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Observation of element distribution in potassium deficient barley (*Hordeum vulgare* L.) leaf by energy dispersive X-ray fluorescence analysis

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Abstract. An energy dispersive X-ray fluorescence (EDXRF) method for the qualitative analysis of calcium (Ca), chlorine (Cl), sulfur (S), phosphorus (P), silicon (Si), and magnesium (Mg) against potassium (K) in barley leaf is presented. Leaf samples associated with K deficiency symptoms were collected from standing barley plants grown on long term sewage sludge compost receiving plots. Then leaves were deep washed in deionized distilled water and dried by ironing. Dried barley leaf sample irradiation was accomplished with an X-ray obtained from an X-ray tube focused on an area of <100 μm in diameter of the sample specimen surface. EDXRF provided sufficient sensitivity for relative concentrations of Ca, Cl, S, P, Si. The distribution pattern of Cl, P and S was homogeneous in the order Cl> S> P, based on the density of droplet integrals which were evenly distributed in the sample leaf. In contrast, the distribution pattern of Ca, K, and Si was categorized in the order Ca> K> Si, focused on vein visibility. However, no relationship existed between K distribution and its characteristics symptoms such as necrotic lesions. However, such lesions were fairly visible with Si distribution. Based on X-ray transmitted images of K deficient barley leaf for relative concentrations of Ca, Cl, S, P, Si, it was concluded that potassium deficiency does not affect the distribution in leaves of other elements needed for plant growth and development.

Keywords: Barley; Mineral element; Potassium; X-ray fluorescence.

Abbreviations: EDXRF, energy dispersive X-ray fluorescence; **Ca,** calcium; **Cl,** chlorine; **S,** sulfur; **P,** phosphorus; **Si,** silicon; **Mg,** magnesium; **K,** potassium.

Introduction

The use of chemical plant analysis for assessing the nutritional status of plants is an old practice. However, recently it has been attracting plant scientists, and more specifically plant nutritionists, due to rapid instrumental developments associated with effective analytical techniques. EDXRF is a powerful analytical technique that allows for simultaneous determination of several elements in biological samples (Valkovic, 1980). For analysis, early workers used whole plants. But workers now generally prefer plant parts such as leaves, roots, and fruits sampled at specific growth stages, thereby determining the nutrient concentration ranges in these plant parts associated with nutrient deficiency, sufficiency, and excess (Bould et al., 1960). Among the various indexes of plant nutrient status, total leaf nutrient concentrations has been of the best use (Chapman, 1966). Plant leaf analysis by X-ray fluorescence (XRF) can provide rapid, precise, and adequate nutrient content data for diagnostic purposes (Kenneth et al., 1992 and Kocman et al., 1991). However, plants are live systems and high or low concentrations of certain nutrient elements can cause surprising (Romero and Tinaut, 1984) and contradictory (Romero, 1987) results.

In addition, there has been an increasing interest in the agricultural application of sludge because of its organic matter, N, P, and other nutrients (Sommers, 1977; Suss, 1979). However, the low potassium (K) content in sewage sludge is one of the major constraints in this regard. In light of this, a field study was undertaken to quantify the effects of the low K content of sewage sludge on induction of K deficiency in barley grown on long term sludge receiving plots. Our investigation confirmed that long term sewage sludge application induces K deficiency (Miah, 1997). Moreover, to our knowledge, no findings on mineral element distribution as it relates to K deficiency on barley leaf grown on long term sludge receiving soil have been reported so far.

In this study we examined whether or not K deficiency affects the distribution, absorption, and function of other nutrient elements on barley when grown on long term sludge applied soil. We also wanted to know if leaves analyzed by EDXRF with sufficient sensitivity would help to determine element specific nutrient disorder symptoms. In this article, we describe the distribution patterns of Ca,

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Cl, S, P, Si, and Mg against K as observed with EDXRF. Subsequently, we also examined whether characteristic K deficiency symptoms such as white necrotic lesions and brown necrotic patches are associated with K distribution or not.

We hope such findings contribute to a better understanding of the movement and distribution of mineral elements under a specific elemental insufficiency in plants thereby simplifying the interpretion of nutrient interaction relationships through nutrient concentrations and plant growth studies.

