Occurrence and Remediation of Pollutants in the Environment

Guest Editors: Núria Fontanals, Rathinam A. James, Yong Sik Ok, Malini Balakrishnan, and Jimmy T. Efird
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In recent years, advances in human society have involved the use of large amounts and varieties of xenobiotics in various areas of our life, which certainly leads to their release into the surrounding environment. The occurrence of pollutants in air, water, and soil in turn affects biota and human health. In addition, each pollutant could undergo structural changes through various transformation and degradation pathways. In light of the possible carcinogenicity, neurotoxicity, and/or endocrine disrupting properties of some of these pollutants as well as their metabolites and transformation products, it is necessary to identify and quantify them at low concentration levels, besides identifying efficient approaches for their removal in treatment plants.

Our special issue aims at addressing novel analytical methods to determine pollutants in environment as well as develop novel strategies for their efficient removal during the treatment of polluted samples. The selected research manuscripts illustrate different research areas in the environmental field that are mainly focused on the determination, fate, and remediation of pollutants in sorted matrices.

Different kind of pollutants was treated in the studies covering from metals to pharmaceuticals or to nutrients. Thus, the sequential extraction of cadmium from soil was addressed by T. Honma et al., who discovered the relationship between chemical forms of cadmium in soil and properties in contaminated and uncontaminated paddy soils. Veterinary antibiotics (including different tetracyclines and sulfonamides) were also determined in sediments and soil samples in the study conducted by Y. M. Awad et al. They also correlated the presence of such antibiotics with antibiotic resistance genes (ARGs), which should be further monitored to ensure public health. The release of volatile fatty acid generated, when food leachate alone or mixed with animal manure was anaerobically digested, was investigated by D.-J. Lee et al., who used the concentration of volatile fatty acid as important parameter to control and manage the anaerobic digestion.

Other studies, instead of determining different pollutants, proposed alternative strategies to remove pollutants during treatment. Thus, D. J. Lee et al. evaluated different hybrid constructed wetlands with different ventilation methods (including natural and electric ventilation) in order to enhance the nutrient removal (mainly nitrogen and phosphorous content) in conventional domestic sewage from agricultural villages. The outcomes from this study recommended an improved ventilation system via an electric fan air blower with renewable energy of solar and wind power for the nutrient removal. A. Abdel-Megeed and A. Tahir also investigated the reduction of phosphorous pollution from poultry waste by supplementing phytase enzyme in broilers feed, so that the nonrenewable inorganic phosphorous for sustainable agriculture is preserved. M. Zhang et al. tested biochar as an alternative sorbent to activated carbon (AC) for the adsorptive removal of trichloroethene (TCE). At the end, AC showed better efficiency to remove TCE from water; nevertheless, biochar is still a good alternative due to its
cost-effectiveness. L. Zhu evaluated the performance of clean and fouled nanofiltration (NF) membranes in the rejection of organic micropollutants (particularly, polycyclic aromatic hydrocarbons, PAHs, and phthalic acid esters, PAEs). After all, suitable membrane and sample conditions were found in order to achieve an enhancement in rejection.

We believe that this special issue will be an important source of information for researchers from several disciplines covering the interdisciplinary of the environmental field.

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Monitoring Antibiotic Residues and Corresponding Antibiotic Resistance Genes in an Agroecosystem

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Antibiotic resistance genes (ARGs) have been commonly reported due to the overuse worldwide of antibiotics. Antibiotic overuse disturbs the environment and threatens public human health. The objective of this study was to measure the residual concentrations of veterinary antibiotics in the tetracycline group (TCs), including tetracycline (TC) and chlorotetraacycline (CTC), as well as those in the sulfonamide group (SAs), including sulfamethazine (SMT), sulfamethoxazole (SMX), and sulfathiazole (STZ). We also isolated the corresponding ARGs in the agroecosystem. Four sediment samples and two rice paddy soil samples were collected from sites near a swine composting facility along the Naerincheon River in Hongcheon, Korea. High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was employed with a solid-phase extraction method to measure the concentration of each antibiotic. ARGs were identified by the qualitative polymerase chain-reaction using synthetic primers. SAs and their corresponding ARGs were highly detected in sediment samples whereas TCs were not detected except for sediments sample #1. ARGs for TCs and SAs were detected in rice paddy soils, while ARGs for TCs were only found in sediment #2 and #4. Continuous monitoring of antibiotic residue and its comprehensive impact on the environment is needed to ensure environmental health.

1. Introduction

Veterinary antibiotics are generally used as additives to maintain animal health and to promote animal growth. In the USA, approximately 12,500 tons of antibiotics is used for livestock production every year [1, 2]. A large amount of antibiotics in the form of active pharmaceutical ingredients has been used in animal husbandry and on fish farms because of their high efficiency to promote growth or control disease [3]. However, > 80% of the antibiotics used are excreted as active metabolites in feces and urine [2]. Subsequently, the excreted antibiotic residues are delivered to the surrounding environment, resulting in elevating antibiotic concentrations [4–6]. The antibiotic residues of four antibiotic groups including tetracyclines (TCs), sulfonamides (SAs), macrolides (MLs), and ionophores are detectable in water and sediment near the mixed-landscape of the Cache La Poudre River watershed [7]. The occurrence of antibiotic residues along a water system is more critical because they are highly mobile [7]. Release of antibiotics into the environment leads to the strains of pathogenic antibiotic-resistant bacteria [8–11]. For example, TC resistance genes have been reported in water samples collected from wastewater treatment plants near swine production facilities in the USA [8, 10]. Sengeløv et al. [12] also detected antibiotic resistance genes (ARGs) against TC, MLs, and streptomycin in bacteria isolated from five farmlands treated with swine manure slurry. Rysz and
Alvarez [13] insisted that dissemination of ARGs severely degrades environments biochemically and should be considered a pollutant.

Once antibiotic residues enter bacterial cells in the environment via passive diffusion, they inhibit bacterial growth [14]. TCs, including TC, chlortetracycline (CTC), oxytetracycline (OTC), doxycycline (DXC), and minocycline (MNC) inhibit protein synthesis in Gram-positive and Gram-negative bacteria by preventing the binding of aminoacyl-tRNA molecules to the 30S ribosomal subunit [14]. Bacterial resistance to these antibiotics occurs by two mechanisms: (i) the multiantibiotic-resistance pump and (ii) conferring of bacterial resistance [14, 15]. Antibiotic research related to resistance genes has been confined to culturable bacteria isolated from pharmaceutically originating wastewater. The cultural isolation method is the most commonly employed; however, only a fraction of actual microbiota in systems containing ARGs can be determined using this method [16]. According to ARG occurrence in environments affected by animal waste, the polymerase chain-reaction (PCR) method is highlighted to quantify genes conferring resistance to selective antibiotics. Several studies have attempted to quantify ARGs by isolating DNA [11, 17]. Knapp et al. [18] showed that isolated DNA from five long-term soil series (over 60 years) was very informative regarding ARG abundance and their resistance to antibiotics. They also found that ARGs have increased sharply in the environment from 1940 to 2008. Differently designed primers are needed to detect antibiotic bacterial resistance. Bacterial resistance to different types of antibiotics was primarily mediated by synthetic primers, such as tet(A), (E), tet(G), tet(M), tet(O), tet(Q), and tet(S), for TCs [11, 19] and sul(I) and sul(II) for SAs [5].

To understand the relationship between antibiotics and corresponding ARGs, seasonal monitoring of veterinary antibiotics is needed due to the variation of climatic features in Korea and the overused annual consumption of antibiotics compared to other countries [20]. Korea has high-intensity rainfall and a large temperature difference between summer and winter seasons due to the geographical monsoon impact [21]. This climate condition can lead to the mobilization of antibiotics, owing to the contamination of surrounding environment. A continuous monitoring of antibiotics has been performed near concentrated animal farming operations (CAFOs) in Korea and antibiotics were detected in environment as mentioned in our previous studies [20, 22].

This study was conducted to further evaluate the presence of veterinary antibiotic residues released into the environment and to identify ARGs in environmental components such as sediment and soil possibly affected by a swine manure-based compost facility.

2. Materials and Methods

2.1. Sampling. The sampling sites were located in Hongcheon, Gangwon Province, Korea, which were assumed to be affected by antibiotic release from a swine manure composting facility (37° 34′ 28″ N, 127° 52′ 26″ E). Specific descriptions of the sampling sites are provided in Table 1. Sampling was done in March 2009. The average temperature was 17.7 °C and total precipitation was 95.8 mm [23]. Sediments were sampled based on the distance from the composting facility of 0.2, 0.5, 1, and 1.5 km as sediment sample #1, #2, #3, and #4, respectively, along the Naerincheon River. Paddy soils were collected from sites (a) directly applied with swine manure for agricultural purposes as soil #1 and (b) only irrigated using a water source from Naerincheon River as soil #2. Specifically, sediment and soil samples were collected at a depth of 0–20 cm. Four subsamples were collected from each site and these subsamples formed a composite sample. The sediment and soil samples were air-dried and then passed through a 2-mm sieve before analysis. The current study is a part of a comprehensive monitoring (since April 2008) of antibiotics in water, sediment, and soil near swine composting facility [20, 22].

2.2. Antibiotic Extraction and Quantification. Antibiotic residues were extracted from the sediment and soil samples using the method described by Kim and Carlson [24] and Ok et al. [20] and were quantified by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (API 3000, Applied Biosystems, Foster City, CA, USA). Recovery and the limit of quantification were determined. Briefly, to extract TCs and SAs, 1g of sediment or soil sample was added to a 50 mL polypropylene centrifuge tube with 20 mL of McIlvaine buffer at pH 4 buffer solution and 200 μL of 5% Na2EDTA, followed by 20 min of shaking at 400 rpm before centrifugation for 15 min at 4,000 rpm (Centrifuge FLETA 5, Hanil Science Industry, Seoul, Korea). The supernatant was filtered through a 0.2 μm glass fiber filter. The extraction process was repeated, and the extracts were combined in a 40 mL vial for solid-phase
Table 2: Conditions for high performance liquid chromatography-tandem mass (HPLC-MS/MS) spectrometry.

| Equipment            | Column temp. | MS/MS (TSQ Quantum Ultra, Thermo) |
|----------------------|--------------|----------------------------------|
| LC condition         | Column flow rate | 15°C                             |
|                      | Injection volume | 300 μL min⁻¹                      |
| Mobile phase         | A: 99.9% water + 0.1% formic acid |
| Gradient             | B: 99.9% ACN + 0.1% formic acid  |
| Ion source           | A: 96% + B: 4% (0 min)          |
| Spray voltage        | B: 96% + B: 4% (30 min)         |
| Vaporizer temp.      | A: 70% + B: 30% (29 min)        |
| Drying gas flow      | A: 96% + B: 4% (30 min)         |
| Drying gas and nebulizer gas | 10.0 L min⁻¹                |
| Sheath gas pressure  | Nitrogen gas                  |
| Aux gas pressure     | 40 psig                        |

extraction (SPE). SPE was employed to retain antibiotics on the cartridge so they could be effectively extracted with MeOH [25]. Due to the wide range in pH, hydrophilic-lipophilic balanced cartridges were used for the antibiotic extraction and preextractants were purified on solid matrices [25]. Electrospray ionization was also applied to quantify antibiotic substances using HPLC-MS/MS in positive mode. The detailed information and mobile phase conditions are summarized in Table 2.

