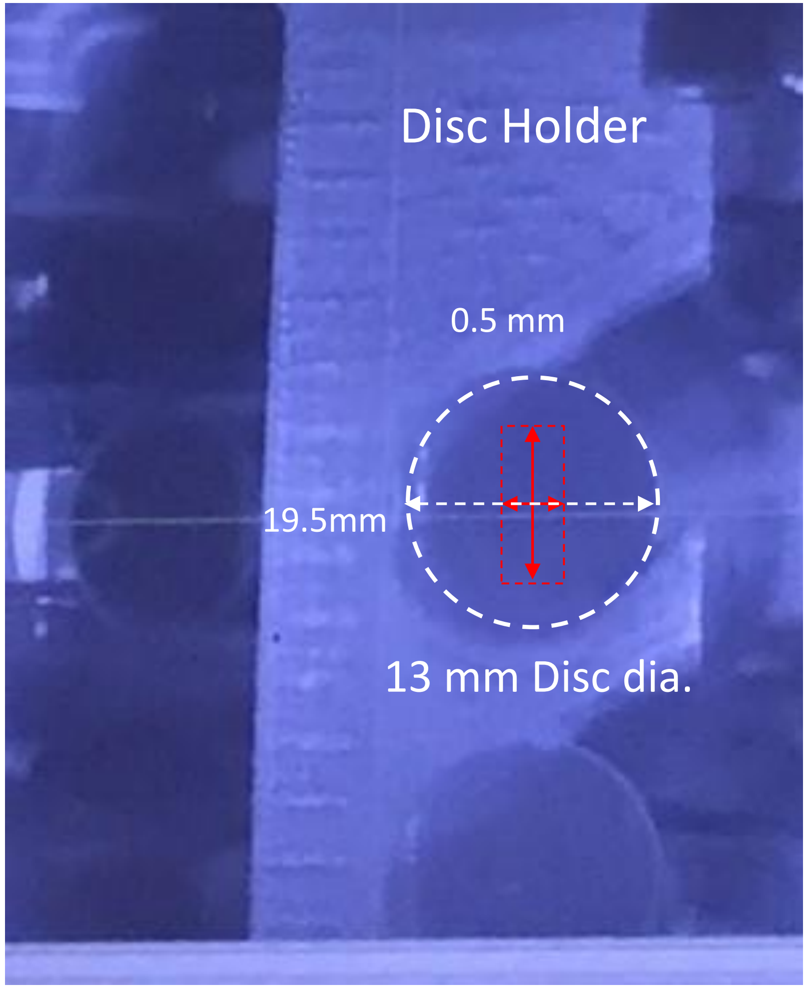
→

3 pellets/sample
13 mm dia; 0.7 mm thick

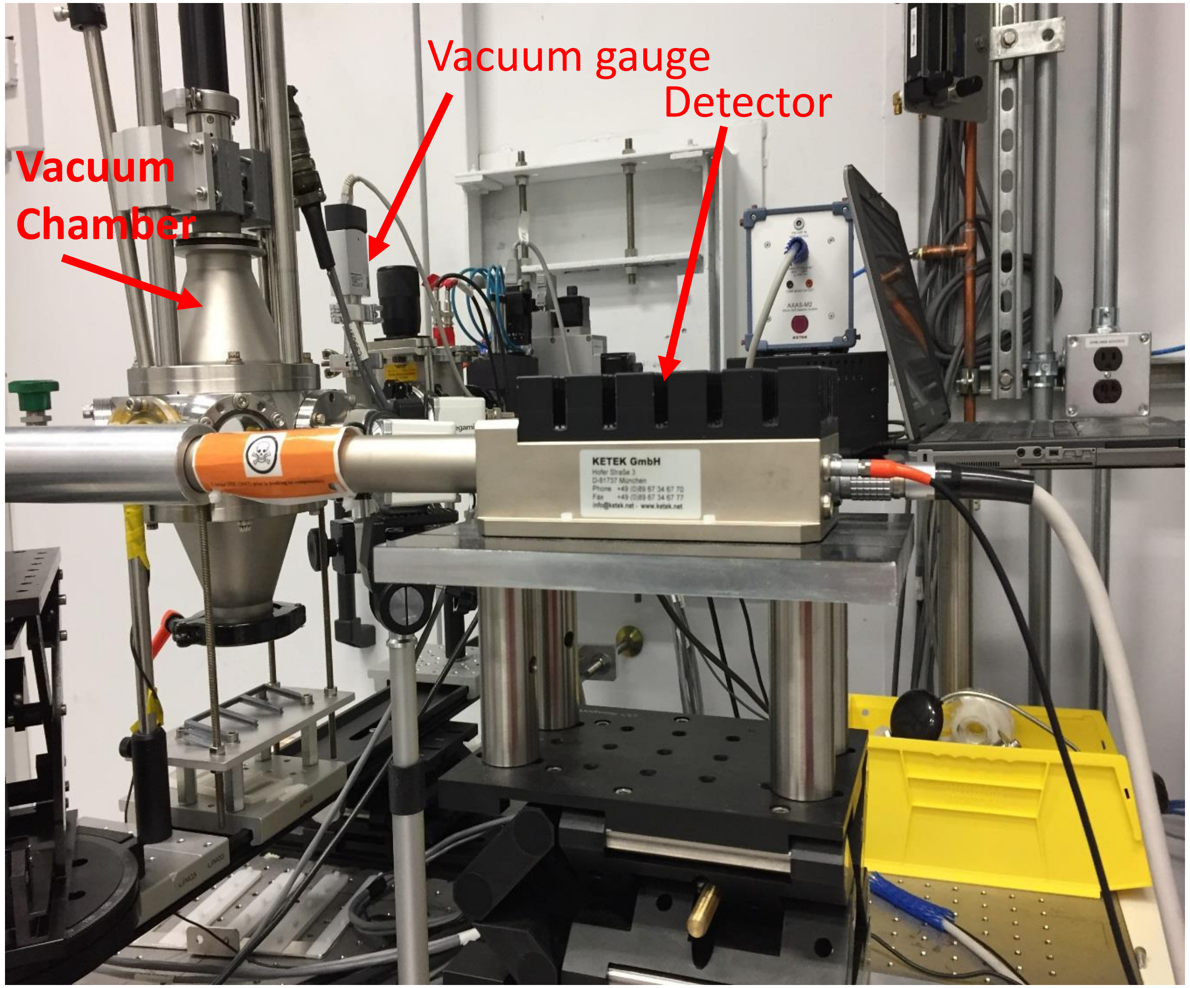
Preparation of standard calibration curves

- Pea starch as base matrix
- Preparation of master stock – mixing compounds of iron, selenium, zinc, calcium, manganese, copper and potassium in pea starch
- Dilutions from master stock
- Three pellets /dilution
- XRF spectra recorded at CLS

