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Quantitative Analysis of Toxic Trace Metal in Gallstone by ICP-OES

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Abstract

Quantitative analysis of toxic trace metal such as Cu, Cr, Cd, Fe, Zn, Pb and Ni by ICP-OES and its role in the etiology and pathogenesis of gallstone namely cholesterol gallstones, pigment gallstones and mixed gallstone. The study has been performed on 29 gallstones. This study was provided data of the main toxic and trace metal in gallstone of human being. Concentration of these elements are varying according to supersaturation of cholesterol, settling of solution and nucleation of human gallbladder bile. To establish the correlation of the levels of toxic trace element in stone of gallstone patient by PCA & also evaluate the demographic characteristic. For the study of correlation statistically, author used PCA analysis performed by Minitab with the help of eigen value, eigen vector and plots. It was noticed that the percentage of copper and zinc was higher in pigment stone than in mixed stone and cholesterol stone. The most important conclusion was that heavy elements such as Pb present in the samples of smoking patients.

Keywords: Cholecystectomy, ICP-OES, Minitab, Correlation, PCA Analysis

Introduction

Major components in the formation of gallstones are cholesterol, bile pigments, proteins, mucus and cellular debris, calcium and many other trace elements including iron, phosphorus, chromium, nickel, copper, zinc, manganese, strontium, lead & others. Gallstones are formed as a result of solids setting out of solution. Factors which have been implicated in the formation and precipitation of gallstones include constitutional elements, bacteria and reflux of antral and pancreatic fluid, hormones and bile stains. Increased fertility, female sex, obesity, ageing are some of the risk factors [1] which shows the complex interaction of genetic, environmental and metabolic risk factors [2]. Because of climatic and other environmental conditions are typical for certain geographical regions, trace elements status may play a crucial role in the development of gallstone disease. Many studies have been done in the past regarding the trace elements play an important role in the formation of gallstones [1-30].

Al-Kinani et al. [4] investigated the presence of minor and trace elements in gallstone and bile and discussed the role of trace elements in the formation of gallstones using three different techniques namely NAA, PIXE and XRF. NAA of five gallstones was used for the quantitative analysis of 19 elements with Z>11. The concentrations of Cl, K, Ca, Mn, Fe, Cu and Zn were determined by PIXE and those of Mg, P, S, K and Ca by XRF. In most cases the concentrations were in the range of a few ppb to a few hundred ppm. The spatial distribution of the component elements was obtained using XRF with a scanning electron microscope and the results demonstrated that calcium was present in all the stones. The analysis of gallbladder from four patients showed that the calcium and other elements play an important role in stone

formation. Infrared spectroscopy showed that the calcium salts were present in gallstones in three compound forms: calcium carbonate, calcium phosphate and calcium bilirubinate. Reasons for the cause of calcium precipitation were discussed.

The scrutiny of the literature demonstrates that in most of the studies atomic absorption spectroscopy (AAS) is preferred for the investigation of the samples. However, for AAS relatively long analysis time, limited multi-element capability and complex and analyte dependent sample preparation is required. Since ICP is fast and multi-elemental detection technique and therefore, it gives rapid and reliable information about the trace metal metabolism in human body. So it is gaining popularity in clinical field. The aim and objectives of this study is to quantitatively analyze the levels of the various trace metals in gallstones of the patients and the prevalence of the different types of gall stones in this region of North India.

In the present analytical study, an assessment of selected trace elements (Cd, Cr, Cu, Fe, Ni, Pb, Zn) has been used microwave dry digestion method with ICP-OES in the gallstone samples of cholelithiasis patients. Author also discuss PCA analysis and correlation between toxic trace metal using Minitab and result interpretate with the help of eigen value, eigen vector and plots.

Materials and Methods

Sample Collection- The study has been performed on 29 patients admitted to the Department of Surgery, Motilal Nehru Medical College, Prayagraj, India. The samples were collected from the admitted cholelithiasis patients of Department of Surgery, Motilal Nehru Medical College, Prayagraj, India. There author collect the 29 gallstone samples. Gallstones were extracted from the surgically removed gallbladder. After sampling, samples were stored duly labeled with relevant codes related to the donor's name, age, gender, eating and drinking habits and social and general health status. All the records were compiled on regular proforma at the time of sampling. Then gallstone was washed by deionized water after they had been extracted from patient to remove/clean all the traces of blood and other possible contaminations.