2.3. Heterotrophic Plate Counts on Antibiotic-Selective Media. Each 1g of moist sediment/soil sample was diluted in sterilized water and agitated for 30 min, followed by a 100-fold serial dilution. Aliquots (100 μL) of the serially diluted sample were spread directly onto the surface of R2A agar media (Difco, Sparks, MD, USA), which contained various antibiotics or no antibiotic as a control to enumerate and isolate resistant bacteria. Specifically, the media contained antibiotics of 30 mg L⁻¹ TC, 70.55 mg L⁻¹ CTC, 45.55 mg L⁻¹ OTC, 281.8 mg L⁻¹ SMT, 50.4 mg L⁻¹ SMX, or 45 mg L⁻¹ STZ. A concentration that was five times greater than the reported average LD₅₀ value was used for the water-soluble antibiotics such as TC, CTC, and STZ, whereas the maximum amount that dissolved readily in water when added to melted agar was used for the insoluble antibiotics such as SMX, SMT, and OTC [5]. Each treated plate was incubated at 30°C for 48 h, followed by incubation for 1 week in the dark at room temperature. Colony forming units (CFUs) were enumerated at the end of the culture period [26].

2.4. DNA Extraction and Purification. DNA was extracted from 0.5 g of sediment or soil sample using a FastDNA SPIN kit (QBiogene, Carlsbad, CA, USA). The extracted DNA was purified using a GeneClean SPIN kit (QBiogene) to minimize PCR inhibition. The concentration of DNA before/after purification and recovery were determined.

2.5. Primer Design. Specific primers for nucleotide sequences encoding the TC- and SA-resistant genes were designed based on the GenBank Database (http://www.ncbi.nlm.nih.gov/). Seven sets of primers obtained from verifiable subjected products were generated as shown in Table 3.

2.6. Detection of ARGs Using Qualitative PCR. PCR was performed to identify the TC and SA ARGs encoding ribosomal protection. We used a Bio-Rad kit (Hercules, CA, USA) in a reaction mixture with a final volume of 20 μL consisting each of 2 μL of the x10 buffer, 2.5 mM dNTP mix, 0.4 μM each primer, 1.75 units of Taq DNA polymerase, and 50 pmol of DNA template (Takara Bio, Shiga, Japan). Amplification was conducted using a PTC-100 thermal cycler (Bio-Rad) to subject samples to conditions of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, 30 s annealing at 55.9°C for Sa genes, 60°C for tet(W), 50.3°C for tet(O), 56°C for tet(S), 43.9°C for tet(B), or 43.9°C for tet(T). Extension was done at 72°C for 30 s with a final extension at 72°C for 7 min. The PCR products were visualized on a 0.8% agarose gel using a Gel Doc 1000 apparatus (Bio-Rad).

3. Results and Discussion

3.1. Antibiotic Concentrations. The concentrations of TCs and SAs in the sediment and soil samples are shown in Figure 1. TC was only detected in sediment #1 (0.39 μg kg⁻¹) (Figure 1(a)). TC and CTC antibiotic residues were detected in both soils #1 and #2, and the maximum concentrations of TC (0.93 μg kg⁻¹) and CTC (6.00 μg kg⁻¹) were observed in soil #1. This result shows that the antibiotic residues in soils are more long-lasting than those in sediment because of lower mobility in soils. No OTC was found in any sample. For instance, stability of TCs is controlled by abiotic and biotic factors with a range of 1–419-day half-lives in
Table 3: Polymerase chain-reaction (PCR) primers for tetracycline- (TC-) and sulfonamide- (SA-) resistant gene classes.

| Gene | Primer | Sequences          | Annealing temp. (°C) | Amplicon size (bp) |
|------|--------|--------------------|----------------------|--------------------|
| tet(S) | tetS-FW† | GAAAGCTTACTTACGAGTAC | 50                   | 169                |
|      | tetS-RV‡ | AGGAGTATCTACATATTAC |                       |                    |
| tet(T) | tetT-FW | AAGGTTTATATATAAAGTG | 46                   | 169                |
|      | tetT-RV | AGGTTATCTATATTACATAC |                       |                    |
| otr(A) | otrA-FW | GGCATYCTGGCCACGT | 66                   | 212                |
|      | otrA-RV | CCCGGGGCTGTCGTASAGG |                       |                    |
| sul(I) | sulI-FW | CGCACCGGAAAATCCTGCAC | 55.9                 | 163                |
|      | sulI-RV | TGAAGTCCGCGCGAAGGTCG |                       |                    |
| sul(II) | sulII-FW | TCCGTTGAGGGCCGTATCTGG | 60.8                 | 191                |
|      | sulII-RV | CGGGAATGCCATCTGCCGTAG |                       |                    |
| sul(III) | sulIII-FW | TCCGTTCAGCGAATTGGTGCAG | 60                   | 128                |
|      | sulIII-RV | TCGTTCAGCGCTTACACCAGC |                       |                    |
| sul(A) | sulA-FW | TCTTGAGCAAGCACTCCAGCAG | 60                   | 229                |
|      | sulA-RV | TCCAGCCCTAGCAACCACATGC |                       |                    |

†Forward.
‡Reverse.

Figure 1: Average concentration of (a) tetracyclines (TCs) and (b) sulfonamides (SAs) in sediment and soil samples collected along the Naerincheon River downstream of a swine manure composting facility.

aquatic systems [27]. In current study, TCs were below the detection limit in sediment samples and this might be due to their strong sorption affinity to aluminum oxide, Fe oxides, organic carbon, and clay particles in soil [20, 27, 28]. In particular, Al₂O₃ and Fe₂O₃ promote the dehydration of TC to anhydrotetracycline (AHTC), epimerization of TC, and formation of Al-TC and Fe-TC complexes [29–31]. In addition, Rubert [27] revealed that organic matter (humic and proteinaceous substances) can absorb TCs in soil and can form complexation of TC in the presence of cations such as Ca, Cu, Al, and Fe. Additionally, sorption of TCs was pronounced in rice paddy soils due to high cation exchange capacity by 9.24 and 8.90 cmol(+)/kg, respectively, for both soils as mentioned in our previous findings [20, 31–33].

The maximum concentrations of SMT (17.68 µg kg⁻¹) in sediment #4, SMX (10.24 µg kg⁻¹) in sediment #2, and STZ (8.34 µg kg⁻¹) in sediment #1 were found (Figure 1(b)). Similar to the TCs, a higher concentration of SAs was observed in soil #1 than those in soil #2. Additionally, the direct application of swine manure onto paddy soils (i.e., soil #1) contributed to the longevity of antibiotic residues in soils compared to that of indirect application via irrigation water possibly contaminated with antibiotics (i.e., soil #2).

These findings were in accordance with our previous study [20, 22] that the concentrations of TCs, including CTC, TC, and OTC, in sediment samples collected along the Naerincheon River are very low or below the detection limit. We showed earlier that the antibiotic residues of SAs, including SMT, SMX, and STZ, are highly detectable in sediment, indicating concentration levels of 38.60–70.32, 8.91–12.20, and 23.68–40.31 µg kg⁻¹, respectively [20]. Our results for the trend of TCs and SAs concentrations in sediment and
in March with 1.5
fourth highest temperature record since 1973 was observed
conditions and type of antibiotic. It was noteworthy that the
flow conditions, and water quality related to geographic
in water are strongly influenced by precipitation, water level,
[24] found that the concentration levels of antibiotic residues
antibiotics from CAFOs. Ok et al. [20] and Kim and Carlson
fow r a t ec a u s et h ed i l u t i o ne ff e c to ft h er e l e a s e dv e t e r i n a r y
pot en ti a le ff e c t so fr a i n f a l ld u r i n gw i n t e rs e a s o na n dh i g h-
cipitation or temperature based on sampling season [24]. The
discrepancy may be explained by seasonal variations in pre-
the concentrations were much lower than their study. This
soilsampleswerequitesimilartoourpreviousstudy [20], but
‡
soil samples were quite similar to our previous study [20], but
the concentrations were much lower than their study. This
discrepancy may be explained by seasonal variations in pre-
cipitation or temperature based on sampling season [24]. The
potential effects of rainfall during winter season and high-
flow rate cause the dilution effect of the released veterinary
antibiotics from CAFOs. Ok et al. [20] and Kim and Carlson
[24] found that the concentration levels of antibiotic residues
in water are strongly influenced by precipitation, water level,
flow conditions, and water quality related to geographic
conditions and type of antibiotic. It was noteworthy that the
fourth highest temperature record since 1973 was observed
in March with 1.5 C higher than normal mean temperature,
while the annual precipitation in Hongcheon was 1000.4 mm,
based on weather information from the Korea Meteorological
Administration [21, 34]. This also can be contributed to the
degradation of TCs in solid matrices (animal manure and
soil). Under soil acidic condition, OTc was epimerized in
swine manure and formed degradation products such as 4-
epi-OT and epi-N-desmethyl-OT [27, 35]. Ingerslev et al. [36]
found that biodegradation of TCs was the main mechanism
in sludge by Ascomycetes fungi [37] and Streptomyces species;
however, sorption and transformation of TCs commonly
occurred in soil [27, 32]. Our study confirmed that
the transformation and stability of TCs in sediment and soil
are dependent on light, temperature, and physiochemical
properties of the matrix [38].

