

Article

Aerobic and Anaerobic Biological Degradation of Pharmaceutically Active Compounds in Rice Paddy Soils

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Featured Application: Environmental management for the use of sewage sludge in rice paddy fields.

Abstract: One of the concerns against the use of sewage sludge for agricultural purposes is emerging contaminants contained in sewage sludge. Most of the studies on biological degradation of pharmaceutically active compounds in agricultural land were carried out with water-unsaturated soils under relatively aerobic conditions. In this study, the degradation of pharmaceuticals mainly including non-steroidal anti-inflammatory drugs (NSAIDs) was investigated in Asian rice paddy soils that are flooded in anaerobic condition. The experimental results showed that the concentrations of the target pharmaceuticals excluding the exception of naproxen were poorly decreased in anaerobic condition. On the other hand, the microbial communities of the soils contained the aerobic degraders of clofibric acid and diclofenac, which are generally persistent in biological wastewater treatment. The higher degradation rates in aerobic condition suggest the possibility of enhanced degradation of pharmaceuticals by supplying oxygen with plowing anaerobic rice fields or with drying the field in off-season for farming.

Keywords: paddy soil; pharmaceutically active compounds; sewage sludge; wastewater treatment

1. Introduction

Sewage sludge contains a wide spectrum of organic and inorganic contaminants which are separated from the liquid phase in wastewater treatment. The main disposal routes are incineration, landfill, composting, and the use in construction depending on regional situations and local regulation. Agricultural use of sewage sludge can reduce the use of chemical fertilizers as well as mitigate problems resulting from unwise processing of the sludge [1]. Although agricultural use of sewage sludge reaches 50% in Denmark [2], only 15 % of the annual generation of sewage sludge was utilized for agricultural and gardening purposes in Japan, where rice cultivation is very active, according to a statistical survey by Japanese government in 2017. It is necessary to deliberate on various environmental impacts with rice cultivation to increase the use of biosolids [3].

One of the concerns against the use of wastewater byproducts for agricultural purposes is emerging contaminants contained in wastewater and sewage sludge. Pharmaceuticals and personal care products are frequently detected with a concentration range 10–10,000 ng/dry-g in primary sludge and in secondary sludge [2,4–6]. Pharmaceuticals in treated and untreated wastewater are also of concern in wastewater reuse for agricultural purposes [7]. The concentrations of pharmaceuticals in soils irrigated with reclaimed wastewater was in the range from less than 1 ng/g to several hundred

ng/g [8,9]. Antibiotics and antibiotic resistance in microbial community are of emerging concern in the agricultural environment receiving sewage sludge and reclaimed wastewater [10,11].

Due to ubiquitous presence of pharmaceuticals in agricultural soils receiving sewage sludge, the fate of pharmaceuticals in sewage sludge treatment processes before agricultural application has been investigated [5]. The effect of type of sludge treatment (aerobic or anaerobic) on the concentrations of residual pharmaceuticals in stabilized sludge was reported to be compound-dependent [12,13], although the composting process has been shown to be effective for the degradation of various pharmaceuticals [14]. Taking all available information into account, it is anticipated that a considerable part of pharmaceuticals still remain in biosolids even after sludge treatment processes.

Non-steroidal anti-inflammatory drugs (NSAIDs) are often focused as target compounds in environmental studies because of the high residual concentrations in sewage sludge in spite of their relatively water soluble nature [2]. A study on the degradation of pharmaceuticals in four US agricultural soils reported half-life times of 1–20 days for ibuprofen, naproxen, and diclofenac [15]. The removal of NSAIDs in reed beds for sludge treatment was reported to be high, although complete separation of removal into adsorption, biodegradation and transformation to other compounds was difficult [16]. The fate of NSAIDs in plants and crops after taking up pharmaceuticals from reclaimed water and sewage sludge have been actively investigated [17]. Nonionic pharmaceuticals were concentrated on leaves at higher concentrations than ionic pharmaceuticals on the occasion of the irrigation of root vegetables with treated wastewater [18].

Limited information is available for the fate of xenobiotics in paddy fields except for agricultural chemicals [19]. Although several studies showed that the degradation of NSAIDs in agricultural soils can be expected to a certain extent [15], it is not clear whether the previous findings on the degradation can be applied to rice paddy fields, that are flooded in anaerobic condition. In addition, Asian soils have to be examined for the degradability of pharmaceuticals because the highest rice producers are Asian countries. The fate of pharmaceuticals in water-saturated different soils taken from various paddy fields was investigated in this study. Six NSAIDs and one metabolite of lipid regulators were selected as target compounds in this study due to their wide use and their high residual concentrations in sewage sludge.