Digestion of gallstone- Microwave-assisted dry acid digestion is a rapid and higher efficient method for breaking down of complex biological matrices of gallstones to analyse their composition. Comparatively, this method utilizes microwave energy to accelerate the digestion process by reducing time & reagent consumption and leading to less waste, improved reproducibility and accuracy in elemental analysis compared to conventional digestion techniques.

Firstly, author collect gallstone samples and dry them at ~100°C to remove moisture. Then, crush or grind the samples into a fine powder to increase surface area for digestion and accurately weigh 0.5 gm powdered gallstone. Place the sample in a specialized high-pressure microwave digestion vessel. Add the selected acid mixture in controlled proportions (5 mL HNO_3 + 2 mL H_2SO_4). Seal the vessel to maintain pressure and prevent acid loss. Heat in a microwave digestion system at programmed temperatures typically 220°C for a set time 20 minutes to increase efficiency. Allow the vessel to cool before opening to prevent pressure-related accidents. Transfer the digested solution to a volumetric flask and dilute with deionized water.

ICP-OES Analysis- The final solution is then analysed using ICP-OES. The sample is introduced into the plasma, where high temperatures (around 10,000 K) cause the elements to emit light at characteristic wavelengths. These emissions are detected and quantified by the ICP-OES instrument. The intensity of light emitted by each element corresponds to its concentration in the sample. The results can be compared to calibration standards to determine the concentration of various toxic trace metals (such as copper, iron,

zinc, nickel, cadmium, lead etc.) in the gallstone sample. This method can provide detailed insights into the mineral composition of gallstones, which may help in understanding their formation and potentially offer insights into treatments or preventive strategies.

Observations

Table 1: Type of the various studied gallstones

Number of Cholesterol Stone	7
Number of Pigmented Stone	15
Number of Mixed Stone	7

The concentration of various trace elements determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) in gallstones.

Table 2: Concentration of trace elements in gallstone samples of cases (mg/l)

Patient	Sample	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Patient 1	CS1	NT	NT	4.982	0.865	NT	0.099	0.109
Patient 2	CS2	NT	NT	NT	NT	NT	NT	NT
Patient 3	CS3	NT	NT	NT	NT	NT	NT	NT
Patient 4	CS4	NT	NT	NT	NT	NT	NT	NT
Patient 5	CS5	NT	NT	0.899	NT	NT	0.061	0.032
Patient 6	CS6	NT	NT	1.91	0.312	NT	NT	NT
Patient 7	CS7	NT	NT	0.056	NT	NT	NT	0.027
Patient 8	PS1	NT	NT	NT	0.005	NT	NT	0.009
Patient 9	PS2	NT	NT	NT	0.011	NT	NT	NT
Patient 10	PS3	NT	NT	0.008	0.108	NT	NT	0.013
Patient 11	PS4	NT	NT	NT	3.29	NT	NT	0.04
Patient 12	PS5	NT	NT	NT	2.3	NT	NT	NT
Patient 13	PS6	NT	NT	0.007	0.117	NT	0.097	0.023
Patient 14	PS7	NT	NT	NT	0.762	NT	NT	0.016
Patient 15	PS8	NT	NT	NT	NT	NT	NT	0.068
Patient 16	PS9	NT	NT	NT	NT	NT	0.091	0.41
Patient 17	PS10	NT	NT	0.471	2.16	NT	NT	NT
Patient 18	PS11	NT	NT	NT	3.16	NT	NT	NT
Patient 19	PS12	NT	NT	0.078	1.29	NT	NT	0.025
Patient 20	PS13	NT	NT	0.249	2.17	NT	0.019	0.029
Patient 21	PS 14	NT	NT	0.456	1.71	NT	NT	0.011
Patient 22	PS15	NT	NT	0.314	0.87	NT	NT	0.017
Patient 23	MS1	NT	NT	0.245	0.291	NT	NT	0.005
Patient 24	MS2	NT	NT	0.211	0.391	NT	NT	0.006
Patient 25	MS3	NT	NT	1.286	0.105	NT	NT	NT
Patient 26	MS4	NT	NT	0.344	0.08	NT	NT	0.117
Patient 27	MS5	NT	NT	0.186	0.098	NT	NT	0.018

Patient 28	MS6	NT	NT	0.08	0.089	NT	NT	0.016
Patient 29	MS7	NT	NT	0.112	NT	NT	0.016	0.406

Abbreviation:
CS: Cholesterol Gallstone, PS: Pigment Gallstone, MS: Mixed Gallstone, NT: Not Traceable

Results and Discussion

The study was performed on 29 gallstone formers. Among which 29 patients were taken as cases having gall stones disease.