Materials and Methods

Sludge Application and Crop Cultivation

Two kinds of sewage sludge composts named S and H were applied separately to a crop field consisting of volcanic ash at 10 t/ha for 17 years beginning in 1978. Of the two, S sludge compost was prepared from a mixture of sewage sludge and sawdust in the city of Tendo, Japan. H sludge compost was prepared from a mixture of sewage sludge and rice husks in the city of Akita, Japan. To fulfill selected objectives, three plots—designated as chemical fertilizer-treated F plot, S sludge compost received S plot, and H sludge compost applied H plot—were prepared in the Tanashi experimental field of the University of Tokyo, Japan. Each had an area of 270 m^2 (length: 60 m and breadth: 4.5 m). On these plots, corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.) were grown in each year in summer and winter, respectively, after 1978. However, rye (*Secales cereale* L.) was grown in all the plots in winter of 1978. Chemical fertilizers and S and H sludge composts were applied at the time of sowing as summer and winter dressings. However in F plot, no fertilizer was applied in the initial two years of the experiment and then only chemical fertilizers were applied continuously beginning in the summer of 1980 with 240 kg ha^{-1} of N, 360 kg ha^{-1} of P_2O_5 and 320 kg ha^{-1} of K_2O. While S and H sludges were applied continuously from the start of the experiment as summer and winter dressings. It should be mentioned here that repressive plant growth with nutritional deficiency symptoms such as white necrotic lesions and brown necrotic patches resembling K deficiency had been noticed in these S and H plots since 1988. Further, K deficiency in these S and H plots was confirmed by some preliminary pot experiments.

Properties of Volcanic Ash and Sewage Sludge Composts Used

A humic volcanic ash (0–10 cm depth) called Ando soil lying beyond the reach of flood water in the center of Japan was used in this study. The soil had a pH of 6.2, total C (5.2%), total surface soil Zn and Cu (137 and 119 mg kg^{-1}) and total subsoil Zn and Cu (165 and 171 mg kg^{-1}), exchangeable Ca and Mg (10.1 and 2.5 cmol kg^{-1}), CEC (26.1 cmol kg^{-1}) and a soil texture of silt loam. Since S and H sewage sludge composts have coagulant fermenting properties, samples of used sludge were placed in plastic bags, each containing 20 kg of material, for analysis of their main physico-chemical properties as shown in Table 1.

Sampling of Soil and Plant

The soil was sampled from the F, S and H plots with standing barley crop showing K deficiency in the sludge compost-receiving S and H plots. Specifically, 5 kg of soil was collected from the surface layer (0–10 cm) at three separate sites in each plot. Simultaneously, three plants at each respective plot were harvested 10 cm above the ground. Then a dried composite sample of both soil and plant were prepared by mixing the individually collected soil and plant samples of the respective plots drying under sunlight (soil) and in an oven at 70°C (plant) to be used for analysis.

Leaf Sample Collection and Preparation for EDXRF Analysis

Fully expanded leaves of 1996 winter barley exhibiting characteristic K deficiency symptoms were collected from standing crop plants of H plot at their peak growth stage. As repressive plant growth with nutritional disorder symptoms resembling K deficiency had been noticed in both of S and H plots since 1988, so to fulfill the selected objectives, leaf samples of H plot for this investigation were selected impartially. Simultaneously, we also analyzed the barley leaf for relative concentrations of Ca, Cl, S, P, Si, and Mg against K looking for differences in the trend of distribution of the analyzed elements between control (F) and the K deficient H plot. K deficiency symptoms only and always occurred in sludge dressed S and H plots. Then the collected barley leaves were deep washed in deionized distilled water and dried flat by ironing for quick elemental analysis with EDXRF.

Chemical Analysis

The Ando soil (volcanic ash) was passed through a mesh (0.5 mm), dried at 40°C, and analyzed for total C, total K, exchangeable Ca and Mg, CEC, and soil texture after Chino et al. 1992. Soil pH was, meanwhile, measured in water suspension (1:2) by a pH meter.

Sewage sludge composts were air dried, ground and passed through a 0.85 mm screen. They were analyzed for water content, pH, and selected chemical composition according to Sims (1990) by atomic absorption spectrometry (Model Z-9000, Hitachi Co., Japan). Results are shown in Table 1. The pH was measured in water suspension (1:2.5) by a pH meter.

