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Energy-dispersive X-ray fluorescence – A tool for interdisciplinary research

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Abstract. Trace elements have been at the focus of attention for decades with considerable emphasis on their role in biology and biomedical sciences, environmental sciences, geology, archaeology and material sciences. They comprise a large number of elements, some having essential physiological functions, whereas others are toxic, mutagenic or carcinogenic. A few even have antiproliferative and anticarciniogenic properties. The advent of various instrumental techniques and sophisticated instrumentations has made their detection to very low limits possible, making this a very important multidisciplinary study. Among these techniques the energy-dispersive X-ray fluorescence (EDXRF) technique is being widely used for trace element detection in various fields of science. Keeping the importance of trace elements in mind, the Kolkata centre of UGC-DAE Consortium for Scientific Research initiated several research schemes in different fields of trace element research using various techniques, EDXRF being one of the main techniques. A Xenemetrix (erstwhile Jordan Valley) EX 3600 EDXRF spectrometer is being used to carry out the research. This presentation aims to highlight some of the very recent applications of EDXRF in the study of the role of trace elements in pre-cancerous tissues, medicinal plants and also in some environmental studies.

Keywords. Energy-dispersive X-ray fluorescence; trace elements; oral cancer; biomonitoring; air pollution.

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1. Introduction

As scientific knowledge in a wide range of disciplines has advanced, researchers have become increasingly aware of the need to link disciplinary fields to more critical questions which can be fully answered, or to facilitate application of knowledge in specific areas. Interdisciplinary research (IDR) is a mode of research by groups or individuals that integrates information, data, techniques, tools, perspectives, concepts, and/or theories

from two or more disciplines or bodies of specialized knowledge to advance fundamental understanding or to solve problems whose solutions are beyond the scope of a single discipline or area of research practice. In the 20th century, after the discovery of sophisticated analytical techniques, much importance has been given to the elemental studies. The elemental studies are done in various fields of scientific studies ranging from material science to forensic science, environmental science to medical/health science, geology to archaeology and none-the-less vast areas of life science research. Several sophisticated techniques, namely AAS, PIXE, XRF, ICP-MS, ICP-AES and NAA, are used for the analysis of elements present in minor quantity at the level of parts per million or parts per billion or in other words trace elements. With this motivation the Kolkata Centre of UGC-DAE Consortium for Scientific Research has initiated several research schemes in the fields of trace element sciences, using various techniques, EDXRF being one among them. Some of these works are discussed in this presentation.

2. Experimental set-up

The set-up consists of a Xenemetrix (previously Jordan Valley) EX-3600 EDXRF spectrometer. This consists of an X-ray tube with a Rh anode as the source of X-rays with a 50 V, 1 mA power supply, Si(Li) detector with a resolution of 143 eV at 5.9 keV Mn X-ray and 10-sample turret enables mounting and analysing 10 samples at a time. The quantitative analysis is carried out by the in-built software *nEXT*.

3. Application of EDXRF

3.1 *EDXRF for trace elemental studies in cancer*

The annual incidence of oral cancer in India is higher than in other parts of the world. In India the annual incidence of oral cancer is 16 per 1,00,000 persons [1]. Smoking, drinking and chewing have been positively associated with oral lesions such as submucous fibrosis, leukoplakia and oral lichen planus which have the potential for malignant transformation [2,3]. Leukoplakia (LKP) is a white patch or plaque that cannot be characterized clinically or pathologically as other diseases and which is not associated with any physical or chemical causative agent except the use of tobacco [4]. Leukoplakic lesions chiefly occur in the 5th, 6th and 7th decades of life, whereas, oral submucous fibrosis (OSF) is a chronic, indigenous, progressive, scaring, high risk pre-cancerous condition of the oral cavity and oropharynx. Higher occurrence of oral leukoplakia and cancer are observed in OSF patients and it is believed to be an important risk factor for oral cancers among the youth [5]. Prevalence of leukoplakia in India varies from 0.2 to 5.2% [6]. Among those who consume alcoholic beverages alone, the prevalence of leukoplakia is higher compared to other lesions. Current research activities throughout the world have shown alteration of elemental constituents as one of the marker factors of different pathological diseases, including malignant and pre-malignant lesions. In this perspective, the present work was designed to assess the trace elemental profile in blood by EDXRF, in patients with leukoplakia and submucous fibrosis.