The reason for the higher concentrations or mobility
of SAs compared to TCs is that SAs are likely moving a
further distance from the composting facility because of
lower organic carbon-normalized sorption coefficient (Koc)
and the lower hydrophobicity [24, 31]. Hu et al. [39] also
showed that SAs have a range of distribution coefficients
(Kd) of 0.9–18.1 mL g−1, indicating high solubility in water
compared to other types of antibiotics.

### 3.2. Antibiotic-Resistant Bacteria

Total bacterial counts indicating antibiotic resistance (CFUs×102)
are shown in Table 4. Total bacterial counts in the sediment samples
decreased with increasing distance from the swine composting facility as a
release source of antibiotics. This result indicates that the
antibiotic-resistant bacteria were present close to the antibi-
otic contamination source. The total enumeration of CFUs
was much higher without antibiotics than with antibiotics,
ranging from 179 to 241 CFU×102 g−1 for sediments and 298
to 316 CFU×102 g−1 for soils. The total bacterial count for SAs
was 8.67–145.33 CFU×102 g−1 for sediments and 10.33–36.33
CFU×102 g−1 for soils. These results indicate that the density
of culturable heterotrophic bacteria was generally higher
in sediment than that in soil. However, no bacteria were
detected in any samples grown in the presence of TCs except
in sediments #2 and #4, indicating a very low population. This
result agrees with a study by Pepper and Gerba [26] showing
that SA-resistant bacteria are present in greater abundance
than TC-resistant bacteria.

### 3.3. PCR Assay for ARGs

The occurrence of ARGs for
TCs and SAs is shown in Table 5 and Figure 2. The results
showed that SA-resistant genes including sul(I), sul(II), and

### Table 4: Antibiotic-resistant bacteria in CFUs isolated from sediment and soil samples cultured on R2A agar plates with/without antibiotics after a 24 h incubation at 30°C.

| Antibiotic          | Sed. #1   | Sed. #2   | Sed. #3   | Sed. #4   | Soil #1  | Soil #2  |
|---------------------|-----------|-----------|-----------|-----------|----------|----------|
| Control             | 217.00e   | 241.00e   | 193.00e   | 179.00f   | 298.00e  | 316.00e  |
| Tetracycline (TC)   | ND        | 2.00e     | ND        | 0.33b     | ND       | ND       |
| Chlortetracycline (CTC) | ND    | 0.67b     | ND        | ND        | 1.00a    | ND       |
| Sulfamethazine (SMT) | 145.33a   | 126.67ab  | 99.33b    | 50.33c    | 36.33c   | 33.33c   |
| Sulfamethoxazole (SMX) | 52.33b   | 88.33b    | 39.00d    | 14.33d    | 10.33d   | 19.33d   |
| Sulfathiazole (STZ)  | 99.33a    | 79.33a    | 13.67b    | 8.67b     | 22.33b   | 12.33b   |

5 Different letters in each row indicate a significant difference at 0.05.

### Table 5: Polymerase chain-reaction (PCR) identification of antibiotic-resistant strains using different tetracycline (TC) and sulfonamide (SA) primers.

| Primer | Sed. #1 | Sed. #2 | Sed. #3 | Sed. #4 | Soil #1 | Soil #2 |
|--------|---------|---------|---------|---------|---------|---------|
| tet(S) | —†      | o‡      | —       | o       | —       | —       |
| tet(T) | —       | o       | —       | —       | o       | —       |
| otr(A) | —       | —       | —       | o       | o       | o       |
| sul(I) | —       | o       | o       | o       | o       | o       |
| sul(II) | o     | o       | o       | o       | o       | o       |
| sul(III) | o     | o       | o       | o       | o       | o       |
| sul(A) | o       | o       | o       | o       | o       | o       |

† Absent.
‡ Present.
sul(A) were present in all sediment and soil samples except sul(I), which was not detected in sediment #1. However, TC-resistant genes including tet(S), tet(T), and otr(A) were only found in sediments #2 and #4 and soil #1. These findings agree with the study by Pei et al. [5] who quantified four SA- and five TC-resistant genes in sediments collected along the Cache La Poudre River using both culture-based and PCR techniques. Auerbach et al. [11] also reported a wide variety of TC-resistant genes in different wastewater samples collected in the USA.

It was noteworthy that wastewater from CAFOs and the application of compost or animal manure to rice paddy soils play a significant role in generating ARGs for SAs in both sediments and soils due to the accumulation of veterinary antibiotics. Similarly, ARGs for TCs were found in rice paddy soils. Similar to our results, previous studies reported that ARGs were generated at higher levels near CAFOs than background and associated with human and animal diseases, including different pathogenic bacteria such as Salmonella and Shigella isolates [40]. For example, they found a significant correlation between the occurrence of methicillin-resistant Staphylococcus aureus (MRSA) in pigs and pig farmers in USA, Canada, and Europe [40, 41]. Thus, the findings of current study agree with previous studies [4, 5, 8, 11, 40, 41] and demonstrate that antibiotic use in CAFOs is highly correlated with the fate and transport of ARGs in surrounding environment. In a survey by Peak et al. [42], a strong correlation between antibiotics and ARGs was identified.

4. Conclusions

This study was conducted to investigate the residual concentrations of selected TCs and SAs and to isolate corresponding ARGs in the environment. Higher concentrations of SAs in sediment and soil samples were found compared to those of TCs. A culture-based technique and PCR were successfully used to demonstrate TC- and SA-resistant genes in the environment. Findings of current study revealed that the widespread antibiotic use in CAFOs in Korea has the potential to generate ARGs as emerging contaminants in solid environmental matrices. Monitoring ARGs in surrounding environments is encouraged to ensure public health. Free-antibiotic swine industry in Korea is recommended to reduce the environmental risks of veterinary antibiotics.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Adsorptive Removal of Trichloroethylene in Water by Crop Residue Biochars Pyrolyzed at Contrasting Temperatures: Continuous Fixed-Bed Experiments

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Biochar (BC) has attracted great attention as an alternative sorbent to activated carbon (AC). Objective of this study was to determine trichloroethylene (TCE) removal by soybean stover BC pyrolyzed at 300 (BC300) and 700°C (BC700) in continuous fixed-bed column. Columns packed with BC300, BC700, and AC reached breakthrough time in 1.1, 27.0, and 50.7 h, respectively. BC700 had higher TCE adsorption capacity than BC300 due to its higher surface area, nonpolarity, and aromaticity. The sorption capacities of AC (774.0 mg g⁻¹) and BC700 (515.1 mg g⁻¹) were 21.6 and 14.4 times higher than that of BC300 (35.9 mg g⁻¹). The lower desorption rate of TCE from BC300 than BC700 and AC may be attributed to the strong binding/partition of TCE to the noncarbonized part of BC. Thomas model also adequately described the adsorption data indicating interphase mass transfer. Overall, AC showed best efficiency for removing TCE from water in column experiments. However, although sorption and desorption capabilities of BC700 were a little lower than AC, it is still a good alternative for AC to remove organic contaminants such as TCE from water due to its cost-effectiveness.

1. Introduction

Char, a solid material produced from carbonaceous biomass, is emerging as an alternative to activated carbon (AC) with lower cost and environmental advantages. Char commonly appears under uncontrolled natural conditions through partial or complete carbonization of biomass such as wood, manure, or leaves [1–3]. Biochar (BC) is a name developed in conjunction with soil science and related to carbon sequestration in soils [4–6]. Biochar means black carbon derived from biomass pyrolysis and closely resembles activated carbon with a structured carbon matrix and a medium-to-high surface area. Biochar has a wide range of chemical compositions and surface properties depending on biomass type and pyrolysis temperature [7]. Several studies have already reported the effect of pyrolysis temperature on sorption properties of biochars [8, 9]. Higher temperature-pyrolyzed-biochar possesses high surface area, carbon content, aromaticity, and hydrophobicity, which lead to the increase of sorption capacity towards contaminants, especially for the nonionized chemicals [10]. Lower temperature-pyrolyzed-biochar was believed to be effective for polar organics and heavy metals due to abundant polar groups on biochar surface [10]. Because of its high efficiency and capacity to adsorb organic contaminants, it has also been spotlighted as an excellent adsorbent [10, 11] for water and wastewater treatment. In
comparison with conventional activated carbon, BC may be economically preferable with less energy requirements and no pre- or postactivation processes during manufacturing. The estimated break-even price for BC is US $246 t\(^{-1}\), which is approximately 1/6 of commercially available AC (~US $1500 t\(^{-1}\)) [11, 12]. It is also environmentally beneficial by converting/recycling of organic wastes via pyrolysis. However, substantial understanding is required to ensure efficiency of BC to remove organic contaminants from water/groundwater.

Trichloroethylene (TCE) is a widely used chlorinated solvent in industry that is released into the atmosphere as vapor [13]. It contaminates surface water or groundwater via direct discharge or leaching from disposal operations [14]. TCE has been identified as a prior environmental pollutant by the US Environmental Protection Agency [15]. Groundwater contamination by TCE commonly occurs worldwide in many industrial and urban areas. A severe level of TCE (1.52 mg L\(^{-1}\)) has been detected from groundwater at the industrial complex in Wonju city, Korea, with typical values ranging from 0.01 to 1.52 mg L\(^{-1}\) [16]. According to the Korea Ministry of Environment, the maximum permissible level (MPL) of TCE is 0.03 mg L\(^{-1}\) for residence and 0.06 mg L\(^{-1}\) for industrial areas. However, TCE concentrations in city groundwater are 50 times greater than the MPL [17, 18].

Sorption is one of the most popular and widely used technologies for depuration of groundwater [11, 19]. Various sorbents such as activated carbon, biomass, zeolite, and resins have been conventionally used to decontaminate water [15, 19–21]. However, there is a need to explore low-cost, effective, and environmentally friendly materials to purify contaminated groundwater. In this context, BC could be a strong candidate for TCE removal due to the advantages mentioned above. Most sorption studies have been confined to batch type equilibrium studies [11, 15]. However, data from batch type sorption experiments is insufficient particularly in column operations where contact time is inadequate to achieve equilibrium and may lead to low sorption efficacy of BC [11, 13, 20]. Therefore, sorption studies in columns should be performed to understand real application potential. Continuous fixed-bed column studies have been used very effectively for large-scale wastewater treatment operations [21, 22]. Other techniques involving destruction of TCE by ozonation, catalytic oxidation/reduction, and use of nano-zero-valent metals are generally associated with the formation of daughter substances that may cause more negative impacts [23]. The objectives of this study were to evaluate the performance of BCs derived from soybean stover, pyrolyzed at different temperatures, for removing TCE from water using a fixed-bed continuous flow column compared with AC. Thomas model was employed to evaluate the sorption and desorption properties of AC and the BCs.

