Fate of nonylphenol and 17β-estradiol contained in composted sewage sludge after land application

M. Minamiyama, S. Ochi and Y. Suzuki

ABSTRACT

Many environmental problems caused by endocrine disrupters (EDs) have been reported. Because little is known about the fate of EDs accumulated in sewage sludge, we carried out a study to clarify the fate of EDs in composted sludge after its application to soil. Nonylphenol (NP) and 17β-estradiol (E2) were measured for leachate and soil. High concentrations of NP and E2 were detected in the leachate at the early stage, but they decreased rapidly. Also, the high contents of NP and E2 in soil decreased significantly within 300 days. Because the decrease of NP and E2 in the soil was much larger than that of NP and E2 in the leachate, there must have been a physicochemical or biological decomposition mechanism in the soil layer. We also tried to clarify the transfer of NPs to plants from compost. In the experimental conditions of this study, the transfer of NPs to plants from compost was not observed.

Key words | 17β-estradiol, composted sewage sludge, lysimeter, nonylphenol, vegetables

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INTRODUCTION

In recent years, there have been many reports on environmental problems caused by endocrine disrupters (EDs) discharged as trace chemicals in many countries. In Japan, the Ministry of Construction carried out a national survey on the EDs pollution of river waters and treated wastewater in FY1998 (MOC 1999). Some trace chemicals suspected to be EDs were detected at almost all surveyed points, and relatively higher concentrations were found in treated wastewater. A further survey was carried out by the Ministry of Land, Infrastructure and Transport in FY2000, and showed that the removal ratios of these EDs in the wastewater treatment process were 70–99% (MLIT 2001).

On the other hand, little is known about the fate of EDs in the sewage sludge treatment process and the fate of EDs accumulated in sewage sludge when the sludge is used for agriculture. Figure 1 shows the trend of sludge utilization in Japan (Takahashi 2003). The amount of agricultural utilization was approximately 280 kt of dry solid (DS) in FY2000. Assuming that the amount of nonylphenol (NP), which is strongly suspected to be an ED, was 25.5 mg/kg-DS in the sewage sludge compost, approximately 7 tons of NP were spread in Japan via sewage sludge. Therefore, it is necessary to clarify the fate of EDs in composted sewage sludge after land application and to study countermeasures against EDs in the sewerage system. We carried out lysimeter experiment to clarify the fate of NPs and E2 from compost to leachate. We also carried out plant cultivation experiments to clarify the transfer of NPs to plants from compost.

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MATERIALS AND METHODS

Investigated endocrine disrupters

Nonylphenol (NP) and 17β-estradiol (E2) were selected as target compounds because these trace compounds were detected in higher amounts at many surveyed points during the national survey. Figure 2 shows an example of the structural formula of NP, which is an end product of the degradation of nonylphenol polyethoxylates (NPnEO) (Giger *et al.* 1984) which have been widely used as

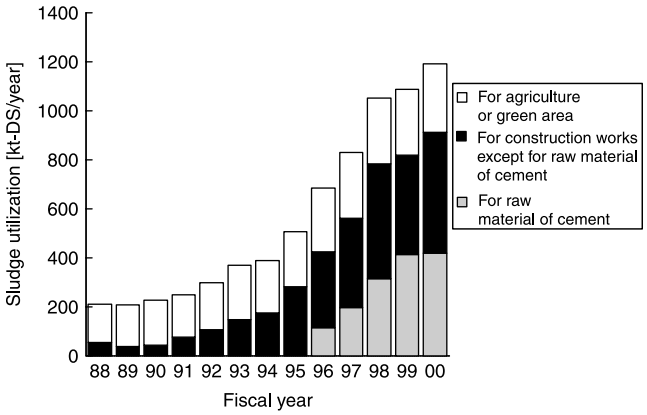

Figure 1 | Trend of sewage sludge utilization in Japan.

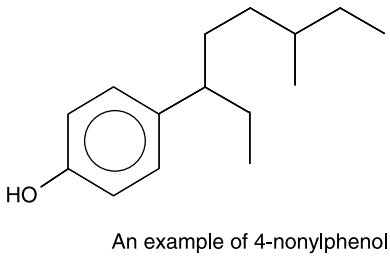

Figure 2 | Structual formula of nonylphenols.

detergents. Figure 3 shows the structural formula of E2, which is one of the estrogens of humans.

