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ENERGY DISPERSIVE X-RAY FLUORESCENCE MATRIX ANALYSIS FOR NUTRIENTS IN FENUGREEK PLANT–SOIL SETUP INFLUENCED BY BOTH FERTILIZATION AND SOIL TEXTURE

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In energy dispersive X-ray fluorescence (EDXRF) analytical studies of samples, the absorption and enhancement of analyte X-rays, collectively called matrix effects, complicate the relation between intensity of analyte X-rays and its concentration. Earlier, the absorption and enhancement relative terms have been derived from the built-up experimental relations of analyte X-ray counts with XRF fundamental parameters and the parameters of the experimental setup for each selective and enhanced X-rays. Now, the terms are implemented on the determined amounts of potassium and calcium nutrients in plants and pot soils from an experiment performed in the lab by growing fenugreek plants on the soils with variable contamination levels and applied fertilizers. The variation pattern of the terms with respective nutrient contents is found to be affected by the basic nature of soils. The pattern shows the high sensitivity of the terms to the plant's behaviour in soil and reflects the picture of supressed benefits of applied fertilizers to the heavy-metals contaminated soils.

Keywords: *energy dispersive X-ray fluorescence, matrix effect, absorption and enhancement terms, plant nutrients, soil contamination.*

Introduction. Based upon the EDXRF technique, elemental analysis of a composite identifies its constituents and their amounts from energies and intensities of their characteristic X-rays. An inherent defect enters the picture due to the process of absorption of characteristic X-rays of the analyte (element of interest) in the target itself if they are capable of exciting the X-rays of some other element. As a result, the analyte's X-rays are absorbed, and the other element's characteristic X-rays are strongly enhanced. Absorption and enhancement of interelemental X-rays, which are matrix effects, complicate the relationship between intensities of the characteristic X-rays and the analyte concentration. To overcome these difficulties, numerous methods in the literature [1–3] have been proposed for compensation, correction, or evaluation of these interelement effects in samples with unknown dark matrices. Bansal and Mittal [4, 5] and Bansal et al. [6] followed an approach in which absorption and enhancement relative terms were derived from the built-up experimental relations for analyte X-ray counts with XRF fundamental parameters and the parameters related to experimental setup for each selective and enhanced X-rays. The relations were verified experimentally with synthetic samples and applied to plants and soil samples.

Nutrients in plants are required for plants' growth and survival. There are 20 macro- and micronutrients necessary for optimum plant growth. Macronutrients are needed in plants in large amounts while the micros are needed in small amounts. The soil chemistry distinguishes heavy metals as a special group of elements because of their toxic effect on plants at high concentrations. According to ecologists, heavy metals/metalloids form the following series according to their hazard degree in soils; Se > Tl > Sb > Cd > V > Hg > Ni > Cu > Cr > As > Ba [7]. But the sequence may vary from place to place as the concentration of metals varies and hence their threat also. Moreover, soil properties are affected by past land use, current activities on the site, and nearness to pollution sources. Human activities have added organic and inorganic additives to the soils. Addition of domestic waste and chemicals used for commercial and industrial purposes is the major sources of

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contamination [8]. Moreover, the applications of different quantities of nutrient fertilizers to the soils during growth of saplings lead to variations in nutrient amounts in the plants [4].

Here, to explore the implementation of absorption and enhancement terms in plant–soil systems influenced by fertilizers and soil texture, a pot experiment has been performed on a plant–soil setup, and EDXRF studies were made on the plant and soil samples.

In the search for plants for the present studies, fenugreek (*Trigonella foenum graceum* L.) is found to be a self-pollinating, old medicinal plant that is commonly used as a traditional food. It is a rich source of potassium, calcium, iron, β-carotene, and other vitamins. It is capable of accumulating considerable amounts of heavy metals and could be used to clean up heavy-metal-contaminated sites [9, 10]. Fenugreek has been used as a bio-indicator of environmental pollution of soils [11–13]. Therefore, a plant–soil setup has been designed for fenugreek to depict the behavior of potassium and calcium nutrients in plant–soil systems with variable amounts of these nutrients added to low and highly contaminated growth media (soils). Two soils from sewage and factory sites were selected as they were found highly contaminated and one was from a garden of the university campus as less contaminated for the experiment with fenugreek.

Potassium and calcium are essential macronutrients of plants. Potassium is a primary [14–16], and calcium [17, 18] a secondary, macronutrient. Potassium in plants performs a number of important functions related to enzyme activation, neutralization of negative charges, maintenance of cell turgor, and plant growth and organ movement [19]; Ca^{2+} is an intracellular messenger molecule involved in many signal transduction pathways in plant. It is needed as a counter-cation for inorganic and organic anions in the vacuole and as an intracellular messenger in the cytosol. Thus, calcium is a critical constituent of a plant cell wall [20]. Potassium and calcium interactions with other soil contents involving contaminants as well as with each other are useful to study and understand plant physiology in certain environmental circumstances [21, 22]. Therefore, for the present studies, macronutrients potassium and calcium are tracked. The details of pot experiment, elemental analysis of pot yields, and outcomes of the experiment are given in the following sections.

Experiment. The selection of soils is made to estimate the properties of the population or entire field. Keeping that in mind, soil samples from three sites: city sewage SS, waste water effluent of a mechanical factory (manufacturer of automotive parts) ES, and garden inside the authors' university campus OS were collected after removing the uppermost layer containing pebbles, grass, etc. A soil layer of 15–20 cm was taken as sample with the help of a wooden tool to avoid any metallic contamination and was collected in clean polybags.