To determine total K content in soil and plant of the F, S and H plots mentioned above, either 0.5 g of dry soil or 1 g of dry plant (aerial portion) material was digested with an acid mixture-HNO_3:$HClO_4$:H_2SO_4 (10:4:1). Before digestion, the sample acid suspension was incubated overnight at room temperature. After digestion, the sample solution was dried and dissolved in 0.5 N HNO_3

Table 1. Physico-chemical properties of S and H sewage sludge composts used in the experiment.

Compost	pH (H_2O)	Elemental content (g kg^{-1})								Water content (%)
		C	N	P	K	Ca	Mg	Zn	Cu	
S	6.1	410	21	14.5	0.7	7.4	4.1	0.7	0.1	49
H	6.5	253	17.9	8.4	4.7	10	3.7	0.6	0.1	64

and then filtered through Toyo paper No. 5C (soil) and 5A (plant). The filtrates were used for analysis of total K content by atomic absorption spectrometer (Mode Z-9000, Hitachi Co., Japan). Total K content in soil of the F, S and H plots were 1.6, 1.2, and 1.1 g kg^{-1}, respectively, while total K content in plants of the F, S and H plots were 53.8, 10, and 15.5 g kg^{-1}, respectively.

EDS (Energy Dispersive Spectroscopy) Analysis

Sticking on the aluminum stub, Mg, Si, P, S, Cl, K and Ca in dried barley leaf samples coated with a 20 nm-thick layer of carbon using a vacuum evacuator (Hus 5 GB, Hitachi Co., Japan) were determined by a Hitachi 2400 scanning electron microscope (SEM, S-2400, Hitachi Co., Japan) with a Kevex (level 4) energy dispersive spectroscopy. Amounts of these elements are shown in Table 2.

EDXRF Analysis

Dried barley leaves were analyzed for Ca, Cl, S, P, Si, Mg against K distribution by EDXRF (Model EMAX-5770, Horiba Co., Japan). Intact dried barley leaf sample irradiation was accomplished by focusing an X-ray tube on an area of <100 µm in diameter of the specimen surface for 600 s. EDXRF, provided with a high purity silicon detector having no spectrometer attachment, emits the first X-ray beam of 10 µm in diameter, excites the elements (excitation-FWHM:full width half maximum; 144 KEV-100cps at 1 KEV) in a very limited area 100–1000 µm in diameter on the surface of the sample, and detects the intensity of the secondary X-ray discharged from the excited specific element in the limited area after diffraction of the X-ray fluorescence through the grating. The practical resolution limit of this method is 1 mm ø. Characteristic X-ray fluorescence emitted from the element contained in the barley leaves is detected using an energy dispersing X-ray detector separately according to energy or the wave length of the X-ray fluorescence emitted. At the same time, the image of the element-specific fluorescence X-ray transmitted through barley leaf is observed. However, no standards were prepared for completion of the X-ray program corresponding to fluorescence readings since the analysis was a qualitative one. In fact, the X-ray analytical microscopy developed by Horiba Co., Japan has some limitations on the quantitative analysis of biological specimens as it lacks a certified or recommended system for standard calibrations.

Table 2. EDS analysis of Mg, Si, P, S, Cl, K and Ca in barley leaf of control and K deficient H plot.

Plot designation	Elements	Leaf (weight percent)
Control	Mg	0
	Si	11.97
	P	1.86
	S	2
	Cl	24.04
	K	46.07
	Ca	14.06
H	Mg	0.75
	Si	18.15
	P	3.95
	S	8.89
	Cl	10.38
	K	30.03
	Ca	27.83

Results

Mechanism of Occurrence of K Deficiency in Barley

K_2O content of H and S sludges are 0.57% and 0.09% (Table 1). Amount of H and S sludges added for one cropping is 57 kg and 9 kg per hectare. Well established is the fact that usual K_2O content in crop plant is 1–2% and crop yield is 10 t/ha. So annual crop removal of K_2O is 100 kg on conditions that K content in plant is 1% and crop yield is 10 t/ha. Thus in a two-crop pattern as in the present investigation, annual K_2O removal is 200 kg while the biennial addition of H and S sludges are 114 and 18 kg, respectively. Naturally H and S sludge receiving plots had an induced K deficiency owing to an accumulated K_2O hungriness derived from S and H sludge application containing low K_2O. White necrotic lesions and brown necrotic patches distinctly visible in the margins and tips of old and mature leaves confirmed K deficiency in barley. The K deficiency symptoms of barley leaf in H plot are shown in Figure 1.