Female to male ratio in gallstone former- 3:1

Cases having multiple stones- 8

Cases having single/two stones- 21

Average size of gall stones was in range of 8mm-24mm

The color of the studied gallstones was, white, yellowish white, chocolatey brown, green and black; depending on their type as cholesterol, mixed and pigmented gallstones.

ICP-OES Performance- The result of various trace elements such as Cu, Zn, Fe, Pb, Cd, Ni and Cr were analyzed using ICP-OES due to its sensitivity, precision, and ability to handle complex matrices and concentration of traceable element shown in observation table 2. The observation table 2 shows that Cd, Cr and Ni were not traceable.

Role of trace metal in gallstone formation- Increased biliary Cu level act as prenucleation factor, So by excretion of Cu resulted in super saturation of bile and form the gallstone. Oral administration of Zn salt resulted in a significant decrease of serum bilirubin level, as a result of inhibition of entrohepatic circulation of bilirubin as a result of biliary and intestinal bile pigment excretion. So, by supersaturation of Zn in the bile from pigment gallstone. Fe play an important role in many metabolic roles including enzymatic reaction. Fe deficiency increased transferring in the bile of patient. Transferrin is a powerful cholesterol prenucleator. In hepatic sterol metabolism HMG-CoA reductase and cholesterol 7α-hydroxylase is the two enzymes responsible for cholesterol and bile salt secretion respectively. Iron is required for the correct action of cholesterol 7α-hydroxylase. Relative deficiency may reduce the activity of cholesterol 7α-hydroxylase without affecting HMG-CoA reductase activity. This produces imbalance between cholesterol and bile salts excretion resulting in cholesterol saturation and favoring crystal precipitation leading to gall stones. Besides this imbalance in the level of serum and hepatic iron also can lead to pigment gall stones. Therefore, iron deficiency may enhance cholesterol gallstone formation by altering hepatic enzyme function, biliary motility and cholesterol crystal nucleation.

Statistical Tool: The computer software Minitab was used to analyze results statistically. PCA explain diversity, grouping, correlation, pattern & summary statistics in obtain result by ICP-OES. PCA explained with the help of eigen value, eigen vector and plots. Correlation coefficient for toxic trace metal (r-value) was used to interpret observed data of cholesterol gallstone, pigment gallstone and mixed gallstone.

Score plot explained that PCA1 and PCA2 is implicated on the gallstone data. So, data is significant for the gallstone analysis. Score plot (Figure 1) shows that distance between samples have been significant difference. In other words, distance between samples directly proportional to the significant difference. The content of various trace toxic metal in mixed type stones and pigment stones is very similar, the reason of which is that the composition of mixed type stones and pigment stones is more similar in comparison to cholesterol gallstone.

Loading plot (Figure 2) and correlation coefficient (Figure 3) explains on the basis of 29 gallstone sample data that the positive correlation occurs between Cu, Zn, Pb and Fe. Cholesterol gallstone also explain the positive correlation between Cu, Fe, Pb and Zn. But in the pigment gallstone and mixed gallstone shows the negative correlation.

Table 3. Eigen analysis of the Correlation Matrix Score

Eigenvalue	2.5689	1.4311	0.0000	0.0000
Proportion	0.642	0.358	0.000	0.000
Cumulative	0.642	1.000	1.000	1.000

Table 4. Eigenvectors

Variable	PC1	PC2	PC3	PC4
Cu	0.527	0.447	0.394	-.606
Fe	-.390	0.652	0.471	0.447
Pb	0.546	-.404	0.521	0.516
Zn	0.521	0.460	-.592	0.408