2. Materials and Methods

2.1. Soil Samples

The degradation of pharmaceuticals was investigated in six soil samples taken at four different locations. Humic andosol (2–10 cm in depth, water content 42% by weight) was taken at a rice paddy field at Ome, Tokyo, Japan in flooded season (September 2018). Two samples of gray lowland soil (2 cm to 10 cm in depth) were taken at a rice paddy field in Kawagoe, Saitama, Japan in flooded season (Jun 2017, water content 61% by weight) and dry season (Dec 2017, water content 15% by weight). Two samples of light-colored andosol with two different depths (surface top soil (0–2 cm, water content 50% by weight) and subsurface top soil (2–10 cm, water content 55% by weight) were taken at a flooded iris garden, Hachioji, Tokyo, Japan in Oct 2017. Wet andosol (2–10 cm in depth, water content 41% by weight) was taken at rice paddy field in Tokamachi, Niigata in flooded season (June 2018). Water contents of the soil samples were measured from weight losses by drying in an oven at 105 °C overnight.

2.2. Target Pharmaceuticals

The target pharmaceutically active compounds in this study were clofibric acid (CA), gemfibrozil (GFZ), ibuprofen (IBP), fenoprofen (FEP), ketoprofen (KEP), naproxen (NPX), diclofenac (DCF), and indomethacin (IDM). CA is a metabolite of lipid regulators clofibrate, etofibrate, and etofyllinclofibrate. Other IBP, FEP, KEP, NPX, DCF, IDM are commonly used NSAIDs.

These compounds are weak acids, and exist as anions in a neutral pH range, which is an important factor considering sludge-water partitioning of the compound [20].

2.3. Degradation Experiment

In the case of humic andosol sample taken at Ome, pH of the soil samples was adjusted at 5, 7, and 9 by adding hydrochloric acid or sodium hydroxide, whereas original pH (5.8–6.7) was not controlled for other soils. In order to evaluate clearly the potential degradability of pharmaceuticals, the target pharmaceuticals were added to the samples before incubation at a known concentration. Each flask contained 4 g (wet weight) soil and 25 mL of pharmaceutical solution containing the seven pharmaceuticals at initial individual concentrations of 200 µg/L, glucose (100 mg/L), and yeast extract (25 mg/L) as carbon sources and nutrients. Oxygen in the solution was purged in advance with nitrogen gas not to inactivate the anaerobic bacteria in the soil samples. The prepared slurry was then incubated at 20 °C for 14 days on a shaker (60 rpm) to keep the slurry at a completely mixed state. Half of the flasks were capped with breathable sponge to keep the slurry aerobic, while others were completely sealed to provide anaerobic environments. The samples were kept in dark to avoid the effect of photo-degradation. Dissolved oxygen (DO) concentration after incubation was measured by a DO sensor (SG9, Mettler–Toledo AG, Switzerland). Concentration of the pharmaceuticals were measured at day 1, day 7, and day 14 by using whole volume of the slurry in a flask among three flasks prepared at day 0 for one test condition. The sterile samples with soil (121 °C, 20 min in an autoclave) were prepared as references to evaluate the contribution of biological degradation separately from adsorption and abiotic reactions. The pharmaceutical residual ratios were calculated by measured pharmaceutical dose in the flask divided by 5 µg, which was equivalent to the initial pharmaceutical dose ($200 \mu\text{g/L} \times 25 \text{ mL} \times 0.001 \text{ L/mL}$).

2.4. Analytical Method for Target Pharmaceuticals

The target pharmaceuticals in the liquid and solid phases were separately quantified by GC/MS method after derivatization by pentafluorobenzyl bromide (PFBBBr) [21,22]. In brief, 25 mL slurry samples were separated into liquid and solid phases by centrifugation (2,000 rpm, 10 min). The target substances in the liquid phase were concentrated by a C₁₈ solid phase extraction column (InertSep Slim] C₁₈ 500 mg, GL-science, Tokyo, Japan) and eluted by ethyl acetate and methanol. After derivatization by PFBBBr, 1 µL in toluene was injected to a GC/MS (GC-2010 PARVUM2, Shimadzu corp., Kyoto, Japan; column: DB-5 MS; temperature: 100–260 °C). Among several methods proposed for the recovery of non-steroidal anti-inflammatory drugs, ultrasonic solvent extraction method was chosen in this study with slight modifications [23]. In brief, the pharmaceuticals in the sludge phase were extracted by 10 min ultrasonication after adding a mixture of 20 mL methanol and 1 mL HCl. The extracted pharmaceuticals were analyzed in the same way as the method for the liquid phase after diluting the extract with 10 times by pure water. The concentrations of the pharmaceuticals were calculated by taking recovery during the extraction steps into account by the addition of 2,3-dichlorophenoxyacetic acid (2,3-DPAA) as a surrogate [21].

