Relative statistics of Metals in a Smoker’s Gallbladder Stones using X-Ray Fluorescence

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Abstract. In this work, the X-ray fluorescence (XRF) technique was used to investigate the concentration of elements in gallbladder stones for smokers and non-smokers. Many elements have been detected in the non-smoker gallstone samples. The most important elements include calcium (Ca), potassium (K), phosphorous (P), magnesium (Mg), sodium (Na), iron (Fe) and lead (Pb). However, significantly, concentrations of the most toxic elements have been found within the group of the smokers. The maximum concentrations of toxic elements such as lead (Pb), cadmium (Cd) and cobalt (Co) were found in people older than 60 years. Furthermore, the minimum concentrations of the trace elements calcium (Ca), phosphorous (P) and sodium (Na) were detected in this age group. The relative statistics of Ca concentration in smokers’ and non-smokers’ were studied, and these groups were divided according to age. It was concluded that the maximum Ca concentration for non-smokers was found in the 20- to 40-year age group when compared with the other groups.

Keywords: Gallbladder stone smoker & non-smoker, smoking related, XRF

1. Introduction
X-ray fluorescence is generated by among other processes, the ionization of the inner shell of an atom. However, the probability that it will occur is small and is also dependent on the atomic number of the atom. Most of the excitation energy is translated into heat, which is the reason that XRF instruments always need to be more or less cooled. If ionization of an inner shell does occur, i.e., an electron is removed, then the hole is filled by an electron from a higher energy shell. The energy difference is released as X-ray fluorescence radiation and is characteristic for the element. Depending on which transition takes place, it is called K, L or M radiation. Instrument technology depending on the method of detection, instrument technology is divided into energy dispersive (EDXRF) and wavelength dispersive (WDXRF) X-ray fluorescence analysis [1].

The XRF technique can be used for multi-elemental analyses. The XRF method has been utilised to determine trace element concentrations in a wide range of samples, for instance biochemistry samples, chemical samples, and archaeological samples. However, the XRF as a portable system frequently lacks the ability to analyse large samples (larger than 1g), and samples with a diameter of 10 mm or more [2]. Therefore, these large samples should be converted to a homogeneous powder. Moreover, the XRF system
is often unable to detect elements with an atomic number larger than 92. In the present work, the EDXRF technique was used for the detection of element concentration in stones samples [3].

Urinary stone disease, also known as urolithiasis or renal calculi [4], causes significant pain and may lead to accidental renal failure. It has influenced the human species since 4,800 B.C [5,6]. Stones of urine are solid pellets found in three urinary pathway sites, namely the ureters, bladder, and kidneys. Finlayson reviewed numerous geographic surveys around the world and reported high or low prevalence areas across the country [7]. In addition, a number of scientists conducted a survey of a particular area for a particular period and attempted to understand the peculiarity of stones of urine for the respective areas [8-12]. Furthermore, the aspects of age and sex in the composition of urinary stones was determined by Daudon et al. [13]. Pathogenic agents that are responsible for the synthesis of stones are diet, involving fluid-intake transfer of ions within the renal and intestinal track, and metabolic disorders. Moreover, geographical zone, hot climate, water quality, bacteria, drug-induced factors, stress, and occupation are all external agents that could lead to stones of urine. A considerable study in this field is being done to understand the three phases of the formation of stones. These phases are crystal aggregation, crystal growth, and heterogeneous diversity, as well as the role of modifiers (promoters, inhibitors, and complex) in the phenomenon of stone synthesis [14]. To understand the influence of causative and environmental agents on stone growth, it is essential to know the constituents of it. The composition of stones changes with time within the same patient and also from one patient to another.

The effects of smoking nicotine is the most significant component of more than 4,000 potential toxic materials in tobacco products. It is the major chemical constituent responsible for the addiction to tobacco, and it seems to mediate the hemodynamic impacts of smoking and is embroiled in the pathogenesis of many diseases [15]. However, researchers have also shown the harmful impacts of smoking on oral health. Moreover, a clinical research study noticed that smokers had a higher incidence of severe and moderate periodontitis and a higher incidence and extent of attachment loss and gingival stagnation than non-smokers, proposing poor periodontal health in smokers [16]. With regard to the interface of bone implants, the adverse effects of tobacco smoke reflect a series of indirect and direct impacts on the metabolism of bone [17,18,19].

The aim of present work is to study the concentration of elements in Gallbladder stone using X-Ray Fluorescence Technique according to age, gender and smoking related.