XRF Parameters



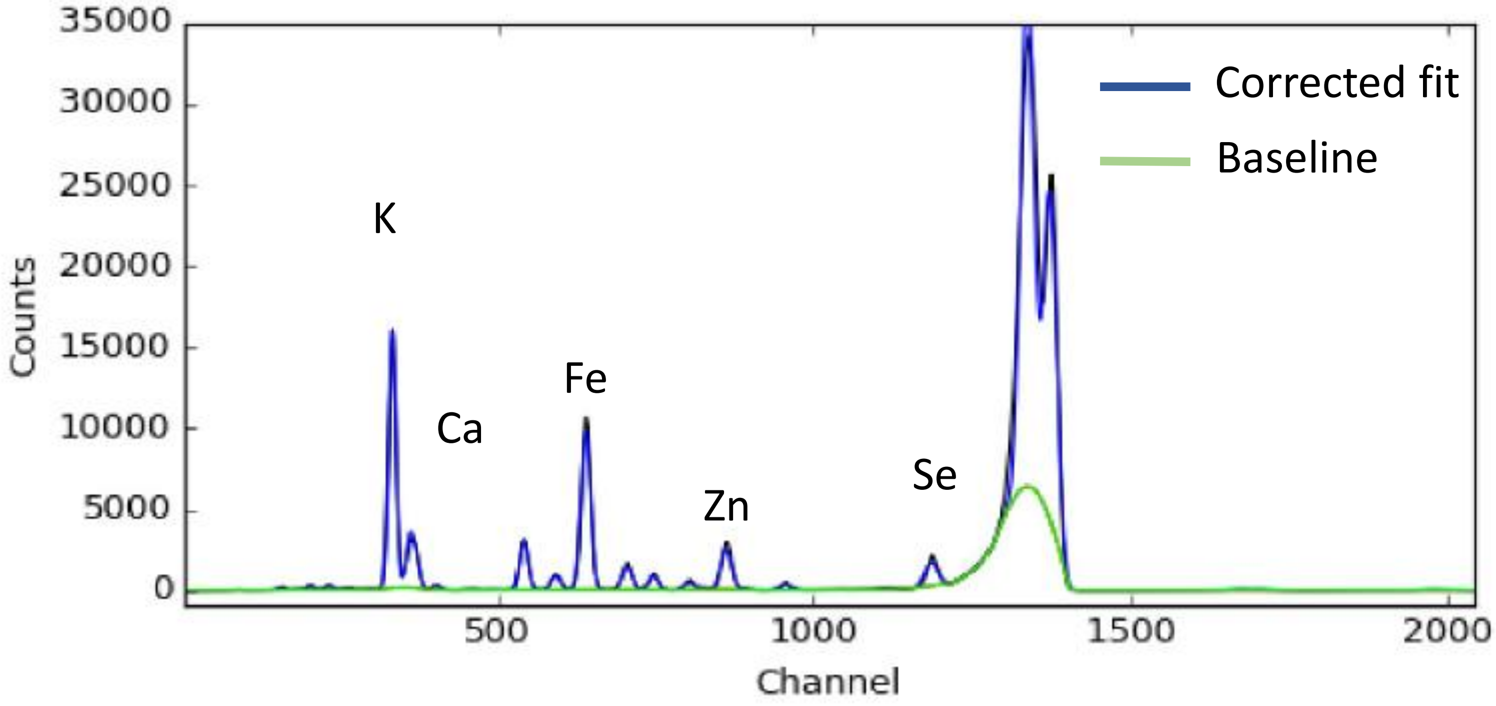
Sample disc and measured area



XRF End station

Data Processing

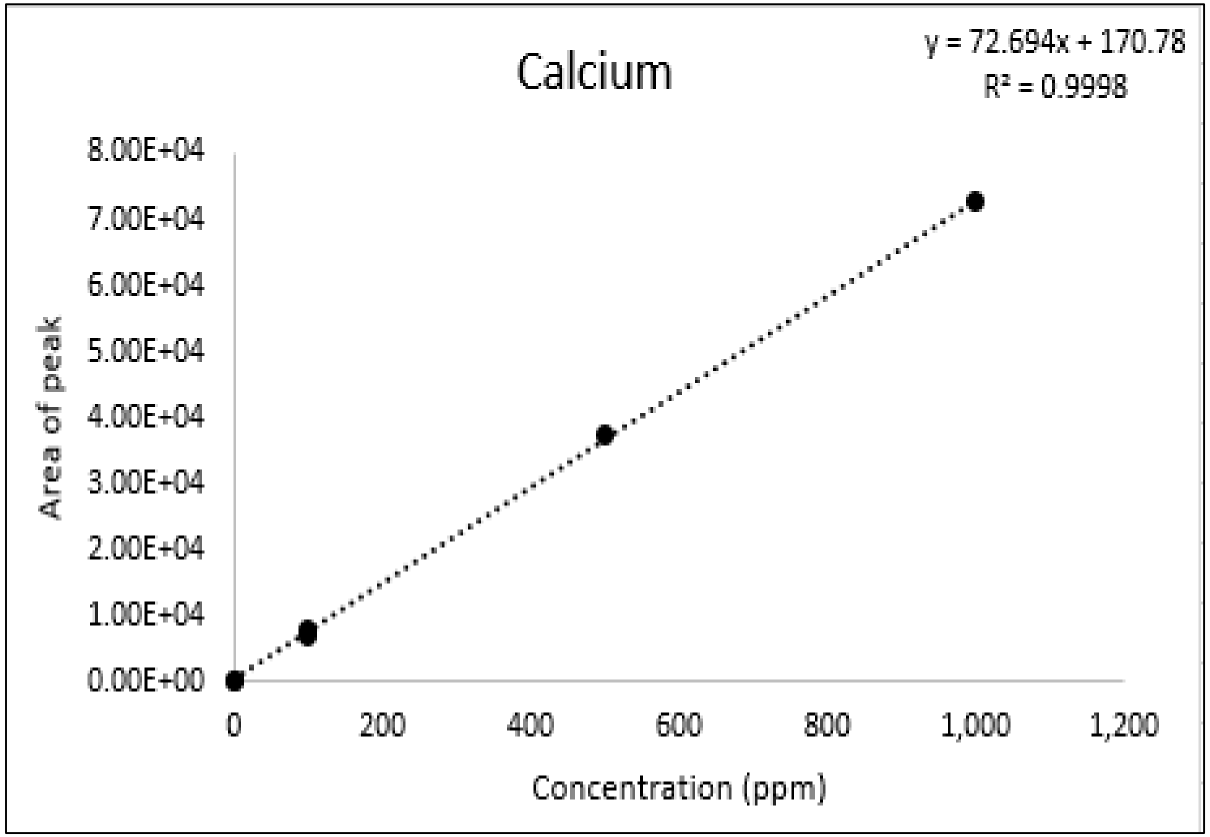
Corrected peak area calculation of element specific Emission- PyMca software

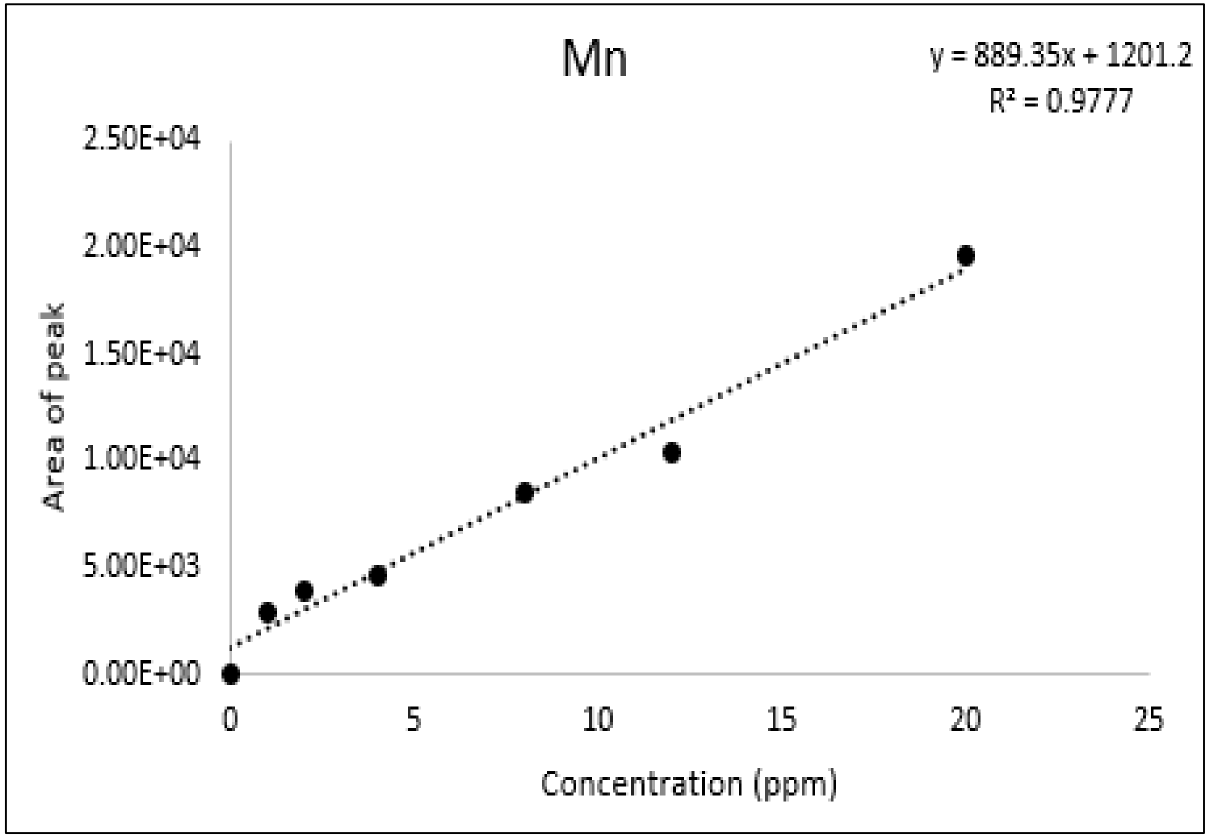


Data Processing

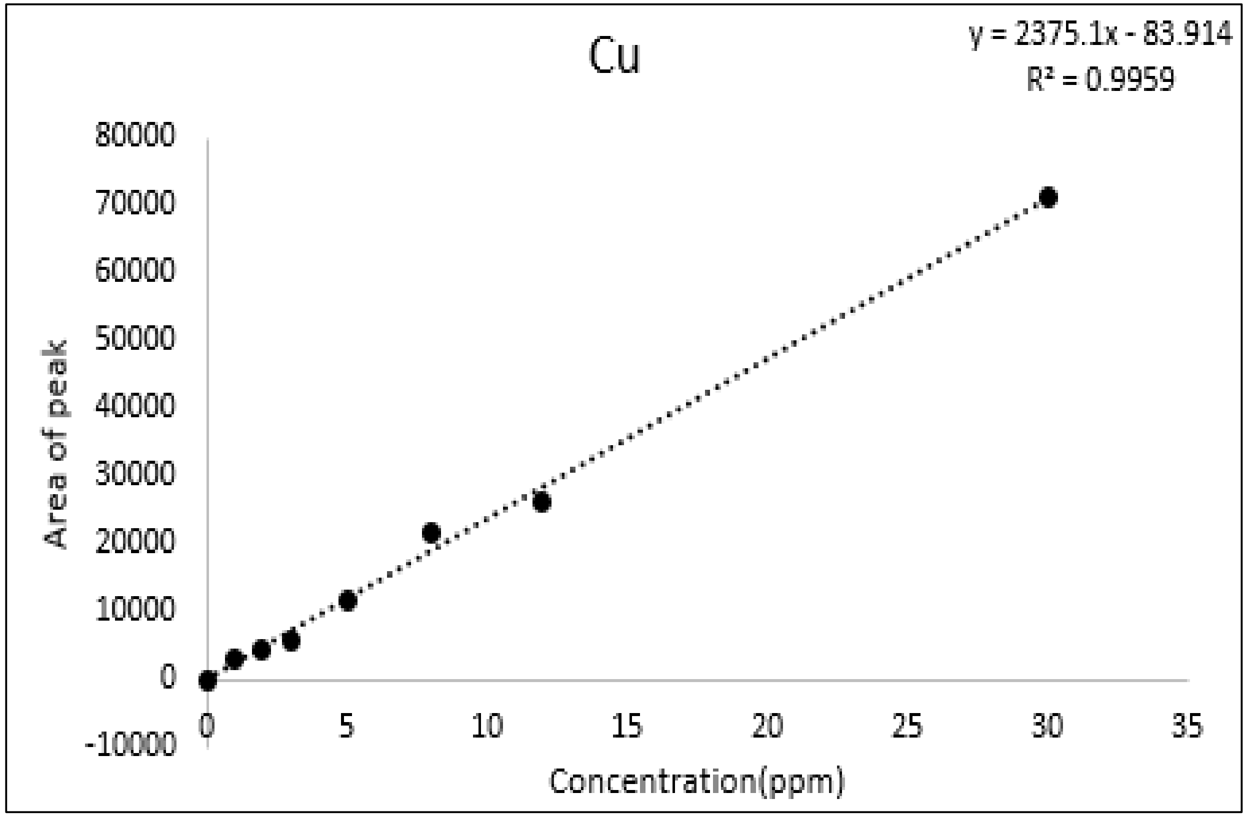
- Average I_0 values were calculated for 13 spectra per pellet
- Peak areas normalized to highest I_0 energy value
- Correlating area of peaks to respective mineral concentrations
- The quality of calibration curves - (R^2) value
- Standard equations were obtained