The thick briquette targets of soils samples were exposed to high flux 18 keV photons from the microfocus X-ray fluorescence beamline (BL-16) of the Indus-2 synchrotron radiation facility at RRCAT, Indore. In the spectra (Fig. 1), along with the *K* peaks of K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, and Zn, the *K* peaks of Se, Rb, and Sr, and *L* peaks of Pb and Hg are observed. Factory soil is rich in elemental Cr, Ni, and Fe, whereas sewage soil contains Fe, Cu, Zn, Hg, As, Se, and Sr in higher amounts than the other two soils. From the peak heights, the comparative elemental amounts can be estimated for the three soils, e.g., potassium content is highest in factory soil, followed by normal soil, and lowest in sewage soil. But for calcium, the trend is exactly reverse. Sewage and factory soils are higher in Fe amounts than normal soils.

For pot experiment, each of the soils was mixed with sand and clay soil in equal proportions [5, 6, 23] and were filled in 20 earthen pots in each set with a total of 60 pots for the three soils. The experiment was held in a spacious airy laboratory room of dimensions ~24 × 21 × 12 ft with proper natural daylight (daytime luminance of 150–200 Lux) and no artificial luminance at night; 80–100 uniform sized seeds of fenugreek were germinated in each pot. The experiment was carried out for 40 days. The pots were watered with 200 mL water when required.

During the growth period, average length, color, and texture of saplings were regularly monitored. On the 15, 21, and 35th day after sowing of seeds, fertilizations with $CaCO_3$ (calcium fertilizer) and KCl (potassium fertilizer) were done (Table 1). For each soil, nine pots numbered POT-1 to POT-9 were treated with 1, 5, 10, 15, 20, 25, 30, 40, and 50 mM $CaCO_3$ solution. The other set of nine pots (POT-11 to POT-19) were treated with similar concentrations of KCl, and one pot (POT-10) was treated with 20 mM solutions of both $CaCO_3$ and KCl fertilizers. POT-0 was left untreated.

Sample Preparations. After 40 days of seed sowings, the fenugreek saplings were cut with stems above the soil surface, and the roots were extracted from the pot soils by manual extraction [24] and washed under running water to remove the soil and foreign material (if any) on the plants. The plants were dried at room temperature for two days and in an oven at 60–70°C for 5–6 h for a consecutive two days. The dried fenugreek plants were then ground in an electric grinder and electric agate mortar and pestle. The thick pellets of samples [13] were prepared by pressing a weighed amount of fine powder of the material in a die with a 25 tons semiauto hydraulic pressing machine to obtain pellets of 2.5 cm diameter. For analysis, the collected soils from individual pots were dried at 70–80°C for 5–6 h for 4 days. The dried soil samples were sieved through a

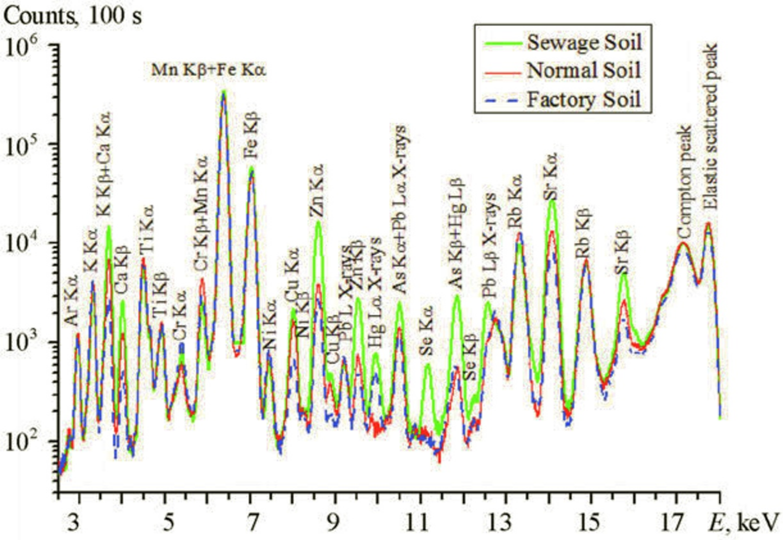

Fig. 1. Comparison spectra of three soils exposed by 18 keV photons for 100 s each.

sieve with mesh number 300 having aperture width 53 μm. The sieved samples were also pressed in the die to prepare pellets of 2.5-cm diameter.

Methodology. In a thick sample *S*, the amount δ of one of its constituents *x*, can be determined with the existing analytic method based on the XRF technique for thick samples [4–6, 23]. The method involves, in turn, selective excitation of analyte element *x* in sample *S* and in its two reference materials. The first reference material is analyte *x* itself or its compound *X* with *n* atoms of *x* per molecule. The second reference S_p is a mixture of *S* and its first reference material in a known ratio. If δ′ is the amount of the first reference *X* added to a unit amount of *S* for S_p preparation, the amount δ is determined using the formula [25]

$$\delta = n\,\frac{M_x}{M_X}\,\delta'\,\frac{\left[\dfrac{N_x^X(i_x)}{N_x^{S_p}(i_x)} - 1\right]}{\left[\dfrac{N_x^X(i_x)}{N_x^S(i_x)}\right] - \left[\dfrac{N_x^X(i_x)}{N_x^{S_p}(i_x)}\right]}\,, \tag{1}$$

where M_x is the atomic weight of analyte *x*, M_X is the molecular weight of analyte compound *X*, and i_x is the incident energy for selective excitation of *x*, N_x^S are the X-ray counts from *S*, S_p, and *X* materials.

