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Improvement of ELISA analysis for soil residue analysis by reducing soil matrix effects

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Improvement of the commercially available fenitrothion-ELISA kit for soil residue analysis was examined. Recovery trials were conducted with 5 soils spiked at 0.5 µg/g soil. Interference of soil matrix was not adequately removed by the addition of synthetic zeolite in extraction, although it reduced Ca²⁺ concentration. Ultrafiltration at 9 kDa after dilution of the soil extracts gave enough results to compare with those obtained by gas chromatography. It was suggested that overestimation in ELISA was caused by high-molecular-weight humus-like compounds in soil extracts.

Keywords: ELISA, fenitrothion, soil matrix, synthetic zeolite, ultrafiltration.

Introduction

Pesticide residue monitoring of environmental samples by enzyme-linked immunosorbent assay (ELISA) facilitates a rapid, easy, and low-cost method for the analysis of numerous samples. Conventional methods, such as gas chromatography (GC) and high performance liquid chromatography (HPLC), are typically employed for the analysis of many pesticides. Extensive cleanup of the extract before detection by chromatography creates a lengthy analysis. In ELISA, the burden of many samples is not a problem since extensive cleanup with organic solvent and the use of solid phase extraction (SPE) are typically excluded to cut the analysis time. These advantages as a screening tool for identifying and categorizing large numbers of samples before GC and HPLC analyses have been commended by various authors.^{1,2)}

In our previous study, we used a commercially available fenitrothion-ELISA kit, designed for residue analysis of crop samples, for soil residue analysis, but recovery by ELISA was highly biased in some soil samples, and it was suggested that soil matrices, such as high-molecular-weight organics and divalent cations, influenced the ELISA reaction to overestimate recovery.³⁾

Zeolites are inorganic materials with large surface areas and well-defined internal structures of uniform cages, cavities or channels. Chemically, the compound is a crystalline aluminosilicate hydrate containing alkaline metal or alkaline earth metal. There are more synthetic than natural zeolites, but both have useful advantages as ion exchangers with a high surface area and adsorption capacity, which make them useful for removing heavy metals, water softening, and the control of malodors in waste water treatment.⁴⁻⁷⁾ In this study, we used a highly sensitive ELISA kit to enable higher dilution to reduce the soil matrix effect, and then considered the feasibility of employing synthetic zeolite and ultrafiltration in ELISA analysis.

Materials and Methods

1. Chemicals and Reagents

An analytical standard (98% purity) of fenitrothion (*O*, *O*-dimethyl *O*-3-methyl-4-nitro-phosphorothioate) was purchased from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan). Milli-Q® water (Nihon Millipore K. K., Tokyo, Japan) was used in the preparation of 9% methanol and for the dilution of soil extracts. A fenitrothion-ELISA kit was purchased from HORIBA, Ltd. (Kyoto, Japan). The kit was exclusively for analysis of crop samples, and its sensitivity range of detection was 0.15–2 ppb. Zeolite, synthetic A-4 powder, was purchased from WAKO.

2. Soil samples

Five soils were selected based on the results of previous study in which samples had overestimated (>120%) fenitrothion recoveries,³⁾ these were Honjo B, Izumo, Koryo, Jinzai, and Takano A. The samples were of sandy type and had higher Ca²⁺ and Mg²⁺ concentrations among other non-sandy soils. The corresponding physico-chemical characteristics of the samples are listed in a previous paper.³⁾

3. Soil preparation and extraction

Ten gram air-dried soil samples were spiked in triplicate with fenitrothion standard solution in acetone at 0.5 µg/g dry soil in a 50-ml Teflon FEP (fluorinated ethylene propylene) tube, and flushed gently with N₂ gas to expel the solvent. The soil samples were thoroughly mixed by gentle agitation and then placed inside the draft chamber until the solvent was completely evaporated. The extraction procedures were described previously.³⁾ The soil-methanol extract was fractionated for ELISA and GC analyses. An aliquot was diluted 100 times with Millipore water and methanol to attain 10% methanol for ELISA to reach the target concentration. The remaining methanol extract was used for GC analysis as described previously.³⁾

4. Extraction with methanol containing zeolite

The fenitrothion-spiked soil sample at 0.5 µg/g dry soil was added with 20 ml of methanol–distilled water (9 : 1) mixed solution containing 1.5 g zeolite, and 0.2 g NaCl.⁸⁾ The extraction fol-

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lowed the above procedure. An aliquot of the extract was taken and diluted for ELISA, and the remainder was used for GC analysis. The experiment was conducted in triplicate.

5. Ultrafiltration of soil extract

The diluted soil-methanol extracts were filtered with High-Performance Centrifugal Concentrators (Apollo 9kDa, Orbital Biosciences, Topsfield, USA) to remove high-molecular-weight soil organic matter prior to ELISA analysis.

6. ELISA and GC analyses

The analysis of fenitrothion in the ELISA plate was performed according to the manufacturer's instructions. The 0.15 and 2 ppb standard solutions were used for preparation of the standard calibration line. A model GC-14B with a flame thermoionic detector (Shimadzu, Kyoto, Japan) was used for GC analysis. The other detailed procedures for ELISA and GC analyses are described in a previous paper.³⁾

7. Ca^{2+} and Mg^{2+} ions in soil extract

Concentrations of co-extracted soluble calcium and magnesium ions in soil-methanol extracts were determined using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICPS 2000, Shimadzu). The extract was evaporated on a hot plate and the residue was dissolved in 0.1 M HCl solution and then filtered in a GC50 filter (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) prior to analysis.