3. Results

3.1. Recovery of Pharmaceuticals from Sterile Soil Samples

Table 1 shows the residual ratios of the target pharmaceuticals after 14 days of incubation in sterile slurry with the breathable sponge caps. Table 2 shows the same ratios in sterile slurry with the sealed caps. Both dissolved fraction and soil-associated fraction were taken into account for the calculated residual ratios. The lowest residual ratio in the breathable cap condition (Table 1) was 73% (FEP case for Kawagoe Sum soil), if the cases with DCF and IDM were excluded. In the case of DCF and IDM, the recovery became low probably due to irreversible adsorption caused by their hydrophobic nature in both conditions of the breathable and sealed caps. The average recovery ratios were 107% (CA),

95% (IBP), 88% (FEP), 85% (NPX), and 99% (KEP) in the breathable cap condition and 103% (CA), 90% (IBP), 98% (FEP), 89% (NPX), and 94% (KEP) in the sealed cap condition. IDM was excluded from the following sections in this paper because of its unstable recovery judging from the large standard deviation in the recovery for the sterile samples. Although the average recovery ratios of DCF were as low as 69% (the samples with breathable sponge caps) and 59% (the completely sealed samples), DCF results were included in the following sections, because of small standard deviations regardless of the origins of soil samples. The residual ratios obtained in these sterile samples were acceptable for the validation of the ultrasonication method for the recovery of the target pharmaceuticals from soils to methanol even after 14 days of incubation. The results also indicated the upper limits of the contribution of abiotic degradation of these compounds in soils. The small difference in the residual ratios between the breathable and sealed caps showed a minimal contribution of microorganisms passed through the breathable caps to the degradation of the target pharmaceuticals.

Table 1. The residual ratios (%) of pharmaceuticals in sterile slurry with the breathable sponge caps.

	CA	IBP	FEP	NPX	KEP	DCF	IDM
Kawagoe Sum	98	92	73	74	93	74	49
Kawagoe Win	104	91	88	84	134	84	40
Shoubuen surface	113	99	87	84	90	63	70
Shoubuen underlayer	124	113	96	91	96	66	77
Niigata	95	77	99	98	102	74	102
Ome	113	99	85	77	83	55	23
Average (n = 6)	107	95	88	85	99	69	60
Standard Deviation	11	12	9	9	18	10	28

Table 2. The residual ratios (%) of pharmaceuticals in sterile slurry with the sealed caps.

	CA	IBP	FEP	NPX	KEP	DCF	IDM
Kawagoe Sum	113	95	117	119	118	72	74
Kawagoe Win	105	91	99	96	105	58	37
Shoubuen surface	100	90	118	74	80	56	77
Shoubuen underlayer	111	103	101	99	105	69	88
Niigata	93	76	83	76	86	48	38
Ome	95	86	72	69	72	51	30
Average (n = 6)	103	90	98	89	94	59	57
Standard Deviation	8	9	18	19	17	9	25