Distribution of Elements in Control and in K Deficient Barley Leaf

The results of analytical data of EDS in barley leaf of control and K deficient H plot are shown in Table 2. From the view point of trends in distribution pattern of the elements analyzed in barley leaf, there was no difference between the control leaf and that of the K deficient H plot except with respect to Mg, which was beyond detectable limit in the control sample.

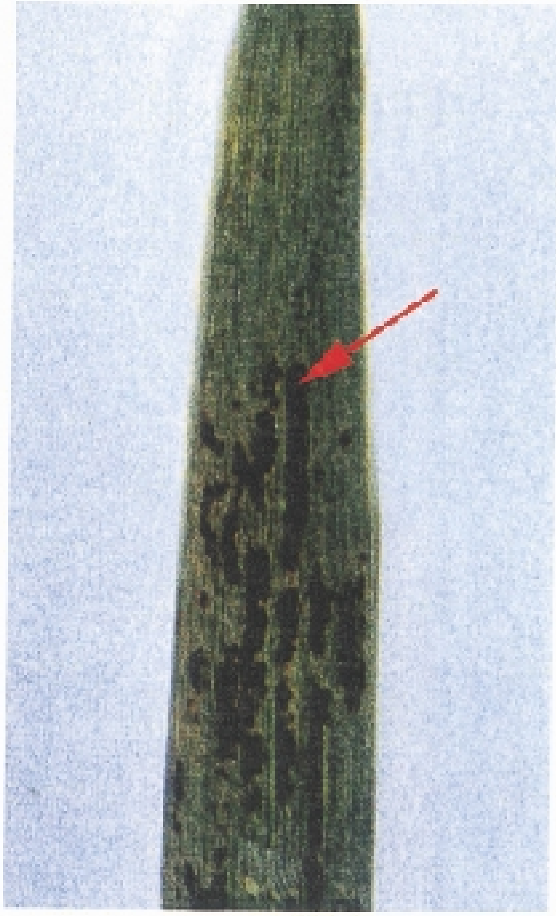

Figure 1. Fairness of the occurrence of K deficiency in barley leaf of H plot.

Distribution of Elements in K Deficient Barley Leaf

Based on X-ray characteristics of transmitted images in K deficient barley leaf, the images of element distribution of Ca, K, S, P, Si and Mg are displayed in Figure 2. However, EDXRF provided sufficient sensitivity for relative concentrations of all the elements analyzed except Mg. From a diagnostic standpoint, relative concentration of Mg as shown in Figure 2, was thus far beyond the recognizable level. As pictured, it was clear that the distribution pattern of P, S and Cl was homogeneous, based on the density of droplet integrals which were evenly distributed on the sample leaf. The homogeneity of P, S and Cl followed the order Cl>S>P. In contrast to P, S and Cl, distinct veins were associated with the distribution of K, Ca and Si, and vein visibility followed the order Ca>K>Si. Thus there were two broad categories of element distribution in K deficient barley leaf of the H plot: One was the distribution of Cl, S and P, and the other was the distribution of Ca, Si and K. However, there was a clear difference in the distribution of Ca and Cl within one leaf. Namely, Ca was more densely distributed in the veins while Cl was evenly distributed between the veins and other parts of leaf. Thus P and S follows the distribution of Cl with regard to density of droplet integrals and their degree of smoothness, and Si is followed by Ca distribution. K distribution was between these two types. On the other hand, no relationship existed between K distribution and its characteristic deficiency symptoms such as white necrotic lesions and brown necrotic patches. Instead these symptoms were fairly visible with Si distribution.