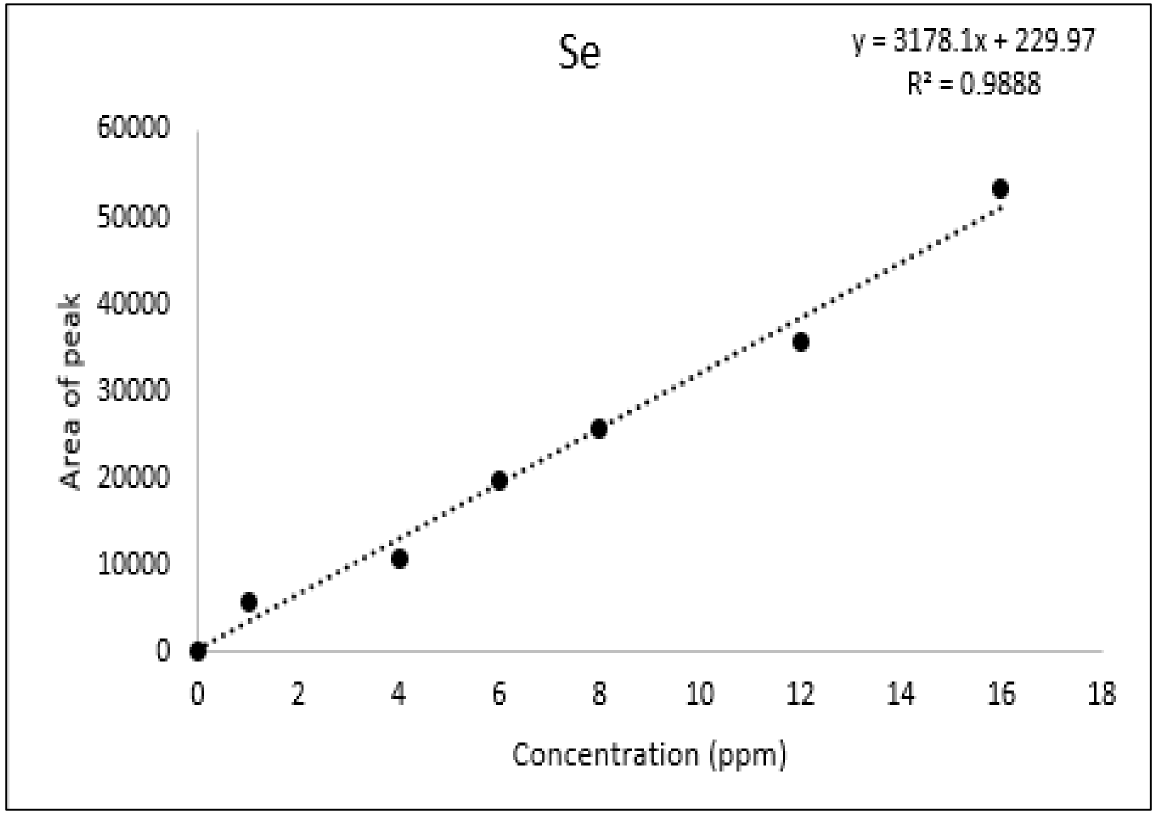
Calibration Curves (Ca & Mn)



