Arsenic distribution and speciation around rice roots

Health risks associated with the long-term, low-dose uptake of arsenic (As) have been of great concern in Southeast Asia, where rice is a staple crop. Among the agricultural crops, rice cultivated in flooded soil is the greatest contributor to inorganic As uptake through food. This is because As is present as As(III), which is mobile under the anaerobic condition in flooded soil [1]. When a soil is aerobic, As is less mobile because As(V) is strongly sorbed to mineral soil components such as Fe(III) hydroxide minerals (Fig. 1(a)). When anaerobic conditions develop in flooded soils, Fe(III) in Fe minerals is reduced to Fe(II), which is soluble. Consequently, the arsenic associated with Fe minerals is released into the solution as the adsorption phase is lost (Fig. 1(b)). However, soil in the vicinity of rice roots is aerobic because oxygen is supplied from above the ground to the root surface through the root aerenchyma (Figs. 1(b-d)). Therefore, dissolved Fe(II) is oxidized and deposited as Fe(III) hydroxide in the vicinity of the root surface (Figs. 1(b,c)). The Fe(III) hydroxide deposited around rice roots is referred to as Fe-plaque (Fig. 1(c)).

The significant correlation between the spatial distributions of As and Fe near rice roots indicates that As sequestration in Fe-plaque occurs [2]. Because Fe-plaque on rice roots has a greater affinity to As(V) than As(III), the oxidation of As(III) in the rhizosphere promotes the sequestration of As in Fe-plaque. Arsenic uptake from roots is partly restricted by Fe-plaque, but the degree of restriction strongly depends on the speciation of As. We observed the microscale distribution and speciation of As in the vicinity of rice roots in paddy soil by X-ray fluorescence mapping and X-ray absorption spectroscopy.

In a typical cultivation cycle of paddy rice in Japan, the soil is flooded for 5 months. The floodwater is drained approximately 10 days before harvesting and thereafter the soil redox condition gradually shifts from anaerobic to aerobic. Soil blocks containing rice roots were collected from a flooded paddy field and under an aerobic condition 1 month after harvesting. Soil blocks were freeze-dried, embedded in epoxy resin, and cut into thin sections with a thickness of 80 μm. The microscale As and Fe distributions in the thin sections were determined by synchrotron microbeam X-ray fluorescence (μXRF) with an excitation energy of 12.5 keV at BL4A of PF KEK. Arsenic *K*-edge μ-X-ray absorption near edge structure (μXANE) spectra were collected from various locations on the Fe-plaque and soil particles. The proportions of As(III) and As(V) were calculated by linear combination fitting using the standard XANES spectra of $NaAsO_2$ (As(III)) and Na_2HAsO_4 (As(V)). In addition, roots from rice seedlings that were grown under flooded conditions were removed from soil and frozen in liquid nitrogen. Transverse sections (50 μm in thickness) of the roots were prepared with a cryomicrotome (CM1850, Leica, Wetzlar, Germany) and were frozen until analysis. μXRF maps of the frozen root sections and As *K*-edge μXANES spectra were obtained by maintaining a frozen thin section under a stream of cryogenic N_2 gas (Cryojet XL, Oxford Instruments, UK) at **BL37XU** in SPring-8.

Optical microscope observation showed that Fe-plaque (shown as a dark-red stain around roots) coated the roots (Fig. 2(a)) but it did not noticeably cover the root apex when the roots were taken from flooded

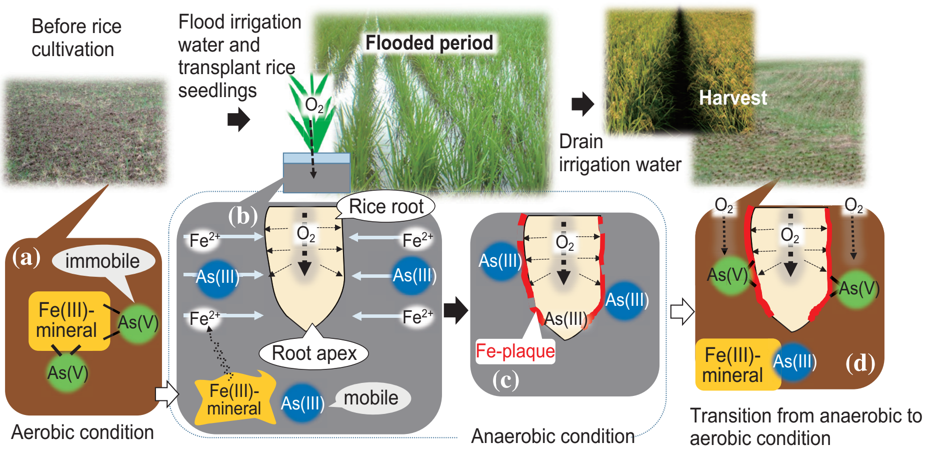

Fig. 1. Behavior of arsenic during rice cultivation in paddy field.

soil (Fig. 2(e)). The root apex is responsible for active solute uptake. The arsenic distribution map provided the evidence that As and Fe were absorbed by the root apex (Figs. 2(e,f)). The μXANES analysis indicated that the proportions of As(III) in the root interior and on the Fe-plaque did not differ significantly. Approximately 80% of the total As was in the form of As(III). This result suggested that As(III) was present near the root despite the occurrence of aerobic conditions in its vicinity. The presence of Fe-plaque can act as a barrier to As(V) absorption by roots but enhance As(III) absorption. Therefore, Fe-plaque may not reduce the absorption of As(III) by rice roots that are grown in flooded soil [3]. The absence of Fe-plaque around the root apex also allowed As absorption.