2. The Experiment part

2.1. Sample preparation
All gallstones (30 samples) were collected from a urologist in Karbala after surgery on the patient or after an automatic emptying of the stone. Details of the collected samples from different patients are shown in Figure1. All stones were dried for a week and powdered before being used for the measurement. Multiple stones from the same patient were dried and powdered separately and then analytical measurements were made.

The elemental concentration of samples was studied by XRF. The samples were prepared by grinding the gallstone samples with a mechanical mortar, then pressing 5g of the powder using a piston with a pressure of 3.5 tons to make a disk with a 1cm diameter.
2.2. X-ray fluorescence
The XRF spectrometer used for this study is the Spectro Analytical Instruments, Kleve, Germany, Model 2010. It utilises an X-ray tube working at a voltage of 44.69kV and a current of 0.55mA with a Pd target. Figure 2 shows the typical XRF spectroscopy. Special software was used to analyse the secondary X-ray emitted by the samples in order to identify their elemental content.

Figure 1. Number of gallstone samples for female and male gender.

Figure 2. The experimental setup for the spectroscopy of XRF (Energy dispersive).

The elemental concentration of gallstone samples was studied using XRF in air. Figure 3 illustrates the gallstone samples after grinding and pressing.
3. Results and Discussion

3.1. Non-smoker gallstones
The XRFS results for the gallstone samples are presented in Tables 1, 2 and 3. Ca is the maximum element concentration in these samples and other elements like P, Na, Mg, Fe and Pb appeared in different ratios. Additionally, heavy metal content was determined in the gallstone samples and were found in different concentrations. These included sulphur (S), silicon (Si), aluminium (Al), copper (Cu), gallium (Ga), arsenic (As), strontium (Sr) and titanium (Ti). The results show the correlations between the concentration of elements and the person’s age that are presented in Tables 1, 2 and 3. A lower concentration of Ca, Na and P was found with a decrease in age. The concentrations of Mg and Pb were found in higher concentrations with an increase in age [20].

3.2. Smokers’ gallstones
XRF has been employed to analyse the element content in smokers’ gallstones. Tables 4 to 6 illustrate the elements that were identified in the smoker’s sample using XRF. Elements such as Na, Ca, P, Fe, Mg, Pb, Cd, Cr and Co were identified. The proportion of these elements varies with age, where the highest concentrations of the toxic elements Co, Cd and Pb appear in older smokers when compared with younger smokers, while the concentration of trace elements Ca, Na, P and Fe decrease with age. The comparison between smokers and non-smokers tells us that these trace elements decrease in smokers when compared to non-smokers, while the toxic elements Co, Cd and Pb increase in smokers when compared with non-smokers. In addition, other elements such as As, Sr, molybdenum (Mo) and niobium (Nb) appeared in high concentrations in the smokers’ samples when compared with non-smokers’ samples. This result is due to the cigarettes smoking [18].
Table 1. XRF results for the age group of 20 to 40 years.