The study subjects were selected from the patients visiting the OPD of Dr R Ahmed Dental College and Hospital, Kolkata, India. Blood samples from patients (RI) and from orally supplemented (for two months) patients (RII) were collected from peripheral veins and freeze dried, then powdered and pellets were prepared for EDXRF study. Differential variation in elemental concentration has been observed in patients (both untreated and treated) and also with respect to control. Reversal trend has been observed between the concentration of certain elements (figure 1a) in patients of OSF and LKP with respect to control in different age groups, which indicates specific metabolic activity involvement of those elements (Ni, Ca, Co, Fe, Cr, V and Se) in the two different types of pre-cancerous lesions. Such variation may be attributed to some connection with the etiology of different types of lesions. Depletion in Fe concentration has been observed in OSF patients with respect to control in the age group of 30 years and less and 30–40 years. However, Fe concentration was found to be more in patients in the age group of 40 years and more. Interestingly, Zn supplementation has resulted in an increase of Fe concentration in all the age groups of OSF patients. In LKP patients, depletion in Fe concentration in relation to control has been observed in all age groups. Also, Zn supplementation has no effect on the concentration of Fe in LKP patients in the age group of 30 years and less and 30–40 years. However, Zn supplementation has resulted in the reduction of Fe concentration in LKP patients in the age group of 40 years and more.

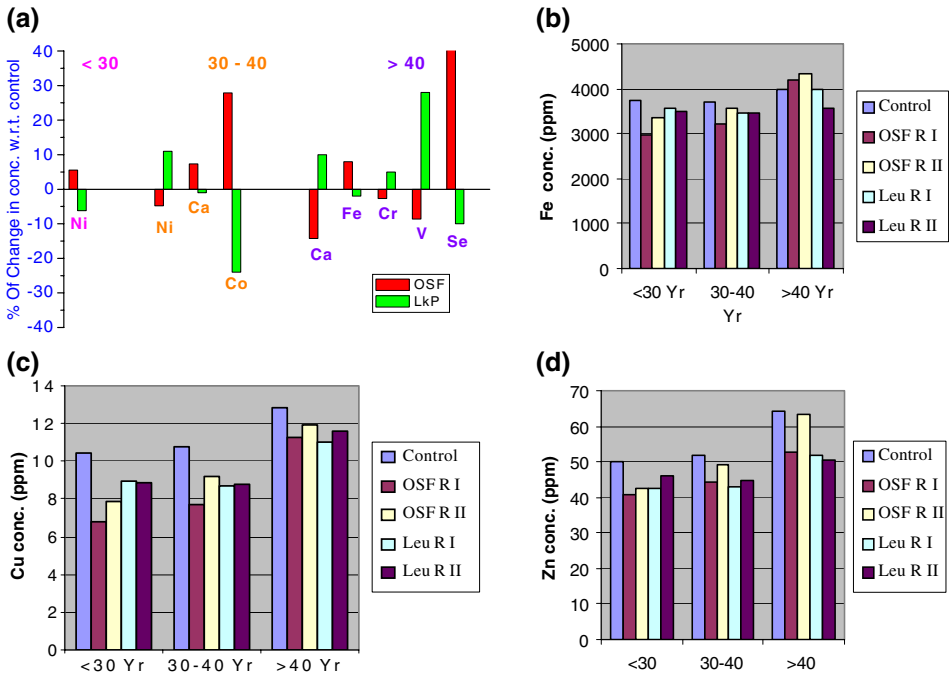

Figure 1. Elemental concentrations: (**a**) reversal trend of element concentration in OSF and LKP, (**b**), (**c**) and (**d**) Concentration of Fe, Cu and Zn, respectively in control, OSF and LKP (both untreated and treated) with oral supplementations.

Similarly, depletion of Cu has been observed in all age groups of both OSF and LKP patients. However, Zn supplementation has resulted in the reversal of Cu depletion in all age groups of both OSF and LKP patients except in LKP patients in the age group of 40 years and more. Zn concentration depleted in all age groups of both OSF and LKP patients, moreover Zn supplementation has resulted in the reversal of Zn depletion in all age groups of both OSF and LKP patients except in LKP patients in the age group of 40 years and more.

3.2 *EDXRF in environmental studies*

3.2.1 *Biomonitoring using lichens.* Lichens are organisms consisting of fungi and algae or cyanobacteria. They are recognized as being very sensitive to air pollution. During the last 40 years numerous studies have been devoted to assess the effect of air pollution on lichens. One major area of research is the analysis of elemental profile of lichen, which provides an idea of pollutant burden in atmosphere of a particular area. In India, the study of lichens and its use for the estimation of different metal contents in air is not as extensive as in other countries, excepting a few studies done recently [7,8]. The purpose of the present study is to gather information regarding the accumulation of heavy metals in a commonly growing lichen species *Parmelia caperata* in and around Kolkata.