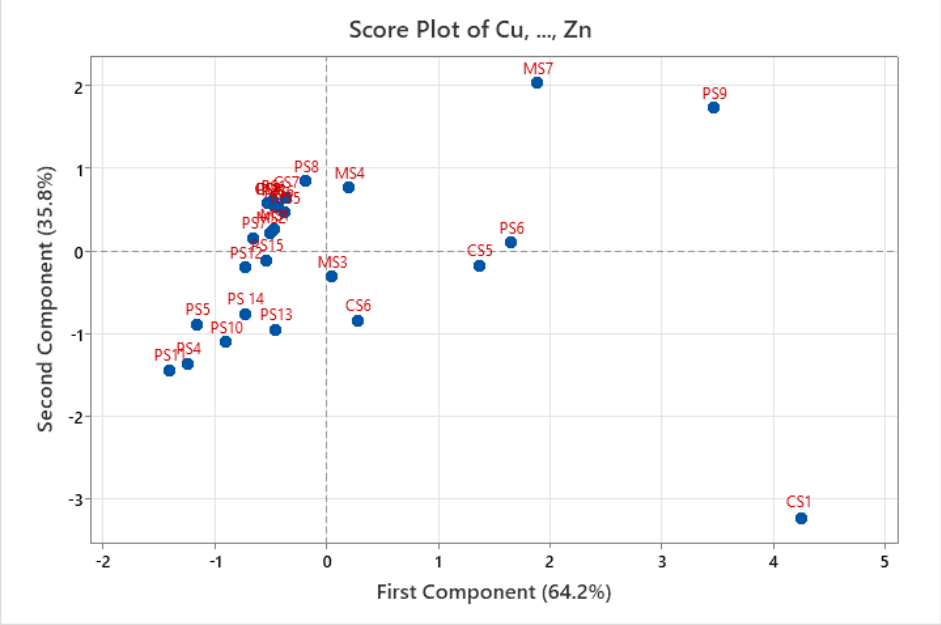

Figure 1: Score plot for Cu, Fe, Pb & Zn concentration in 29 samples of gallstone sample from people of different ages, classes and some of whom were smoker

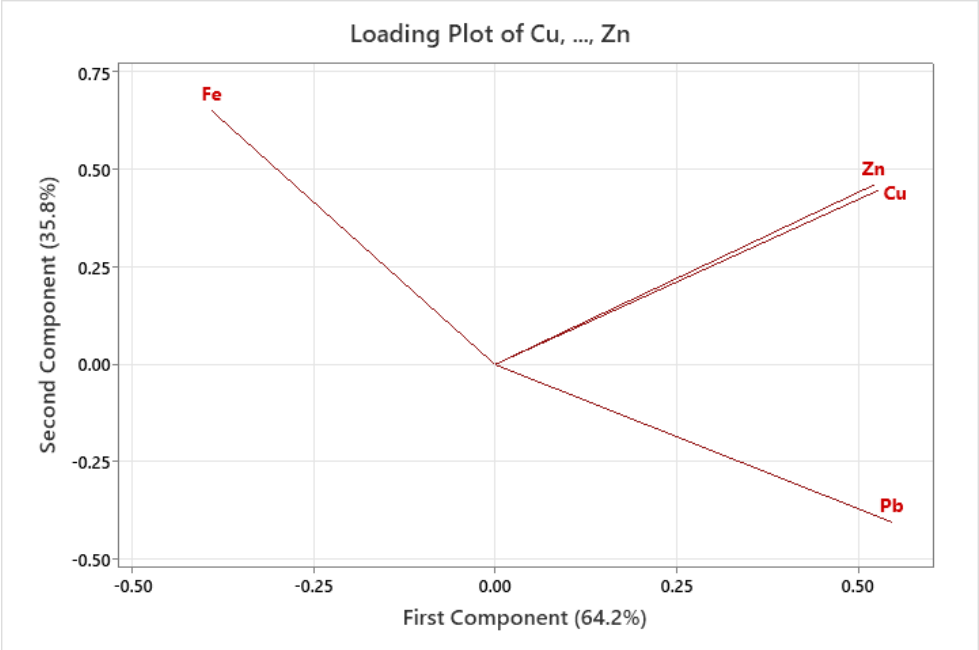

Figure 2: Loading plot for Cu, Fe, Pb & Zn concentration in 29 samples of gallstone sample from people of different ages, classes and some of whom were smoker