Absorption and enhancement terms. In sample *S*, if the X-rays of analyte *a* of fractional amount δ excites the X-rays of other element *c* of fractional amount λ, then it causes absorption of *a* X-rays and enhancement of *c* X-rays, called matrix effects, and disturbs the linearity between the X-ray intensity and analyte amount in *S*. For *S* irradiation with i_a photons for selective excitation of *a* and recording of X-ray spectra in the detector in a 90° reflection geometry setup, $N_a^S(i_a)$ are the X-ray counts under the photo peak after their absorption in the substrate of *S*, and $N_c^S(i_a)$ are the enhanced X-ray counts under the *c* photo peak in *S* in the same excitation.

The amount δ/λ of elements *a*/*c* in the *S* of unknown substrate is determined from relation (1) with selective excitation of analyte *a*/*c* in *S* and their two references *A*/*C* and Sp(*a*/*c*) with i_a/i_c photons. The relative absorption term $\dfrac{\mu^S_{a/c}(Ab)}{\mu^{A/C}_{a/c}(Ab)}$ for *a*/*c* in *S* from i_a/i_c selective excitation are given as [7, 8]

$$\frac{\mu^S_{a/c}(Ab)}{\mu^{A/C}_{a/c}(Ab)} = \delta/\lambda\,\frac{M_{A/C}}{n/\ell M_{a/c}}\left[\frac{N^S_{a/c}\left(i_{a/c}\right)}{N^{A/C}_{a/c}\left(i_{a/c}\right)}\right]^{-1}, \tag{2}$$

where $\mu^S_{a/c}(Ab) = \mu^S_{a/c}(i_{a/c}) + \mu^S_{a/c}(e_{a/c})$, with $i_{a/c}$ being the incident energy and $e_{a/c}$ being the emitted energy; μ^S are the absorption coefficients of the *S* material for *a*/*c* X-rays.

The enhancement term $\left(\dfrac{\mu_c^S(En)}{\mu_c^C(En)}\right)/G$ for c in i_a excitation for S is

$$\left(\frac{\mu_c^S(En)}{\mu_c^C(En)}\right)\Big/G = \frac{\lambda M_C}{l M_c}\left[\frac{N_c^S(i_a)}{N_c^C(i_a)}\right]^{-1}, \tag{3}$$

where A/C are the known compounds of the element a/c with n/l atoms of a/c. For a particular combination of i_a (incident energy), e_a (emitted a X-ray energy), and a and c (analytes), $G = \sigma_a^{i_a}\sigma_c^{e_a} / \sigma_c^{i_a}$ is constant and independent of elemental amounts δ and λ. Here $\sigma_a^{i_a}$ is the production cross section for a X-rays at incident energy i_a; $\sigma_c^{e_a}$ is the production cross section for c X-rays at emitted a X-ray energy e_a, and $\sigma_c^{i_a}$ is the production cross section for c X-rays at incident energy i_a, with σ's in barns (cm^2), and G's dimensions become cm^2.

Experimental Observations and Their Analysis. *Elemental analysis.* For potassium, the first reference material was KNO_3, and for calcium it was CaO. The second reference for each, potassium and calcium, was obtained by mixing the sample and its first reference materials in a known ratio. For each detection the pellets of plant/soil sample and its two references were irradiated in the single reflection geometry with the X-rays from Low Power Neptune X-ray tube with Rh anode and the emitted X-rays spectra of targets were recorded with an Amptek X123 spectrometer having Si PIN detector with 0.5 mil Be window and of dimensions 6 mm^2/500 μm having resolution 145 eV at 5.959 keV Mn X-rays. The X-ray flux from the tube was adjusted to keep the dead time losses <10%. To determine the potassium and calcium amounts in fenugreek plant and soil samples, selective excitations of sample and its two reference materials were done with tube voltages/filament current at 4 kV/0.3 mA (for potassium) and 5 kV/0.2 mA (for calcium). Typical spectra of fenugreek plant sample at 4 kV/0.3 mA and 5 kV/0.2 mA are given in Fig. 2.

The fractional amounts of potassium and calcium in plant and soil samples were calculated using the recorded counts under potassium and calcium photo peaks in relation (1) and are listed in Table 1.

Absorption terms. The absorption ratio for Ca/K in fenugreek plant and soil samples as $\mu_{\text{Ca/K}}^{\text{Fenu (Ca/K)}}(Ab)\big/\mu_{\text{Ca/K}}^{\text{CaO/KNO}_3}(Ab)$ for the α fraction of calcium and the β fraction of potassium is derived from relation (2) with $n = \ell = 1$ for CaO and KNO_3 as

$$\frac{\mu_{\text{Ca/K}}^{\text{Fenu (Ca/K)}}(Ab)}{\mu_{\text{Ca/K}}^{\text{CaO/KNO}_3}(Ab)} = (\alpha / \beta)\,\frac{M_{\text{CaO/KNO}_3}}{M_{\text{Ca/K}}}\left[\frac{\text{N}_{\text{Ca/K}}^{\text{Fenu}}}{\text{N}_{\text{Ca/K}}^{\text{CaO/KNO}_3}}\right]^{-1}. \tag{4}$$

The evaluated absorption terms from selectively excited Ca/K X-ray counts plotted against the determined α/β in fenugreek samples are given in Fig. 3.

