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Introductory Chapter: Tracelement Effects in Biological Applications Using Photon Induced (EDXRF), Proton Induced (PIXE) and Synchrotron Induced (EXAFS) X-Ray Spectrometry

Daisy Joseph

1. Introduction

Heavy metals are dangerous to human health. Heavy metals are Sb, As, Bi, Cd, Ce, Cr, Co, Cu, Ga. Trace elements occur in natural environments in small amounts and when present in sufficient concentrations, are toxic to living organisms [1]. Trace elements enter the ecosystems via direct discharges from industrialization processes, sewage sludge, atmospheric deposits and agricultural practices including application of pesticides or fertilizers [2–4]. They can be transferred from sediments to benthic organisms and then become a potential risk to human consumers through the food chain [5]. We have a X-ray emission spectrometer. Energy Dispersive X-ray Fluorescence and Proton Induced X-ray Emission, in which trace elements (micronutrients as well as toxic elements) were analysed.

2. Application of EDXRF and PIXE in biosciences

The large number of applications of XRF in our laboratory has been carried out in Biosciences. They can be summarized as follows.

2.1 Detection of trace elements in Indian spices

Concentrations of K, Ca, Mn, Fe, Cu, Zn, Rb and Sr were determined in Indian Spices namely pepper, clove, cardomon, cinnamon, and cumin using Cd^{109} radioisotope source induced XRF. The levels of K and Ca were highest in clove and cinnamon. Rubidium and Strontium were found in all spices except cinnamon. Chromium and titanium were found only in pepper [6].

2.2 Determination of mercury and arsenic in Indian ayurvedic medicines using EDXRF

Elemental concentration in some herbal medical products, produced by different *ayurvedic* pharmacies in India, was determined using Energy Dispersive X-Ray

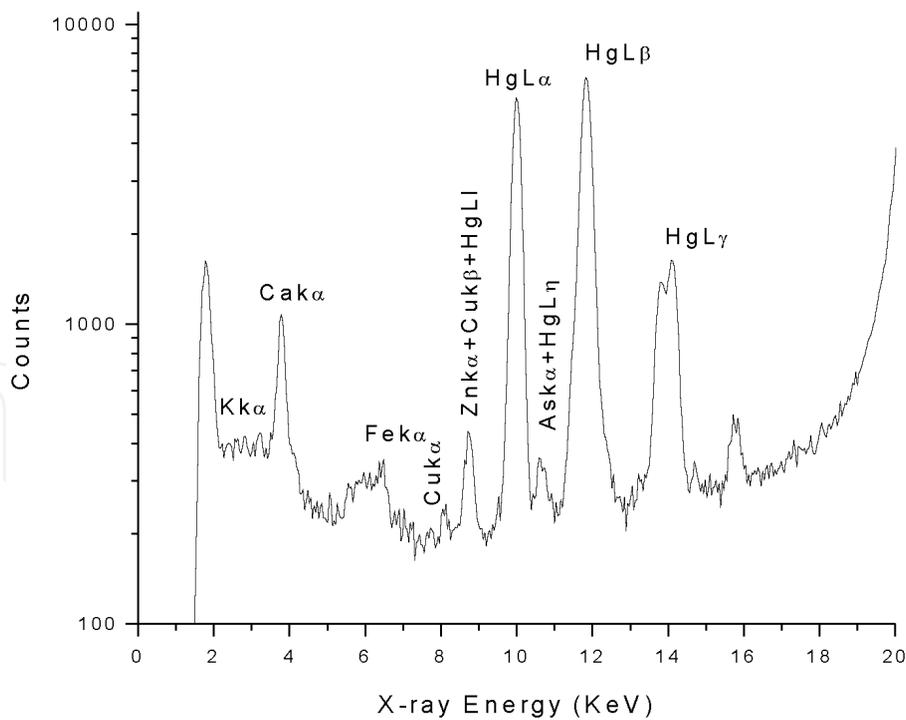


Figure 1.
A typical EDXRF spectrum of an Indian ayurvedic medicine Balguti Kesaria.

Fluorescence Spectrometry (EDXRF). It is mandatory to look into the effects of these heavy metals being administered in the body functions before taking them over a long period of time (**Figure 1**) [7].

