Application of Electronprobe X-Ray Microanalysis to the Localization of Chemical Elements within and around Rice Roots Grown in Soils under Submerged Condition

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The electronprobe X-ray microanalyser (EMA) is a kind of scanning electron microscopes equipped with an X-ray detecting system. By using this apparatus, it is made possible to localize chemical elements in a microarea of a sample section and to observe the morphology of it at the same time[1,2,3]. Not only thin sections but also rather thick ones like soil blocks can be used for that purpose.

Thus, EMA may successfully be employed in studies on the *in situ* localization of chemical elements in soil with growing plant roots. It is expected that the distribution of chemical elements in such a microarea like soil-root interface can also be determined, although only few reports have been published on this aspect[4,5,9,10]. However, low sensitivity and poor resolution of EMA and the lack of development of proper techniques for sample preparation have prevented us from getting valuable information on such a micro-distribution of chemical elements. Although estimations of the sensitivity and the resolution of EMA have been reported so far to be fairly good[6], chemical elements in soils and plants at an average concentration of 1000 ppm or less can not practically be localized, and the distribution of chemical elements within a particle of a diameter of 1–2 μ or less can not be determined according to the author's experience.

Preparation of sample specimen is one of the difficult problems in EMA analysis. The original distribution of the elements in the section as well as the original morphology of it should be kept unchanged in the process of sample preparation and analysis. Further, the sections prepared must have electric conductivity and resistance against high vacuum condition of EMA. Various problems still remain unsolved for the preparation of the soil section satisfying these requirements.

In the present paper, the author's results of the application of EMA to soil sections containing rice roots, that were prepared by two different methods, i.e., the resin-embedding method[9] and the frozen-section method,[10] will be described briefly.

Results of analysis of the section prepared by the resin-embedding method [9]

In this method, the soil block was dehydrated with ethanol and then impregnated with epoxy resin. After polymerization of the resin, the block was sliced into the sections of 1 mm thick with a diamond saw. The cutface was wet ground on silicone carbide paper disks and finally polished with diamond paste of 1 μ grade.

The longitudinal view of a rice root grown in the soil of high zinc content (300 ppm) is shown as a secondary electron image in Plate 1.