### 2. Materials and Methods

**2.1. Biochar Production and Characterization.** Soybean stover collected from a local agricultural field in Chungju city, Korea, was used as raw feedstock for producing the BCs. Ground feedstock was placed in a ceramic crucible with a lid and pyrolyzed in a muffle furnace (MF 2IGS, Jeio Tech, Seoul, Korea) increasing at 7°C min\(^{-1}\) under limited oxygen conditions. Two different peak temperatures, that is, 300 and 700°C, which were selected as representatives of low and high pyrolysis temperatures, were adapted to carbonize each feedstock, and they were held for 3 h followed by cooling to room temperature inside the furnace. The BCs were denoted as BC300 and BC700 based on pyrolysis temperature. The commercially available AC was used in our study. BCs and ACs were ground and passed through 2 mm sieve prior to use. The modified proximate and ultimate analyses proposed by McLaughlin et al. [24] were employed to characterize the BCs and AC. The elemental composition including C, H, N, and O was determined by dry combustion using an elemental analyzer (EA1110, CE Instruments, Milan, Italy). These data were used to calculate molar ratios of H/C and O/C. Specific surface area was determined by the Brunauer-Emmett-Teller method of N\(_2\) adsorption at 77 K (ASAP 2010 v 5.02 H, Micrometrics, Norcross, GA, USA) with 6 h degasification at 473 K prior to analysis.

**2.2. Column Experiments.** Fixed-bed continuous flow sorption experiments were conducted in a Plexiglass column with an inner diameter of 1.8 cm and length of 9.8 cm. 5 g of BCs and AC was placed in the columns. TCE was commercially purchased from Wako Pure Chemical Industries, Japan, with purity of 99.5%, and the TCE solution (100 mg L\(^{-1}\)) was pumped as influent through the column from top at 9 mL min\(^{-1}\) with a peristaltic pump. Effluent samples were collected from the outlet of the column at different time intervals. The column was stopped when the effluent TCE concentration became nearly equal to the influent TCE concentration. After exhausting the column of TCE, the saturated sorbents were eluted with 25% methanol at relatively low flow rate (4 mL min\(^{-1}\)) compared to the sorption experiment. Commercially available AC was also used for column sorption and desorption experiments to compare the efficiency of BCs in TCE removal.

**2.3. Analysis of TCE.** A high performance liquid chromatograph (SCL-10A, Shimadzu, Tokyo, Japan) equipped with an autosampler (SIL-10AD, Shimadzu) and UV-VIS detector (SPD-10A, Shimadzu) was used to analyze the aqueous TCE concentration. A reverse-phase Sunfire C18 column (Waters, Milford, MA, USA; 4.6 × 250 mm) was employed in a column oven (CTO-10AS, Shimadzu) heated at 40°C for the separation. The mobile phase was a mixture of 85:15 (v v\(^{-1}\)) acetonitrile and water at a flow rate of 1.0 mL min\(^{-1}\). A 10 μL sample aliquot was injected into the column, and absorbance was measured at 214 nm. The detection limit was 1.4 mg L\(^{-1}\), and the recovery of TCE was 98.13%.

**2.4. Analysis of Column Data.** Fixed-bed continuous flow column performance was evaluated from the breakthrough curve expressed as the ratio of effluent TCE concentration (C\(_e\)) to influent TCE concentration (C\(_i\)) as a function of flow time (t) for a given bed height. Effluent volume (V\(_{ef}\)) was calculated from

\[ V_{ef} = Qt_{total} \]

(1)
where $Q$ is the volumetric flow rate (mL min$^{-1}$) and $t_{\text{total}}$ is the total flow time (min).

Total adsorbed quantity of TCE ($q_{\text{total}}$) was calculated from

$$q_{\text{total}} = \frac{QA}{1000} = \frac{Q}{1000} \int C_{\text{ad}} dt,$$

where $A$ is the area under the breakthrough curve that can be obtained by integrating the adsorbed concentration ($C_{\text{ad}}$) versus $t$ plot.

The total amount of TCE sent to column ($M_{\text{total}}$) is calculated from

$$M_{\text{total}} = \frac{C_{i}Qt_{\text{total}}}{1000}.$$

Column performance was evaluated from total removal of TCE, which was calculated by

$$\text{Total removal} \, (\%) = \frac{q_{\text{total}}}{M_{\text{total}}} \times 100.$$

Column capacity or equilibrium TCE sorption ($q_{\text{eq}}$) at the end of total flow time was calculated from

$$q_{\text{eq}} = \frac{q_{\text{total}}}{X},$$

where $X$ is the weight of sorbent (g) used in the column.

The process parameters were also determined for the design of larger-scale column studies. The time at which $C_c$ increases from a steady state, known as breakthrough time ($t_b$), and the column exhaustion time ($t_e$) when $C_c$ becomes equal to $C_i$ were recorded and used to calculate the overall sorption zone ($\Delta t$) as follows:

$$\Delta t = t_e - t_b.$$

Critical bed length also known as length of the mass transfer zone ($Z_m$) was calculated from the following equation:

$$Z_m = Z \left(1 - \frac{t_b}{t_e}\right),$$

where $Z$ is bed height (cm).

The Thomas model was applied to the column experimental data to model the breakthrough behavior of TCE sorption onto the BCs. The linearized form of the Thomas model is given as follows:

$$\ln \left(\frac{C_i}{C_c} - 1\right) = \frac{k}{Q} (q_{\text{ad}}X - C_i V_{\text{ef}}),$$

where $k$ is the rate constant (mL min$^{-1}$ mg$^{-1}$) and $q_{\text{ad}}$ is the maximum solid-phase concentration (mg g$^{-1}$). $k$ and $q_{\text{ad}}$ can be determined from a plot of $\ln((C_i/C_c) - 1)$ versus $V_{\text{ef}}$ at a given flow rate.

The amount of TCE desorbed ($q_{\text{total,desorbed}}$) was calculated from the area under the elution curve (desorbed concentration ($C_{\text{de}}$) versus $t$), and elution efficiency ($E$) was calculated from

$$E \, (\%) = \frac{q_{\text{total,desorbed}}}{q_{\text{total,sorbed}}} \times 100.$$

### 3. Results and Discussion

#### 3.1. Characteristics of the BCs and AC

Table 1 presents the proximate and ultimate analyses results of the soybean stover derived BCs at two different pyrolysis temperatures compared with AC. Temperature strongly influenced the BC yields and properties. The decrease in the BC yield at high pyrolysis temperature was mainly due to a greater loss of volatile matter. Ahmad et al. [10] reported a decrease in BC yield with increasing pyrolysis temperature. This was further supported by the greater loss in mobile matter at 700°C compared to that at 300°C. In contrast to mobile matter, the resident matter, which indicates the fixed or nonbiodegradable matter, increased with increasing pyrolysis temperature.

Changes in the elemental composition of the BCs were also observed. Total C contents of the BCs increased with pyrolysis temperature. In contrast, H, N, and O contents decreased in BC700 than those in BC300. Compared with the BCs, AC has the highest C content and lowest H, N, and O contents. BC700 exhibited high aromaticity and lower polarity than BC300 as indicated by the low molar H/C and O/C ratios, which could be related to high carbonization and low hydrophilicity at high temperature [1]. AC had much lower H/C and O/C ratios and higher C content compared with those of the BCs, indicating higher aromaticity, hydrophobicity, and lower polarity. The higher temperature derived biochar (BC700) had higher surface area (420.3 m$^2$ g$^{-1}$) and pore volume (0.19 cm$^3$ g$^{-1}$) than BC300 (5.61 m$^2$ g$^{-1}$ surface area and 0 cm$^3$ g$^{-1}$ pore volume). We conclude that the loss of mobile matter in the soybean stover during pyrolysis at higher temperature created more empty space in the residue than that at lower temperature, which increased the surface area and pore volume of the BCs [25]. However, AC had the largest surface area and pore volume.
3.2 Column Studies. The breakthrough curves of TCE sorption onto BC300, BC700, and AC are shown in Figure 1. The bed heights of the columns \((Z)\) were 5.8, 4.8, and 3.5 cm for BC300, BC700, and AC, respectively, corresponding to 5 g of sorbent in each column. The sorption process parameters calculated from the breakthrough curve are presented in Table 2. It was predicted that \(t_b\) for BC300 was 1.1 h whereas it was 27.0 h for BC700. This indicated that the column packed with BC300 began to saturate much earlier than that of BC700 and reached exhaustion after 10.5 h, whereas BC700 column had an exhaustion time \((t_e)\) of 66.3 h. The maximum \(t_b\) value of 50.7 h was observed for AC with \(t_e\) of 95.5 h. The adsorbent bed height \((Z)\) affects the efficiency of a column [26]. However, the greater \(Z\) for BC300 (5.8 cm) compared to that of BC700 (4.8 cm) and AC (3.5 cm) did not enhance the efficiency of BC300. Consequently, the critical bed length \((Z_m)\) required to obtain the breakthrough time was lower for BC700 (3.5 cm) than that of BC300 (5.2 cm), indicating the shorter mass transfer zone of TCE in the column packed with BC700. This further indicates that the BC700 column had greater capacity for cycling TCE sorption because of the greater difference between \(Z\) and \(Z_m\) (1.9 cm) compared to that of the BC300 (0.6 cm) and was similar to AC.

Column performance was evaluated based on the total TCE removal percentage and total TCE uptake \((q_{eq})\) by the sorbents. BC700 resulted in 68.4% removal of TCE compared to 30.0% removal by BC300 (Table 2). Similarly, the \(q_{eq}\) value was extremely high for BC700 (515.1 mg g\(^{-1}\)) than that of BC300 (35.92 mg g\(^{-1}\)). These sorption process parameters for the BC packed columns clearly indicate the high performance efficiency of BC700 compared to that of BC300. In contrast, the column packed with AC outperformed the BCs with a removal efficiency of 72.1% and 774.0 mg g\(^{-1}\) uptake of TCE. This could be explained by the presence of a more noncarbonized fraction in BCs than in AC, which could lower the sorption of TCE onto the relatively less carbonized fraction in the BCs [27]. Moreover, higher surface area and pore volume of BC700 and AC was one of the reasons for the higher sorption capacity. However, higher hydrophobicity of BC700, indicated by the lower molar ratio of O/C and higher C content, also resulted in higher sorption capability to the relatively hydrophobic TCE by BC700 [II].