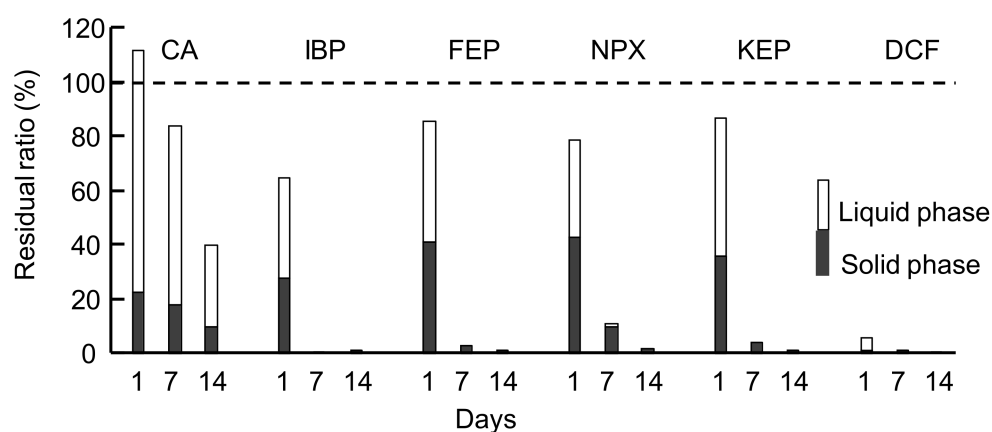
3.2. Dissolved Oxygen after Incubation

The residual dissolved oxygen (DO) concentrations after 14 days of incubation in the flasks with breathable sponge caps were above 7.3 mg/L, indicating the samples were kept under aerobic condition. On the other hand, dissolved oxygen was below the detection limit (0.1 mg/L) for the samples in the sealed flasks except for the surface top-soil sample taken at the iris garden (0.7 mg/L), indicating most of the samples were kept under anaerobic condition. The reason for the presence of oxygen in the exceptional sample would be insufficient sealing and/or the soil history of the exposure to aerobic condition before sampling.

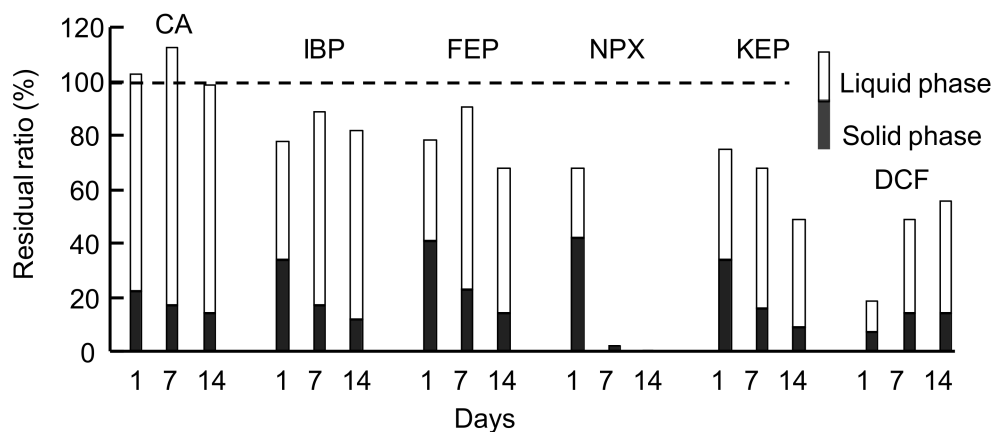
3.3. Incubation Time Required for the Evaluation of Degradation

Figure 1 shows time-dependent changes of pharmaceutical residual ratios in humic andosol slurry (Ome) under aerobic condition (a) and anaerobic condition (b). Higher degradation ratios were observed in aerobic condition compared with those in anaerobic condition. Figure 1 also shows that 14 days of incubation were long enough to observe aerobic degradation of the target compounds in soil slurry, while longer incubation time may be needed to assess the degradability in anaerobic degradation. The residual ratio of DCF at day 1 in the aerobic condition was less than 10%, suggesting

the necessity of the sampling between day 0 and day 1 to obtain exact half-life times of this compound. The results also showed almost no anaerobic degradation of CA, IBP, FEP, and KEP. The persistence of DCF was also observed under anaerobic condition judging from the residual concentration after 14 days of incubation, although the measured residual ratios of DCF in anaerobic condition were even increased during incubation. The inconsistency in the measured concentrations of DCF might be due to the low recovery of DCF from soil, as mentioned in the earlier section, although other chemical or biological factors may have influenced the concentration. On the other hand, more than 95% of the initial concentrations of IBP, FEP, NPX, KEP, and DCF were degraded in aerobic condition during 14 days of incubation. It is noted that more than 60% aerobic degradation was observed for CA by this slurry, although the rate of degradation was the slowest among the target pharmaceuticals.



(a) Aerobic condition.



(b) Anaerobic condition.

Figure 1. The residual ratios of pharmaceuticals as parent compounds with elapsed time by humic andosol (Ome) under (a) aerobic conditions and (b) anaerobic conditions.

3.4. Effect of Soil Type on the Degradation

Sterile slurry samples were prepared in this study as references to estimate the contribution of biological degradation separately from adsorption and abiotic reactions in the decrease of the target compounds. The differences in the residual ratios between the sterile and nonsterile samples indicate the contribution of biological degradation. In this study, the term “degradation” means degradation at least as parent compounds, because no transformation products were measured. The residual ratios as shown for 14 days in Figure 1 (humic andosol slurry, Ome) were plotted with the results on the sterile

references in Figure 2. Figures 3–5 shows the residual ratios in slurry samples containing other soils in the same way.