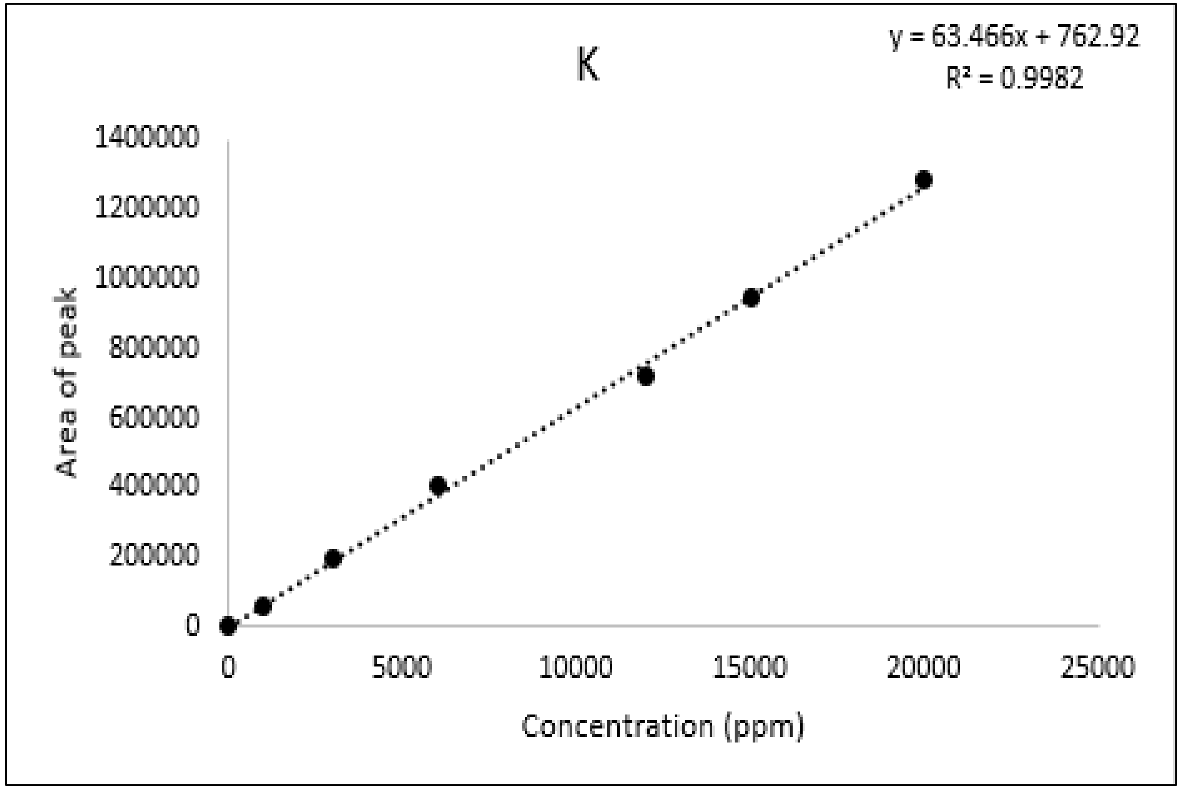


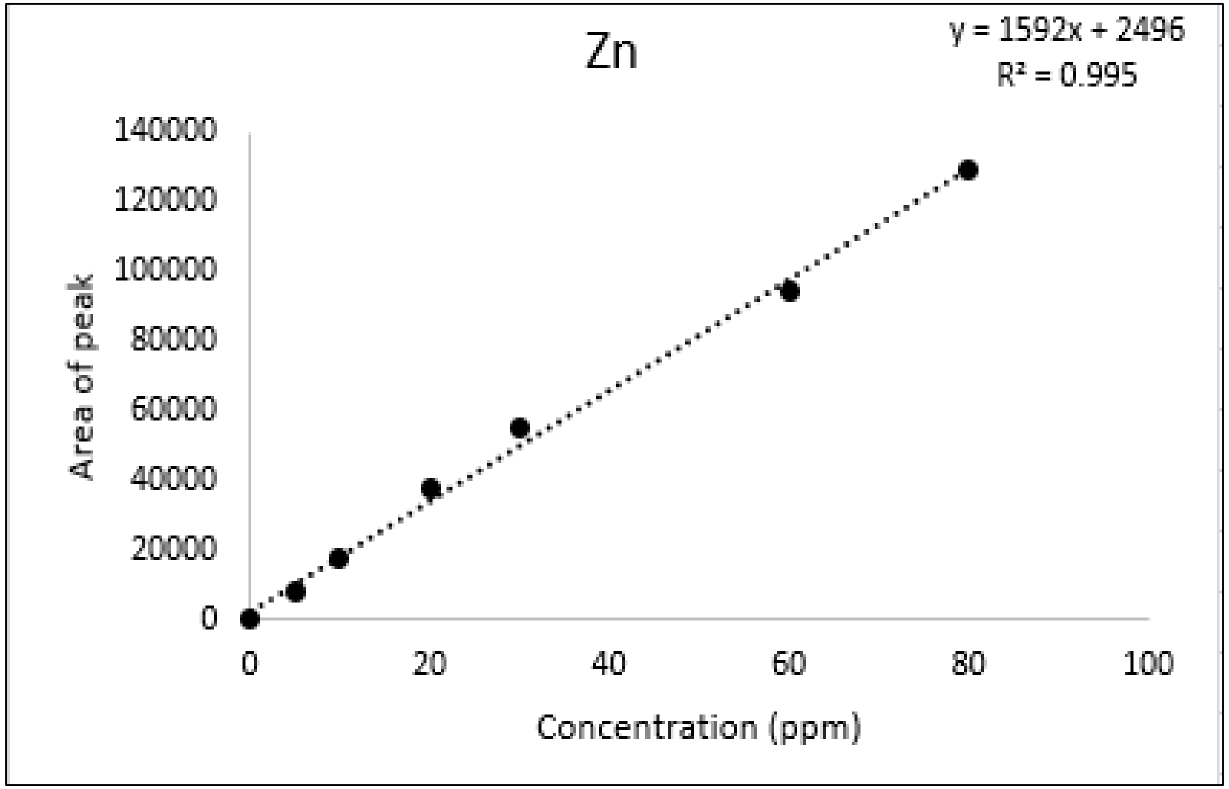
Calibration Curves (Cu & Se)



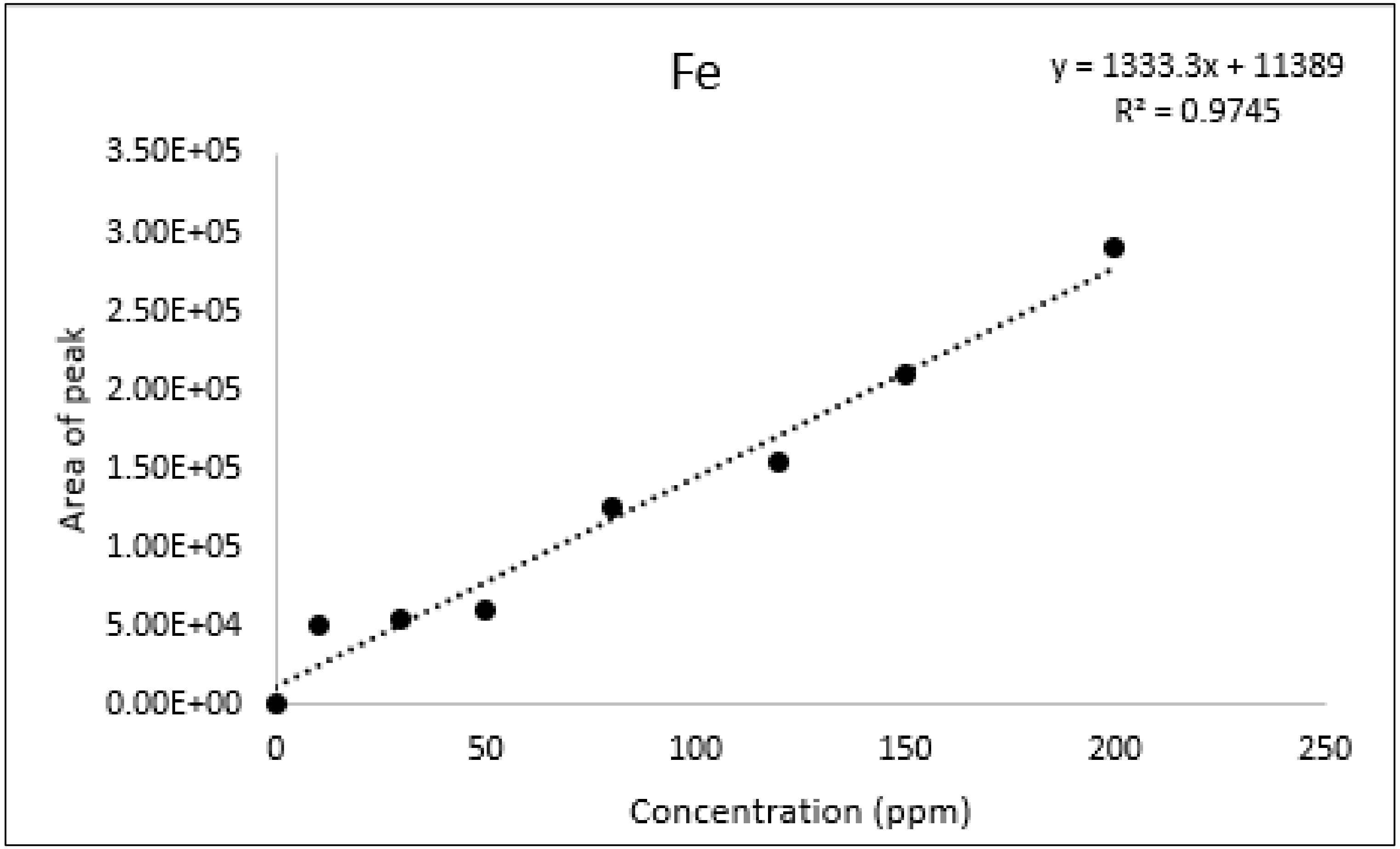


Calibration Curves (K & Zn)