From the variation pattern of the absorption terms with respective contents, a search was made for an empirical relation between the evaluated absorption ratios and analyte amounts. Different-order polynomial fits in amounts α/β were tried on the term $\ln\left[\mu_{\text{K/Ca}}^{\text{Fenu (Ca/K)}}(Ab)\big/\mu_{\text{Ca/K}}^{\text{CaO/KNO}_3}(Ab)\right]$. Following the criteria of lowest powers of amount and close agreement between the actual and generated values, the polynomial fits for plant and soil samples were selected. The trends in plant and soil samples follow the pattern

$$\ln\left[\frac{\mu_{\text{Ca/K}}^{\text{Fenu (Ca/K)}}(Ab)}{\mu_{\text{Ca/K}}^{\text{CaO/KNO}_3}(Ab)}\right] = \sum_{j=-p}^{q} A_j\left[[\alpha]^j/[\beta]^j\right], \tag{5}$$

where A_j's are the coefficients of fit for absorption of K X-rays of Ca/K in fenugreek plant and soil samples, and p and q indicate the order of highest negative and highest positive integer values of j that are characteristics of each pattern. The obtained empirical fit relations and mean absolute percent deviations D_p [26] are given in Table 2.

Enhancement terms. For enhanced potassium K X-ray studies, in the selective excitation of Ca in fenugreek samples at 5 keV, counts $N_{\text{K}}^{\text{Fenu (Ca)}}$ under the potassium K photo peak were collected and normalized with potassium K X-ray counts $N_{\text{K}}^{\text{KNO}_3}$ in standard KNO_3 at 5 keV. The enhancement term $\left[\dfrac{\mu_{\text{K}}^{\text{Fenu (Ca)}}(En)}{\mu_{\text{K}}^{\text{KNO}_3}(En)}\Big/G\right]$ for K in fenugreek samples derived from relation (3) is

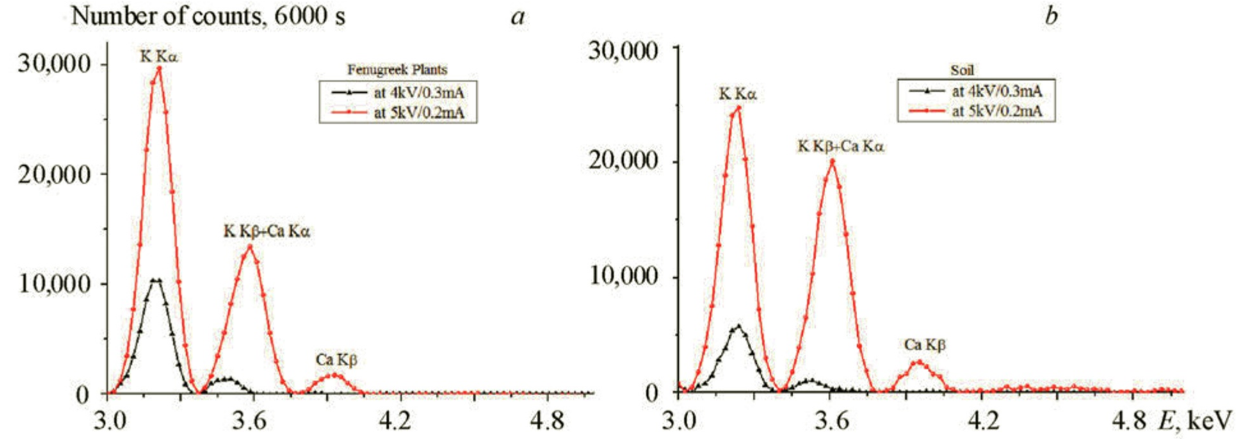

Fig. 2. Typical spectrum of fenugreek (a) plant and (b) soil sample taken with X-ray tube voltage/filament current at 4 kV/0.3 mA and 5 kV/0.2 mA.

$$\frac{\mu_{\mathrm{K}}^{\mathrm{Fenu}}(En)}{\mu_{\mathrm{K}}^{\mathrm{KNO_3}}(En)}\Bigg/G = \frac{\beta M_{\mathrm{KNO_3}}}{M_K}\,\alpha\left[\frac{N_{\mathrm{K}}^{\mathrm{Fenu}}}{N_{\mathrm{K}}^{\mathrm{KNO_3}}}\right]^{-1}. \tag{6}$$

The evaluated terms empirically correlated with the evaluated α/β amount in fenugreek samples and plotted against α/β for both plants and soils are shown in Fig. 4.

The general relation found for $\ln\left[\frac{\mu_{\mathrm{K}}^{\mathrm{Fenu\,(Ca)}}(En)}{\mu_{\mathrm{K}}^{\mathrm{KNO_3}}(En)}\Bigg/G\right]$ versus (α/β) for fenugreek samples is independent of the X-ray counts,

$$\ln\left(\left(\frac{\mu_{\mathrm{K}}^{\mathrm{Fenu\,(Ca)}}(En)}{\mu_{\mathrm{K}}^{\mathrm{KNO_3}}(En)}\right)\Bigg/G\right) = \sum_{j=-p}^{q} E_j\,[\alpha]^j\Big/[\beta]^j\,, \tag{7}$$

where E_j's are the coefficients of fit for enhancement of potassium K X-rays in the samples.