Epiphytic lichens, *Parmelia caperata*, were collected from a site close to an industrial area with moderately high traffic load (Botanic Garden, S1), a residential site with heavy traffic volume (Salt Lake, S2), a semi-urban area with light industries and low traffic volume (Budgebudge, S3) and relatively unpolluted semi-urban area far away from main motorways (Baruipur, S4). The elemental analysis of the same lichen samples was carried out using Jordon Valley Ex-3600 EDXRF spectrometer.

EDXRF analysis of *P. caperata* collected from the four selected sites in both the seasons (summer and winter) showed the presence of Al, S, K, Ca, V, Cr, Mn, Fe, Ni, Cu, Zn, Pb (table 1). Interesting variation was noted in the concentrations of the detected elements in the same lichen species depending on the site of collection. The present study shows that the epiphytic lichen *P. caperata* can be used as an effective monitor of air pollution level in Kolkata and surrounding areas. This study gives the pioneering report of elemental profile of lichen from Kolkata. Site-dependent differences in the concentrations of analysed elements reflect the prevailing levels of atmospheric pollution in the respective areas. The present data provide baseline information for carrying out future studies related to changing air quality in the area. The work strengthens the possibility of utilizing elemental analysis of lichen species for passive long-term biomonitoring of air pollution.

3.2.2 *Characterization of dust particulates.* It has been established that leaves and exposed parts of a plant generally act as persistent absorbers in a polluted environment [9]. Trees act as sink and thus reduce the concentration of dust in air. The hypothesis that plants are important particulate traps is supported by the evidence obtained from studies dealing with trace elements, pollen, spore, salt, dust and unspecified particles [10]. Trace elements, especially heavy metals, are most commonly associated with fine particles in contaminated atmosphere. Trace element investigations conducted in roadsides,

Table 1. Mean element concentration ± standard deviation (ppm) of epiphytic lichen *P. caperata* collected from different sites in and around Kolkata by EDXRF.

Element	Botanic Garden (S1)	Salt Lake (S2)	Budgebudge (S3)	Baruipur (S4)
Al	2361 ± 167	630 ± 36	1640 ± 362	456 ± 31
S	5571 ± 361	4520 ± 678	3123 ± 245	4929 ± 278.9
K	3324 ± 228	2443 ± 536	2433 ± 64	3405 ± 224
Ca	3211 ± 311	2665 ± 367	1167 ± 81	1361 ± 94
V	9.8 ± 1	5.4 ± 1.3	7.1 ± 1.1	2.3 ± 0.1
Cr	10 ± 2	5.4 ± 0.1	7.5 ± 0.4	8.5 ± 0.6
Mn	65 ± 5	35 ± 4.4	56 ± 2.7	57 ± 2.3
Fe	3093 ± 231	1833 ± 20	2222 ± 400	2040 ± 37
Ni	10 ± 2	9 ± 0.7	8 ± 1.8	5.5 ± 0.3
Cu	9 ± 1	7.6 ± 0.4	6.6 ± 1.3	12 ± 1
Zn	47 ± 4	54 ± 1	50 ± 1.2	62 ± 2
Pb	22 ± 4	20 ± 3.7	12 ± 2.5	7 ± 1

industrial and urban environments demonstrated a significant burden of particulate heavy metals accumulated on vegetative surfaces [11]. It was reported that the surface of a variety of city trees possessed substantial accumulation of certain metals, particularly lead, zinc, iron [12] and aluminum [13]. The present study deals with elemental profiling of dusts deposited on roadside tree canopies in some areas of Kolkata, India. In this study the extent of metal deposition was analysed using EDXRF.

The plant species selected for the present study are: (i) *Terminalia catappa* (TC), (ii) *Bauhinea variegata* (BV), (iii) *Acacia auriculiformis* (AA), (iv) *Alstonia scholaris* (AS), and (v) *Ficus benghalensis* (FB). The surfaces of the leaves collected from two different sites were swabbed using Whatman filter papers, which were used as targets for elemental analysis using EDXRF technique. The results showed the chemical signatures of metals like Si, V, Cr, Mn, Fe, Cu, Zn and Pb on the dust particles collected from leaf surfaces. The concentrations of different elements varied within species from different locations. The variation in elemental concentrations among the species at the same location was also observed. Present study shows that elemental profiling of dust particles deposited on leaf surfaces can be used for passive monitoring of air pollution. This preliminary study on characterization of dust particulates accumulated on leaf surfaces can further be used in larger scale to screen the plants based on their dust interception capacity for air pollution abatement (see figure 2).