| Sample Name | Description | Element | Norm.Int | Concentration | Abs.Error |
|-------------|-------------|---------|----------|---------------|-----------|
| NaN2O       | Sodium      | 11      | 306.8806 | 7.897 %       | 0.049 %   |
| MgO         | Magnesium   | 12      | 185.8240 | 1.345 %       | 0.017 %   |
| Al2O3       | Aluminum    | 13      | 0.0000   | <0.0036 %     | 0.0 %     |
| SiO2        | Silicon     | 14      | 917.8023 | 1.135 %       | 0.008 %   |
| P2O5        | Phosphorus  | 15      | 43157.1734 | 25.62 %   | 0.02 %    |
| SO3         | Sulfur      | 16      | 6280.0728 | 2.675 %       | 0.003 %   |
| ClO          | Chlorine    | 17      | 2333.1385 | 0.8966 %     | 0.0003 %  |
| KO2         | Potassium   | 18      | 0.0000   | <0.0039 %     | 0.0 %     |
| CaO         | Calcium     | 20      | 12785.4417 | 43.73 %    | 0.04 %    |
| TiO2        | Titanium    | 22      | 1.3234   | 0.0074 %      | 0.0017 %  |
| V2O5        | Vanadium    | 23      | 0.0000   | 0.0056 %      | 0.0056 %  |
| Cr2O3       | Chromium    | 24      | 3.3355   | <0.0015 %     | 0.0 %     |
| MnO         | Manganese   | 25      | 1.5339   | 0.00126 %     | 0.00027 % |
| Fe2O3       | Iron        | 26      | 209.6043 | 0.198 %       | 0.00055 % |
| CuO         | Cobalt      | 27      | 5.0276   | 0.00715 %     | 0.00061 % |
| NiO         | Nickel      | 28      | 6.1256   | 0.00171 %     | 0.00008 % |
| CuO         | Copper      | 29      | 6.0804   | 0.00162 %     | 0.00009 % |
| ZnO         | Zinc        | 30      | 721.3867 | 0.0722 %      | 0.00026 % |
| Ga          | Gallium     | 31      | 4.4283   | 0.0035 %      | 0.00055 % |
| Ge          | Germanium   | 32      | 0.0000   | <0.0001 %     | 0.0 %     |
| As2O3       | Arsenic     | 33      | 0.0000   | <0.0005 %     | 0.0 %     |
| Se          | Selenium    | 34      | 0.0000   | <0.0001 %     | 0.0 %     |
| Bi          | Bromine     | 35      | 5.8198   | 0.00022 %     | 0.00002 % |
| Rb2O        | Rubidium    | 37      | 5.9752   | 0.00115 %     | 0.00002 % |
| SrO         | Strontium   | 38      | 1311.4654 | 0.03113 %  | 0.00007 % |
| Y           | Yttrium     | 39      | 0.0000   | <0.00005 %    | 0.0 %     |
| ZrO2        | Zirconium   | 40      | 0.0000   | <0.00014 %    | 0.0 %     |
| Nb2O5       | Niobium     | 41      | 0.6584   | 0.00022 %     | 0.00006 % |
| MoO2        | Molybdenum  | 42      | 3.7817   | 0.000363 %    | 0.00006 % |
| Ag          | Silver      | 47      | 0.7397   | 0.00026 %     | 0.00010 % |
| C3          | Cadmium     | 48      | 1.1757   | <0.00020 %    | 0.0 %     |
| SnO2        | Tin         | 50      | 4.2569   | 0.00033 %     | 0.00005 % |
| ZrO2        | Zirconium   | 51      | 3.0910   | 0.00037 %     | 0.00007 % |
| Te          | Tellurium   | 52      | 0.0000   | <0.00030 %    | 0.0 %     |
| I           | Iodine      | 53      | 0.0000   | <0.00033 %    | 0.0 %     |
| Cs          | Cesium      | 55      | 0.0000   | <0.00040 %    | 0.0 %     |
| Ba          | Barium      | 56      | 0.0000   | <0.00020 %    | 0.0 %     |
| La          | Lanthanum   | 57      | 0.0000   | <0.00022 %    | 0.0 %     |
| Ce          | Cerium      | 58      | 0.0000   | <0.00020 %    | 0.0 %     |
| Hf          | Hafnium     | 72      | 0.0105   | <0.00010 %    | 0.0 %     |
| Ta2O5       | Tantalum    | 73      | 24.9536  | 0.00833 %     | 0.00019 % |
| WO3         | Tungsten    | 74      | 56.5285  | 0.0156 %      | 0.00023 % |
| Hg          | Mercury     | 80      | 0.0000   | <0.00010 %    | 0.0 %     |
| Th          | Thorium     | 81      | 1.4887   | 0.00015 %     | 0.00002 % |
| PbO         | Lead        | 82      | 0.3803   | 0.0058 %      | 0.00005 % |
| Bi          | Bismuth     | 83      | 0.0000   | <0.00010 %    | 0.0 %     |
| Th          | Thorium     | 90      | 4.3762   | 0.00026 %     | 0.00003 % |
| U           | Uranium     | 92      | 5.3988   | <0.001 %      | 0.0 %     |

Sum of concentration 81.95 %
Table 2. XRF results for the age group of 40 to 60 years.