Lysimeter experiment to clarify the fate of NPs and E2 from compost to leachate

To reveal the fate of EDs in composted sludge after its application to soil, the lysimeter method was used.

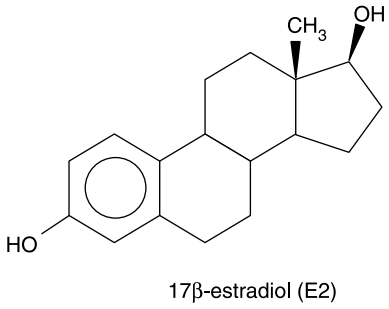

Figure 3 | Structual formula of estrogens.

Lysimeters

Four stainless-steel lysimeters were set outside. A schematic diagram of the lysimeters is shown in Figure 4. The lysimeters differed in soil layer conditions such as addition of sewage sludge compost or chemicals (Table 1).

The source of the composted sewage sludge was dewatered digested sludge of the conventional activated sludge process in the Tsuruoka Municipal Wastewater Treatment Plant located in Yamagata prefecture, Japan. Soil was red soil taken in Ishioka area, Ibaraki prefecture, Japan.

The amount of compost added to the soil was approximately 6 kg-DS/m^2. According to prefectural guidelines for agricultural use of sewage sludge compost, which provide for example 0.5 kg-DS m^{-2} $year^{-1}$ for agricultural use and 1 kg-DS m^{-2} $year^{-1}$ for vegetable field use, this amount was rather high. However, it was anticipated that if the usual amount of composts was used, then the concentration of NP or E2 in the leachate would be too low to detect, so this high amount was applied.

Sampling methods

Samples were taken from leachate and soil for two years and eight months.

The leachate samples were taken after each major rainfall. Figure 5 shows the amount of rainfall and

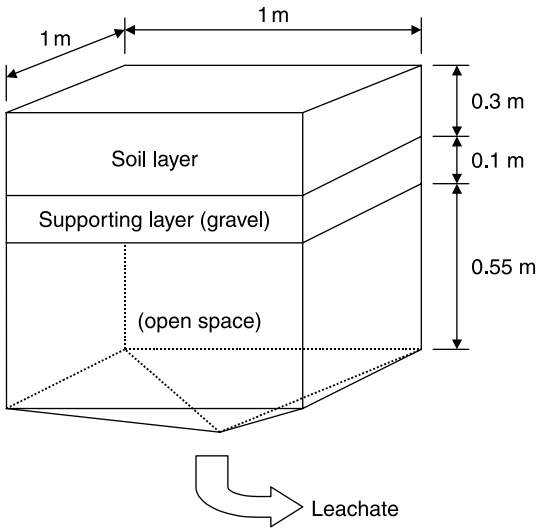

Figure 4 | Schematic diagram of a lysimeter.

Table 1 | Experimental conditions of soil layer in each lysimeter

	Conditions of soil layer	
Case 1	Soil	(control)
Case 2	Soil + Chemicals(NP, E2)	
Case 3	Soil + Compost	
Case 4	Soil + Compost + Chemicals (NP, E2)	

Added chemicals: NP = 500 mg/lysimeter, E2 = 17 mg/lysimeter.

volume of leachate in case 3. The total amount of leachate during the experiment was 6.3–8.2 times the volume of the soil layer.

Core samplers made of acrylic resin were used to take soil samples. The diameter of the core sampler was 40 mm. Two or three core samples were taken each time. In the early stage of this experiment, the soil samples were taken approximately every two months. After one year, the sampling interval had to be extended greatly, because few good points for taking soil samples remained.

Analytical methods

The NP and E2 in samples were measured using the Japan Sewage Works Association (JSWA) analytical methods for wastewater and sewage sludge (JSWA 2001).

The analytical method for NP was as follows. Each leachate sample was filtered using a glass fiber filter that had approximately 1 μm pores. NP in suspended solids on the filter was extracted with an ultrasonic extraction method using acetone. NP in the mixture of the filtrate and the extract was added to a Sep-Pak PS-2 (Waters Corp.), and extracted with methyl acetate. The extracted NP was ethylated and saponified, followed by a clean-up process using a florisil column, and then the NP was detected with GC/MS (Shimadzu QP-5050 A, Japan).