3.3 *EDXRF in agriculture*

Plants require different elements generally referred to as macronutrients and trace elements. The increase of nutrients in the food is a challenge for every researcher. Also, application of radiation has revolutionized current day research in the fields of agricultural science and food technology. Previous literatures indicated that the uptake and translocation of mineral nutrients within the plant could be affected by elevated UV-B radiation

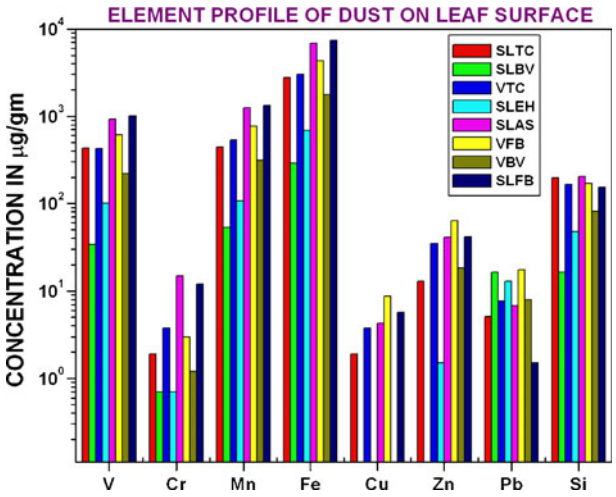

Figure 2. V, Mn, Fe, Si, Cr, Cu, Zn, Pb elemental profiles of dust particulates deposited on the leaf surfaces.

[14,15]. However, we have employed gamma irradiation to check the alteration in the essential and nonessential element content in root, stem, leaf and fruit. The present study was designed to investigate elemental profiling of different parts of *Cicer arietinum* L. plant using EDXRF technique.

The seed grains of *Cicer arietinum* L. were irradiated using the method described by Cuero *et al* [16]. The seeds were exposed to ^{60}Co gamma source at SINP, Kolkata, India using absorbed doses of 1.15, 0.10, 0.15 and 0.20 kGy. The elemental concentrations of root, stem, leaf and fruit of F3 generation plants were measured using EDXRF technique (figure 3). Variation in the uptake or accumulation of different mineral nutrients noted in the different irradiated seeds highlights the response of the respective plant type to the stress experienced at the developing stage according to functional needs of the respective parts. Most of the essential elemental concentrations were increased in the dose range of 0.10–0.15 kGy. This highlights the specific adaptive response of the plant type in terms of maintenance of its micronutrients homeostasis, which may have some correlation with the genetic make-up of the respective plant. Significance of such stimulation correlated with yielding ability of the plant concerned is discussed in the light of newer aspect of agricultural research. Further study is needed to quantify the nutritional value of harvested crop.

4. Conclusion

EDXRF has a multidimensional application and can be used effectively in studying the trace elemental profile in different types of systems and matrices – biological, environmental, agricultural sciences etc. The EDXRF spectrometer at UGC-DAE CSR, Kolkata Centre can be used for both thick (pellet) and thin samples effectively with appropriate

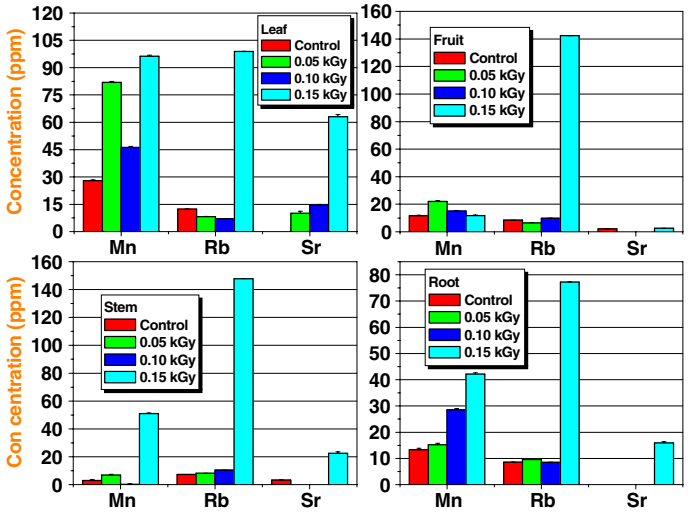

Figure 3. Concentration of Mn, Rb and Sr in different plant parts.

sample preparation. EDXRF is definitely a very useful tool for multidisciplinary research for trace element sciences. An editorial in *Science* [17] noted, "The time is upon us to recognize that the new frontier is the interface, wherever it remains unexplored… . In the years to come, innovators will need to jettison the security of familiar tools, ideas, and specialties as they forge new partnerships."

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