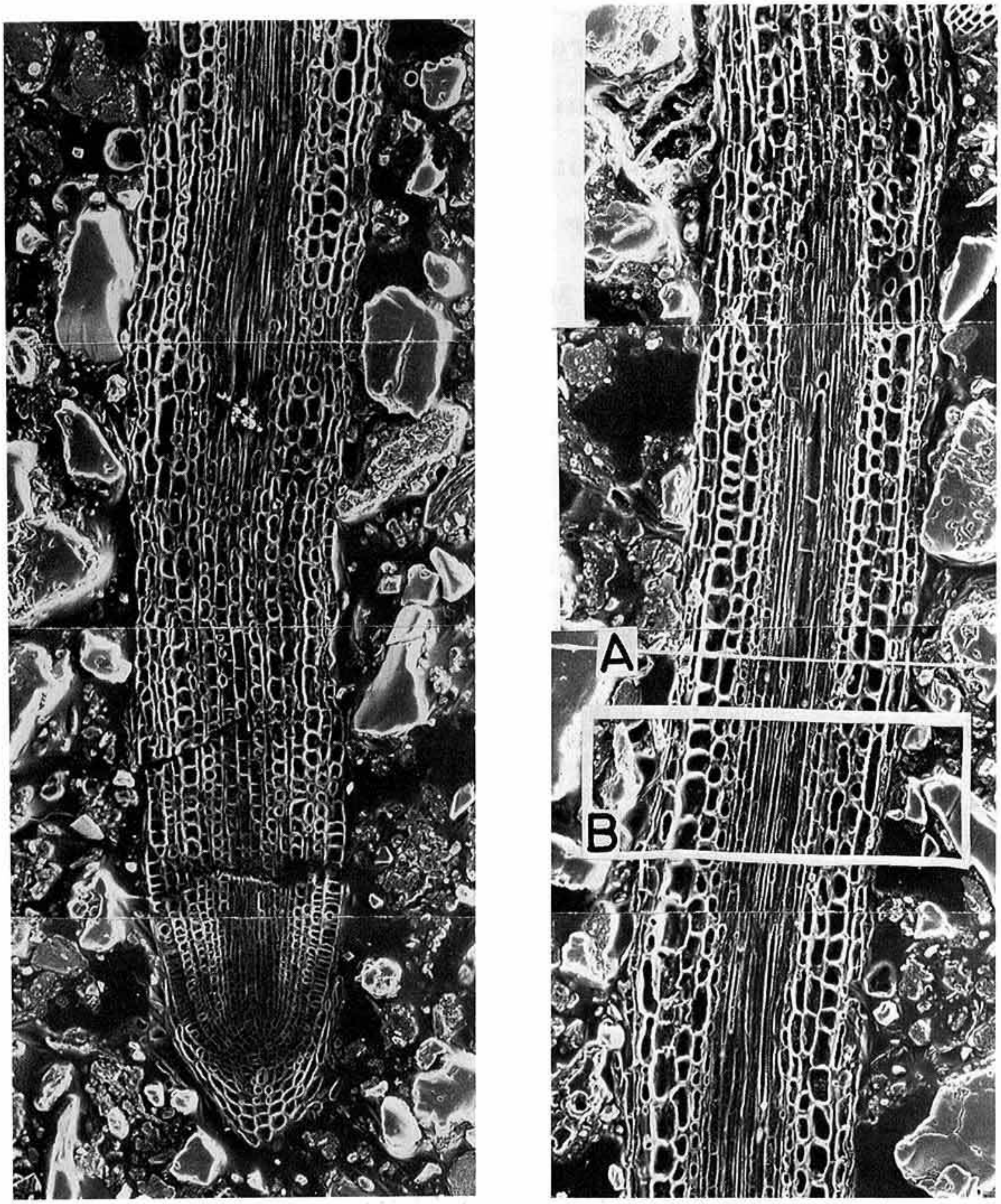

Plate 1. A longitudinal view of the rice root in the soil. The straight line lettered A indicates the line along which the chemical element concentration was determined by line analysis. The area lettered B was analysed for distributions of Zn, P, S and Ca.

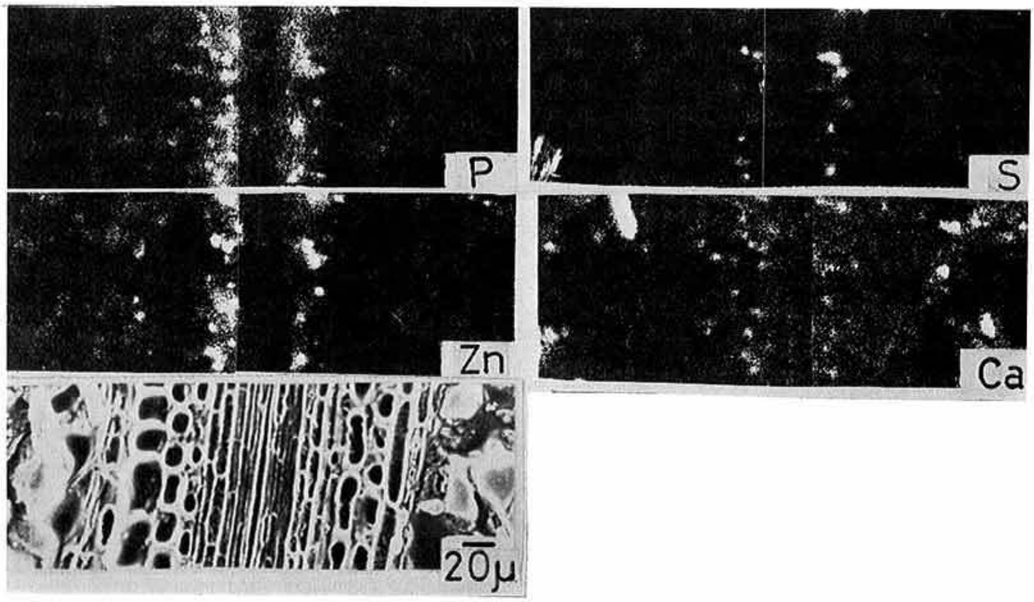

Plate 2. X-ray distribution images of Zn, P, S and Ca in the area B shown in Fig. 1, with a secondary electron image of the area