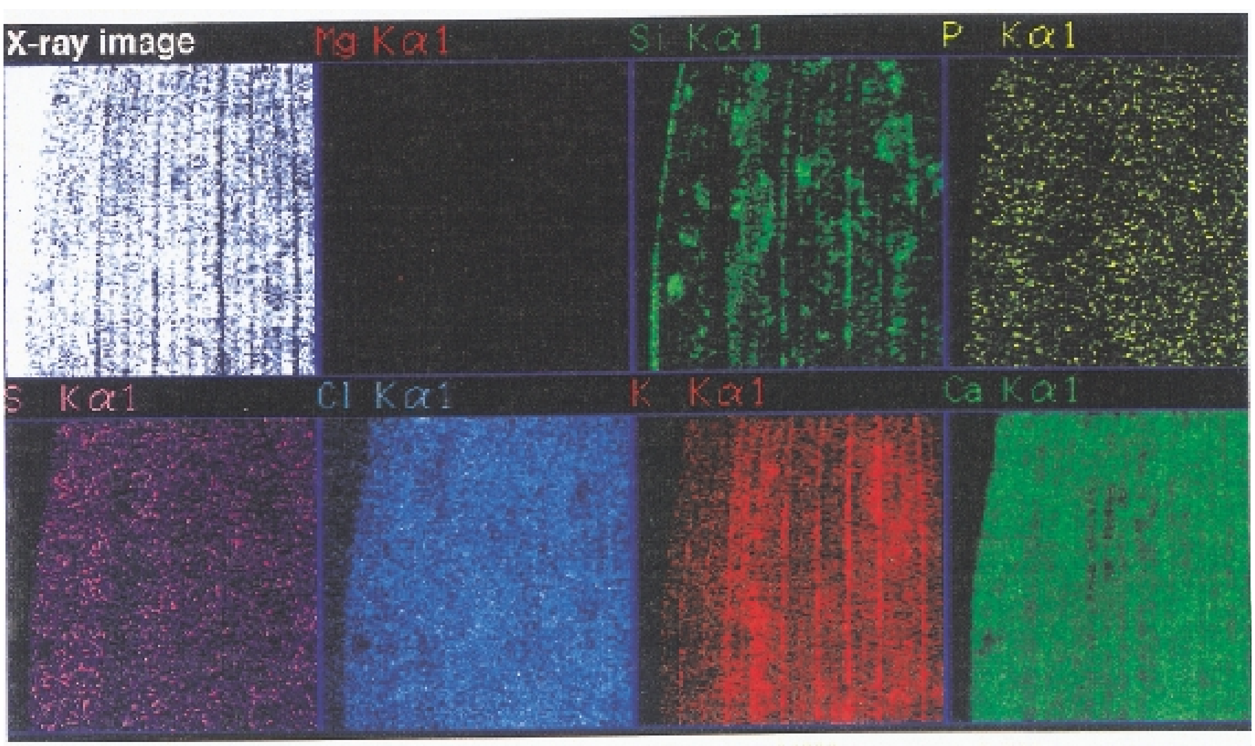

Figure 2. Distribution of relative concentration of Ca, K, Cl, S, P, Si and Mg in potassium deficient barley leaf of H plot.