NP in soil was extracted with an ultrasonic extraction method using methanol, followed by a clean-up process using hexane saturated by methanol, 5% sodium chloride solution, dichloromethane, and water. The NP in the extract was ethylated and saponified, followed by a clean-up process using a florisil column, and then the NP was detected with GC/MS.

The analytical method for E2 was as follows. Each leachate sample was filtered using a glass fiber filter that had approximately 1 μm pores. E2 in the filtrate was added to a Sep-Pak C18 cartridge (Waters Corp.), and extracted with methanol. E2 in suspended solids on the filter was extracted with an ultrasonic extraction method using methanol. Both extracts were mixed and concentrated with a rotary evaporator, then dried. The residue was dissolved in dimethyl sulfoxide (DMSO). E2 in DMSO was detected with the enzyme linked immunosorbent assay (ELISA) method. The 17β-estradiol ELISA kit (Assay Designs, Inc.) was used.

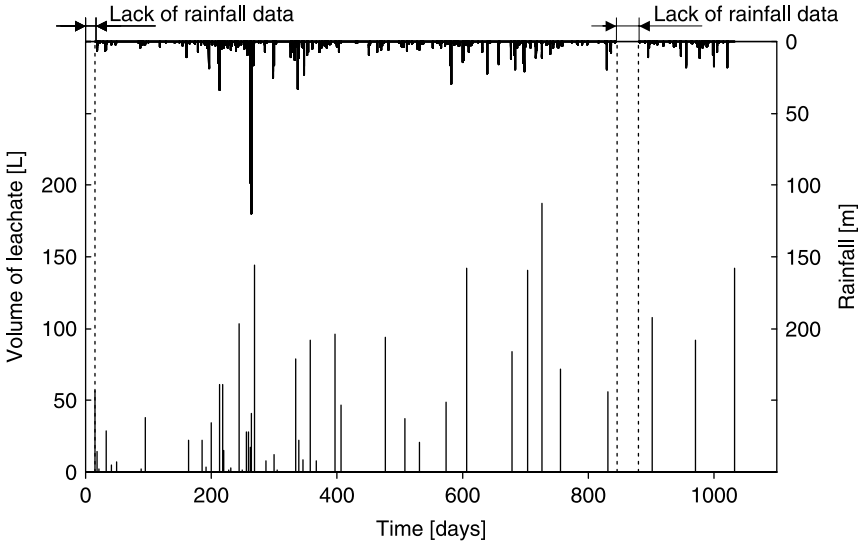

Figure 5 | Volume of sampled leachate and amount of rainfall.

E2 in soil was also extracted with an ultrasonic extraction method using methanol. The methods were almost same as those of E2 in the suspended solids in leachate.

The analytical methods used in this experiment have some problems, because ELISA reacts with other substances such as estrone (E1) (Komori *et al*. 2004). When the amounts of estrogens in sewage sludge compost were measured using LC/MS/MS, E2 was not detected but E1 was detected in the compost samples. However, because the analyzed data were continuous, the values obtained using ELISA were treated as the amounts of E2.

Total organic carbon (TOC) was measured with a TOC analyzer (Shimadzu TOC-5000 A, Japan).

Plant cultivation experiments to clarify the fate of NP and NPnEO from compost to plants

In order to clarify the biomagnification of NPs, the plant cultivation experiments were conducted. The komatsuna (*Brassica camperstris*) as a leaf vegetable and the radish (*Raphanus sativus*) as a root vegetable were used for the experiments.

Cultivation method

To set up the basic cultivation condition, the testing method for toxicity of fertilizer on cultivated plant (MAFF 1984) was used as reference. The stainless steel container (312 mm × 520 mm × 134 mm in height) was filled up with the mixture of soil, boiling stone, and sewage sludge compost. Soil was red soil taken in Ishioka area, Ibaraki prefecture, Japan. The boiling stone was mixed with the red soil by 3:1. The source of the composted sewage sludge was dewatered raw sludge that ferric chloride and lime were used for sludge conditioning. The amount of the compost added to the soil was 1,430 mg/container as nitrogen equivalent in the low mixing ratio condition and 8,580 mg-Neq./container in the high mixing ratio condition. The low mixing ratio condition was decided because the condition was good as for growth of komatsuna in a preliminary cultivation experiment. Because the high mixing ratio condition was not good as for growth of plants, it was not appropriate to set higher mixing ratio conditions. Calcium superphosphate, ammonium sulfate, and potassium chloride were used for adjustment for nitrogen, phosphate, and potash, respectively. The amounts of these added chemical fertilizers were 1,001 mg/container as N, P_2O_5, and K_2O, respectively.