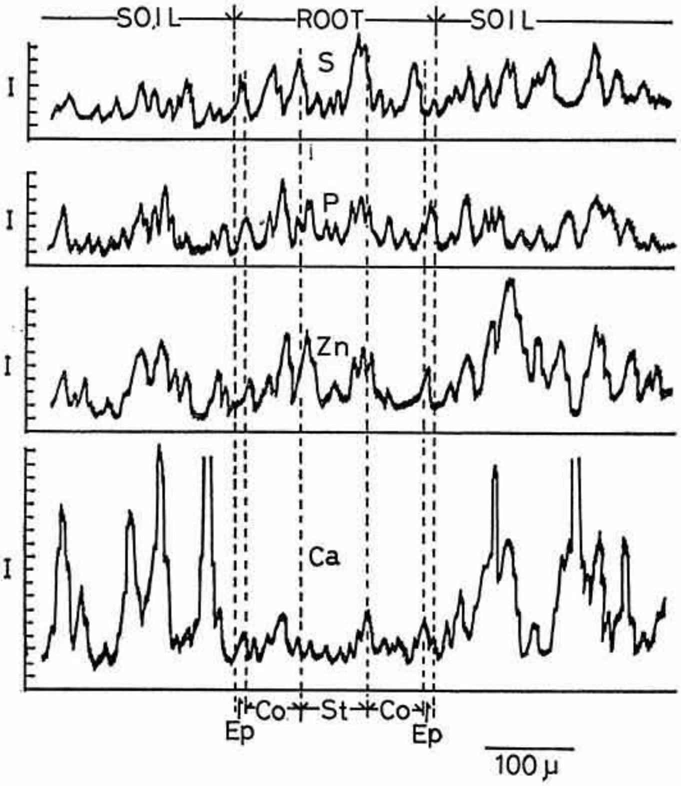

Plate 3. Results of line analysis on the distribution of chemical elements along the line A in Plate 1.

Lunt and Beals reported that the density of soil particles is higher at the immediate vicinity of root than in other areas due to root interception7). In Plate 1, however, such a difference in the density of soil particles is not observed. The author grew the rice root under the submerged condition, while Lunt and Beals under the upland condition. This difference in the cultural condition might have caused the different results. Under the submerged condition, the pressure developed on the root surface by the compaction effect of the expanding root may reach to a distant place without causing a reduction of intensity in a liquid phase. Thus, it can be said that the effect of the root interception on the absorption of ions is negligible under the submerged condition.

The distributions of Zn, P, S and Ca in an area in the region of maturation of this root are shown as X-ray distribution images in Plate 2 with the secondary electron image of the area. As the sample was fully dehydrated with ethanol, water soluble elements like potassium were mostly eluted, and hence not detected. In other words, only immobile elements were determined. All the elements detected are shown to be concentrated around the endodermis and the pericycle layer of the stele. Small accumulation of each element is also seen around the epidermis. Zn and P appear to be often in particles at the same sites in the root, which suggests the co-precipitation of Zn with P in the root tissue. This co-precipitation may occur only when Zn is added at a rather high concentration to the medium. The distribution of elements at the immediate vicinity of root is essentially the same as that in other areas (Plate 3).