3.3 Determination of Sorption Rate Constants. The behavior of a sorption column was modeled using the simpler and more tractable Thomas model. This model is frequently used because of its simplicity, the lack of numerical simulations, and immediate practical benefits [22]. The rate constant \((k)\), maximum solid-phase concentration \((q_0)\), and the correlation coefficient \((R^2)\) values for BC300, BC700, and AC are presented in Table 3, while the predicted nonlinear regressions of the Thomas model are shown in Figure 1. The \(R^2\) values calculated from the nonlinear regressions of the Thomas model were 0.965, 0.977, and 0.987 for BC300, BC700, and AC, respectively, indicating that the experimental data was well fitted to the Thomas model. The \(k\) value was higher for BC300 (0.091 mL min\(^{-1}\) mg\(^{-1}\) for linear and 0.056 mL min\(^{-1}\) mg\(^{-1}\) for nonlinear regression) than for BC700 (0.018 mL min\(^{-1}\) mg\(^{-1}\) for linear and 0.011 mL min\(^{-1}\) mg\(^{-1}\) for nonlinear regression) and AC (0.009 mL min\(^{-1}\) mg\(^{-1}\) for both linear and nonlinear regression), indicating that BC300 achieved the maximum sorption of TCE within a short time. These predictions are in agreement with the relatively low \(t_b\) value in BC300 than those...
in BC700 and AC (Table 2). As expected, the $q_0$ value was higher for AC (1212 mg g$^{-1}$ for linear and 1436 mg g$^{-1}$ for nonlinear regression) and BC700 (682.8 mg g$^{-1}$ for linear and 961.4 mg g$^{-1}$ for nonlinear regression) compared to BC300 (45.81 mg g$^{-1}$ for linear and 78.02 mg g$^{-1}$ for nonlinear regression), which also appeared in the observed experimental $q_{eq}$ values given in Table 2. The fitness of the Thomas model to the experimental sorption data presumed that the TCE sorption onto BCs was controlled by interphase mass transfer, but axial dispersion may be an important factor determining the rate limiting step [22].

The greater efficiency of the column packed with AC and BC700 for removing TCE from water could be related to its specific properties such as high surface area (758.9 m$^2$ g$^{-1}$ for AC and 420.3 m$^2$ g$^{-1}$ for BC700) and low molar H/C (0.085 and 0.19) and O/C (0.057 and 0.14) compared to those of BC300 (Table 1). High aromaticity (as indicated by low molar H/C) and low polarity (as indicated by low molar O/C) of BC700 control TCE sorption from water [11].

### 3.4. TCE Desorption

After the columns were exhausted from TCE, they were eluted with 25% methanol to desorb the TCE from column to evaluate the recovery rate of each sorbent. Figure 2 shows the elution curves for TCE desorption from saturated BC300, BC700, and AC at the eluent flow rate of 4 mL min$^{-1}$. A relatively low flow rate was applied for the desorption process compared to sorption to impart more contact time of the eluent with TCE and also to obtain maximum desorption with less eluent consumption. A sharp increase in TCE desorption was observed in the beginning for all sorbents, which then gradually decreased with time. A similar trend was reported in several other desorption studies [21, 28]. Elution efficiency was 57.9% for BC300, 69.2% for BC700, and 82.8% for AC. The relatively low elution efficiency for the BCs compared to AC may presumably be due to the strong binding of TCE to the BCs [11]. As mentioned above, low temperature pyrolyzed BCs contain a larger noncarbonized fraction than high temperature pyrolyzed BCs and AC, which led to the multiple sorption mechanisms. Chen et al. [27] found that sorption of organic contaminants to biochar consists of two parts: partition to noncarbonized fractions and adsorption to the carbonized fraction of the biochar. As a result, sorption of TCE on AC and BC700 may have been predominantly contributed by surface adsorption as they had much higher surface areas and lower noncarbonized fractions. Therefore, TCE was much easier to be contacted by eluent and easier to remove. Sorption of TCE on BC300 may have also significantly contributed by partitioning to the noncarbonized fractions, which was not easy to contact with eluent and harder to remove.

Overall, AC showed best efficiency for removing TCE from water in column experiments. Although sorption and desorption capabilities of BC700 were a little lower than AC, it is still a good alternative for AC to remove organic contaminants such as TCE from water due to its cost-effectiveness.

### 4. Conclusions

Biochar derived from soybean stover pyrolyzed at 700°C and AC outperformed biochar pyrolyzed at 300°C for removing TCE from water in continuous fixed-bed columns. High surface area, low polarity, and high aromaticity were involved in the greater efficiency of AC and BC700 than that of BC300. The sorption capacities of AC and BC700 were 20.5 and 13.3 times higher than that of BC300. The Thomas model well described the column sorption data, indicating that the sorption of TCE onto BCs and AC is controlled by interphase mass transfer. A comparison of the efficiency of the BCs for removing TCE from water suggests that BC700 is cost-effective comparable to AC. The lower desorption rate of TCE from BC300 than BC700 and AC may be attributed to the strong binding/partition of TCE to the noncarbonized part of BC. Future studies, such as BC surface activation and modification, are needed to further improve the sorption capacity of BCs.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Rejection of Organic Micropollutants by Clean and Fouled Nanofiltration Membranes

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The rejection of organic micropollutants, including three polycyclic aromatic hydrocarbons (PAHs) and three phthalic acid esters (PAEs), by clean and fouled nanofiltration membranes was investigated in the present study. The rejection of organic micropollutants by clean NF90 membranes varied from 87.9 to more than 99.9%, while that of NF270 membranes ranged from 32.1 to 92.3%. Clear time-dependence was observed for the rejection of hydrophobic micropollutants, which was attributed to the adsorption of micropollutants on the membrane. Fouling with humic acid had a negligible influence on the rejection of organic micropollutants by NF90 membranes, while considerable effects were observed with NF270 membranes, which are significantly looser than NF90 membranes. The observed enhancement in the rejection of organic micropollutants by fouled NF270 membranes was attributed to pore blocking, which was a dominating fouling mechanism for loose NF membranes. Changes in the ionic strength (from 10 to 20 mM) reduced micropollutant rejection by both fouled NF membranes, especially for the rejection of dimethyl phthalate and diethyl phthalate by NF270 membranes (from 65.8 to 25.0% for dimethyl phthalate and 75.6 to 33.3% for diethyl phthalate).

1. Introduction

Due to the growing demand for high quality water, applications employing membrane processes for water treatment have increased rapidly. Nanofiltration (NF) and reverse osmosis (RO) are promising membrane technologies that have been recognized as reliable and affordable techniques for the production of high quality water from nontraditional sources such as polluted surface water and secondary treated effluent, which require the removal of organic micropollutants [1–6]. The rejection mechanism of organic micropollutants by NF has been investigated in previous studies, and researchers agree that steric hindrance (or size exclusion) is the most important mechanism of uncharged organic micropollutant rejection [7, 8]. Nevertheless, other micropollutants and membrane physicochemical properties may also influence the separation behavior. The results from various investigations showed that the adsorption of hydrophobic neutral compounds to membranes enhanced rejection and adsorption, which increased almost linearly with the distribution coefficient (LogD), although the observed enhancement was most likely limited to a relatively short time scale [9, 10]. In many previously reported publications, the removal of organic micropollutants by NF has been described; however, only a few studies have been devoted to the time frame prior to rejection, and the rejection efficiency of an NF membrane for a hydrophobic compound will be overestimated if it is determined during short-term experiments [7, 11].

The fouling of NF membranes changes the membrane characteristic and affects the rejection of micropollutants. Natural organic matter (NOM) such as humic acid is one of the major causes of membrane fouling. Numerous studies have reported the physical and chemical aspects of NF membranes fouled by humic acid [12–15]. The influence of membrane fouling on the rejection of micropollutants by NF has also been studied. Membrane fouling can either increase or decrease the rejection of organic micropollutants by NF membranes [5, 13, 16, 17]. The mechanisms of
the different effects of fouling on the rejection of micropollutants have been illustrated. For instance, Nghiem and Hawkes [8] observed a considerable increase in rejection of trace organics by loose NF membrane under fouled conditions. The observed rejection increase was attributed to pore blocking. After fouled with sodium alginate, Yangali-Quintanilla et al. [7] observed a decline in rejection of hydrophobic neutral compounds by NF200 membrane; however, they attributed the general trend of decreasing rejection to the phenomenon of “cake-enhanced concentration polarization.” More recently, Shen et al. [18] investigated the influence of solute-solute interactions on hormone rejection during nanofiltration and observed that solute-solute interactions between humic acid (HA) and micropollutants improved micropollutant rejection and decreased micropollutant adsorption to membranes. HA sorption was attributed to enhanced water permeability due to the opening of charged membrane pores. In summary, many studies on the effects of membrane fouling on the NF of organic micropollutants have been reported, but the results of the aforementioned studies are highly variable. Certainly, the variety of commercially available micropollutants and membranes is the most probable cause of the observed discrepancies in previous results.

Recently, polycyclic aromatic hydrocarbons (PAHs) and phthalic acid esters (PAEs) have been frequently detected at higher concentrations in surface water [19–22]. Due to their toxicity, mutagenicity, and carcinogenic potential, PAHs are included in the priority list of pollutants of the US EPA and the European Union [23]. PAEs are a group of chemicals with high environmental relevance due to their production rates and ecotoxicological potential. Various adverse effects, including those on the reproductive and endocrine systems of crustaceans and amphibians, have been reported [24–26]. Thus, the occurrence and removal of PAHs and PAEs have become a significant focus in the field of water treatment. PAHs and PAEs are difficult to remove by conventional water treatment technology; however, until now, little information on the removal of PAHs and PAEs by NF membranes has been provided. Furthermore, dimethyl phthalate and diethyl phthalate present low hydrophobicity, while all PAHs are highly hydrophobic. Thus, the nanofiltration of PAHs and PAEs can improve the understanding of the relationship between the hydrophobicity of compounds and rejection by NF.

The aim of the present study was to investigate the rejection of PAHs and PAEs by NF membranes and to explain the effects of polymeric NOM on the removal efficiency. Filtration experiments were carried out on three types of PAHs and PAEs, including dimethyl phthalate, diethyl phthalate, acenaphthylene, phenanthrene, and pyrene, using two different commercial NF membranes.