| Symbol | Element   | Norm.Int | Concentration | Abs.Error |
|--------|-----------|----------|--------------|-----------|
| Na2O   | Sodium    | 299,6145 | 7.231%       | 0.047%    |
| MgO    | Magnesium | 263,6008 | 1.847%       | 0.017%    |
| Al2O3  | Aluminum  | 0.0000   | 0.0033%      | 0.000%    |
| SiO2   | Silicon   | 998,0100 | 1.133%       | 0.007%    |
| P2O5   | Phosphorus| 374,337,542 | 20.231%   | 0.20%     |
| S03    | Sulfur    | 6481.9742 | 2.661%     | 0.05%     |
| Cl     | Chlorine  | 2403.1140 | 0.1747%    | 0.002%    |
| K2O    | Potassium | 0.0000   | 0.0013%      | 0.001%    |
| CaO    | Calcium   | 11567.1899 | 30.458%    | 0.41%     |
| TiO2   | Titaniuim | 1.5557   | 0.0083%      | 0.003%    |
| V2O5   | Vanadium  | 0.0000   | 0.0059%      | 0.007%    |
| Cr2O3  | Chromium  | 4.3819   | 0.00221%     | 0.000%    |
| MnO    | Manganese | 5.1471   | 0.0032%      | 0.000%    |
| Fe2O3  | Iron      | 257,4709 | 6.149%       | 0.001%    |
| Co     | Cobalt    | 9.0659   | 0.0128%      | 0.001%    |
| Ni     | Nickel    | 9.4249   | 0.0016%      | 0.001%    |
| CuO    | Copper    | 11.0832  | 0.00086%     | 0.002%    |
| ZnO    | Zinc      | 1018.9100 | 0.292%     | 0.002%    |
| Ga     | Gallium   | 0.0199   | 0.0035%      | 0.002%    |
| Ge     | Germanium | 0.0000   | 0.0035%      | 0.001%    |
| As2O3  | Arsenic   | 0.0000   | 0.0000%      | 0.001%    |
| Se     | Selenium  | 0.0000   | 0.003%       | 0.000%    |
| Br     | Bromine   | 9.2588   | 0.0018%      | 0.001%    |
| Rb2O   | Rubidium  | 9.4507   | 0.0002%      | 0.002%    |
| SrO    | Strontium | 2665.2536 | 0.0633%    | 0.001%    |
| Y      | Yttrium   | 0.0000   | 0.0031%      | 0.001%    |
| ZrO2   | Zirconium | 0.0000   | 0.0016%      | 0.001%    |
| Nb2O5  | Niobium   | 0.8772   | 0.00327%     | 0.002%    |
| Mo     | Molybdenum| 5.1685   | 0.00077%     | 0.000%    |
| Ag     | Silver    | 0.9559   | 0.00025%     | 0.000%    |
| Cd     | Cadmium   | 4.1150   | 0.0031%      | 0.001%    |
| SnO2   | Tin       | 6.6820   | 0.0029%      | 0.002%    |
| Sb2O5  | Antimony  | 4.9122   | 0.0039%      | 0.001%    |
| Te     | Tellurium | 0.0000   | 0.0028%      | 0.001%    |
| I      | Iodine    | 0.0000   | 0.0034%      | 0.001%    |
| Cs     | Cesium    | 0.0000   | 0.0044%      | 0.001%    |
| Ba     | Barium    | 0.0000   | 0.0019%      | 0.001%    |
| La     | Lanthanum | 0.0000   | 0.0021%      | 0.001%    |
| Ce     | Cerium    | 0.0000   | 0.0022%      | 0.001%    |
| Hf     | Hafnium   | 0.2048   | 0.0013%      | 0.001%    |
| Ta2O5  | Tantalum  | 38.7803  | 0.0123%      | 0.001%    |
| WO3    | Tungsten  | 86.2844  | 0.0144%      | 0.002%    |
| Hg     | Mercury   | 0.0000   | 0.0003%      | 0.000%    |
| TI     | Thallium  | 2.2846   | 0.0013%      | 0.002%    |
| PbO    | Lead      | 14.3864  | 0.0138%      | 0.001%    |
| Bi     | Bismuth   | 0.0003   | 0.0024%      | 0.002%    |
| Th     | Thorium   | 6.3623   | 0.0031%      | 0.002%    |
| U      | Uranium   | 15.1166  | 0.0033%      | 0.001%    |

Sum of concentration: 73.91%
Table 3. XRF results for the age group above 60 years.