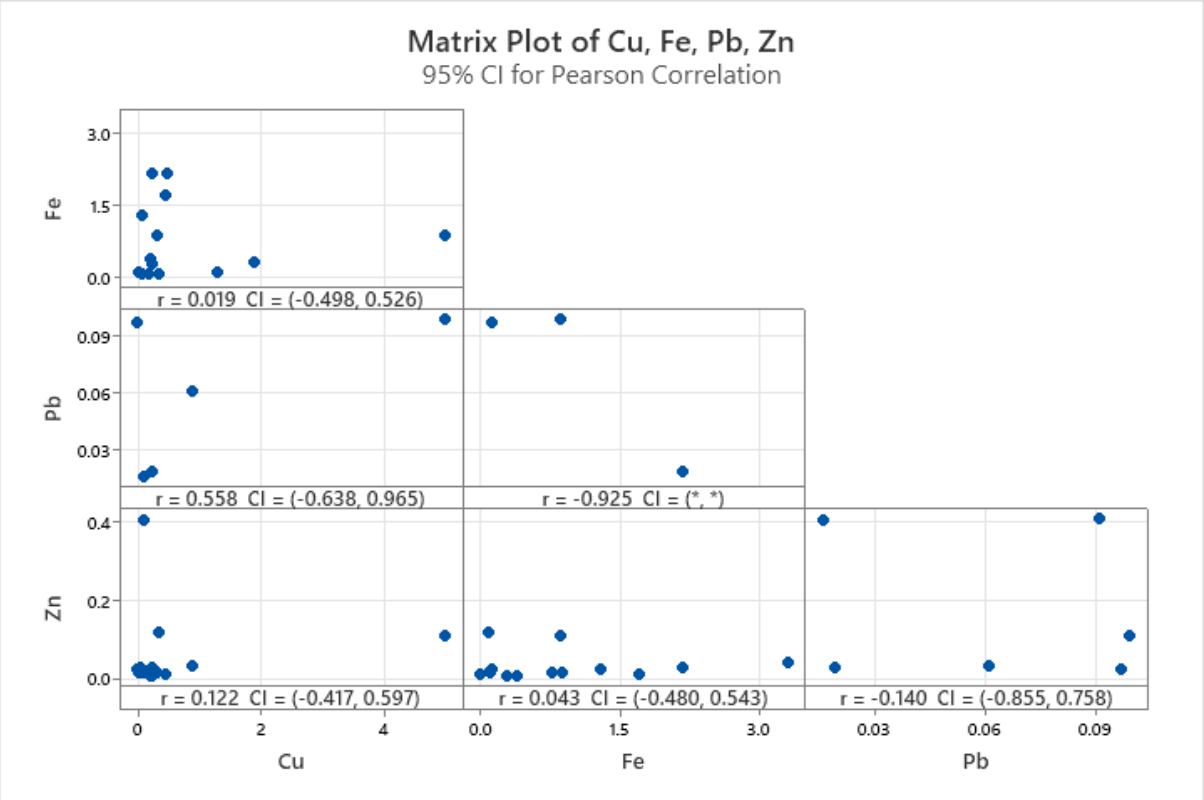

Figure 3: Correlation coefficient between Cu, Fe, Pb & Zn concentration in 29 samples of gallstone sample from people of different ages, classes and some of whom were smoker