The different polynomial fits and their mean absolute percent deviations D_{p} for fenugreek samples are given in Table 3.

Results and Discussion. The present experimental setup and the methodology used with synthetic samples has already been quoted [27]. In this work, for quantification of elements in plant and soils with an unknown dark matrix, extra care has been taken in handling, weighing, mixing, grinding, and preparation of the samples. The experimental error with <1% counting statistics comes out to 7%. To be on the safe side, the maximum error in determined amounts is listed as 14% in Tables 1.

For convenience, the plants and soils, OS, ES, and SS are named according to their growing media as normal, factory, and sewage. respectively. The health condition of the fenugreek plants was noted on the last day of the pot experiment (Table 4). Table 4 data predicts the low level of potassium fertilizers and the high level of calcium fertilizers that are effective for normal soil plants. For factory soil plants, both the potassium and the calcium fertilizer effects are in reverse order to those of normal soil, and the overall fertilizer effect is negative. In the case of sewage soil, high calcium and highest potassium fertilizers seem effective, but the overall fertilization effect is less negative compared to that on factory soil plant.

The spectra in Fig. 1 point to the relative qualities of the soil in terms of its potassium and calcium macro nutrients; potassium is highest in factory soil, followed by normal soil, and is lowest in sewage soil. But the calcium trend is exactly opposite. The average values of the determined fractions for these elements in pot soils (Table 1) also exhibit the same kind of picture.

The variation in nutrient contents with fertilizers in different pot soils and plants can be summed up by the parameter: the percentage variance w.r.t the mean listed in the last row of Table 1. The percentage variance for potassium and calcium

TABLE 1. Determined Fractional Amounts of K/Ca (with 14% error) in Dried Fenugreek Plants and Pot Soils (OS, ES, and SS) from the Treatments with K/C Fertilizers, along with the Mean and Variance Percentage with Respect to Mean of Fractional Amounts for Each Set of 20 Pots

Pot No.	K amounts						Ca amounts					
	OS (normal)		ES (factory)		SS (sewage)		OS (normal)		ES (factory)		SS (sewage)	
	Soil	Plants	Soil	Plants	Soil	Plants	Soil	Plants	Soil	Plants	Soil	Plants
0	0.0174	0.0572	0.024	0.013	0.0201	0.0153	0.0132	0.0096	0.0086	0.0188	0.0181	0.0113
1	0.0250	0.0377	0.0426	0.0084	0.012	0.0209	0.0129	0.0076	0.0105	0.0265	0.0154	0.011
2	0.0230	0.0203	0.0249	0.0181	0.0255	0.0111	0.0182	0.016	0.0096	0.0324	0.0224	0.0111
3	0.0233	0.0247	0.0262	0.0211	0.0235	*	0.0167	0.0135	0.0133	0.0095	0.0223	*
4	0.0228	0.0286	0.0298	0.0348	0.0318	0.0157	0.0165	0.0115	0.0152	0.0157	0.0265	0.015
5	0.0195	0.0175	0.0265	0.0395	0.0183	0.0308	0.0191	0.0133	0.0122	0.013	0.0253	0.007
6	0.0359	0.0197	0.0271	0.0162	0.0158	0.0156	0.0213	0.0163	0.0124	0.0103	0.0379	0.0125
7	0.0227	0.0229	0.0328	0.0255	0.0163	0.0169	0.0319	0.0144	0.018	0.0156	0.0326	0.0098
8	0.0302	0.0298	0.0296	0.0175	0.0247	0.0128	0.0276	0.0166	0.0107	0.0196	0.0351	0.0117
9	0.0371	0.0139	0.0279	0.0247	0.0157	0.0169	0.029	0.0258	0.0226	0.0138	0.035	0.0094
10	0.0313	0.0376	0.024	0.056	0.029	0.0174	0.0199	0.0123	0.012	0.0134	0.0241	0.0139
11	0.0357	0.0292	0.0253	0.0058	0.0276	0.013	0.0225	0.0118	0.011	0.01	0.0148	0.0144
12	0.0278	0.0238	0.0291	0.0116	0.0242	0.0083	0.0162	0.0081	0.0119	0.0121	0.0168	0.0075
13	0.0264	0.024	0.0181	0.0052	0.022	0.014	0.0196	0.0087	0.0138	0.0141	0.0168	0.0096
14	0.0191	0.0372	0.0359	0.0206	0.0359	0.011	0.017	0.0075	0.0112	0.0137	0.0293	0.0136
15	0.0262	0.032	0.0317	0.0192	0.0182	0.0098	0.0136	0.0124	0.0109	0.0117	0.0205	0.0105
16	0.023	0.0281	0.0392	0.0089	0.0313	0.0212	0.0176	0.0131	0.0108	0.0155	0.0186	0.004
17	0.0373	0.0148	0.0315	0.0113	0.0098	0.03	0.0168	0.0057	0.01	0.0173	0.0134	0.0057
18	0.035	0.0745	0.0408	0.0111	0.0237	0.0111	0.0159	0.0107	0.0107	0.0142	0.0158	0.0116
19	0.0169	0.0044	0.0427	0.0138	0.0266	0.0216	0.0193	0.0062	0.0082	0.014	0.0152	0.0084
Mean	0.0268	0.0274	0.0305	0.0191	0.0226	0.0165	0.0192	0.0121	0.0122	0.0156	0.0228	0.0104
Variace Percentage w.r.t. mean	0.1595	0.8266	0.1421	0.7722	0.1971	0.2204	0.1298	0.1669	0.0856	0.1857	0.2455	0.0799