| Sample Name | Symbol | Element | Norm.Int | Concentration | Abs.Error |
|-------------|--------|---------|----------|---------------|-----------|
| 11 Na20      | Na     | Sodium  | 285.3537 | 6.345%        | 0.049%    |
| 12 MgO       | Mg     | Magnesium| 352.0723 | 2.550%        | 0.017%    |
| 13 Al2O3     | Al     | Aluminum| 0.0000   | 0.0031%       | 0.002%    |
| 14 SiO2      | Si     | Silicon | 1.427.5638| 1.121%        | 0.007%    |
| 15 P2O5      | P      | Phosphorus| 3530.1533| 16.984%       | 0.21%     |
| 16 SO3       | S      | Sulfur  | 8789.5217| 2.562%        | 0.004%    |
| 17 Cl        | Cl     | Chlorine| 2473.4671| 1.704%        | 0.002%    |
| 19 KO        | K      | Potassium| 0.0000   | 0.0011%       | 0.001%    |
| 20 CaO       | Ca     | Calcium | 9850.0100| 30.624%       | 0.042%    |
| 22 TiO2      | Ti     | Titanium| 2.1428   | 0.0052%       | 0.003%    |
| 23 V2O5      | V      | Vanadium| 0.0000   | 0.0062%       | 0.006%    |
| 24 Cr2O3     | Cr     | Chromium| 3.2194   | 0.0024%       | 0.001%    |
| 25 MnO       | Mn     | Manganese| 14.6086  | 0.002%        | 0.002%    |
| 28 Fe2O3     | Fe     | Iron    | 250.9407 | 0.119%        | 0.001%    |
| 27 CuO       | Cu     | Cobalt  | 16.3159  | 0.014%        | 0.001%    |
| 29 NiO       | Ni     | Nickel  | 57.7356  | 0.0014%       | 0.002%    |
| 30 CuO       | Cu     | Copper  | 5.5559   | 0.00067%      | 0.001%    |
| 31 ZnO       | Zn     | Zinc    | 861.8102 | 0.0103%       | 0.001%    |
| 32 Ga        | Ga     | Gallium | 75.40    | 0.0037%       | 0.00%     |
| 33 Ge        | Ge     | Germanium| 0.0000   | 0.0018%       | 0.001%    |
| 33 As2O3     | As     | Arsenic | 0.0000   | 0.0011%       | 0.002%    |
| 34 Se        | Se     | Selenium| 0.0000   | 0.0007%       | 0.001%    |
| 35 Br        | Br     | Bromine | 79.9261  | 0.0014%       | 0.001%    |
| 37 Rb2O5     | Rb     | Rubidium| 85.0230  | 0.0022%       | 0.002%    |
| 38 SrO       | Sr     | Strontium| 32.8957  | 0.0782%       | 0.001%    |
| 39 Y         | Y      | Yttrium | 0.0000   | 0.002%        | 0.001%    |
| 40 ZrO2      | Zr     | Zirconium| 0.0000   | 0.0019%       | 0.000%    |
| 41 Nb2O5     | Nb     | Niobium | 75.19    | 0.0031%       | 0.002%    |
| 42 Mo        | Mo     | Molybdenum| 44.287   | 0.0007%       | 0.001%    |
| 47 Ag        | Ag     | Silver  | 108.78    | 0.00022%      | 0.000%    |
| 48 Cd        | Cd     | Cadmium | 112.41    | 0.0037%       | 0.000%    |
| 50 SnO2      | Sn     | Tin     | 57.9355  | 0.0027%       | 0.001%    |
| 51 Sb2O5     | Sb     | Antimony| 83.69    | 0.0041%       | 0.002%    |
| 52 Te        | Te     | Tellurium| 73.39    | 0.0025%       | 0.001%    |
| 53 I         | I      | Iodine  | 126.9011 | 0.0039%       | 0.001%    |
| 55 Cs        | Cs     | Cesium  | 132.9055 | 0.0048%       | 0.001%    |
| 56 Ba        | Ba     | Barium  | 137.327    | 0.0015%      | 0.001%    |
| 57 La        | La     | Lanthanum| 138.9055 | 0.0022%      | 0.000%    |
| 58 Ce        | Ce     | Cerium  | 140.117    | 0.0024%      | 0.001%    |
| 71 Hf        | Hf     | Hafnium | 178.49    | 0.0015%      | 0.001%    |
| 73 Ta2O5     | Ta     | Tantalum| 180.947   | 0.0058%      | 0.000%    |
| 74 WO3       | W      | Tungsten| 183.841   | 0.0132%      | 0.000%    |
| 80 Hg        | Hg     | Mercury | 200.59    | 0.0002%      | 0.001%    |
| 81 Tl        | Tl     | Thallium| 204.38    | 0.0011%      | 0.001%    |
| 82 PbO       | Pb     | Lead    | 207.21    | 0.0183%      | 0.001%    |
| 83 Bi        | Bi     | Bismuth | 197.286   | 0.0031%      | 0.002%    |
| 90 Th        | Th     | Thorium | 232.037    | 0.0036%      | 0.001%    |
| 92 U         | U      | Uranium | 238.0288  | 0.0004%      | 0.00%     |

Sum of concentration: 63.75%
Table 4. XRF results for smokers’ the age group of 20 to 40 years.