As shown in Figures 2–5, higher residual concentrations were observed under anaerobic condition for most of the target compounds. The exception was NPX, which decreased its concentration at higher rates in anaerobic condition. The higher anaerobic degradation rates of NPX, unlike the cases of other NSAIDs, were consistent with previous reports on anaerobic digestion of sewage sludge [5,13,24], although transformation products of NPX have usually been detected after aerobic and anaerobic treatment [25,26]. No anaerobic degradation of CA was observed. Anaerobic condition significantly suppressed the degradation of FEP and KEP, while moderate suppressions were observed for IBP and DCF.

On the contrary to the cases in anaerobic condition, the concentration of the target pharmaceuticals decreased with relatively higher rates in aerobic condition. DCF was almost completely disappeared in aerobic slurry regardless of the origins of the soils after 14 days of incubation. The high degradability of DCF in this study was quite different behavior from that in aerobic wastewater treatment where DCF is persistent in general [16,27–30]. IBP and FEP were also degraded completely regardless of the origin of soils under aerobic condition, although several metabolites of IBP have been well known in biological degradation [31]. In the case of KEP and NPX, 29–75% of the initial concentrations remained after 14 day of aerobic incubation in the cases of Kawagoe soil (both flooded season and dry season), although the residual concentrations of these compounds were small in the slurry samples containing the other soils, probably because of the difference in the constituents of the soils and the difference in the microbial communities. The degradation of CA was the slowest among the target pharmaceuticals. No obvious aerobic degradation of CA was observed in the soils taken at iris garden and in Kawagoe rice paddy field, reflecting the persistency of CA in biological reactions [6,30,32]. Although the reaction rates were small, the soils taken at Ome and Niigata rice paddy fields degraded CA slowly, which is consistent with the reported slow but steady reaction in the soil environments [33]. According to a previous literature [34], the microbial degraders of CA were highly localized into soils exposed to an herbicide propanil, which has a similar chlorinated aromatic ring molecular structure as CA. The difference in microbial community, affected by the history of the applied herbicides, would have influenced the aerobic degradation of CA. As mentioned in the earlier section on the dissolved oxygen concentration after incubation, the microaerobic environment (dissolved concentration of 0.7 mg/L) was unexpectedly provided instead of the anaerobic environment for the surface top soil sample of the iris garden. Only a slight difference in the degradation spectrum was observed between aerobic and microaerobic environments in this soil.

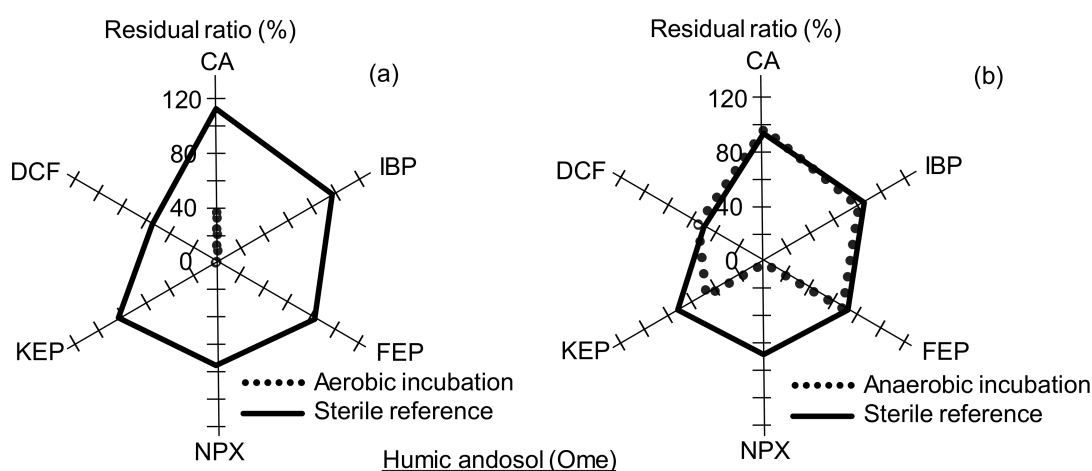


Figure 2. Residual ratios of the pharmaceuticals in humic andosol (Ome) slurry after 14 days of incubation under aerobic (a) and anaerobic (b) conditions.