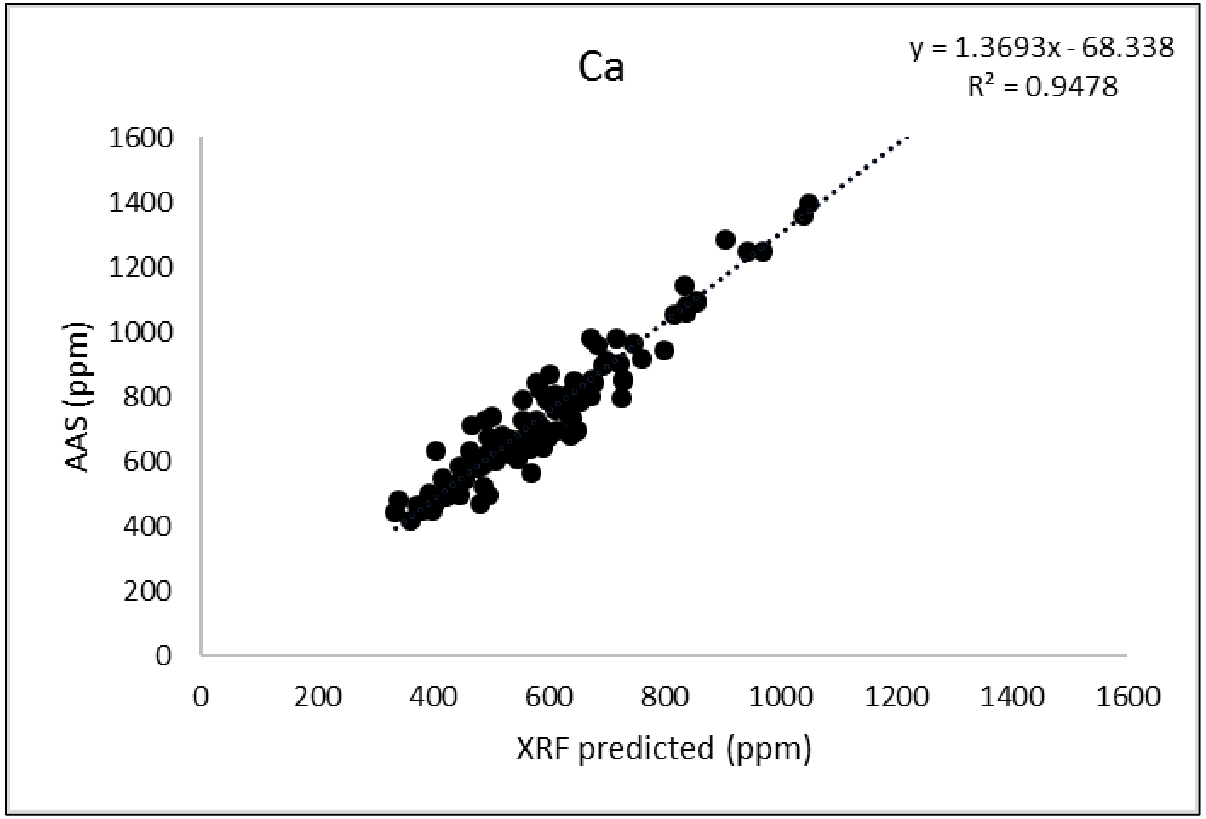


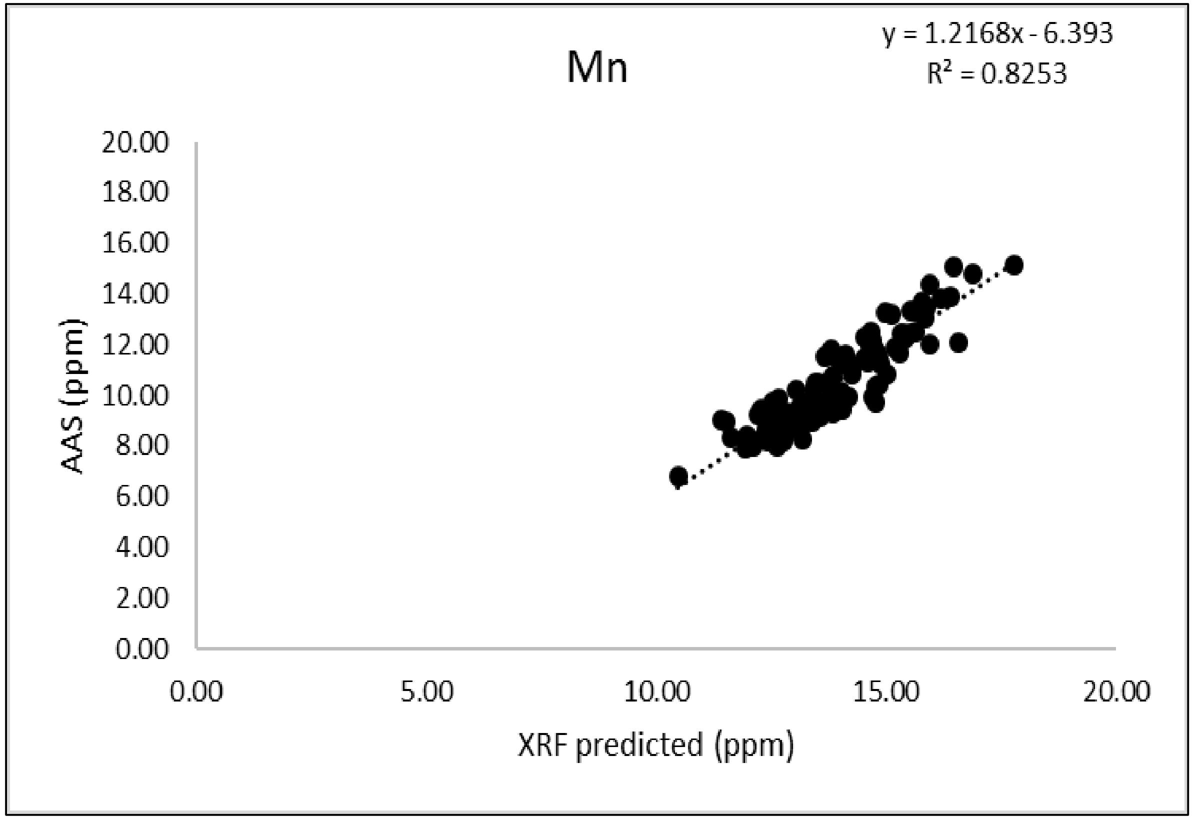


Calibration Curves (Fe)

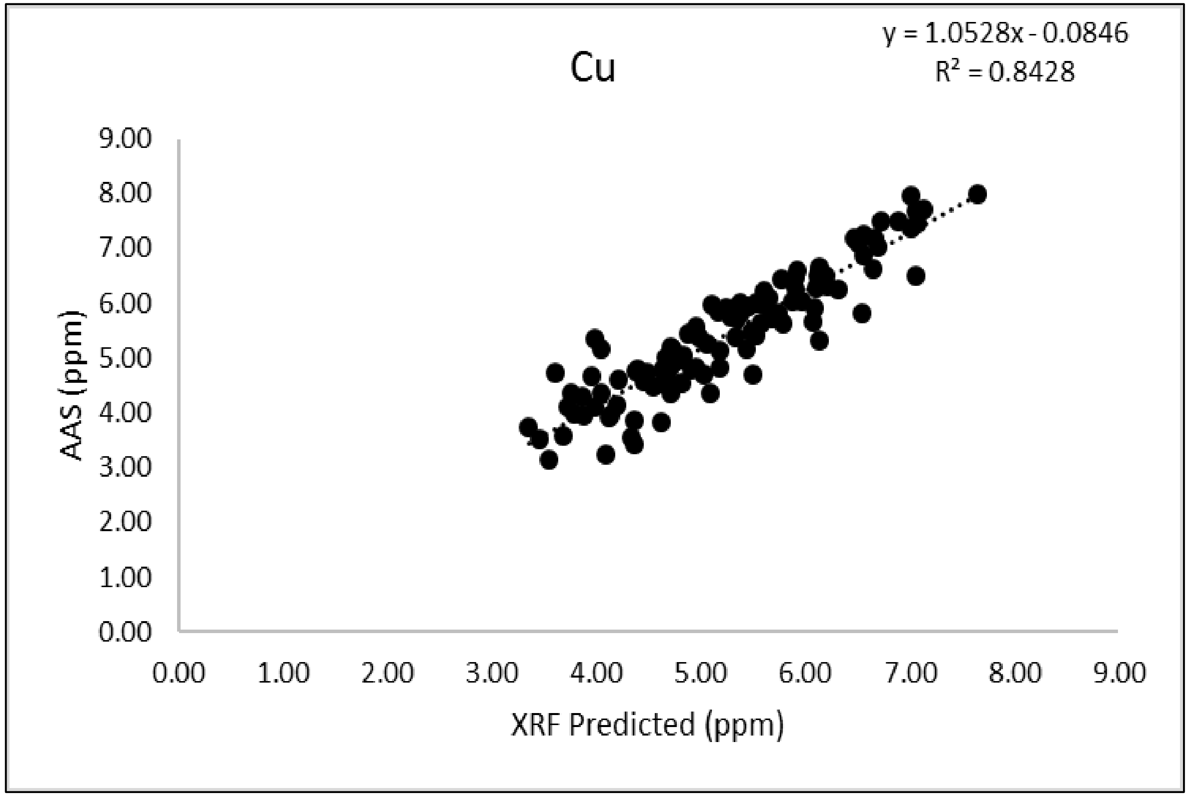


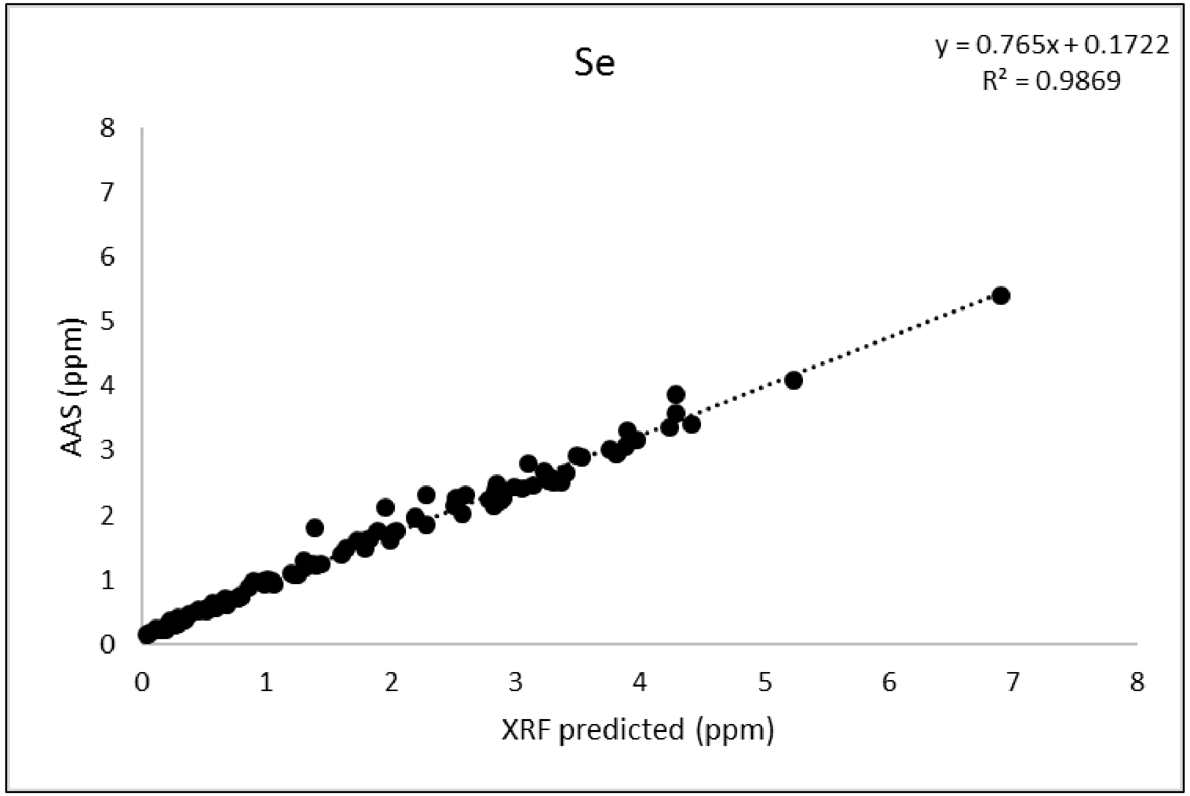
Correlation Between XRF and AAS concentrations



