BIOSTIMULATED REDOX PROCESSES IN SOILS DISINFESTED WITH ETHANOL SOLUTION

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Introduction

The chlorinated fumigants chloropicrin (trichloronitromethane) and 1,3-dichloropropene (1,3-D) are major fumigants used in Japan to control soil-borne diseases in crops such as cucumbers, gingers, tomatoes, melons, green peppers, etc. Although these two fumigants are not recognized as stratospheric ozone-depleting substances such as methyl bromide, their rapid volatilization causes air pollution that is a public health concern. Biological Soil Disinfestations (BSDs) with organic materials such as wheat bran, rice bran or sugarcane molasses have been strenuously examined as an alternative to chemical soil fumigations in Japan. Hitherto known factors for the effects of BSDs are assumed to be decreasing in redox potential (Eh), O_2 concentration and pH, and generated-organic acids such as acetic acid and butyric acid. However, the fungicidal activity in soil amended with the organic materials has not been explained adequately by only these factors. The purposes of our study were to develop the new fumigation technique with low concentration of ethanol solution, and to elucidate the fungicidal mechanisms by evaluating metal ions dynamics in soil solutions under biostimulated anoxic condition with diluted ethanol solution.

Materials and methods

Laboratory experiments were conducted to fill up polypropylene containers (15.4 cm in height, 17.0 cm in width and length with an inner dimension, and ca. 4.45 L with an internal volume) with fresh soils (4 kg, Hydric Hapludand soil at the NIAES, Tsukuba and 6.5 kg Loamy Sand at Sousa, Chiba), and to place each 3 porous cups for soil water sampling and the inoculum bag to depth of 10 cm from the soil surface. Water contents of fresh Hydric Hapludand soil and Loamy Sand were 28.9 and 15.4 %, respectively. Given amounts of diluted ethanol solutions (0.0, 0.5, 1.0 and 2.0 v/v%) were applied at a volume of 1.0 L to each container. For a comparison purpose, 30 g of rice bran and 1.0 L of water were applied to other containers. These containers were maintained at 20 and 30 ^{o}C for 14 days in the incubators.

For sequential changes of physicochemical properties of soils by applying diluted ethanol, we focused on soil pH, Eh and O_2 concentrations in soils or soil waters. Soil pH and Eh were measured with multiple electrodes (Orion 3-Star Portable pH/ORP/Temperature Meter, Thermo Fisher Scientific, Inc.) and oxygen concentrations in soils or soil waters were measured with Oxygen Sensor Spectrometer (USB4000-FL-450 Spectrofluorometer and FOXY-T1000-RTD, Ocean Optics, Inc.) consecutively every 30 minutes.

7 and 14 days after treatment, soil solutions were withdrawn by porous cups and analyzed for organic acids and metals. The amounts of organic acids and metals were analyzed using High-Performance Liquid Chromatography equipped with a conductometric detector (HPLC; SCL-10A, CDD-6A Shimadzu) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES: VARIAN VISTA-Pro), respectively. The inoculum bags were retrieved, and viable pathogen was counted 7 and 14 days after treatment.

Results and discussion

The ethanol seems to act as an initiator to the reduced condition of soils and several phase reaction steps take place (Fig. 1). Acetic acid was detected in ethanol treatment. Concentration of other organic acids was negligibly low. In water treatment, no organic acids were detected (data not shown). These concentrations of organic acids had not sufficient fungicidal activity to the pathogen. Iron (Fe) and Manganese (Mn) are important and abundant element in the soil environment. Microbially stimulated reductive condition with ethanol solution has been linked to the release of Fe and Mn to soil water (Fig. 2). Furthermore, the pathogen was effectively killed in Mn^{2+} and Fe^{2+} solution. Therefore, we suggested Fe^{2+} and Mn^{2+} were important factors that incite cell death of the pathogen in this fumigation technique. In contrast to soil water of the Hydric Hapludand soil, that of Loamy Sand contained high concentrations of Fe and Mn, which raises the question how multiple metal releases are effected by microbial activities under changing redox conditions. Few studies have addressed the potential impact of metal dynamics on fungicidal activity of redox sensitive elements.This study linked fungicidal activity with metal dynamics in field soils under biostimulated reductive conditions with ethanol.

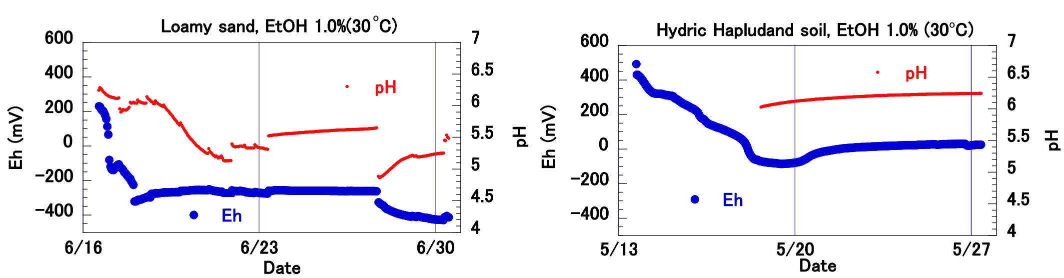

Fig.1 Transitions of Eh and pH treated with 1.0% ethanol solution

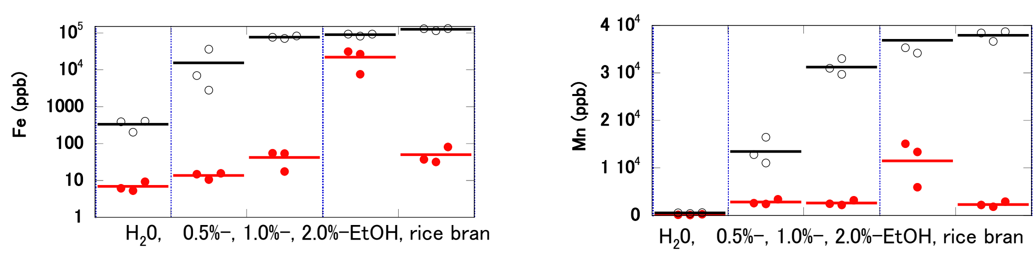

Fig. 2 Fe and Mn concentrations in soil water 14 days after treatment

○ : loamy sand, ●: hydric hapludand soil

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