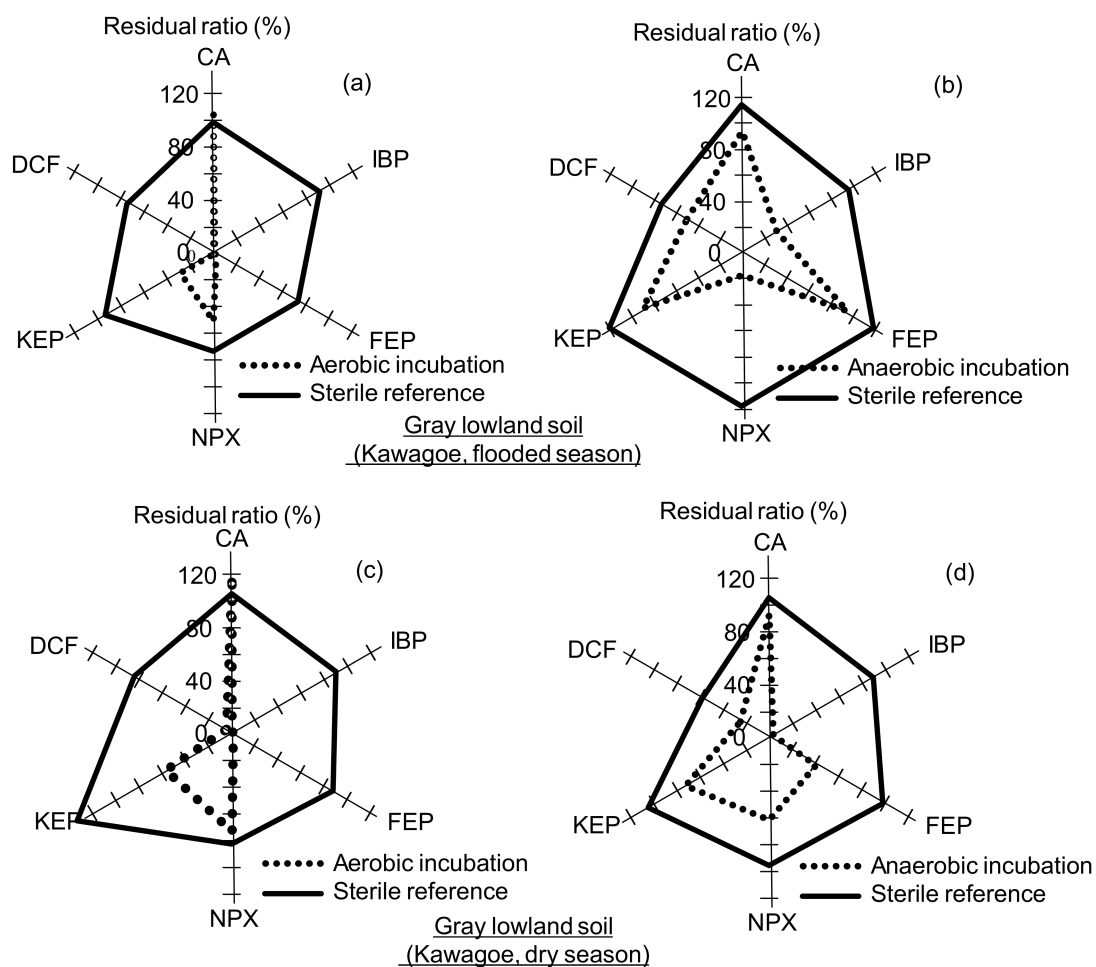


Figure 3. Residual ratios of the pharmaceuticals in gray lowland soil slurry (Kawagoe) taken at flooded season (a,b) and dry season (c,d) after 14 days of incubation under aerobic (a,c) and anaerobic (b,d) conditions.