| Sample Name | Element | Norm.Inv | Concentration | Abs.Error |
|-------------|---------|----------|---------------|-----------|
| Na2O        | Sodium  | 27.5.9881| 5.3 %         | 0.064336  |
| MgO         | Magnesium| 259.5463 | 1.4 %         | 0.022030  |
| Al2O3       | Aluminum| 0.8351  | 0.0331 %      | 0.008685  |
| SiO2        | Silicon | 816.561  | 1.098 %       | 0.025455  |
| P2O5        | Phosphorus| 3387.914 | 1.3 %         | 0.035192  |
| SiO3        | Sulfur  | 5303.433 | 2.233 %       | 0.007566  |
| Cl          | Chlorine| 1966.104 | 1.593 %       | 0.015574  |
| K2O         | Potassium| 0       | 0.00099 %     | 0.013786  |
| CaO         | Cadmium | 10297.35 | 1.2 %         | 0.058947  |
| TiO2        | Titanium| 1.002003 | 0.049 %       | 0.006379  |
| V2O5        | Vanadium| 0       | 0.0058 %      | 0.010069  |
| Cr2O3       | Chromium| 2.01694 | 0.0093 %      | 0.019741  |
| MnO         | Manganese| 3.308841| 0.002718 %    | 0.006959  |
| Fe2O3       | Iron    | 206.8743 | 0.096 %       | 0.007513  |
| CoO         | Cobalt  | 5.828732 | 0.019687 %    | 0.016419  |
| NiO         | Nickel  | 6.056886 | 0.00169 %     | 0.005438  |
| CuO         | Copper  | 7.112059 | 0.0099 %      | 0.004940  |
| ZnO         | Zinc    | 655.0136 | 0.0711 %      | 0.006931  |
| Ga          | Gallium | 3.869851 | 0.00038 %     | 0.004452  |
| Ge          | Germanium| 0     | 0.0003 %      | 0.013218  |
| As2O3       | Arsenic | 0       | 0.0094 %      | 0.020408  |
| Se          | Selenium| 0       | 0.0005 %      | 0.011091  |
| Br          | Bromine | 5.952068 | 0.00225 %     | 0.017556  |
| Rb2O        | Rubidium| 6.094704 | 0.00153 %     | 0.015259  |
| SrO         | Strontium| 7.13377 | 0.0706 %      | 0.020222  |
| Y           | Yttrium | 0       | 0.0006 %      | 0.027239  |
| ZrO2        | Zirconium| 0     | 0.0017 %      | 0.002465  |
| Nb2O5       | Niobium | 0.563907 | 0.0033 %      | 0.014413  |
| Mo          | Molybdenum| 3.321517| 0.0075 %      | 0.008583  |
| Ag          | Silver  | 0.61452  | 0.0021 %      | 0.012272  |
| Sn           | Lead    | 2.643256 | 0.0014 %      | 0.016293  |
| SnO2        | Tin     | 4.295599 | 0.0029 %      | 0.011854  |
| Sb2O5       | Antimony| 3.157832 | 0.0037 %      | 0.011854  |
| Te          | Tellurium| 0   | 0.0028 %      | 0.012858  |
| I           | Iodine  | 0       | 0.0027 %      | 0.015442  |
| Cs           | Cesium  | 0       | 0.0027 %      | 0.002111  |
| Ba           | Barium  | 0       | 0.0002 %      | 0.008778  |
| La           | Lanthanum| 0    | 0.0022 %      | 0.009379  |
| Ce           | Cerium  | 0       | 0.0018 %      | 0.010365  |
| Hf           | Hafnium | 0.18954 | 0.0018 %      | 0.019953  |
| Ta2O5        | Tantalum| 23.64451 | 0.0789 %      | 0.015823  |
| WO3         | Tungsten| 55.45668 | 0.0143 %      | 0.015046  |
| Hg           | Mercury | 0       | 0.0018 %      | 0.008784  |
| Tl           | Thallium| 1.4687  | 0.00014 %     | 0.008636  |
| Bi           | Bismuth | 3.247742 | 0.0024 %     | 0.009068  |
| Th           | Thorium | 4.090064 | 0.00026 %    | 0.001741  |
| U            | Uranium | 9.71784  | 0.00018 %     | 0.009135  |

Sum of concentration: 63.46 %
Table 5. XRF results for smokers’ the age group of 40 to 60 years.