Results of analysis of the section prepared by the frozen-section method10)

A recent advance in a scanning electron microscope has made it possible to observe and analyse directly a frozen specimen without complicated processing like fixation and dehydration. Namely, the soil block is frozen in liquid nitrogen and broken into pieces with a hammer. The pieces containing roots exposed at their broken surfaces are processed and sliced with a razor blade to give flat surfaces, and fixed to a sample holder by freezing in a cold chamber. Water soluble ions like K can be hold and localized in the sections prepared by this method. Direct observation and X-ray microanalysis of a frozen specimen is expected to be a simple and excellent way to minimize the morphological deformation and the disturbance of ion distribution within the tissue.

A secondary electron image of the frozen section of the rice root in the soil is shown in Plate 4. Neither shrinkage nor deformation of the root and the soil is observed.

The distributions of Si, Al and Fe at the area around and within the root were determined by line scannings along the line shown

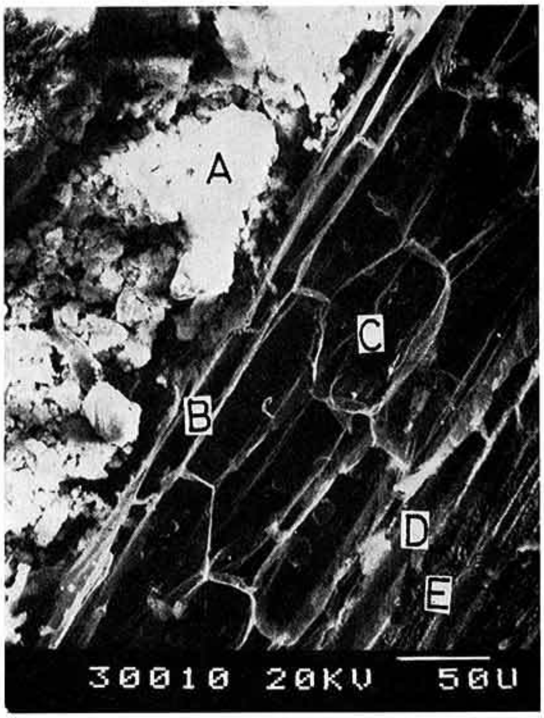

Plate 4. A secondary electron image of the frozen section of the rice root in the soil. Spot analysis was done on the micro area lettered A—E.