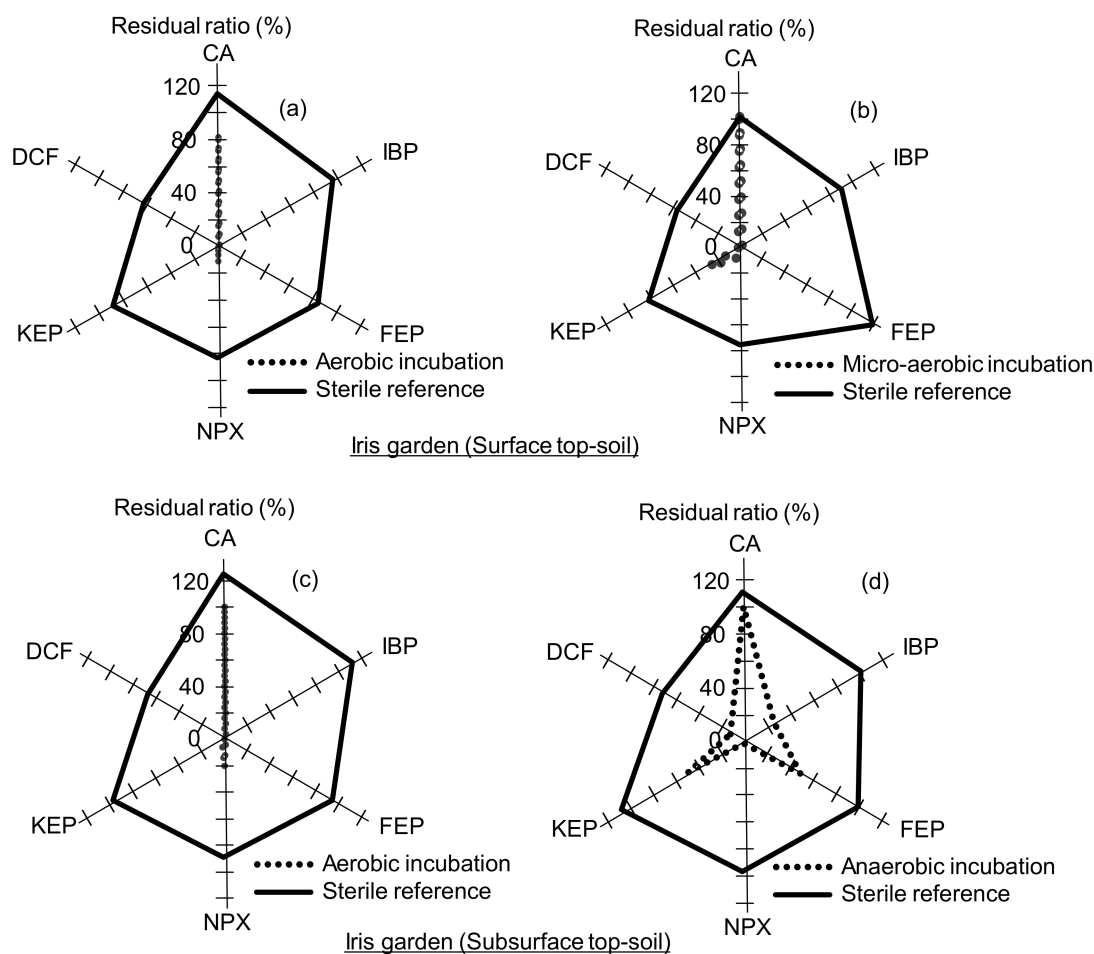


Figure 4. Residual ratios of the pharmaceuticals in slurry after 14 days of incubation under aerobic (a,c), micro-aerobic (b) and anaerobic (d) conditions. The soil was taken at the iris garden from surface top-soil layer (a,b) and subsurface soil layer (c,d).

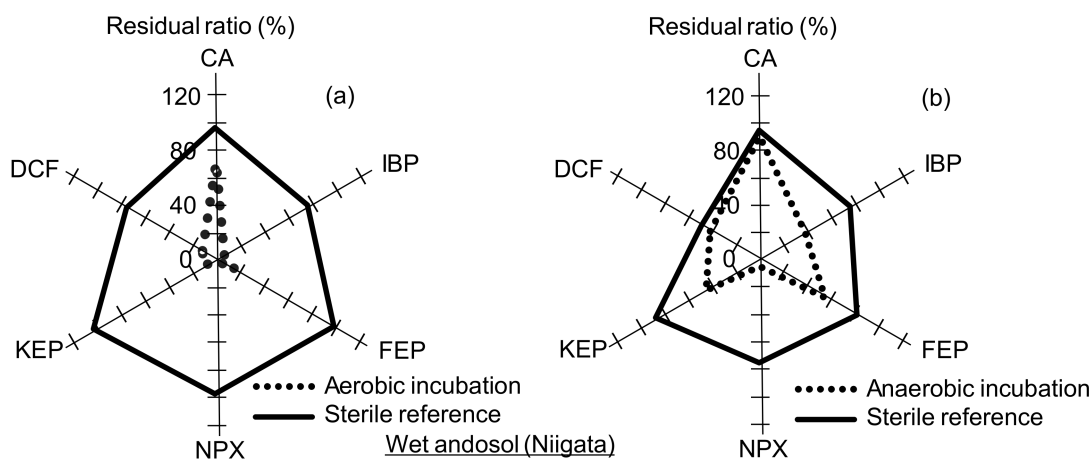


Figure 5. Residual ratios of the pharmaceuticals in wet andosol slurry (Niigata) after 14 days of incubation under aerobic (a) and anaerobic (b) conditions.