2.3 Drought tolerant and susceptible genotypes of sorghum plants

Drought tolerant and susceptible genotypes of sorghum plants were analyzed by EDXRF technique to study the correlation of trace elements with drought tolerance capacities for sorghum plants. Samples prepared from mature seeds, young seedlings and old plants were analyzed using ^{109}Cd radioisotope source. The elements such as K, Fe, Cu, Zn, Rb and Sr and Y were seen to be present in different quantities in various samples. K and Fe concentrations were found to be more in the tolerant genotype as compared to the susceptible type. Concentration of Fe decreased with maturity in the tolerant group while it increased with maturity in the susceptible group. The genotype Arfa Gadamak (AG) showed a distinct abnormality in its young seedling with high level of Zn. In conclusion, the drought tolerant and susceptible genotypes of sorghum genotypes (cultivated in Sudan) exhibited varying levels of trace elements. The drought tolerant genotypes of sorghum seeds exhibited high K and Fe concentrations as compared to susceptible genotypes. In seedlings Fe concentration decreased with maturity in the tolerant group while it increased in the susceptible variety [8].

2.4 PIXE studies of blood Pb levels in children of the Dharavi slum areas in Mumbai

PIXE was used to study lead levels in blood samples of children from Dharavi slum areas. Blood lead levels of children admitted to Sion Hospital, Bombay (India), from the adjoining Dharavi slum areas. Blood samples were collected from 36 children with suspected lead poisoning (indicator was acute anemia) and from 20 control children. The analysis showed that the lead concentration of the patients varied from 0.1 to

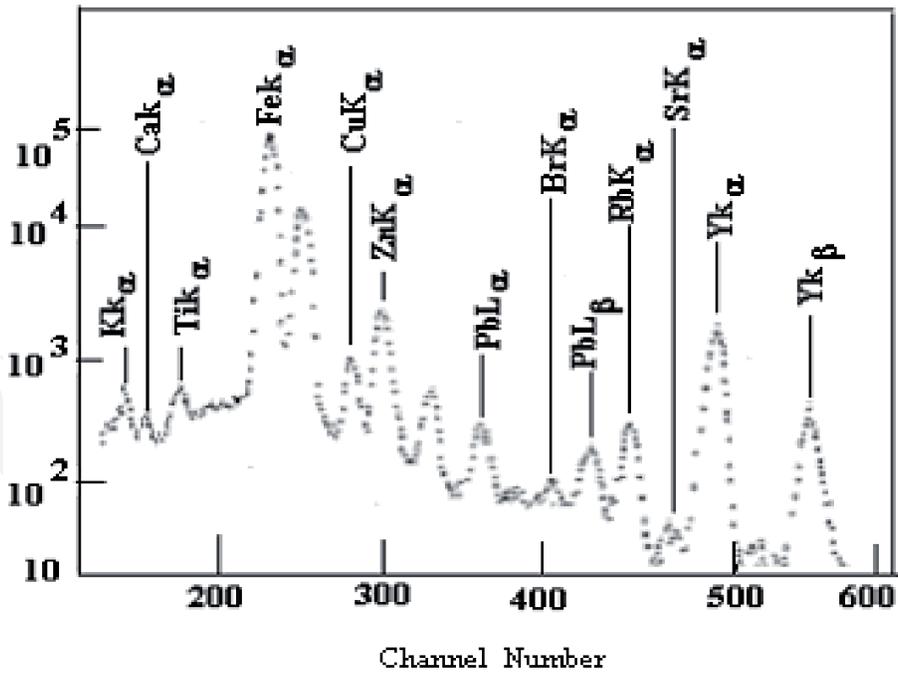
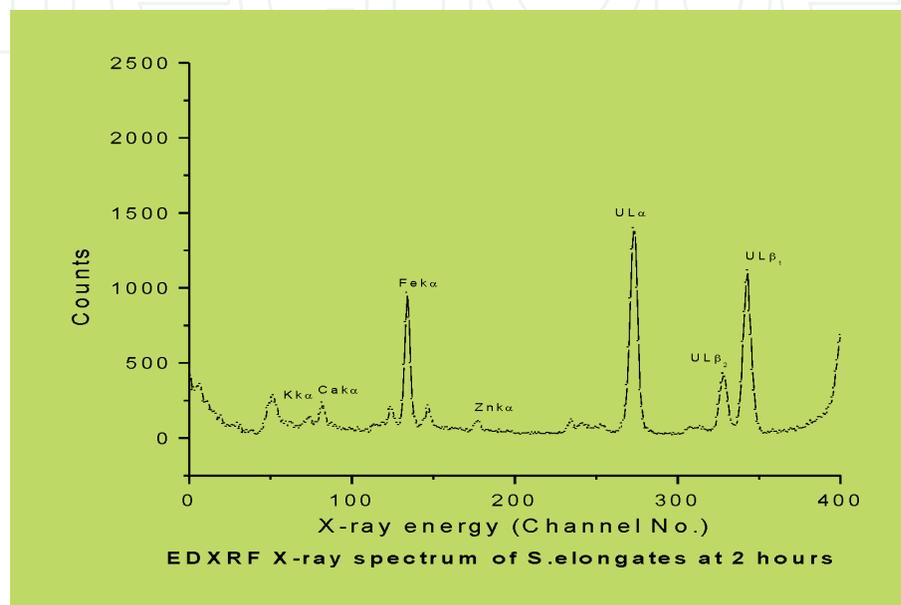