Results and Discussion

One of the advantages generally attributed to immunoassays is the reduction of matrix interferences by diluting sample extracts at a higher rate. In our previous work, overestimation of recovery occurred at 10 times dilution,³⁾ therefore, we examined the effect of higher diluted extracts on the recovery using a more sensitive ELISA plate.

The recovery of fenitrothion from soil samples is shown in Fig. 1. The analysis gave adequate results as the percentage recoveries were within the acceptable range (83–88%) analyzed by GC. In contrast, apparent overestimations of recovery were observed in

ELISA for all soil samples (169–201%), even after 100 times dilution, and they were rather higher than those in our previous study using a low-sensitive ELISA kit with 10 times dilution (127–163%).³⁾ This could be explained by the difference in sensitivity of the antibody on the ELISA plate with soil matrices since two different plates were used. The lack of an effect of a higher dilution rate seems to be due to a change in the ELISA kit.

The addition of zeolite in the process of extraction showed no significant change in the extraction efficiency of fenitrothion as demonstrated in GC analysis, indicating less adsorption of fenitrothion to zeolite (Fig. 1), while in the case of ELISA analysis, the overestimation described above was reduced by the addition of zeolite (137–144%), but still above the range of acceptable recovery. Considering the ion exchange property of zeolite and previous studies showing the effect of Ca^{2+} and Mg^{2+} ions on ELISA analysis, it was expected that the added zeolite adsorbed Ca^{2+} and Mg^{2+} ions in the extracts, and reduced their adverse effects. By adding zeolite, concentrations of Ca^{2+} ion in the soil extracts decreased from 21–84 to 8–36 ppm, and slightly in Mg^{2+} from 2.8–16 to 2.8–12 ppm, suggesting that the added zeolite adsorbed Ca^{2+} ions. In our previous study, the ELISA reaction was affected by the addition of Ca^{2+} at 4.0 ppm, and not at 0.4 ppm. By diluting the extract 100 times, concentrations of Ca^{2+} ions decreased between 0.4 and 4.0 ppm without the addition of zeolite; therefore, the improvement of overestimation could be caused in part by reducing Ca^{2+} ions. In addition, removal of other components by zeolite might contribute to the improvement.

Recoveries in ELISA after ultrafiltration were comparable with those in GC both without zeolite (77–98%) and with zeolite (86–98%) treatments (Fig. 1). It was suggested that overestimation in ELISA was caused by high-molecular-weight (>9 kDa) humus-like compounds in the soil extracts, some of which were removed by zeolite by adsorption.⁹⁾ It was reported that ELISA was susceptible to the presence of humus-like substances,¹⁰⁾ that could adversely and non-specifically affect antibody affinity towards the pesticide analyte. The same effects of ultrafiltration were reported in ELISA analysis for pesticide residues in spinach.^{11,12)} Several ELISA kits were examined, and it was shown that ultrafiltration (UF10000, Waters, Tokyo, Japan) after dilution of extracts

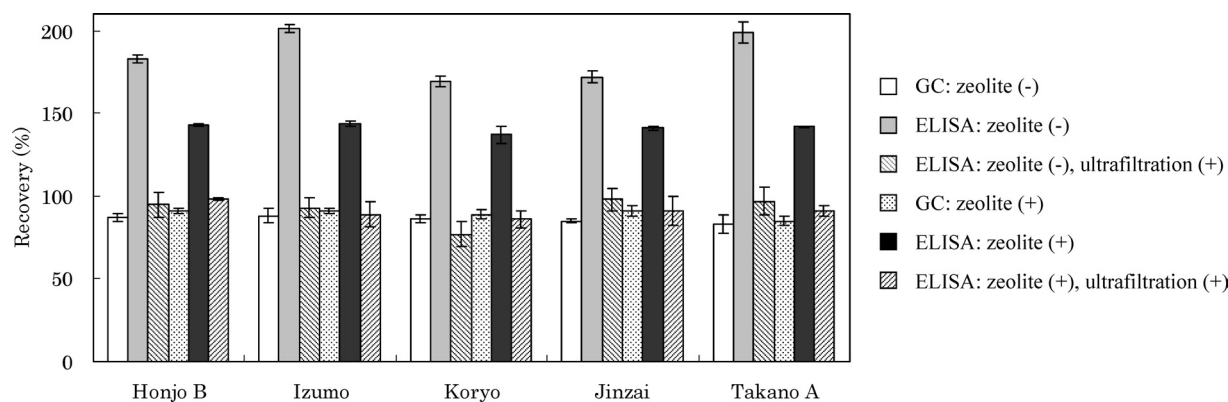


Fig. 1. Recoveries of fenitrothion from soil samples spiked at 0.5 $\mu\text{g/g}$ dry soil extracted with and without zeolite analyzed by GC and ELISA. An aliquot of the diluted extracts for ELISA was ultrafiltrated at 9 kDa. Error bars indicate standard deviation.

improved the overestimation caused by spinach matrix effects. In summary, additional clean-up by ultrafiltration of the diluted extracts most improved ELISA for fenitrothin soil residues analysis.

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