2. Materials and Methods

2.1. Membranes and Chemicals. Two thin-film composite NF membranes, denoted as NF270 and NF90 (Dow FilmTec), were employed in the present investigation. The membranes were received as flat sheet samples and were stored dry at 4°C. Dimethyl phthalate, diethyl phthalate, and dibutyl phthalate, which possessed a purity of 99.5%, were purchased from Sinopharm. Acenaphthylene was purchased from TCI and possessed a purity of 99%. Phenanthrene was purchased from Acros and presented a purity of 98%. Pyrene was purchased from J&K and possessed a purity of 98%. Table 1 shows the key physicochemical parameters of these compounds. Methanol was purchased from Amethyst and had HPLC grade. The feed solutions were prepared by spiking an appropriate amount of dimethyl phthalate and diethyl phthalate from a water-based stock solution into Milli-Q water. For the other chemicals, the stock solutions were prepared by dissolving the compound in methanol. For all of the tested compounds, the experiments were performed at a feed concentration of approximately 500 μg L⁻¹, except for dimethyl phthalate, which was evaluated at a feed concentration of 800 μg L⁻¹. In all of the experiments, three PAHs and three PAEs were mixed in a single feed solution. Humic acid was used in the present study and was obtained from Sinopharm.

2.2. Membrane Test Unit. A laboratory-scale, cross-flow membrane test unit with two parallel cells was used in the current study (Figure 1). The experiments were conducted in recycle mode, in which the retentate and permeate were returned to the feed reservoir to maintain a constant concentration. The membrane cell and tubes were made of stainless steel to minimize compound losses on nonmembrane components of the test system. The feed reservoir (4 L) was made of glass. For each experiment, a new membrane coupon with an effective membrane area of 22.05 cm² was used. The experiments were conducted at ambient temperature (25 ± 1°C).

2.3. Experimental Protocol. Prior to each experiment, the membrane was stabilized at 500 kPa using deionized water until the permeate flux reached a constant value. After
membrane stabilization, water was replaced by the solution in the feed reservoir, which contained the tested compounds. Feed and permeate samples (1 mL each) were obtained at specified time intervals for analysis.

The fouling of membranes and subsequent rejection experimental protocol were conducted in three steps, including compaction, fouling development, and rejection measurement. First, the membrane was compacted using Milli-Q water at 500 kPa for at least 2 h until a stable baseline flux was obtained. A fouling layer was then allowed to develop using a solution consisting of 50 mg L\(^{-1}\) of humic acid, and a stable flux was again obtained prior to the addition of the tested compounds in an electrolyte solution. The electrolyte solution was used to produce a foulant cocktail with a NaCl concentration of 10/20 mM. The fouling layer was allowed to develop until changes in the flux were no longer observed.

2.4. Analysis. A high performance liquid chromatography (HPLC, Aglient 1260) system equipped with a reverse phase C18 column was employed to determine PAHs and PAEs using methanol and water as mobile phase with gradient elution. Acenaphthylene, phenanthrene, and pyrene were determined by fluorescence detector with excitation/emission wavelengths of 225 nm/360 nm, 244 nm/360 nm, and 237 nm/385 nm, respectively. PAEs were determined by ultraviolet detector with wavelength of 230 nm. The limits of detection (LOD) for acenaphthylene, phenanthrene, pyrene, dimethyl phthalate, diethyl phthalate, and dibutyl phthalate were 0.003 mg L\(^{-1}\), 0.006 mg L\(^{-1}\), 0.022 mg L\(^{-1}\), 0.018 mg L\(^{-1}\), 0.007 mg L\(^{-1}\), and 0.019 mg L\(^{-1}\), respectively.

2.5. Measurement of Contact Angle. Contact angle measurements were performed with a DSA10-MK2 contact angle analyzer (KRUS BmbH Co., Germany). The sessile drop method was used to measure the contact angles of deionized water (3 L) on the dried surfaces of the membranes at 25°C. The volume of drop was 5 μL. Images were captured 5 s after introducing the drop and the contact angles were calculated. At least ten measurements on different locations of the membrane sample were performed and averaged to obtain the contact angle of the measured membrane sample. All the results presented were an average data from five membrane samples with standard deviation of the measured values.

3. Results and Discussion

3.1. Membrane Characteristics. The nanofiltration membranes assessed in the present study consisted of a thin active layer on top of a porous polysulfone backing layer. The active
layer of the NF270 membrane was made of semiaromatic piperazine-based polyamides, while the NF90 membrane possessed a fully aromatic polyamide active layer. The two membranes displayed quite distinct characteristics. NF90 was a relatively tight NF membrane with an average pore diameter of only 0.68 nm. In contrast, NF270 can be considered a loose NF membrane (0.84 nm) [13]. Salt rejection tests were carried out to simulate the standard conditions indicated by the membrane datasheets. The average rejection of sodium sulfate by clean NF270 and NF90 membranes was 97.8% and 98.7%, respectively. The pure water permeability and contact angle of clean and fouled membranes are shown in Table 2. As described in the table, NF270 was more permeable than NF90 but provided lower solute rejection efficiencies. The membrane datasheets are presented in Figure 2. The concentration of all six compounds in permeate and contact angle of both membranes fouled with humic acid compared to that of clean membranes.

### 3.2. Variation of Tested Compounds during the Filtration Tests.

The concentration of all six compounds in permeate and feed, as a function of the filtration time by NF270 and NF90 membranes, is presented in Figure 2. The concentration of acenaphthylene, phenanthrene, pyrene, and dibutyl phthalate in the feed solution decreased over time. The observed decrease was likely due to the adsorption of these compounds on the membrane because evaporation and adsorption onto the membrane reached equilibrium. In the current study, NF90 had a larger contact angle and possessed a more hydrophobic surface. An increase in the hydrophobicity was clearly observed by a considerable increase in the contact angle of both membranes fouled with humic acid compared to that of clean membranes.

### 3.3. Rejection of Organic Micropollutants.

The rejection of organic micropollutants by NF90 and NF270 membranes, as presented in Table 3, was determined based on 8 h of filtration. The results showed that the tight NF90 membrane provided significantly higher rejection efficiencies compared to the loose nanofiltration NF270 membrane for all of the tested compounds. The rejection of PAEs by NF270 was significantly lower than that of NF90, which was attributed to the MWCO of the membrane. For neutral compounds with low hydrophobicity, such as acenaphthylene, phenanthrene, pyrene, and dibutyl phthalate, adsorption and charge exclusion were very weak; thus, steric hindrance was the dominant mechanism during nanofiltration. The rejection of compounds with are highly hydrophobic, as reflected by their high log $K_{ow}$ values (>3.0), showed apparent adsorption to the membrane polymeric layer. In contrast, dimethyl phthalate and diethyl phthalate presented low hydrophobicity ($\log K_{ow} < 2.7$), and their adsorption to the membrane polymeric layer was not significant. Based on these results, the rejection efficiency of hydrophobic compounds should be determined after the adsorption of compounds to the membrane reaches equilibrium. In the present study, the rejection efficiency of acenaphthylene, phenanthrene, pyrene, and dibutyl phthalate was determined based on 8 h of filtration.

### Table 2: Comparison of clean and fouled membrane properties.

| Characteristic                  | NF90 Clean | NF90 Fouled | NF270 Clean | NF270 Fouled |
|-------------------------------|------------|-------------|-------------|--------------|
| Contact angle (°)              | 47.3 ± 2.0 | 61.5 ± 2.2  | 26.1 ± 2.1  | 30.8 ± 2.1   |
| Pure water permeability        | 57.2       | 37.9        | 85.5        | 68.2         |

### Table 3: Rejection of organic micropollutants by NF90 and NF270.

| Compound            | NF90 (%) | NF90 (%) | NF270 (%) | NF270 (%) |
|---------------------|----------|----------|-----------|-----------|
| Acenaphthylene      | 92.4     | 91.2     | 86.4      | 87.3      |
| Phenanthrene        | 95.7     | 96.0     | 88.6      | 92.5      |
| Pyrene              | >99.9    | >99.9    | 93.2      | >99.9     |
| Dimethyl phthalate  | 87.9     | 95.0     | 32.1      | 65.8      |
| Diethyl phthalate   | 95.3     | 92.0     | 50.4      | 75.6      |
| Dibutyl phthalate   | >99.9    | >99.9    | 50.0      | 59.6      |
Figure 2: Change in concentration of (a)acenaphthylene, (b)phenanthrene, (c)pyrene, (d)dimethyl phthalate, (e)diethyl phthalate, and (f) dibutyl phthalate during filtration test (applied pressure: 500 kPa).
molecular weights (MW of 194–278 g mol⁻¹) similar to or greater than the MWCO of NF90 (200 Da) showed higher rejection rates. In contrast, for NF270 (300 Da), which possessed a higher MWCO than all of the MWs of the PAEs, lower rejection rates were observed. Moreover, the rejection of dibutyl phthalate, which possessed a higher log Kow than dimethyl phthalate and diethyl phthalate, did not show significantly higher rejection efficiency by NF270. This observation was supported by Yangali-Quintanilla et al. [7], who found that larger hydrophobic neutral compounds (MW 216–272 g mol⁻¹) did not show considerably higher rejection rates by loose NF200 membranes than low MW hydrophilic neutral compounds (MW 151–194 g mol⁻¹) due to the diffusion of hydrophobic neutral compounds across the membrane after saturation. The rejection of all of the PAHs was high by both NF90 and NF270 and ranged from 86.4% to more than 99.9%. The rejection of acenaphthylene and phenanthrene, which possess MWs (154 g mol⁻¹ and 178 g mol⁻¹) that are lower than the MWCO of both NF90 and NF270 membranes, showed greater rejection efficiencies. This result was attributed to the adsorption of compounds to the membrane polymer, as evidenced by a decrease in the feed concentration in the final stages of the experiment. According to Nghiem et al., the adsorption of compounds onto the membrane skin layer is fast and is not a rate-limiting step. Rather, the rate of transport across the membrane is governed by diffusion through the skin (active) layer of thin-film composite NF membranes. Thus, the similar rejection rates of acenaphthylene, phenanthrene, and pyrene by tight NF90 and loose NF270 membranes were attributed to their comparable active layer thicknesses. Similar performances have also been observed for the rejection of natural hormones by NF90 and NF270 membranes [11].