| Sample Name Description | Element | Norm.Int | Concentration | Abs.Error |
|-------------------------|---------|----------|---------------|-----------|
| Na2O                    | Sodium  | 254.2183 | 0.8%          | 0.066409  |
| MgO                     | Magnesium| 321.8160 | 2.3%          | 0.030171  |
| Al2O3                   | Aluminum| 007.29   | 1.02%         | 0.003170  |
| SiO2                    | Silicon | 32346.84 | 52.0%         | 0.034191  |
| SO3                     | Sulfur  | 58927.04 | 2.15%         | 0.007357  |
| Cl                       | Chlorine| 2184.649 | 0.1577%       | 0.017564  |
| K2O                     | Potassium| 000057   | 0.0057%       | 0.021312  |
| CaO                     | Calcium | 0929.05  | 0.053%        | 0.000806  |
| TiO2                    | Titanium| 111337   | 0.044%        | 0.019425  |
| V2O5                    | Vanadium| 0061     | 0.061%        | 0.007303  |
| Cr2O3                   | Chromium| 3129.33  | 0.028%        | 0.016804  |
| MnO                     | Manganese| 367649   | 0.0362%       | 0.015679  |
| Fe2O3                   | Iron    | 164.2198 | 0.10%         | 0.019297  |
| SnO2                    | Tin     | 6.476569 | 0.45%         | 0.005713  |
| CuO                     | Copper  | 7902286 | 0.0077%       | 0.013739  |
| ZnO                     | Zinc    | 7277929 | 0.0218%       | 0.014231  |
| Ga                      | Gallium | 4299834 | 0.0045%       | 0.002927  |
| Ge                      | Germanium| 0006     | 0.006%        | 0.010715  |
| As2O3                   | Arsenic | 00133   | 0.0133%       | 0.019474  |
| Se                      | Selenium| 00007   | 0.0007%       | 0.015019  |
| Br                      | Bromine | 6163409 | 0.00223%      | 0.019686  |
| Rb2O5                   | Rubidium| 6771893 | 0.00171%      | 0.003279  |
| SrO                     | Strontium| 1903753 | 0.0082%       | 0.013426  |
| Y                       | Yttrium | 00004   | 0.004%        | 0.009863  |
| ZrO2                    | Zirconium| 00018   | 0.0018%       | 0.013368  |
| Nb2O5                   | Niobium | 0626504 | 0.0075%       | 0.009023  |
| Mo                      | Molybdenum| 369075 | 0.0099%       | 0.008783  |
| Ag                      | Silver  | 06628   | 0.0019%       | 0.011372  |
| SnO2                    | Tin     | 293925  | 0.0012%       | 0.014129  |
| Sb2O5                   | Antimony| 3508703 | 0.0042%       | 0.005527  |
| Te                      | Tellurium| 00027   | 0.0027%       | 0.001491  |
| I                       | Iodine  | 00031   | 0.0031%       | 0.014513  |
| Cs                      | Cesium  | 00033   | 0.0033%       | 0.015951  |
| Ba                      | Barium  | 00018   | 0.0018%       | 0.000031  |
| La                      | Lanthanum| 00026   | 0.0026%       | 0.001367  |
| Ce                      | Cerium  | 00021   | 0.0021%       | 0.003464  |
| Hf                      | Hafnium | 02106   | 0.002%        | 0.001749  |
| Ta2O5                   | Tantalum| 2627168 | 0.0877%       | 0.001575  |
| WO3                     | Tungsten| 6101742 | 0.0132%       | 0.001390  |
| Ho                      | Holmium | 0002    | 0.002%        | 0.005179  |
| Tl                       | Thallium| 1631890 | 0.0012%       | 0.001912  |
| Pb                       | Lead    | 1027527 | 0.0093%       | 0.009617  |
| Bi                       | Bismuth | 0002    | 0.002%        | 0.001393  |
| Th                      | Thorium | 4544515 | 0.0033%       | 0.001626  |
| U                       | Uranium | 107976  | 0.002%        | 0.002152  |

Sum of concentration: 58.77%
Table 6. XRF results for smokers’ the age group above 60 years.