In the thin sections of soil prepared from the soil collected after rice harvesting, Fe minerals accumulated approximately 100 μm from the root (Fig. 3(a)). The fluorescence intensities of Fe Kβ did not differ between the Fe minerals in the soil matrix and those in the Fe-plaque around the roots (Fig. 3(b)). The fluorescence intensities of As Kα were greater on the Fe-plaque around the roots than on the Fe aggregates in the soil matrix (Fig. 3(c)). The relationships between the As Kα and Fe Kβ intensities clearly indicated the existence of two areas with different the As to Fe ratios. The areas with the greater As to Fe ratio corresponded to areas of Fe-plaque around the roots (Fig. 3(d)). The μXANES analysis indicated that 33% of the As that was associated with Fe minerals at a distance of 100 μm from the root surface was in the form of As(III). In contrast, As(V) was the dominant As species on the Fe-plaque that was attached to the root (Fig. 3(c)). The larger proportion of As(V) on the Fe-plaque after harvesting suggested that the shift to aerobic conditions was faster around the root than in the soil matrix [3].

In summary, Fe-plaque on rice roots did not effectively block As absorption from the roots under a flooded condition because As on the Fe-plaque was present in the form of As(III). After draining the floodwater, As on the Fe-plaque is oxidized faster than that in the soil matrix, thereby Fe-plaque sequestrates As(V).

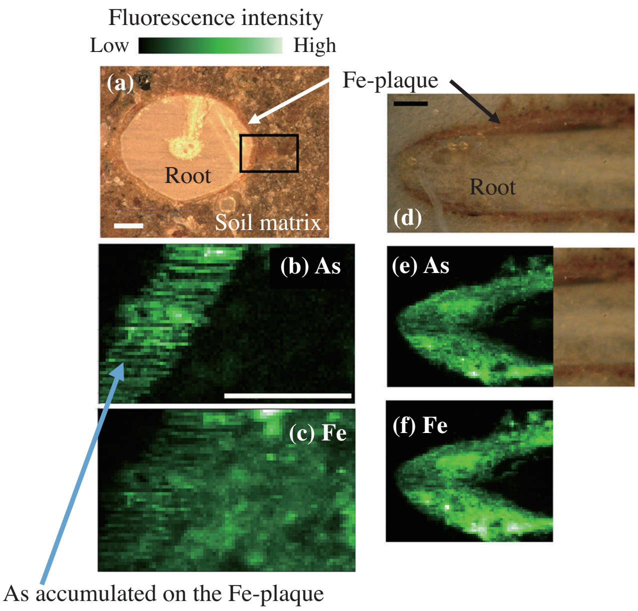

Fig. 2. Light microscope and micro-X-ray fluorescence (μXRF) images taken around rice roots grown under anaerobic condition. Light microscope images **(a, d)**, As images **(b, e)**, and Fe images **(c, f)** of freeze-dried resin embedded cross sections **(a-c)** and the transverse section of a frozen root **(d-f)** are presented. The black boxes in (a) show where μXRF maps were obtained. Black and white bars indicate 500 and 200 μm, respectively.

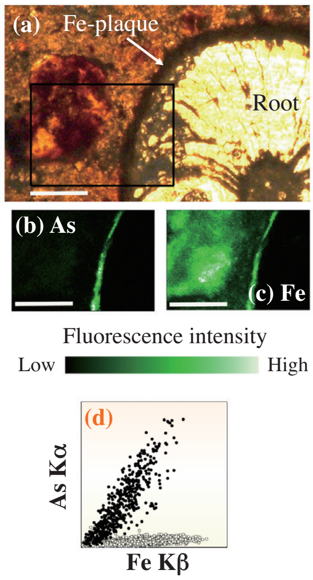

Fig. 3. Light microscope and μXRF images of rice roots collected 1 month after harvesting. Light microscope images **(a)**, As images **(b)**, Fe images **(c)**, and the relationships between the fluorescence intensities of As Kα (b) and Fe Kβ (c) around the roots **(d)** are presented. The black and white circles correspond to location on the Fe-plaque and Fe minerals, respectively (d). The black boxes in (a) show where the μXRF maps were obtained and the white bars indicate 200 μm.

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References

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