Figure 2.
 PIXE spectrum of blood of a lead poisoned child.

6.0 $\mu\text{g ml}^{-1}$. In addition to lead, K, Ca, Fe, Cu, Zn, Se, Br and Rb were also detected simultaneously, of which the concentrations of Fe, Cu, Zn, Se, Rb and Pb were determined. The high blood lead levels of the children from this area may be ascribed to environmental pollution due to heavy vehicular traffic and industrial sources. Pb was found to high even in normal children due to vehicular exhaust containing lead in petrol. **Figure 2** shows the PIXE spectrum of a Pb poisoned child [9].

2.5 Uranium extraction from cyanobacterial cells

Cyanobacterial cell organisms grown in Uranyl nitrate and Uranyl Carbonate were determined for their Uranium uptake by ^{109}Cd induced EDXRF during different time intervals. It was found while elements such as K, Ca, Cr, Mn, Fe and Zn were present in small quantities was seen to be present in significant amounts after 2 hours of uptake and it became saturated in 5 hours after which the uptake reduced and became minimum.



2.6 Tracelement variation in renal failed patients

In a separate experiment of application of PIXE in bio-medical research, blood samples of Patients with Chronic renal failure were analyzed for trace element abnormalities and the results showed marked differences in patients before and after dialyses. PIXE being more sensitive in the low Z region due to its higher cross section is an ideal technique for bio-science applications. The knowledge that small amounts of metals which are needed in the diet goes back several hundred years to the discovery of a requirement for iron. It is well established that some elements such as I, Cu, Mn, Zn, Se and Mb in trace quantity are needed in physiology. Very recently, the six “newer trace elements”, tin, vanadium, fluorine, silicon, nickel, and arsenic, were discovered to have nutritional requirement. Recommended Dietary Allowances (RDA) and requirements have been set for iron, iodine, and zinc. For copper the issuance of an RDA can be established. Only tentative recommendations for the “newer” trace elements in the form of a range of values can be presently proposed. To establish these recommendations more firmly, knowledge of the content of each of these trace elements in the diet is necessary. A detailed study of trace element abnormalities in serum of patients was carried out by PIXE in the case of non-dialyzed, hemodialysed and post-transplantation. Forty-two patients and eight healthy controls selected for this study were grouped on the basis of their Serum Creatinine (SC) levels. Serum samples of these subjects were excited by protons from the Van de Graff accelerator of 2.5 MeV energy and the Characteristic X-rays were detected by Si (Li) detector (**Table 1**).

In the case of renal failed patients the exact mechanism of trace element disturbance is not known. Reduced renal excretion increased oral intake and Globin Insulin (G.I.) absorption and contamination as well as loss across the hemodialysis membranes have been incriminated. Considerable variation in plasma and tissue concentrations of trace elements have been found in different geolgraphical areas due to variation in water and soil content. Patients on dialysis therapy had elevated serum copper levels. Butamante reported similar results and attributed liberation of copper from the dialysis membrane as a cause for hypercupremia. Successful renal transplantation resorted serum copper to normal levels. A well established observation is that serum brooming levels are not different from normal in non dialyzed chronic renal failure patients, but those on dialysis show subnormal levels. Transference of bromine from blood into the