In the komatsuna cultivation experiment, 230 grains/container were seeded and they grew for 56 days. In the radish cultivation experiment, 8 grains/container were seeded and they grew for 42 days. In order to adjust moisture of the container, water was added so that the weight of the whole container was kept constant.

Analytical methods

The NP and NPnEO in plant samples were measured based on the methods reported by Minamiyama *et al*. (2007). Pressurized fluid extraction method (methanol, 2000 psi, 100C, 10min) was used for extraction of NP and NPnEO from samples. In analysis of NP and NPnEO in stalks and leaves of komatsuna and in radish, the solid phase extraction (Sep-Pak plus C_{18}) was performed to clean up the extracted solution. The detection was performed using a HPLC(fluorescent detector). In analysis of NP and NPnEO in roots, separation of roots and soil was tried using the ultrasonic cleaner.

RESULTS AND DISCUSSION

Endocrine disrupters in leachate

Trends of total organic carbons in leachate

Figure 6 shows the concentration of total organic carbons (TOC) in leachate, which was high until about 100 days with the total leachate volume of approximately 100 L and then the TOC decreased rapidly with time.

Trends of concentrations of NP and E2 in leachate

High concentrations of NP and E2 were detected in the leachate at the early stage of the experiment, and decreased rapidly with time (Figure 7). These trends were similar to that of TOC in the leachate. Figure 8 shows the ratios of NP or E2 to TOC. Although these ratios were not constant, the E2/TOC ratio was around 10^{-7}. Therefore, the fate of E2 in the leak-out process was almost the same as that of the TOC substances.

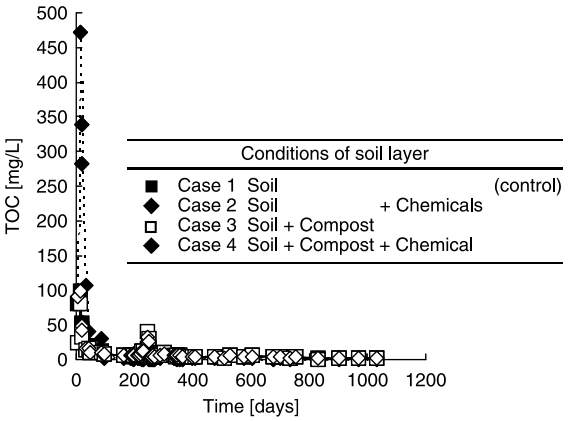

Figure 6 | TOC in the leachate from lysimeter.

Jacobsen *et al.* (2004) reported that NP was not measured in the leachate. In this experiment, the NP was measured in the leachate. The maximum concentration of NP (2.7 μg/L) obtained in this experiment was slightly lower than the proposed water quality criteria of NP for aquatic life (6.6 μg/L for freshwater organisms) which is based on the acute and chronic toxicity of NP (U.S. EPA 2005).

Effect of compost on concentrations of NP and E2 in leachate

The concentrations of E2 in cases 3 and 4 with compost were far higher (over 20 times) than those in cases 1 and 2 without compost at the early stage of the experiment. On the other hand, the concentrations of NP in cases 3 and 4 with compost were only 1.4–1.9 times those in cases 1 and 2 without compost. From the experimental results, it is thought that NP has high affinity to compost and is retained in the compost, on the other hand, E2 in the compost leaked out more easily than NP.

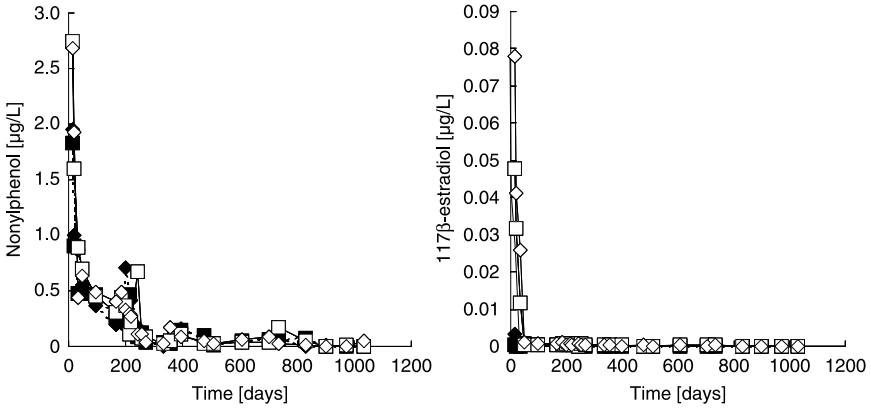

Figure 7 | NP and E2 concentration in the leachate from lysimeter.