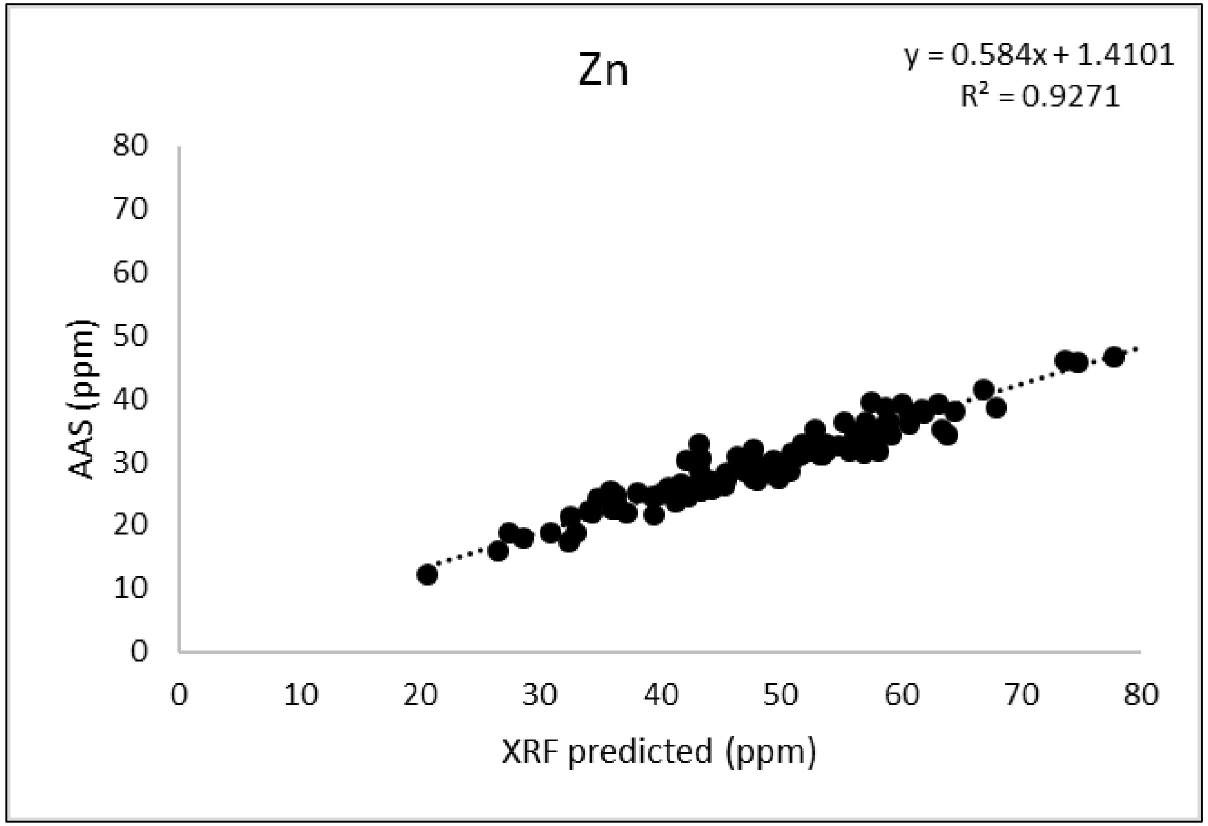


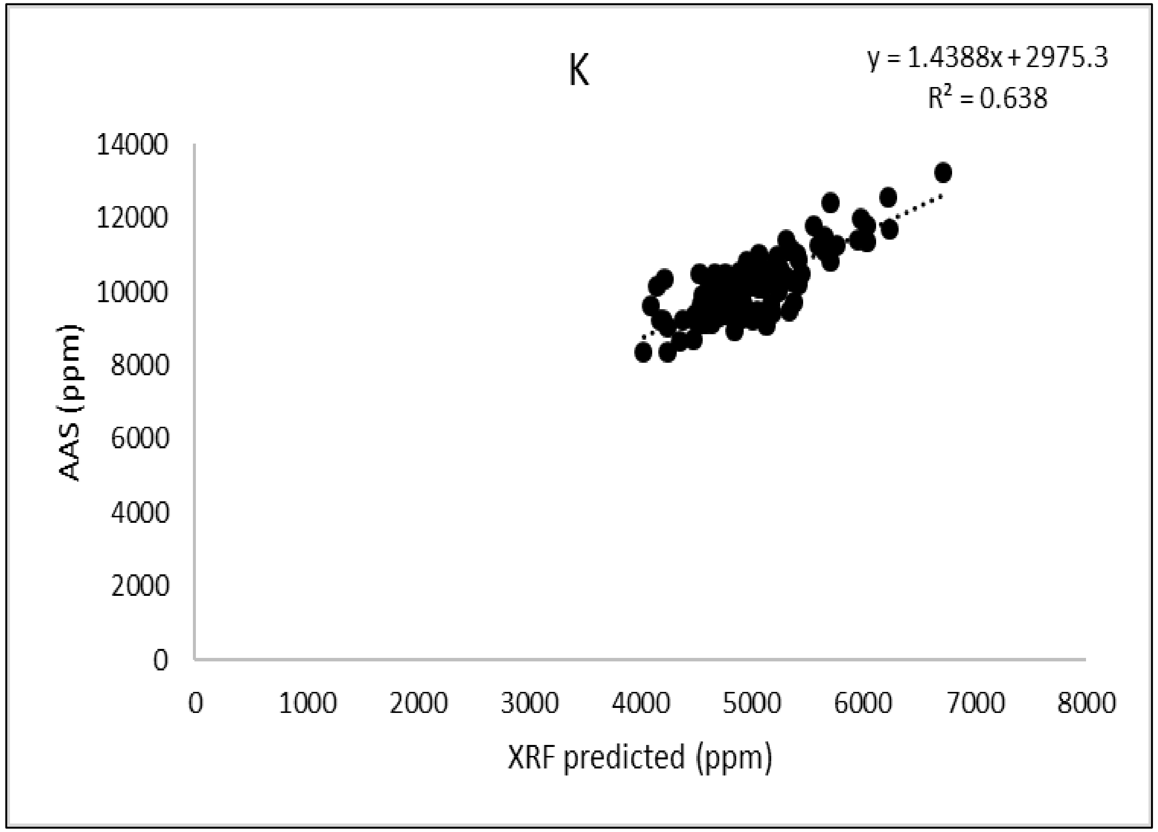
Correlation Between XRF and AAS concentrations



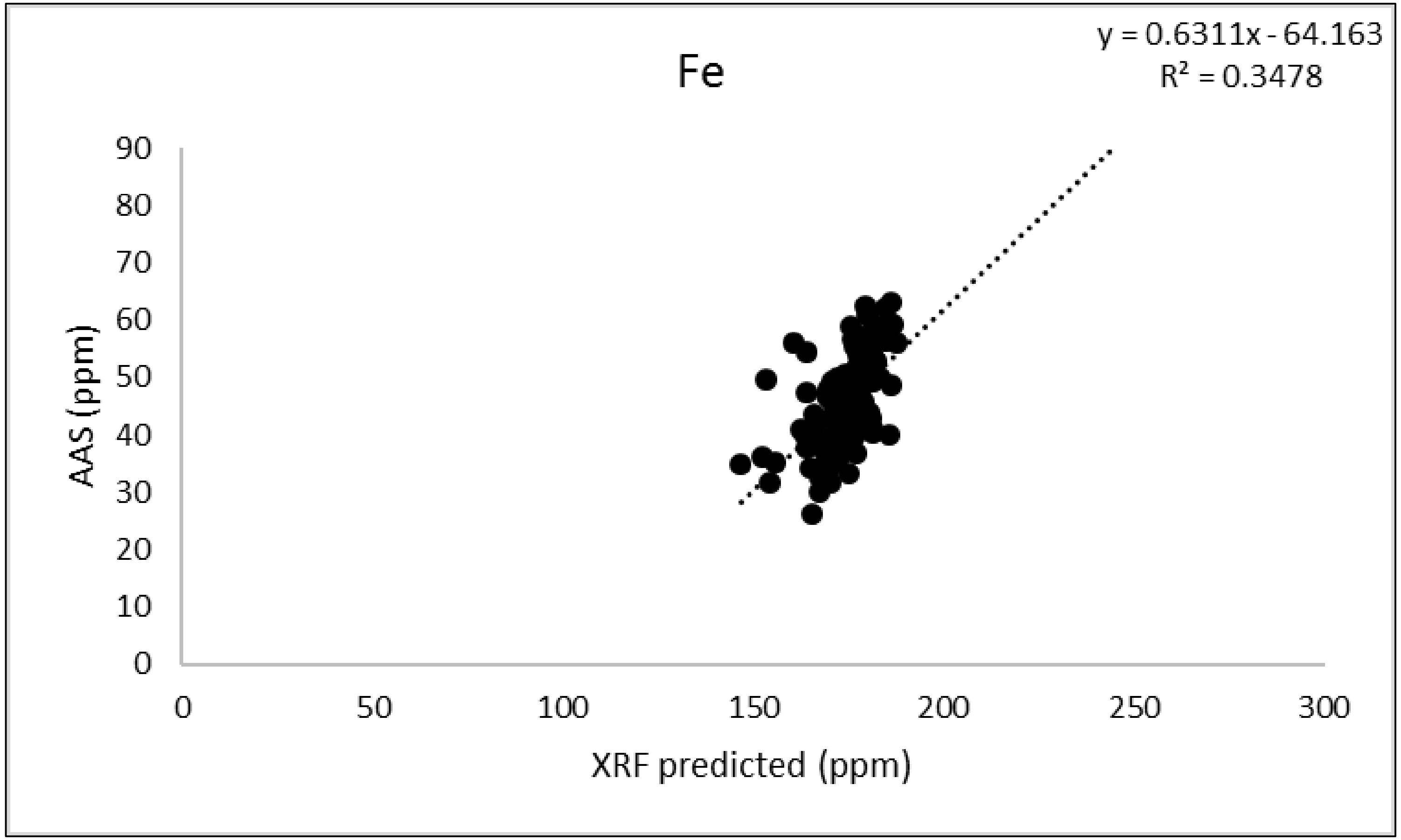


Correlation Between XRF and AAS concentrations





Correlation Between XRF and AAS concentrations



Conclusions

- Standard curves: excellent correlation between Zn, Se, Fe, Mn, Ca and K concentrations and XRF emission counts
- Seed Samples: excellent correlation between XRF emission based prediction of Se, Zn, Ca, Cu and Mn concentrations and AAS results