3.4. Effects of HA on the Membrane Rejection Behavior

3.4.1. Membrane Fouling. The extent of membrane fouling can be described by the normalized permeate flux decline. The ratio of the permeate flux (J) and initial pure water flux (J0) was calculated as an indicator of membrane fouling. Figure 3 presents the normalized permeate flux as a function of time during fouling by HA for NF270 and NF90 membranes. Similar to previous studies on membrane fouling behavior due to organic matter, two distinct fouling stages were observed [8, 28, 29]. A rapid flux decline occurred immediately after HA was introduced to the feed solution. Subsequently, a considerably slower flux decline was observed, and a quasi-steady-state fouling layer fully developed after approximately 12 h of filtration. The NF270 membrane exhibited a lesser decline (20% reduction) due to fouling compared to the NF90 membrane, which presented a 30% reduction in the normalized specific flux. According to an atomic force microscopy (AFM) study performed by Boussu et al., the NF90 membrane surface (mean roughness of 38.8 nm) is rougher than the NF270 membrane (mean roughness of 4.6 nm). Previous studies indicated that rougher membranes are expected to foul to a greater degree than smoother membranes. Thus, the higher flux decline for the NF90 membrane was attributed to its greater membrane roughness.

Compared to rejection by clean membranes, membrane fouling resulted in the modification of membrane properties and affected the rejection of organic micropollutants. A dark brown layer of humic foulant was firmly attached to the membrane surface after all of the fouling experiments. The characterization of fouled membranes showed that there was a significant change in membrane hydrophobicity after fouling (Table 2). The rejection rates of selected PAHs and PAEs by clean and fouled NF90 and NF270 membranes are presented in Table 3. More than 90% of contaminants was rejected by clean NF90 membranes and was not significantly affected by fouling, except for the rejection of dimethyl phthalate, which increased from 87.9% to 95.0% due to fouling. This result was consistent with those of previous studies performed by Nghiem and Hawkes [8]. The observed increase in the rejection of dimethyl phthalate by fouled NF90 membranes was attributed to an enhanced sieving effect. Regarding NF270 membranes, a significant enhancement in the rejection of compounds with low hydrophobicity, such as dimethyl phthalate and diethyl phthalate, was observed (raised 33.6% and 25.2%, resp.) due to fouling. The observed increase in rejection by fouled NF270 membranes, which have a larger pore size (0.84 nm), was attributed to pore blocking [8] and the increased hydrophobicity of fouled NF270 membranes. Furthermore, a small but nevertheless apparent increase in the rejection of the four hydrophobic compounds by NF270 membranes under fouled conditions was also observed, as shown in Table 3.

3.4.2. Effects of the Ionic Strength on Rejection. To study the effect of the ionic strength on rejection, the salt content of the feed solution was adjusted with NaCl (10 and 20 mM) after the
NF membranes were fouled by humic acid. Table 4 shows the rejection of dimethyl phthalate, diethyl phthalate, acenaphthylene, and phenanthrene by the two membranes. For NF90, an increase in the ionic strength of the feed solution slightly decreased the rejection of the compounds. In contrast, the rejection of pollutants by NF270 decreased significantly as the ionic strength increased, especially for dimethyl phthalate and diethyl phthalate. The observed decrease in rejection was attributed to the swelling of membrane pores, a reduction in the compound’s hydrodynamic radius, or a combination of these two phenomena [1, 30, 31]. The charge density of NF membranes depends on the presence of salts at a constant pH (which was maintained in the present study). Adding salt to the solution resulted in a greater concentration of counterions in the electrical double layer at the surface of the membrane pores, which induced pore swelling and electrical double-layer compaction. As a result, the apparent pore radius increased. Alternatively, in a mixed solution of chemicals and salt, a lower apparent volume of hydrated compounds may be observed compared to in the absence of salt, and hydrated compounds can permeate more freely through the membrane. This effect becomes stronger when the salt concentration increases, which explains the observed decrease in the rejection rate with an increase in the ionic strength. This phenomenon was also confirmed by the observed increase in flux due to higher salt concentrations in the feed solution.

3.4.3. Effects of the Transmembrane Pressure on Rejection and Permeate Flux. To investigate the influence of transmembrane pressure on the performance of the membranes, experiments were performed at 500, 600, and 700 kPa. In the present study, dimethyl phthalate, the most hydrophilic compound among the six tested chemicals, was used. Regardless of the mechanism of adsorption, the effect of pressure should be evident. Table 5 shows the rejection of dimethyl phthalate and the permeate flux at different transmembrane pressures for the two membranes. The rejection of dimethyl phthalate decreased with an increase in the transmembrane pressure for both NF90 and NF270 nanofiltration membranes, and the permeate flux increased with an increase in the transmembrane pressure for both membranes. These results are in accordance with those of previous studies [32], which showed that the (initial) fluxes of membranes varied almost linearly with the applied pressure. Nevertheless, different trends were observed during long-term filtration. Overall, the influence of pressure on the performance of NF270 membranes was more pronounced than for NF90.

### Table 4: Effect of ionic strength on rejection of compounds by the two NF membranes (transmembrane pressure 500 kPa, pH 7.5; initial concentration of compounds 0.5–0.8 mg L\(^{-1}\)).

| Membrane/ionic strength | Flux (L m\(^{-2}\) h\(^{-1}\)) | Dimethyl phthalate (%) | Diethyl phthalate (%) | Acenaphthylene (%) | Phenanthrene (%) |
|------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| NF90 10 mM             | 34.6            | 95.0            | 96.0            | 91.2            | 96.0            |
| NF90 20 mM             | 37.1            | 93.0            | 95.0            | 91.2            | 93.1            |
| NF270 10 mM            | 72.3            | 65.8            | 75.6            | 87.3            | 92.5            |
| NF270 20 mM            | 87.3            | 25.0            | 33.3            | 82.4            | 88.6            |

### Table 5: Influence of transmembrane pressure on dimethyl phthalate rejection and permeate flux (the feed solution contained 800 mg L\(^{-1}\) dimethyl phthalate; pH 7.5, 10 mM NaCl).

| Transmembrane pressure (kPa) | NF90 Flux (L m\(^{-2}\) h\(^{-1}\)) | NF90 Rejection (%) | NF270 Flux (L m\(^{-2}\) h\(^{-1}\)) | NF270 Rejection (%) |
|-----------------------------|-------------------------------------|-------------------|-------------------------------------|-------------------|
| 500                         | 43.4                                | 95.8              | 65.2                                | 65.8              |
| 600                         | 57.2                                | 95.0              | 88.5                                | 17.9              |
| 700                         | 66.5                                | 92.5              | 108                                 | 14.0              |

### 4. Conclusion

Rejection of PAHs and PAEs by NF was governed by steric hindrance (or size exclusion) and adsorption to the membrane surface and pore structure. Under both fouled and clean membrane conditions, higher rejection rates of all six selected micropollutants were achieved with the tight nanofiltration NF90 membrane compared to the loose nanofiltration NF270 membrane. Although fouling had a negligible effect on the rejection of micropollutants by the NF90 membrane, considerable effects were observed with the NF270 membrane, which possessed a larger pore size. The observed enhancement in the rejection of micropollutants by fouled NF270 membranes was attributed to pore blocking and hydrophobic interactions between the membrane and organic micropollutants. The rejection of PAHs and PAEs was influenced by the ionic strength and transmembrane pressure, which were affected by the structure and properties of the micropollutants and membrane.

### Conflict of Interests

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the paper. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Research Article

Reduction of Phosphorus Pollution from Broilers Waste through Supplementation of Wheat Based Broilers Feed with Phytase

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The present study was conducted to reduce phosphorus pollution from broilers waste by supplementing phytase enzyme in broilers feed. Two hundred two-week-old broilers (Hubbard) were selected and randomly allocated to three dietary treatment groups, one control group (without phytase) and two trial groups (group A with 300 U/kg phytase and group B with 600 U/kg phytase). Each group was composed of 5 replicates with 10 chicks. Broilers fed the control diet (without phytase) gained weights lower (P < 0.05) than the other treatment groups. A significant increase in body weight gain of group A (28.00 ± 2.97) and group B (29.75 ± 3.45) was observed as compared to control group (26.75 ± 2.78). The feed intake of the birds fed the diets containing microbial phytase 600 U/kg was the highest. Phytase significantly (P > 0.05) reduces excreta P and Ca level. Phytase addition did not affect excreta pH. The presence of phytase in feed mixtures significantly (P > 0.05) improves the body weight gain and feed intake of broiler chickens.

1. Introduction

Phytase is a hydrolytic enzyme that releases phytate phosphorus, which represents 60 to 80 percent of the total phosphorus in plant-based feeds. Due to the lack of phytase in their gastrointestinal tracts, monogastric animals such as swine, poultry, and preruminant calves cannot digest phytate phosphorus. This results in the excretion of high levels of phosphorus in the manure of these animals [1, 2].

The enzyme phytase finds application in the hydrolysis of phytic acid (myo-inositol hexaphosphoric acid) and thus its metal chelating capability is eliminated and nutritional value of defatted oil seed cakes (canola meal, cotton seed meal, soybean meal, wheat, and mung beans) is enhanced. Several studies reported that phytase reduced the ileal flows of endogenous minerals and amino acids in broiler chickens, while phytate increased the excretion of endogenous amino acid [3–5]. The enzyme phytase catalyses the dephosphorylation of phytic acid and its salts, phytates. Supplementation of monogastric animal feed with microbial-derived phytase increases the bioavailability of phytic acid bound phosphate. This facilitates a reduction in the addition of inorganic phosphate to the feed and reduces phosphorus excretion [6].

The objective of this study was to reduce phosphorus excretion from poultry waste, reduce the feed cost of inorganic phosphorus supplementation, and preserve the nonrenewable inorganic phosphorus for sustainable agriculture.

2. Material and Method

2.1. Effect of Phytase on Body Weight and Food Intake Broiler Chicks. All experiments were conducted according to guidelines approved by the Animal Ethics Committee of Lahore College for Women University, Lahore, for the care and use of animals in research.