Discussion

Well established is the fact that crops growing on sludge-receiving agricultural lands face numerous problems such as heavy metal toxicity, N deficiency, K deficiency, acidification, and the like. In the current investigation, we found K deficiency (Figure 1) in barley grown on soil with a long history of sewage sludge application. Total K content of plant and soil mentioned in our results coincided with that reported by Miah (1997), in which the author clearly suggested that under these K concentrations of sludge-receiving agricultural land, the occurrence K deficiency would not be unusual. This confirmed the occurrence of K deficiency in the sludge-receiving H plot as shown in Figure 1 with apparent K deficiency symptoms, namely, white necrotic lesions and brown necrotic patches. In relevance to Ca, Cl, S, P, Si, Mg and K distribution as revealed with EDS, high concentrations of these elements in K deficient barley leaf (Table 2) compared to control would seem to lie in the suggestion of crop removal derived from sludge application since, as a rule, the usual content of these elements in sludges, except for Cl, is high. However, a similar distribution pattern of the elements analyzed in either case confers advantages in that K deficiency does not harm the distribution and function of other essential elements in plants. The present work mainly summarizes the distribution of patterns of Ca, Cl, S, P, Si, and Mg against K distribution in K deficient barley leaf. The homogeneity in the distribution of Cl, S and P confirmed by our analytical results (Figure 2) is consistent with Nakanishi and Matsumoto (1992), who described smooth movement and redistribution to the other tissues of leaf by a transpiration system. Despite the differences in the patterns of Ca and Cl distribution within one leaf, their coupling clearly indicated their involvement in an osmoregulatory function through smooth movement and redistribution to the other tissues. A credible explanation for association of veins with Ca and Cl could be their movement and distribution to the other tissues through transpiration because of carboxyl-Ca-Cl association (Kinzel, 1989). Si distribution through veins, on the other hand, could be attributed to its involvement in decreasing light shading of the dense stands of barley improving leaf erectness (Marschner, 1995) and its protecting effects against insects and pathogens (Heath and Stupmf, 1986) through its dynamic redistribution to other tissues. Thus Si distribution also supports the validity of the preliminary pot experiments of K deficiency through its protecting effects against insects and pathogens. However, Si and P distribution would seem to lie in the suggestion of Grannsmann (1962), who clearly explained the possible mechanisms of the influence of the silicic acid on the uptake of phosphoric acid and other nutrients. Focused on Mg distribution, Knudsen et al., 1981 explained the insufficiency of the sensitivity derived from EDXRF. These authors clearly stated that EDXRF provides adequate sensitivity for Mg in most, but not in all, situations. We suggest, in case of Mg, that sensitivity derived from EDXRF was beyond detectable limit. However, the reason for the association of the characteristic K deficiency symptoms along with Si distribution rather than K distribution remained unclear under this analysis. It is reasonable to ask whether any correlation exists between the determination of elemental contents in plant by traditional methods and by EDXRF. In the current investigation, selected objectives were fulfilled by EDXRF. In contrast, total K concentration both in soil and plant for K deficiency was determined by the traditional method. The existence of a correlation between the traditional method and EDXRF regarding elemental concentrations in plant has been well clarified by Miah (1997). Therefore, EDXRF is a rapid means to determine relative concentrations of plant essential elements as it necessitates only a sampling of plant leaf from a standing crop plant and a flat iron drying followed by deep washing in distilled water thus reducing cost and analysis time. Simultaneously, it was also evident that EDXRF could not detect the characteristic deficiency symptoms of certain elements, such as the white necrotic lesions and brown necrotic patches of K deficiency. Similar distribution trends for Ca, Cl, S, P and Si between control and K deficient barley leaf also suggests that K deficiency does not harm the distribution, movement, or function of other elements essential for plant growth and development. We expect future research to clarify whether differences in K concentration between S and H sludge composts impacts the movement and distribution of the analyzed elements.

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利用能量散射X射線螢光光譜分析法觀測缺鉀大麥(*Hordeum vulgare* L.)葉片之元素分佈

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利用能量散射X射線螢光光譜分析法(energy dispersive X-ray fluorescence, EDXRF)進行大麥葉片內鈣、氯、硫、磷、矽、鎂及鉀等元素的定性分析。具有鉀缺乏症狀的葉片樣本由長期施用污泥堆肥的田區採得，然後葉片以去離子水徹底清洗，再利用熨斗熨燙使葉片乾燥。乾燥後的葉片以X-射線照射葉片樣本表面，照射面積直徑為100 μm。EDXRF對鈣、氯、硫、磷、矽等元素的相對含量有高度的靈敏度。其中氯、磷及硫含量的分佈圖譜是均勻的，依據圖像之密度顯示葉片樣品的均勻程度依序為氯＞硫＞磷。相對而言，葉片中的鈣、鉀、矽的含量分佈與葉脈之辨識程度依序為鈣＞鉀＞矽。然而葉片的症狀如壞疽斑點(necrotic lesions)與鉀的分佈並無相關性，但壞疽斑點處卻發現有相當量的矽存在。根據X射線穿透影像所得知之缺鉀的大麥葉片中鈣、氯、硫、磷、矽等元素的相對含量，研判缺鉀並不致於影響到葉片生長發育所需之其它元素的分佈。

關鍵詞：大麥；礦物元素；鉀；X射線螢光光譜。