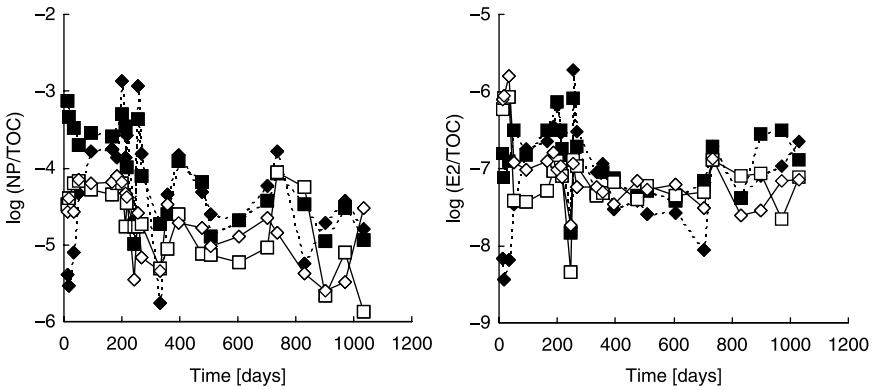

Figure 8 | Ration of NP and E2 to TOC.

Total amount of NP and E2 in leachate

The percentages of total amount of NP and E2 that leaked out during the experiment to the initial content in the soil layer are shown in Table 2. Very small amounts of NP and E2 leaked out from the soil layers, with a lower leakage ratio for NP in the cases with compost.

Endocrine disrupters in the soil layer

The NP and E2 contents in the soil layer of each lysimeter are shown in Figure 9. High contents were observed in the cases with compost, but they decreased significantly to almost the same level as those of the control after 300 days. The NP contents decreased from the beginning of the experiment in cases 3 and 4, and the rate was almost constant as approximately 2.4 μg kg-DS-1 d-1. As for the E2 content, the reduction rate was low until about 200 days, and then the E2 content decreased rapidly.

Mass balance of endocrine disrupters in the lysimeters

The amount of the decrease of NP and E2 in the soil layer was much larger than the amount of NP and E2 that leaked out with leachate. Although the vapor pressure of NP is slightly high such as approximately 0.3 Pa at 25 °C (EU 2002), the occurrence of NP in ambient air might be negligible from the estimation based on the Mackay's fugacity model level I (MOE 2001). Because the vapor pressure of E2 is approximately 3×10^{-8} Pa (Lai 2002), the volatilization of E2 from the soil surface is negligible. Therefore, there must be a physicochemical or biological decomposition mechanism in the soil layer. Because the NP content decreased linearly in the early stage of this study, the decomposition mechanism of NP might be the physicochemical one or biological one by indigenous microorganisms. In the latest research, the possibility of the decomposition of NP by indigenous microorganisms in the compost was suggested (Shoji *et al.* 2006). On the other hand, acclimation of microorganisms was necessary before the E2 decomposition mechanism started.

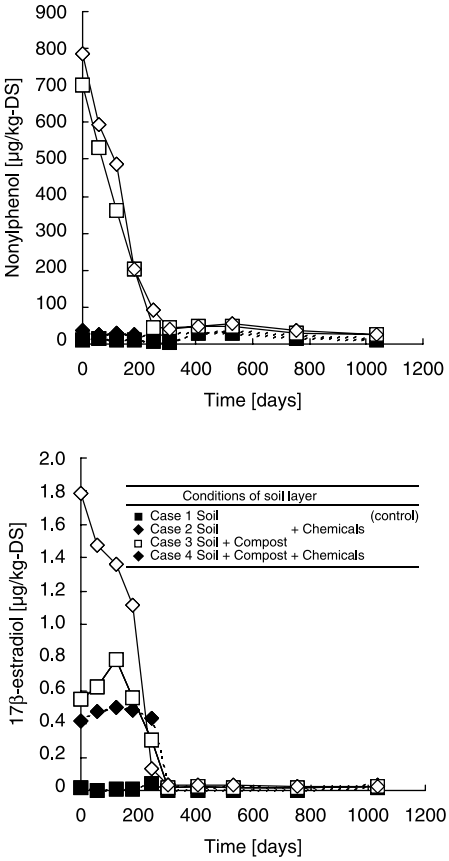

Figure 9 | NP and E2 content in the soil layer of the lysimeter.