*Due to the small amount of sample data, this could not be determined.

is greater for normal plants than for factory plants and lowest for sewage plants. This points to the fact that contamination of soil supresses the benefits of applied fertilizers on the plant's nutrient amounts (especially the organic part in sewage soil; Table 1). The general logic is that the varying concentrations of fertilizers in soil should affect the nutrient concentration in soil accordingly and the movement of nutrients from soil to plant in the same pattern. The requisite pattern is observed in normal soil and plants for calcium fractions with fertilizations above 25 mM. But the toxic constituents of soils are found to suppress the uptake of nutrients even when they are being added, leading to less variations in potassium and calcium amounts in plants. The heavy metals Mo, Zn, Cu, Ni, Co, Fe, Mn, and V predicted in soils from their spectra are required in minute quantities by organisms. However, their excessive amounts seem harmful to plants, while metals such as Pb, Cd, Hg, and As do not affect the organisms but are regarded as the "main threats" to plants [28, 29]. These are easily taken up by plants and cause disturbances in other nutrients uptake [30, 31], which is why the percentage variance is comparably low for factory

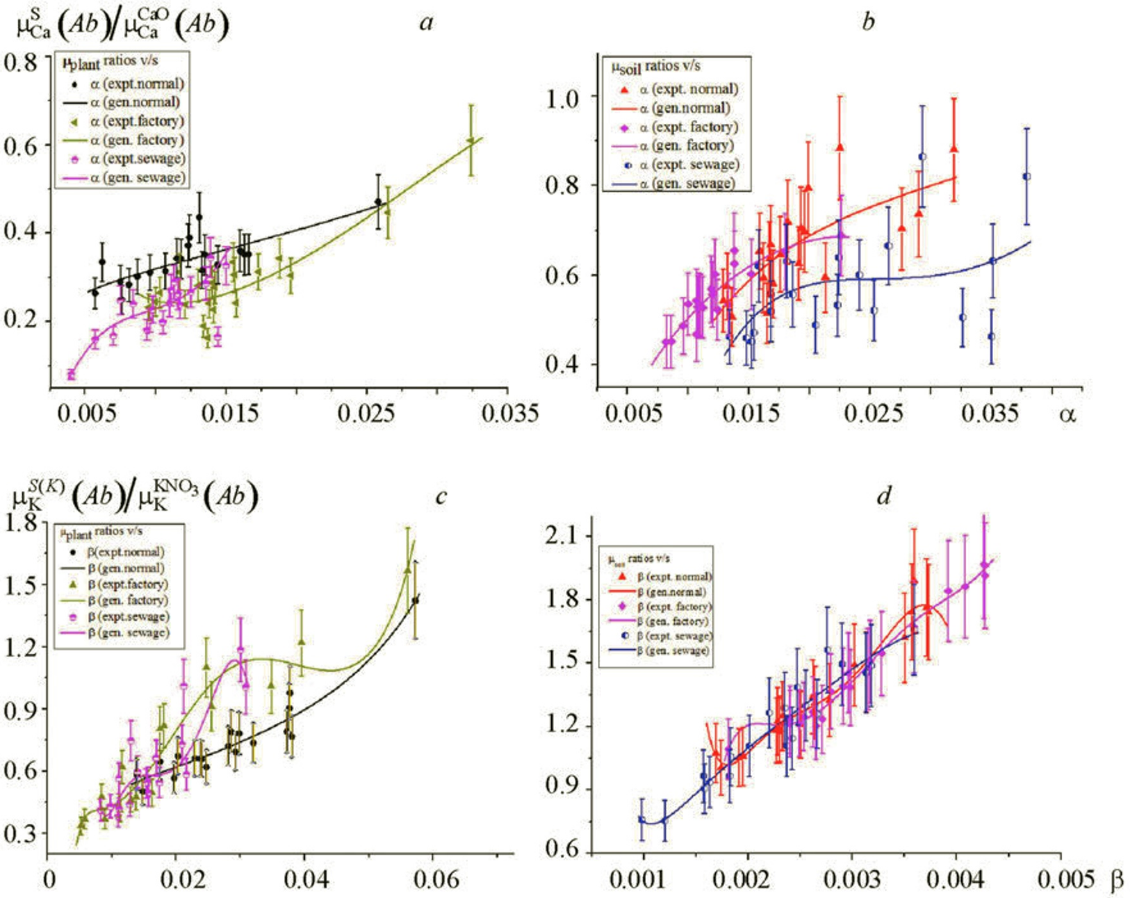

Fig. 3. Plot of fenugreek relative absorption terms for (a) calcium vs. α at 5 keV for plants, (b) calcium vs. α at 5 keV for soils, (c) potassium vs. β at 4 keV for plants, and (d) potassium vs. β at 4 keV for soils.