| Elements | I | II | III | IV | V |
|----------|---------------|---------------|---------------|---------------|---------------|
| Ni | .0167 ± .0164 | 0.034 ± 0.022 | 0.030 ± 0.033 | 0.066 ± 0.051 | 0.042 ± 0.027 |
| Cu | .083 ± 0.16 | 0.83 ± 0.25 | 0.97 ± 0.33 | 1.08 ± 0.37 | 0.71 ± 0.28 |
| Zn | 1.12 ± 0.93 | 1.06 ± 0.25 | 1.30 ± 0.01 | 1.38 ± 0.67 | 1.13 ± 0.47 |
| Se | .035 ± 0.017 | 0.032 ± 0.029 | 0.035 ± 0.011 | 0.033 ± 0.018 | 0.030 ± 0.012 |
| Br | 3.66± .154 | 3.383± 0.241 | 0.599± 0.417 | 0.122± 0.127 | 0.281± 0.255 |
| Rb | .178 ± 0.101 | 0.297 ± 0.177 | 0.246 ± 0.101 | 0.172 ± 0.109 | 0.263 ± 0.114 |
| Sr | .029 ± 0.044 | 0.132 ± 0.088 | 0.204 ± 0.089 | 0.111 ± 0.214 | 0.088 ± 0.082 |
| Pb | .173 ± 0.143 | 0.279 ± 0.137 | 0.347 ± 0.236 | 0.797 ± 0.537 | 0.400 ± 0.260 |

Table 1.

Values in mean ($\mu\text{g/ml}$) \pm S.D. of concentrations of trace elements in various groups.

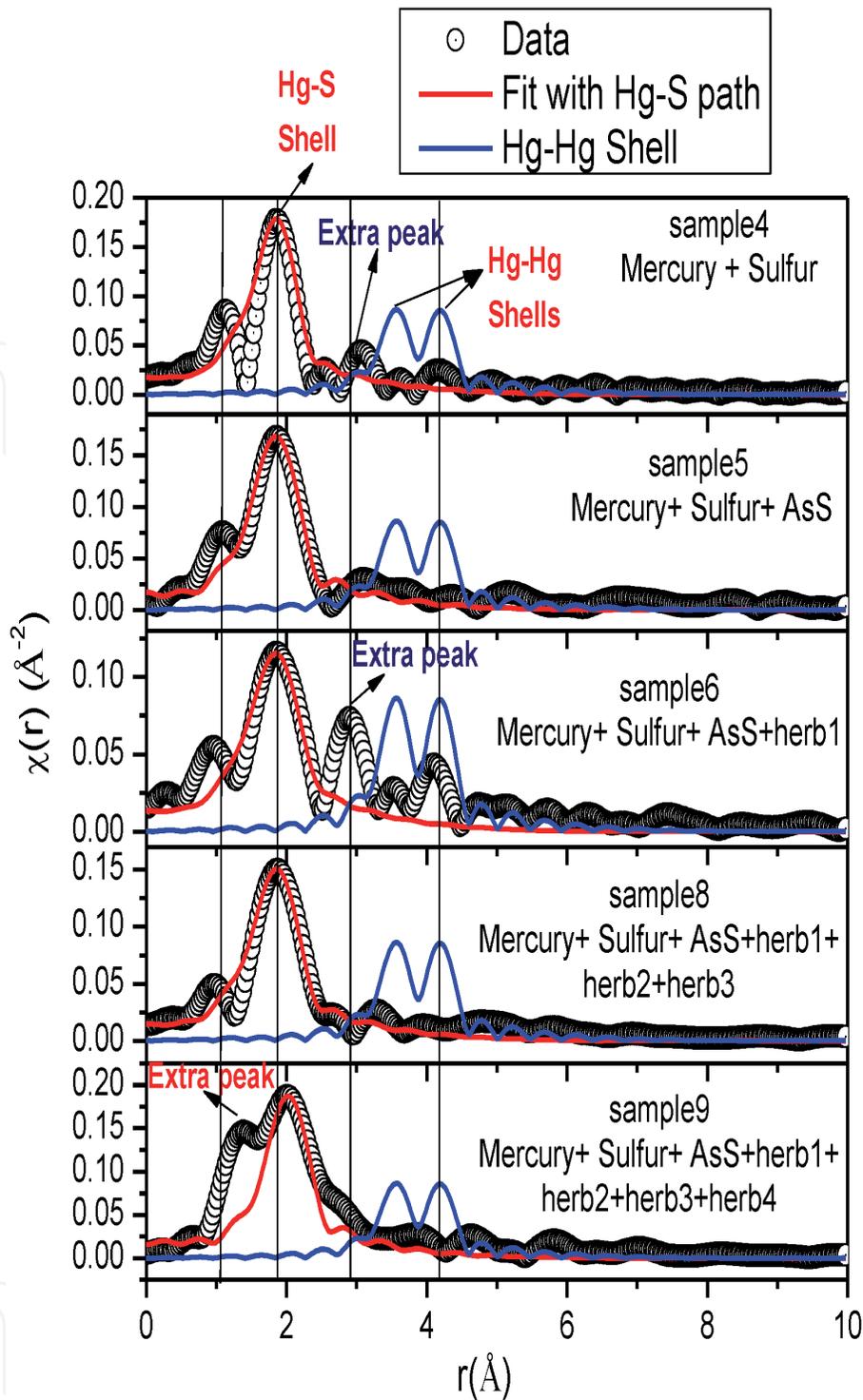
dialysate could be responsible for this deficiency. Transplantation resulted in rise of bromine towards normal. As a result of loss of renal function, Sr which depends on the kidney for elimination is probably retained accounting for the significantly elevated serum concentration of Sr in patients of CRF. Patients on hemodialysis and those who received a successful renal allograft had SR concentration within the normal range. Chronic Renal insufficiency did not result in accumulation of lead in our study similar to that observed by Thomson et al. Contamination of the dialysate or dialysate delivery system possibly resulted in elevation of lead concentration while on dialysis. Following renal transplantation the serum lead levels went down as compared to lead levels while on dialysis, but were still significantly elevated when compared to normal. Mobilization of lead which was sequestered in the tissues while the patient was on dialysis into the serum, to be excreted via the kidneys could explain the high lead levels. It could also be possible that the transplanted kidney has not yet attained a normal function with regard to lead excretion and with passage of time normal serum lead level would be achieved. Low and normal serum zinc levels have been reported in non dialysed patients, but in dialyzed patient elevated subnormal and almost normal concentration have been described. An unrestricted dietary protein intake (45–50 g/day) and normo-proteinemia could explain the lack of hypozincemia studied by Mansouri et al., in patients who were on a protein restricted diet (20–30 g/day) and had significant hypoproteinemia. Our study showed a slight, but non significant elevation of serum zinc in hemodialysed patients, probably as a result of contamination of the dialysate or dialysate delivery system with zinc [10].