Table 2 | Percentage of leaked out EDs (%)

	Addition of sewage sludge compost			
	No		Yes	
	NP	E2	NP	E2
Addition of chemicals				
No	13	8.2	0.45	5.3
Yes	5.0	0.55	0.34	2.5

NPs in plants cultivated using sewage sludge compost

The amounts of NP and NPnEO in plant samples are shown in Table 3. In these experiments, NP was not detected from the plant samples. The amounts of NPnEO in the plant samples were too low to determine quantities. In the experimental conditions of this study, the transfer of NPs to plants from compost could not be observed.

Table 3 | Results of plant cultivation experiments

	Komatsuna						Radish					
	No seed			Seeded 230 grains/container			No seed			Seeded 8 grains/container		
Sewage sludge compost	Without compost	Low mixing ratio condition	High mixing ratio condition	Without compost	Low mixing ratio condition	High mixing ratio condition	Without compost	Low mixing ratio condition	High mixing ratio condition	Without compost	Low mixing ratio condition	High mixing ratio condition
At the start of cultivation												
Soil												
NP	–	–	–	–	–	–	ND	Tr(0.01)	0.06	ND	Tr(0.02)	0.1
NPnEO ($n = 1$–4)	–	–	–	–	–	–	ND	ND	ND	ND	ND	ND
NPnEO ($n = 5$-)	–	–	–	–	–	–	ND	ND	0.05	ND	Tr(0.01)	Tr(0.02)
At the end of cultivation												
Soil												
NP	ND	Tr(0.02)	0.07	ND	ND	0.4	0.04	ND	0.09	0.03	0.03	0.07
NPnEO ($n = 1$–4)	ND	ND	0.3	ND	ND	0.2	ND	ND	ND	ND	ND	ND
NPnEO ($n = 5$-)	0.07	0.1	0.3	0.1	0.2	0.3	ND	ND	ND	ND	ND	ND
Plants												
Foliage (leaf and stalk)												
NP	–	–	–	ND	ND	ND	–	–	–	ND	ND	ND
NPnEO ($n = 1$–4)	–	–	–	ND	ND	ND	–	–	–	ND	ND	ND
NPnEO ($n = 5$-)	–	–	–	ND	Tr(0.4)	Tr(0.4)	–	–	–	ND	ND	ND
Roots												
NP	–	–	–	ND	ND	ND	–	–	–	ND	ND	ND
NPnEO ($n = 1$–4)	–	–	–	ND	ND	ND	–	–	–	ND	ND	ND
NPnEO ($n = 5$-)	–	–	–	Tr(0.7)	Tr(1)	Tr(0.8)	–	–	–	ND	ND	ND

Unit: μg/g-dry, ND: Not Detected, Tr. Trace, –: No data.

CONCLUSIONS

To clarify the fate of EDs in composted sludge after its application to soil, a lysimeter experiment and plant cultivation experiments were carried out.

(1) The two-year eight-month lysimeter experiment revealed that very small amounts of NP and E2 leaked out from the soil layers and that there must be a physicochemical or biological decomposition mechanism in the soil layer. High concentrations of NP and E2 were detected in the leachate at the early stage of the experiment, and decreased rapidly with time. The ratio of NP and E2 leaked out from the soil layers was 0.34–13% and 0.55–8.2%, respectively. High contents of NP and E2 in the soil were observed in the cases with compost, but they decreased significantly to almost the same level as those of the control after 300 days. Because the NP content decreased linearly in the early stage of this study, the decomposition mechanism of NP might be the physicochemical decomposition or biological one by indigenous microorganisms. On the other hand, acclimation of microorganisms was necessary before the E2 decomposition mechanism started.

(2) According to the results of plant cultivation experiments, the transfer of NPs to plants from compost was not observed in the experimental conditions of this study.

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