TABLE 2. Relative Absorption Terms and their Empirical Relations with Amounts α/β and Mean Absolute Percent Deviations D_p in Plants and Soils for Ca at 5 keV and K at 4 keV Photons

Sample	$\ln\left[\frac{\mu^{S}_{Ca}(Ab)}{\mu^{CaO}_{Ca}(Ab)}\right]$ vs. α at (5 keV)		$\ln\left[\frac{\mu^{S}_{K}(Ab)}{\mu^{KNO_3}_{K}(Ab)}\right]$ vs. β at (4 keV)	
	Relation	D_p, %	Relation	D_p, %
Plant (OS)	$\sum_{j=-1}^{2} A_j[\alpha]^j$	7.04	$\sum_{j=-2}^{3} A_j[\beta]^j$	5.81
Plant (ES)		10.28		10.70
Plant (SS)		8.85		11.43
Soil (OS)		8.31		0.12
Soil (ES)		3.69		2.42
Soil (SS)		12.39		4.77

and sewage soils compared to that of normal soil. The overall variation is low in calcium amounts than for potassium in plants. Plant health findings in earlier works also support these heavy element effects. It seems that potassium, being the primary macronutrient to plants, is more prone to these hindrance effects in plant uptakes as compared to the secondary

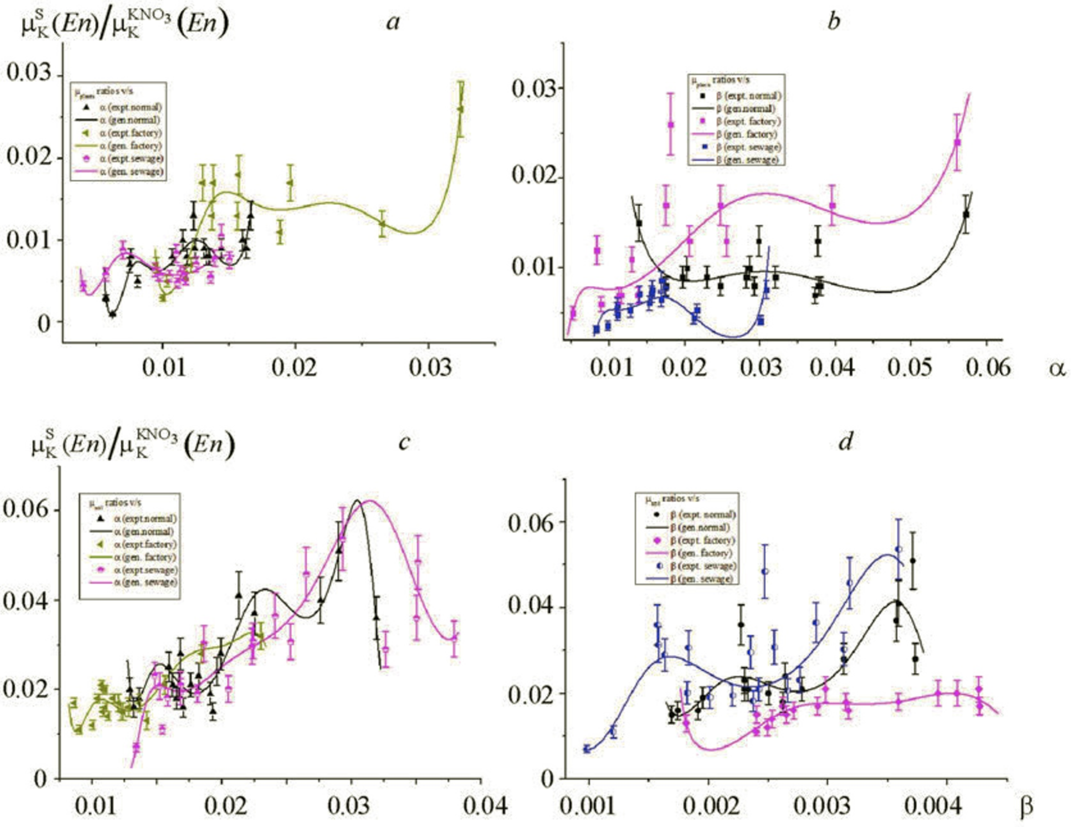

Fig. 4. Plot of fenugreek enhancement terms of potassium K X-rays at 5 keV excitations vs. (a) α and (b) β for plant samples and (c) α and (d) β for soil samples.

macronutrient Ca. These facts are corroborated by the findings of Pesarrakli [32]. The stated facts point to the reliability of the present determined potassium and calcium amounts in both plants and soils as the findings are supported by already established facts.

In matrix-terms analysis of potassium and calcium in plants and soils (Tables 2 and 3), the mean absolute deviations (D_p) are within the 14% error limit for all absorption and almost all enhancement terms, which justifies the reliability of the empirical relations in the present determinations. This further signifies the factuality of the adopted methodology, thereby proving its worth for plant and soil samples with unknown constituents.

Observations and explanations for variation pattern of absorption terms (Fig. 3). Potassium and calcium absorption terms are higher in soils than in plants (Fig. 3), which is justified by the fact that low Z (H, C, O, etc.) organic substrate of plants results in lesser absorption of Ca/ K X-rays as compared to higher Z silicon, the main inorganic constituent of soil. Low average potassium amount of sewage soil (Table 1) leads to less absorption of calcium X-rays as potassium is the best absorber for Ca K X-rays due to their adjacent atomic numbers. This is why the calcium absorption curve for sewage soil lies below the curves of the other two soils. The same explanation is valid for the low absorption term of calcium in sewage and factory plants. The empirical fit patterns for calcium and potassium relative absorption ratios for soils and plants are the same with a p to q order of –1 to 2 for calcium and –2 to 3 for potassium. Here, the soil texture has no special influence on the variation pattern of potassium/calcium absorption terms.

Observations and explanations for variation pattern of enhancement terms (Fig. 4). K enhancement ratios for all the three soils satisfy the fit order (–3 to 4) with α and (–2 to 3) with β.

