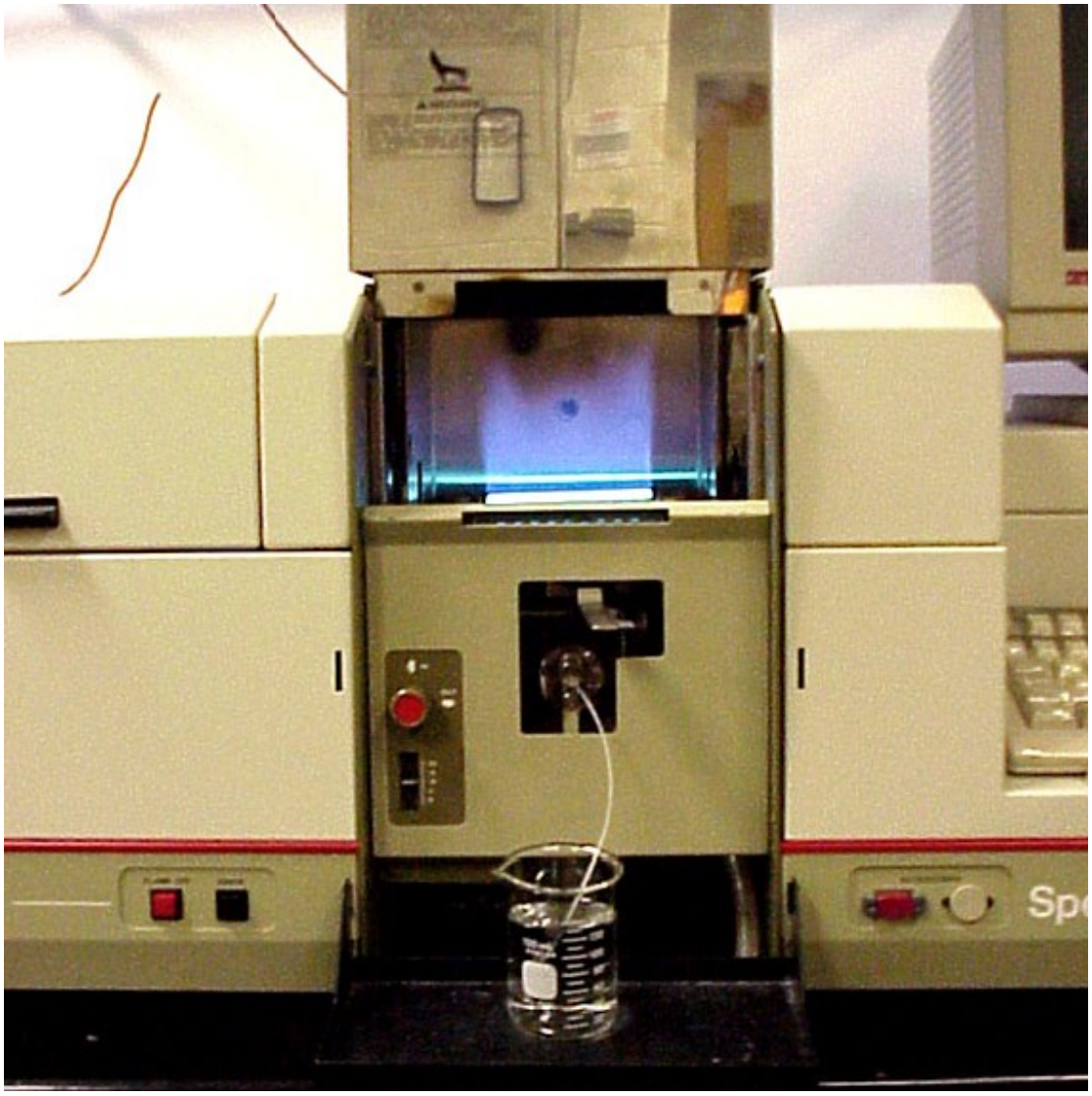
Appendix Three

Mesurement of plasma zinc

Method

The estimation of zinc was carried by atomic absorption spectroscopy device using analytical method for atomic absorption spectroscopy that principally based on the technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on Beer's-Lambert law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbital's (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using Beer's- Lambert law.



Procedure:

Plasma samples dilute 1:5 with deionized water.

Establish instrumental and gas-flow settings and aspiration rate precisely, to optimize signal and minimize background noise. The instrumental settings shown in the table below apply to the instrument we used in this study. Once the aspiration rate is optimized with 10-mL aliquots of water, lock the nebulizer flow adjustment in place. Aspirate glycerol/water solution (5/95 by vol) into the luminescent flame and set the baseline to read 0.000 ±0.001 absorbance *(A).* Take a baseline reading before and after each sample and reset the baseline as required.[48]

- **Instrumental arrangements used for plasma zinc analysis:[48]**

Instrument settings		
Wave length	213.8	
slit	0.7	
Mode	Absorbance,1.0-s reading	
Lamp current	15mA	
Gain	Midscale	
Lamp focus	Grazing burner head	
Burner height	7-7.5 units	
flame	Luminescent, fuel rich	
Gas-flow settings	Flowmeter (ib/in^2)	Pressure
Air	30	54
Acetylene	9	38
Aspiration rates, water,ml/min	6.0±0.1	

- **Standard atomic absorption conditions for zinc:**

Wavelength (nm)	**Slit (nm)**	**Relative Noise**	**Characteristic Concentration (mg/L)**	**Characteristic Concentration Check (mg/L)**	**Linear Range (mg/L)**
231.9	0.7	1.0	0.018	1.0	1.0
307.6	0.7	0.38	79.0	3500.0	___

- **Standard flame emission conditions for zinc:**

flame	**Slit (nm)**	**Wave length (nm)**

Nitrous oxide – acetylene[45]	0.2	213.9

Advantages of atomic absorption spectroscopy:

The advantages of atomic absorption spectroscopy include:

Inexpensive (equipment, day-to-day running), high sample throughput, easy to use and high precision.[47]

Disadvantages of atomic absorption spectroscopy:

The disadvantages of atomic absorption spectroscopy include:

Only solutions can be analyzed, relatively large sample quantities required(1–2mL) ,less sensitivity (compared to graphite furnace), problems with refractory elements.