3.5. Effect of pH on the Degradation and on Partition between Liquid Phase and Solid Phase

Dissociation to ions depending on pH is an important factor in the adsorption of acidic pharmaceuticals to solids [20]. The effect of pH on the aerobic degradation and on solid-liquid distribution was presented in Figure 6. In the acidic pH condition, higher adsorption to the solid

phase was observed, while pharmaceuticals remained in the liquid phase under the high pH condition. The pH dependence on the distribution of these acidic pharmaceuticals between solid and liquid phases was consistent with pKa value (3.0–5.2) of target pharmaceuticals. At pH 7, where the target pharmaceuticals were ionized in the solution, the compounds with low residual ratios were distributed more in the solid phase, while CA (the most persistent compound in the slurry) distributed more in the liquid phase. The relationship between the residual ratio and the distribution suggests that the degradation took place by microorganisms in the liquid phase of the slurry. As to degradation activity, neutral pH condition was preferable for the aerobic degradation of the target compounds, probably because of a high activity of the microorganisms in the slurry. The enhanced removal of the target compounds by promoting uptake of the target compounds in acidic condition through the biological membrane of microorganisms, as suggested in the case of the activated sludge process [35], was not observed for the slurry samples in this study. If long-term enrichments of the microbial community had been conducted to acclimatize the microorganisms to low pH or high pH environments, different results might have been obtained.

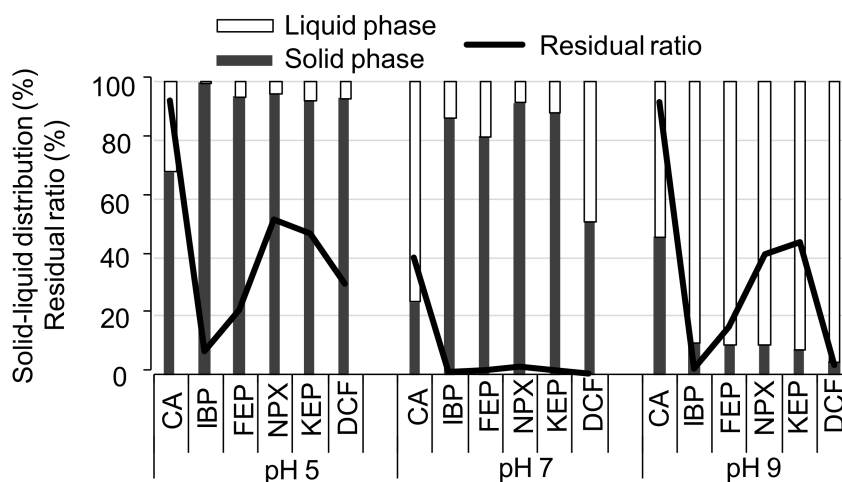


Figure 6. Effect of pH on the residual ratios and solid-liquid distribution of the pharmaceuticals after 14 days of incubation under aerobic condition.

4. Discussion

This study aimed to clarify the factors affecting the degradation of pharmaceuticals in Asian paddy fields, which are characterized with flooded and anaerobic condition. The completely mixed conditions at the state of slurry in this study would have promoted the degradation of the target compounds compared with the conditions in the real paddy fields. The results clearly indicated that aerobic condition promoted the degradation of the target pharmaceuticals except for NPX. Although exact half-life time was not calculated in this study, it is anticipated that most pharmaceuticals were more resistant to biological reactions in anaerobic rice paddy fields compared with the implications of a previous study under water unsaturated condition where relatively shorter half-life times 1–6 days for IBP, 11–18 days for CA, and soil-dependent half-life times (3–20 days) for NPX and DCF were reported in agricultural soils [15].

This study also showed that the soils taken from rice paddy fields contained microorganisms which potentially degrade the target pharmaceuticals if aerobic environment is provided, because low residual ratios of the parent compounds were observed in aerobic condition even when the same soils were incubated. The degradation spectrum of rice paddy soils in aerobic condition was similar to that of aerobic wastewater treatment in that IBP and FEP (at least as parent compounds) are readily biodegradable. In addition, slow but steady degradation of DCF and CA observed in this study implies that the microbial community of rice paddy fields would have higher diversity compared

with that of activated sludge in aerobic wastewater treatment where many studies on biological degradation have been conducted [6,27,28,30–32,35]. The decrease in the concentration of DCF and CA took place in this study without lag-time and without the acclimatization of anaerobic soil to the aerobic environments. Consequently, the higher degradation rates in aerobic condition suggest the possibility of promoted degradation of pharmaceuticals by supplying oxygen with plowing anaerobic rice fields or with drying the field during off-season for farming. Future studies are needed for the fate of transformation products in aerobic and anaerobic reactions in a real rice paddy field, because some of them are anticipated to have persistent nature [25,28,31,34]. To generalize the results, the degradation experiments using pharmaceuticals other than NSAIDs by soils taken in other countries may still be needed.

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