For normal plants, the K enhancement ratios follow the order –3 to 4 for α and –2 to 3 for β, but for sewage plants, these are –2 to 3 for both α/β; for factory plants, the K enhancement ratios show an intermediate trend, –3 to 3 with α and –2 to 3 with β.

Higher degrees of polynomial means frequent up and down variations in the values of the terms, while lower degrees correspond to smooth variations with the amounts. When a lower order of fit for one set changes to a higher order for

TABLE 3. Relative Enhancement Term of Potassium K X-rays at 5 keV for Plants and Soils and its Empirical Relations with α/β along with Mean Absolute Percent Deviation D_p

Sample	$\ln\left[\frac{\mu_\mathrm{K}^\mathrm{S}(En)}{\mu_\mathrm{K}^{\mathrm{KNO_3}}(En)}\right]$ vs. α/β at (5 keV)			
	Relation $\sum_{j=-p}^{q} E_j[\alpha]^j$	D_p, %	Relation $\sum_{j=-p}^{q} E_j[\beta]^j$	D_p, %
Plant (OS)	$\sum_{j=-3}^{4} E_j[\alpha]^j$	11.92	$\sum_{j=-2}^{3} E_j[\beta]^j$	11.04
Plant (ES)	$\sum_{j=-3}^{3} E_j[\alpha]^j$	15.29	$\sum_{j=-2}^{3} E_j[\beta]^j$	19.55
Plant (SS)	$\sum_{j=-2}^{4} E_j[\alpha]^j$	11.74	$\sum_{j=-2}^{3} E_j[\beta]^j$	12.92
Soil (OS)	$\sum_{j=-3}^{4} E_j[\alpha]^j$	9.77	$\sum_{j=-2}^{3} E_j[\beta]^j$	13.52
Soil (ES)	$\sum_{j=-3}^{4} E_j[\alpha]^j$	12.64	$\sum_{j=-2}^{3} E_j[\beta]^j$	12.09
Soil (SS)	$\sum_{j=-3}^{4} E_j[\alpha]^j$	10.17	$\sum_{j=-2}^{3} E_j[\beta]^j$	16.46

the other, it means some external factor (fertilizer) has enter the picture. For plants and soils from all the three sites, the enhancement terms involve polynomial fits of the orders (–2 to 3) for β. But for plants, the fitting trend for the enhancement term is –3 to 4 with α for normal plants, –3 to 3 for factory plants, and –3 to 2 for sewage plants. For factory and sewage plants, enhancement terms with enhancer amount α are relatively more stable than plants from normal soils. This can be seen in the context of excess iron and other heavy metal involvement with macronutrients in the soil and plant. Thus, the fitting pattern tracks the soil contamination and its reflection in the plant's composition. Metallic contamination in soils is in the order sewage > factory > normal. This proves that iron amounts ~50,000–60,000 mg/kg in sewage and factory soils can be toxic for plants [33, 34].

It is established that within one plant species, more than one mechanism can be in operation to combat metal excess. Fenugreek is a monocot plant [35, 36], and there exists ample evidence in the literature [37] to suggest that monocotyledonous species are tolerant to iron toxicities, and the tolerance of these plants is associated with low growth rates [38] that may alleviate direct toxicity by helping to ensure low rates of iron uptake at the cost of reduced macroelemental uptake [14, 39]. Also, the amount of metals like Ni and Cr in fenugreek plant tissues increases with increasing rates of them in soil, along with the fact that Ni supports Fe transfer to plants [40, 41]. The shift in fitting structures, especially in the K enhancement terms with regard to the calcium amounts, can be the outcome of reduced K uptake [42–45] as a result of interference between potassium and iron, which in turn provides stability to the enhancements terms with respect to α for sewage plants and factory plants in the order of their iron toxicity. Sewage soil with higher iron toxicity has comparatively high stability compared to factory soil with moderate iron toxicity and the least stability for normal soil.

TABLE 4. Last-Day Health Condition of the Fenugreek Plants

Pot No.	Fertilization in mM	Normal Plants	Factory Plants	Sewage Plants
0	0	OK	Dead	Weak
$CaCO_3$ treatment in mM				
1	1	Good	Dead	Good
2	5	Good	Dead	Weak
3	10	Good	Good	Weak
4	15	Good	Good	Good
5	20	Weak	Good	Good
6	25	Good	Weak	Good
7	30	Weak	Good	Good
8	40	Good	Weak	Good
9	50	Dead	Weak	Weak
10	20, 20 (Ca, K)	OK	Weak	OK
KCl treatment in mM				
11	1	OK	Good	Dead
12	5	Weak	Good	Weak
13	10	Good	Weak	Dead
14	15	Weak	Weak	Weak
15	20	Good	Good	Dead
16	25	Good	Weak	Dead
17	30	Good	Dead	Dead
18	40	OK	Dead	Dead
19	50	OK	Dead	Good

Conclusions. The similarities of fitting trends in soils may be due to the relative abundance of nutrients in soils being uninfluenced by their mutual interactions, while in plants the variation observed may be the consequence of complex interrelationship between K and Ca uptake under heavy metal stress; moreover, the selective permeability of the root hairs comes into play, and the rate at which they take up nutrient ions is not necessarily proportional to the nutrient concentrations in soils [46]. Also, the chemistry of the soil elements plays an important role in nutrient uptake by the plant.

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