2.7 Synchrotron based EXAFS on Mercury based Indian herbomineral drug.

An Indian herbomineral drug was characterized for its trace elements by radioisotope induced EDXRF. The drug contains minerals like mercury, sulfur and arsenic disulfide, along with herbs. By XRF As, Hg, Fe, Ca were detected. S was not detected as S X-rays get absorbed in the detector window. However AsS and HgS was seen in EXAFS spectra. There is no peak in the experimental data corresponding to the 2nd shell of HgS viz., the Hg-Hg shell (corresponding to the blue lines). It may happen that Hg is not present in the form of HgS in the samples, instead Hg forms bonds with S, C or O present in the herbs. The peaks near 3 Å for samples 4 & 6 and near 1.5 Å of sample 9 might be due to bond formation of Hg with C or O atoms of the herbs. Sample 6 shows maximum disorder and the Hg-S bond length shows an increasing trend from sample 4 to sample 9. A more comprehensive report is underway regarding its structure after a complete analysis of EXAFS spectra.

The XRF results for the herbomineral samples are as follows:

| Sample No | Major analyte | Trace |
|-----------|---------------|-----------|
| Sample 4 | Hg | Y |
| Sample 5 | Hg and As | Fe |
| Sample 6 | Hg and As | Fe |
| Sample 7 | Hg and As | Fe |
| Sample 8 | Hg and As | Fe and Ca |
| Sample 9 | Hg and As | Fe and Cu |



3. Conclusion

Though trace elements are required in minimal quantities their presence in the optimal amount is essential for the normal physiological functioning of the body. They are one of the corner stone's in maintenance of biodynamic of the body. Both, excess and the deficiency states lead to initiation, promotion, and progression to various disease processes. The present paper has thoroughly discussed trace elements, as this area is away from the deserved attention. Thus, a comprehensive understanding of these trace elements is essential and significant for disease control and maintaining optimal health and X-ray Emission Techniques such as EDXRF, PIXE and EXAFS have shown to be good diagnostic tools for determining trace elements in Biological samples.

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