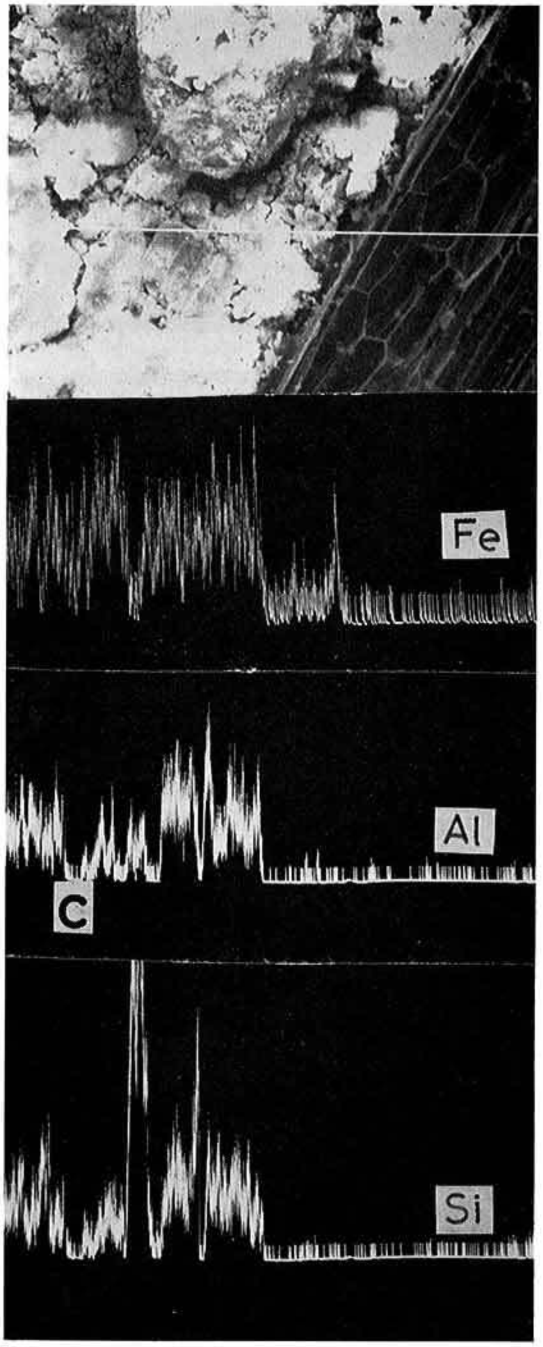

Plate 5. The tracks of line scannings on the distribution of Fe, Al and Si (Plate 5-a, b and c, respectively) along the line shown in the secondary electron image (Plate 5-a).

in Plate 5-a. The changes in the concentration of each element along this line is shown in Plate 5-b, c and d. All these elements were found to be much more concentrated in the soil than in the root. The small but significant accumulation of Fe was found at root surfaces but not at the surface of root hairs. It is postulated that Fe is accumulated at the surface of old parts of rice roots as ferric oxide and hardly at the surface of young active parts such as root tips[8]. Root hairs are also regarded as one of the most active parts of root. The accumulation of Si and Al is confined to the soil area.

Results of spot analysis of areas in the epidermis, cortex, endodermis and the pericycle layer are shown in Plate 6. The peak of characteristic X-ray of K is clearly found in each area. These peaks of K are high at the pericycle layer and the endodermis, while low at the cortex and the epidermis. To the contrary, the peak of Ca is higher at the epidermis than at the endodermis and the pericycle layer. However, in the section prepared by the resin-embedding method Ca was found more concentrated in the endodermis and the pericycle layer than in the epidermis, as mentioned above. The difference between these two sctions in Ca distribution is considered to be attributable to the difference in the sample preparation.

It can safely be said that the elements are likely to be concentrated at the surface layers of the root (the epidermis and the exodermis) and the inner layers (the endodermis and the pericycle layer of the stele). Elements are more concentrated in the surface layers than in the inner layers, but under other conditions they are more concentrated in the inner layers. At which layers, inner or surface, does the element accumulate mor can not be clearly determined. It seems to be influenced by the kind of ions, concentration of ions in the medium, and other factors. In the author's unpublished experiment, the ions of low concentration in the medium were accumulated mainly in the surface layers, while the ions added at high concentration were accumulated in both the surface and inner layers. The mechanism of the ion accumulation and the chemical nature of the