Potential Resolution of Issues

- XRF Vs AAS correlation low for Fe and K in Pea seed samples (not for starch matrix)
- Potential causes of Error:
  - Fe – External noise (Scatter from chamber walls)
  - K – Reabsorption of emitted radiation by matrix
- Potential resolution of Error:
  - Fe – XRF spectra outside of vacuum chamber
  - K – Thinner sample discs
  - Correction for reabsorption

Acknowledgements

Supervisor

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Saskatchewan
GOVERNMENT OF SASKATCHEWAN

UNIVERSITY OF SASKATCHEWAN

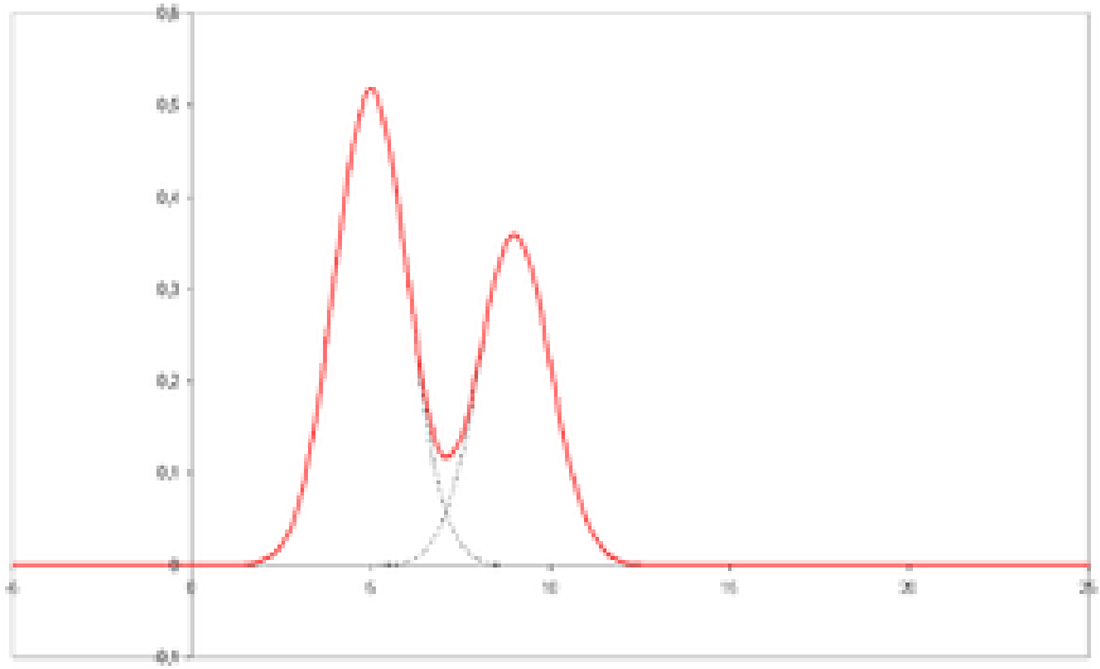
Western Grains Research Foundation

Thank You

Detection Limits

Element	AAS (ppm at 99.9% CI)	XRF (ppm at 99.9% CI)
Se	0.0006	0.016
Zn	0.018	0.2
Cu	0.049	0.35
Mn	0.013	0.5
Ca	0.38	3.5
K	0.084	9.54
Fe	0.105	0.54

Peak Overlap



Fundamental Parameters

FIT | DETECTOR | BEAM | PEAKS | PEAK SHAPE | ATTENUATORS | MATRIX | CONCENTRATIONS | XRFMC

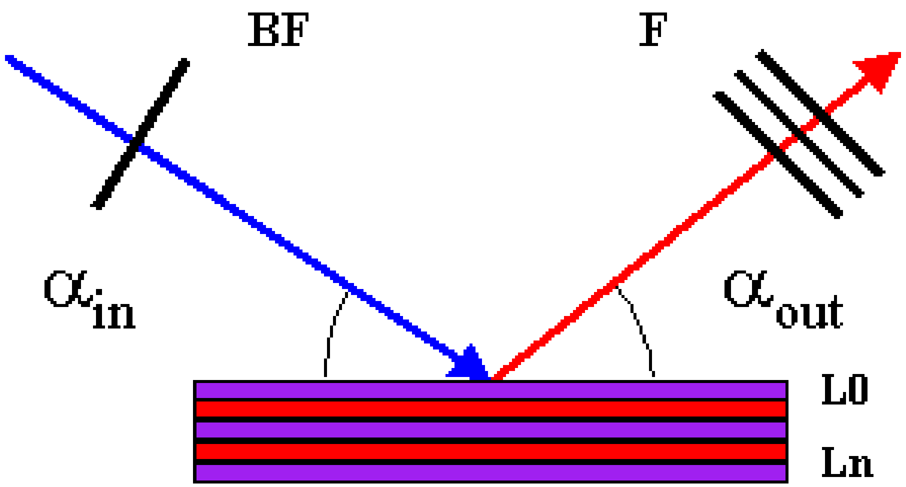
Attenuator	Name	Material	Density (g/cm3)	Thickness (cm)	Funny Factor
☑ Filter 0	air path	Air	0.0012048	8.0	1.0
☑ Filter 1	Be	Beryllium	1.85	0.0025	1.0
☐ Filter 2	window	-	0.0	0.0	1.0
☐ Filter 3	contact	Au1	19.37	1e-06	1.0
☐ Filter 4	sample	Sucrose	1.29	0.07	1.0
☐ Filter 5	Filter 6	Sucrose	1.29	0.07	1.0
☐ Filter 6	Filter 7	-	0.0	0.0	1.0
☐ Filter 7	beryllium	Beryllium	1.85	0.0025	1.0
☐ BeamFilter0	BeamFilter0	-	0.0	0.0	1.0
☐ BeamFilter1	BeamFilter1	-	0.0	0.0	1.0
☐ Detector	Detector	Si1	2.33	0.5	1.0