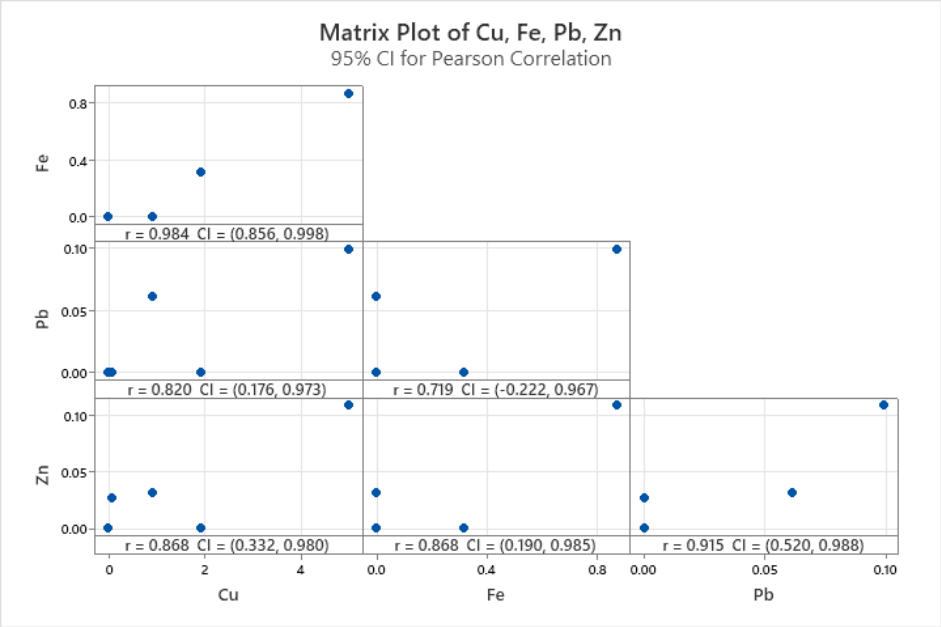

Figure 4: Correlation coefficient between Cu, Fe, Pb & Zn in 7 samples of cholesterol gallstone sample from people of different ages, classes and some of whom were smoker

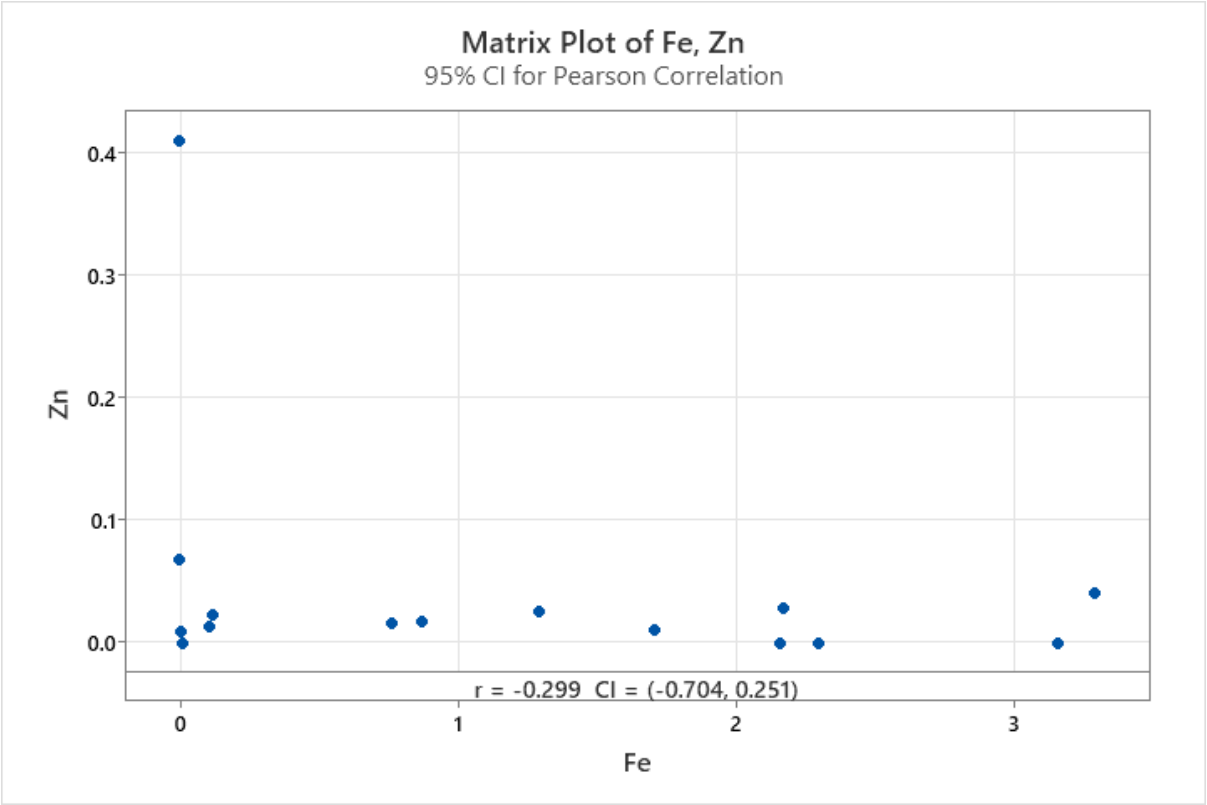

Figure 5: Correlation Coefficient between Fe & Zn in 15 samples of pigment gallstone sample from people of different ages, classes and some of whom were smoker