| Sample Name | Element | Date of Receipt Method | Concentration | Abs. Error |
|-------------|---------|------------------------|---------------|------------|
| Na2O        | Sodium  | 582.7639               | 5             | 0.026333   |
| MgO         | Magnesium| 29012.8               | 5             | 0.020467   |
| Al2O3       | Aluminum| 5940.831              | 5             | 0.014312   |
| SiO2        | Silicon  | 7.64133               | 5             | 0.005375   |
| P2O5        | Phosphorus| 11.422               | 5             | 0.003691   |
| SO3         | Sulfur   | 11.422               | 5             | 0.003691   |
| ClO          | Chlorine | 2.64237               | 5             | 0.007334   |
| K2O         | Potassium| 7.64133               | 5             | 0.005375   |
| MnO         | Manganese| 12.7825              | 5             | 0.017497   |
| Fe2O3        | Iron     | 8.86179               | 5             | 0.00559    |
| CoO         | Cobalt   | 7.64133               | 5             | 0.005375   |
| NiO         | Nickel   | 7.64133               | 5             | 0.005375   |
| CuO         | Copper   | 7.64133               | 5             | 0.005375   |
| ZnO         | Zinc     | 7.64133               | 5             | 0.005375   |
| Ga          | Gallium  | 3.319725              | 5             | 0.006819   |
| Ge          | Germanium| 0.0009                | 5             | 0.005049   |
| As2O3       | Arsenic  | 0.00156               | 5             | 0.002077   |
| Se          | Selenium | 0.0009                | 5             | 0.003884   |
| Br          | Bromine  | 6.94408               | 5             | 0.010873   |
| Rb2O        | Rubidium | 7.10488               | 5             | 0.001763   |
| SrO         | Strontium| 2.883427              | 5             | 0.016282   |
| Y           | Yttrium  | 0.0003                | 5             | 0.019381   |
| ZrO2        | Zirconium| 0.00019               | 5             | 0.019381   |
| Nb2O5       | Niobium  | 0.657892              | 5             | 0.002046   |
| Mo          | Molybdenum| 3.875103              | 5             | 0.003884   |
| Ag          | Silver   | 0.71694               | 5             | 0.020682   |
| Cd          | Cadmium  | 3.703465              | 5             | 0.004746   |
| SnO2        | Tin      | 4.334298              | 5             | 0.006899   |
| Sb2O5       | Antimony| 2.89468               | 5             | 0.01549    |
| Te          | Tellurium| 0.00021               | 5             | 0.004762   |
| I           | Iodine   | 0.0087                | 5             | 0.015931   |
| Cs          | Cesium   | 0.0085                | 5             | 0.008466   |
| Ba          | Barium   | 0.00014               | 5             | 0.01256    |
| La          | Lanthanum| 0.00029               | 5             | 0.005012   |
| Ce          | Cerium   | 0.0031                | 5             | 0.01071    |
| Hf          | Hafnium  | 0.22113               | 5             | 0.013529   |
| Tl           | Thallium| 2.759238              | 5             | 0.013137   |
| WO3         | Tungsten | 59.49268              | 5             | 0.01063    |
| Hg          | Mercury  | 0.00025               | 5             | 0.003072   |
| Th          | Thallium | 1.751343              | 5             | 0.018568   |
| PbO         | Lead     | 17.59082              | 5             | 0.01357    |
| Bi          | Bismuth  | 0.00023               | 5             | 0.014358   |
| Th          | Thorium  | 4.771741              | 5             | 0.008724   |
| U           | Uranium  | 11.33748              | 5             | 0.009648   |

Sum of concentration: 49.25 %
3.3. Relative statistics of element concentration

This section provides a comparison of trace element (Ca, P, and Na) with toxic element concentration in the total gallstones for each group sample classified according to age. Figure 4 shows the relative statistics of element concentration in the non-smoker group. This Figure illustrates that the maximum concentration of Ca, P and Na were found in the 20- to 40- year age group. In addition, this Figure shows that the presence of Pb concentration increases with age.

![Figure 4](image)

**Figure 4.** Concentration of elements in the non-smoker group divided according to age.

Figure 5 shows the concentration of trace elements and toxic elements Co, Cd and Pb in the smoker group at different ages. The maximum concentration of toxic elements Co and Cd were found in the age group above 60 years. The presence of toxic elements in smokers due to habits such as smoking or drinking alcohol is more when compared to the normal presence [21,22]. In addition, the presence of trace...
elements decreases with age, and it was also observed that the trace element concentration was almost higher in non-smokers when compared with smokers. This is due to smoking [18].

Figure 5. Concentration of elements in the smoker group at different ages.

4. Conclusion
Distinguishing between smokers and non-smokers was probable made by using the alteration of the ratios of concentration of the matrix ingredient elements P and Ca, and non-matrix elements Na and Fe. The concentration of matrix elements and non-matrix elements were higher in non-smokers’ gallstones while Mg and Pb were higher in smoker samples. The concentration of several atomic elements in these samples was affected by age. That is, the presence of atomic elements including P, Ca, and Na decrease with age. A positive correlation between the content of Pb and Mg in samples and age was noticed. Thus, significantly, the concentration of the most toxic elements was found to be within the smoker group. The maximum concentration of toxic elements such as Cd, Pb, and Co and the minimum concentration of trace elements Ca, P and Na were found in the older age group (above 60 years).
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