Plot T(filters) * (1 - T(detector)) Efficienty Term

Material Editor

Enter name of material to be defined: Air

FIT | DETECTOR | BEAM | PEAKS | PEAK SHAPE | ATTENUATORS | MATRIX | CONCENTRATIONS | XRFMC



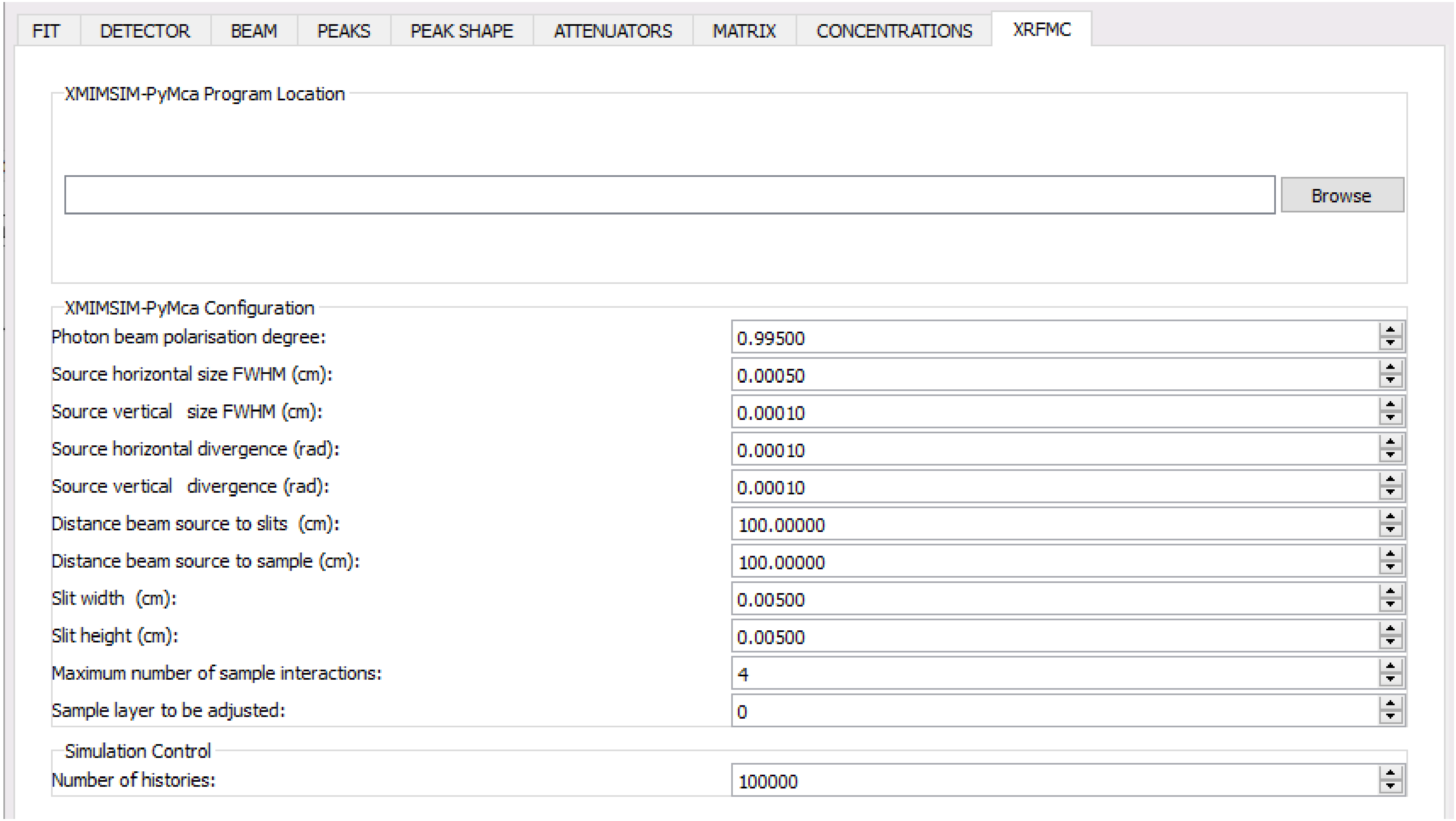
Incoming Angle (deg.): 45

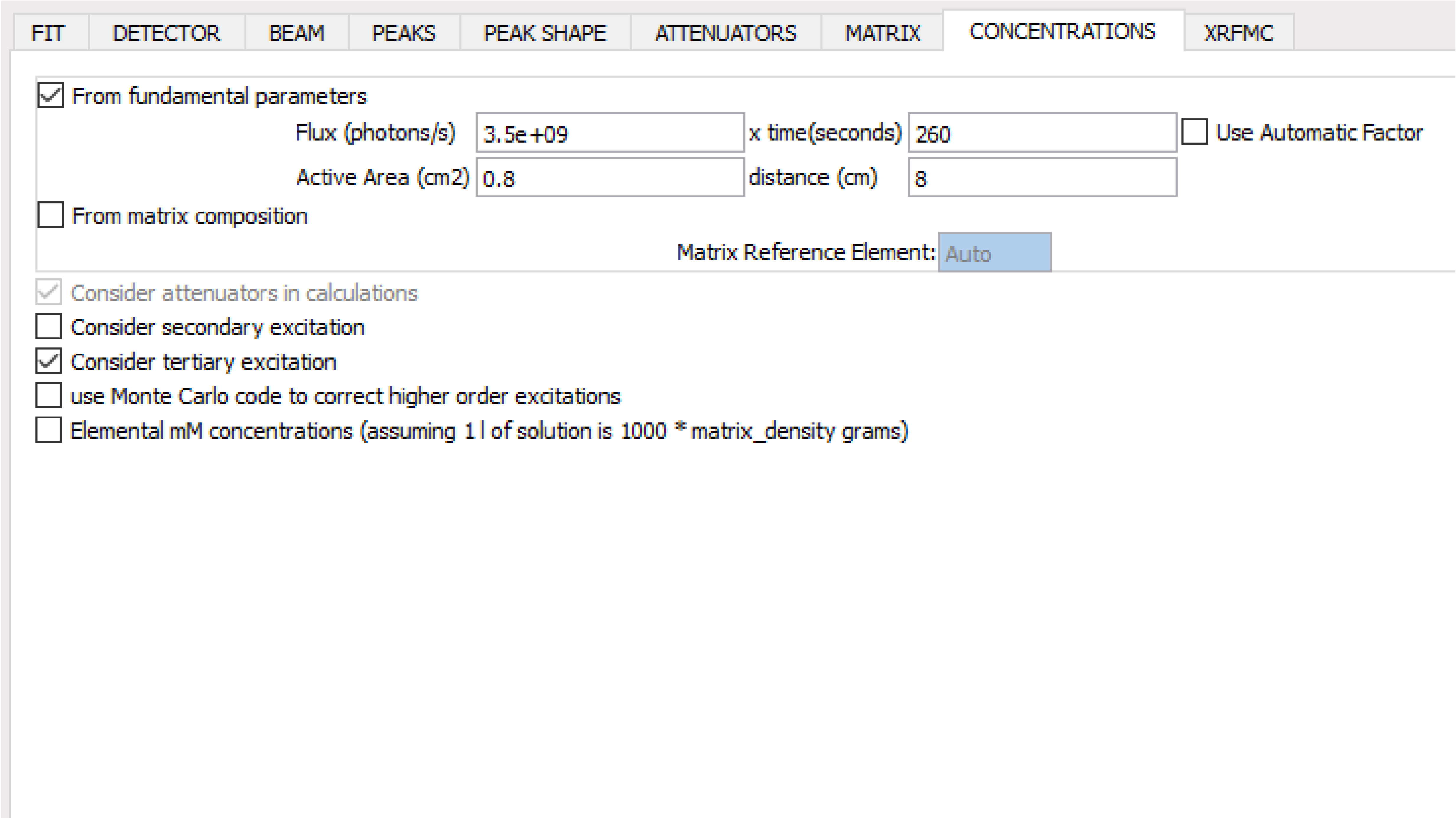
Outgoing Angle (deg.): 45

☐ Scattering Angle (deg.): 90

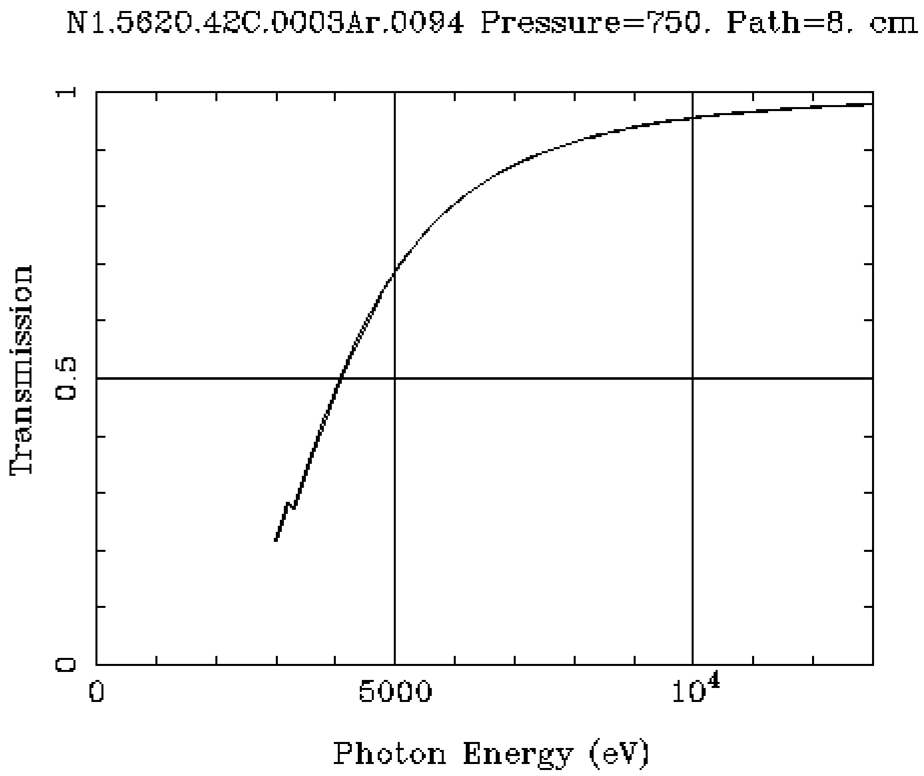
This matrix definition will only be considered if Matrix is selected and material is set to MULTILAYER in the ATTENUATORS tab.

Layer	Name	Material	Density (g/cm3)	Thickness (cm)
☑ Layer0	Layer0	PeaPowder	1.33	0.023
☐ Layer1	Layer1	-	0.0	0.0
☐ Layer2	Layer2	-	0.0	0.0


FIT
DETECTOR
BEAM
PEAKS
PEAK SHAPE
ATTENUATORS
MATRIX
CONCENTRATIONS
XRFMC
XMIMSIM-PyMca Program Location
Browse
XMIMSIM-PyMca Configuration
Photon beam polarisation degree:
0.99500
Source horizontal size FWHM (cm):
0.00050
Source vertical size FWHM (cm):
0.00010
Source horizontal divergence (rad):
0.00010
Source vertical divergence (rad):
0.00010
Distance beam source to slits (cm):
100.00000
Distance beam source to sample (cm):
100.00000
Slit width (cm):
0.00500
Slit height (cm):
0.00500
Maximum number of sample interactions:
4
Sample layer to be adjusted:
0
Simulation Control
Number of histories:
100000


FIT
DETECTOR
BEAM
PEAKS
PEAK SHAPE
ATTENUATORS
MATRIX
CONCENTRATIONS
XRFMC
From fundamental parameters
Flux (photons/s)
3.5e+09
x time(seconds)
260
Use Automatic Factor
Active Area (cm2)
0.8
distance (cm)
8
From matrix composition
Matrix Reference Element:
Auto
Consider attenuators in calculations
Consider secondary excitation
Consider tertiary excitation
use Monte Carlo code to correct higher order excitations
Elemental mM concentrations (assuming 1 l of solution is 1000 * matrix_density grams)

Air Attenuation



K Attenuation Length