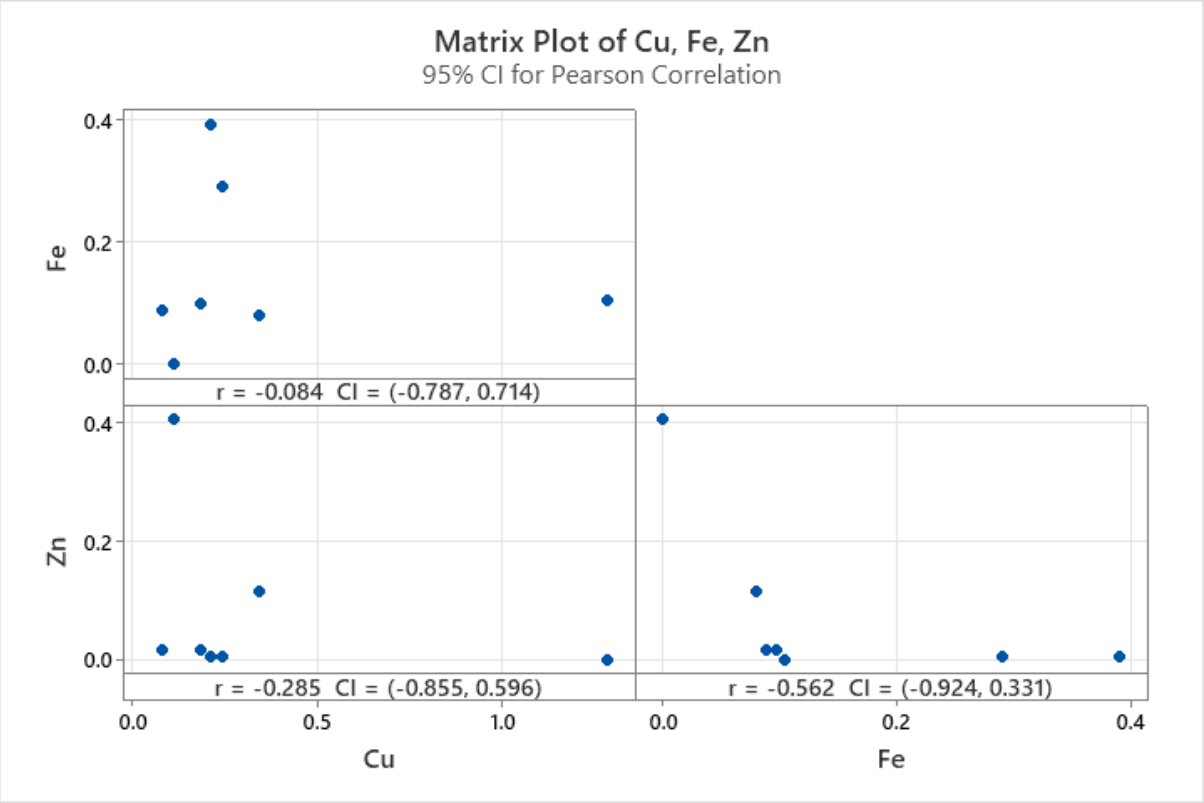

Figure 6: Correlation Coefficient between Cu, Fe & Zn in 7 samples of mixed gallstone sample from people of different ages, classes and some of whom were smoker

Conclusion

Gallstone disease is one of the most prevalent gastrointestinal disorders. Despite decades of researches, the mechanism of gallstone formation remains incompletely understood. The trace elements are believed to play an important role in gallstone formation. Unfortunately, the study on this issue is still rather limited. Age and female sex considered strong risk factors compared to other risk factor. Author found that the proportion of gallstone disease was increased with increasing age and female sex, which might be because estrogen increases biliary cholesterol secretion causing cholesterol supersaturation of bile and pigment stones formed due to infections or hemolytic hematologic conditions. ICP-OES a preferred tool in medical and biochemical studies investigating the elemental composition of gallstones. It was notice that Cu and Zn was higher in pigment gallstone sample in comparison to cholesterol and mixed gallstone. Pb was present in sample of smoking patient. But for the accuracy and precision of the result large number of samples is required

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