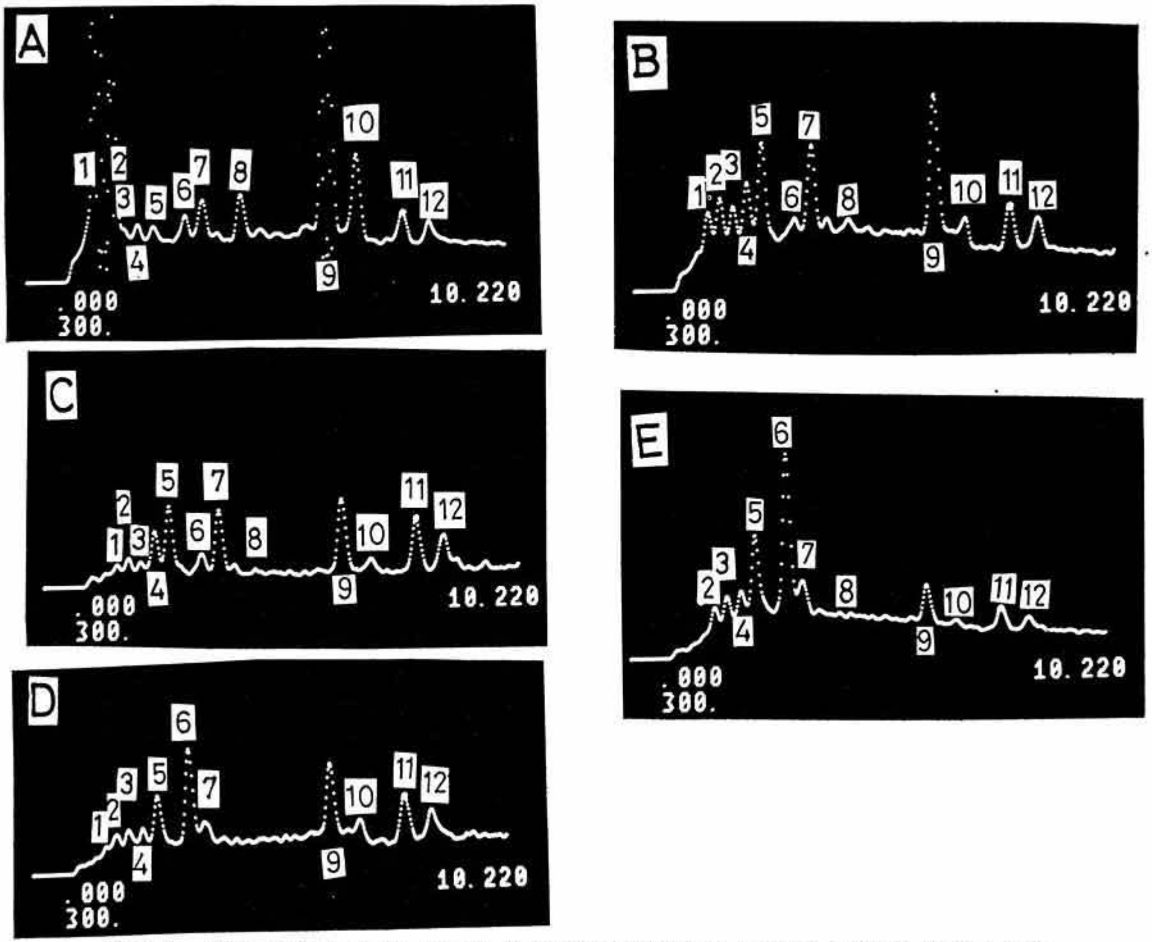

Plate 6. Characteristic X-ray spectra (0–10.22 kev) obtained by spot analysis at the area shown in Fig. 4. The area A shows the soil at the immediate vicinity of the root, B epidermis, C cortex, D endodermis and E pericycle layer of the stele. Peak 1; Al, 2; Si, 3; P, 4; S, 5; Cl, 6; K, 7; Ca, 8; Ti, 9; Fe (K_α), 10; Fe (K_β), 11; Cu (K_α), 12; Cu (K_β).

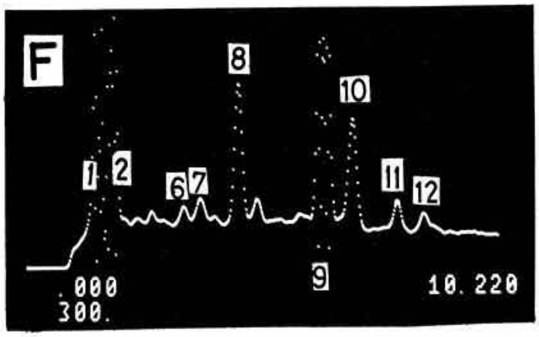

Plate 7. Characteristic X-ray spectrum (0–10.22 kev) obtained by spot analysis at the soil. This spectrum can be compared with the spectrum A in Fig. 6. No significant difference in the distribution of elements is observed.

accumulated elements may be different between these two layers For example, elements which accumulate at the outer layer are more soluble or exchangeable than those at the inner layer. This is the reason why Ca at the surface layer is leached out in the process of dehydration with ethanol.

Again, no specific distribution of an element except Fe was detected at the immediate vicinity of the root (Plate 7).

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