Two experiments were conducted to determine the effect of added microbial phytase on weight gain and feed intake of Hubbard broiler chicks. The broilers were housed in 2 × 4.34-m pens at a local poultry farm in 1 room of a ventilated tunnel house equipped with cool cells and fans. At two weeks
of age, 200 chicks were divided into four groups each of 50 hens. Each group was composed of 5 replicates with 10 chicks each. They were held overnight without feed and water on the day before allotment to treatment. The broilers were then weighed and allotted to treatments in a completely randomized design. The experiment was conducted for 48 days. Both trails consisted of three treatments as follows:

1. Control group: diet contains no phytase enzyme;
2. Group A: low phosphorous diet contains 300 PU/kg diet;
3. Group B: low phosphorous diet contains 600 PU/kg diet.

Ingredients of the diet are shown in Table 1.

Experiment 1. Chicks were weighed on weekly basis till the end of the experiment which lasted for 48 days. Body weight was weekly recorded and weight gain was calculated.

Experiment 2. In EXP 2, feed intake to body weight gain ratio (FCR) was recorded daily.

2.2. Analysis of pH, Ca, and P of Excreta Samples. The excreta were collected in plastic sheets. The excreta samples were mixed and homogenized individually. The pH of 1.0 g of excreta in 10 mL of distilled water was measured using a digital pH meter. The level of ash, Ca, and P in excreta was determined by standard method [7].

2.3. Statistical Analysis. The data on various parameters were tabulated and subjected to statistical analysis using computer software Costat, cs 6204W.exe.

3. Results and Discussion

A significant increase in body weight gain of group A (28.00±2.97) and group B (29.75±3.45) was observed as compared to control group (26.75±2.78). The effect of supplementation of phytase on daily feed intake is presented in Table 2.

It was observed that chicks fed diet supplemented with phytase had significantly superior body weight gains as compared with control group. This indicates the synergetic effect of phytase for improving the growth performance. The improved growth due to phytase supplementation indicates that phytic acid is a growth limiting factor for chicks [8]. Our results are in complete agreement with observation of other workers [9, 10]. This may be due to the improved nutrients absorption especially crude protein which complicates with phytate and inhibit other proteolytic enzymes such as pepsin and trypsin [10–13]. The improvement in body weight gain of chicks fed phytase-supplemented diets could be attributed to the improvement in availability of protein, essential amino acids, metabolisable energy, and minerals for animal growth [8]. Phytase enzyme supplementation improved (P < 0.05) feed intake in broilers fed P-deficient diets. The feed intake of the birds fed the diets containing microbial phytase 600 U/kg was the highest, following the 300 U/kg enzyme group and control group, respectively (Table 2). These differences among the groups were due to the use of microbial phytase enzyme amount in diets. Our findings are supported by other workers [12–14].

The improved feed intake with phytase may be due to release of phosphorus, which is potential for other nutrients to show a higher availability. In particular, positively charged (cationic) minerals such as calcium, zinc, copper, cobalt, iron, magnesium, nickel, and manganese are all known to form complexes with phytate and show higher digestibility values in the presence of phytase. This finding is consistent with other workers [2, 14].

3.1. Effect of Phytase on Phosphorus Content, pH, and Ca in Broiler Chickens’ Excreta. Effects of phytase supplementation on the excreta pH, Ca, and P are presented in Table 3. Dietary treatments have significant effect on excreta pH. Significant reduction of P excretion was observed by phytase supplementation of diet. P reduction was 40% in group B and 28% in group A as compared to control. According to another researcher, the reduction of P excretion was 41% with the low P diet and supplementary phytase [15].

Microbial phytase improved availability of phytate phosphorus in layer diets [16]. In the present study excreta Ca content was 50% reduced as compared to control. Literature is lacking reports on the influence of phytase on availability of

| Feeds          | Control (g/100 g) | Group A (g/100 g) | Group B (g/100 g) |
|----------------|-------------------|-------------------|-------------------|
| Wheat          | 65.00             | 65.00             | 65.00             |
| Soybean meal   | 14.86             | 14.86             | 14.86             |
| Sunflower meal | 7.62              | 7.62              | 7.62              |
| Vegetable oil  | 0.92              | 0.92              | 0.92              |
| Salt           | 0.50              | 0.50              | 0.50              |
| DL-methionine  | 0.12              | 0.12              | 0.12              |
| L-lysine       | 0.17              | 0.17              | 0.17              |
| Vitamin premix | 0.25              | 0.25              | 0.25              |
| Mineral premix | 0.25              | 0.25              | 0.25              |
| Dicalcium phosphate | 1.33         | 1.33              | 1.33              |
| Ground limestone | 8.98            | 8.98              | 8.98              |
| Phytase enzyme (U/kg) | —               | 300               | 600               |

Chemical analysis (DM basis)

| Chemical                | Control (%) | Group A (%) | Group B (%) |
|-------------------------|-------------|-------------|-------------|
| Dry matter (%)          | 89.27       | 89.30       | 89.32       |
| Crude protein (%)       | 15.00       | 15.03       | 15.02       |
| Crude cellulose (%)     | 4.28        | 4.29        | 4.29        |
| Ash (%)                 | 13.65       | 13.63       | 13.62       |
| Ether extract (%)       | 6.04        | 6.06        | 6.03        |
| Calcium (%)             | 3.75        | 3.70        | 3.73        |
| Phosphorus (%)          | 0.42        | 0.41        | 0.42        |
| Methionine (%)          | 0.60        | 0.61        | 0.61        |
| Lysine (%)              | 0.79        | 0.78        | 0.77        |
| Linoleic acid (%)       | 1.83        | 1.82        | 1.84        |

(Source: Hi-Tech Feed, Lahore, Pakistan).
Table 2: Effect of dietary supplementation of microbial phytase on body weight gain and feed intake.

| Age (week) | Body weight gain (g) | Feed intake (g) |
|------------|----------------------|-----------------|
|            | Control Group (A)    | Group (B)       | Control Group (A) | Group (B) |
| 3          | 20                   | 21              | 34               | 33         | 35             |
| 4          | 25                   | 26              | 28               | 47         | 48             | 49             |
| 5          | 29                   | 30              | 33               | 63         | 65             | 67             |
| 6          | 33                   | 35              | 37               | 74         | 71             | 80             |

Table 3: Effects of diet inclusion with two phytase levels (300 and 600 g/100 g) on excreta parameters (pH, Ca, and P).

| Parameter | Excreta pH | Excreta Ca (%ash) | Excreta P (%ash) |
|-----------|------------|-------------------|------------------|
| Control   | 7.59       | 6.15              | 0.78             |
| Group A   | 7.48       | 4.22              | 0.59             |
| Group B   | 7.15       | 3.07              | 0.47             |

Ca in broilers. We suppose that microbial phytase improved availability of Ca.

4. Conclusion

Our findings showed that phytase should be a mandatory feed additive. The use of a fungal phytase as a feed supplement proved effective in alleviating the negative effects of phytate in livestock diets and provided an improvement on feed intake and body weight.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Relationship between Cadmium Fractions Obtained by Sequential Extraction of Soil and the Soil Properties in Contaminated and Uncontaminated Paddy Soils

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The method for the sequential extraction of cadmium from soil was adapted to investigate the relationship between different chemical forms of cadmium in soils and the soil properties of Cd-contaminated and uncontaminated paddy soils. Air-dried soil samples from each field site were sequentially fractionated into five forms: exchangeable Cd, inorganically bound Cd, organically bound Cd, oxide-occluded fraction, and residual Cd. The average and range of soil properties such as pH, total C, total N, CEC, exchangeable Ca, Mg, K, base saturation, available phosphate, particle size distribution, free iron oxide, oxalate extractable Al, and Fe were somewhat similar between uncontaminated and contaminated soils. The average total Cd in uncontaminated and contaminated soils was 0.26 and 0.65 mg kg\(^{-1}\), respectively. The proportions of soil Cd fractions did not differ between the uncontaminated and contaminated soils, although the Cd concentration of several fractions in contaminated soils was statistically higher than those in uncontaminated soils except for residual fraction. The proportion of exchangeable Cd was correlated with the CEC and phosphate absorption coefficient in contaminated soil but not in uncontaminated soil. Thus, soil properties appear to affect the proportions of soil Cd fractions in contaminated soil and should be considered when evaluating soil Cd mobility.

1. Introduction

Under Japan’s Food Sanitation Act, the Cd concentration of unpolished rice must not be higher than 0.4 mg kg\(^{-1}\) on or after February 28, 2011 [1]. The official method for extracting soil Cd to estimate the extent of soil contamination generally uses a 0.1 mol L\(^{-1}\) HCl solution, but the concentration of Cd estimated by this method tends to be unrelated to the Cd concentration of unpolished rice grains [2, 3]. Therefore, various extraction methods have been tested to determine the plant-available Cd concentration in contaminated soil, and the Cd concentrations in some upland plants have shown high correlations with plant-available soil Cd concentrations [4, 5].

Despite this progress, the association of soil properties with the Cd concentration of paddy rice—especially the rice grain—has not been evaluated [2] because the rapid shifts between submerged and drained conditions affect the solubility of soil Cd due to the change in oxidation-reduction potential. Chino [6] reported that most of the Cd taken up by rice plants in the 10 days after heading was transported to the grain but that the final Cd concentration was determined by the oxidation-reduction potential in this period. The Cd concentration of rice grown in paddy soil was greatly affected by the number of days of dry conditions after heading [7]. It is therefore difficult to estimate the Cd concentration of rice grains by analyzing dried soil. Nevertheless, an assessment of potential risk apart from the influence of the water condition of soils is useful for zoning high-risk fields and deciding on countermeasures or remediation.

The sequential extraction of soil Cd can be used to evaluate Cd behavior in soil and its availability to plants.
The exchangeable fraction of Cd (ex-Cd) is much higher in contaminated soil than in uncontaminated soil [8]. Hattori et al. [9] found that it is possible to estimate the Cd concentration of brown rice from the ex-Cd concentration in the soil at the time of heading. This result suggests that the Cd concentration in rice grains may be affected by the ex-Cd concentration in the soil. However, the ex-Cd concentration decreases with increasing soil pH [10], submergence [11], or the application of organic matter [12]. Correlation analysis also revealed that pH was the most dominant soil variable affecting soil solution Cd concentration and sorption and desorption of native and added Cd in 29 New Zealand soils. However, organic matter (OM), cation exchange capacity (CEC), and total soil Cd were also found to be important [13].

Organic matter addition [14] revealed that pH was the most dominant soil variable affecting soil solution Cd concentration and sorption and desorption of native and added Cd in 29 New Zealand soils. However, organic matter (OM), cation exchange capacity (CEC), and total soil Cd were also found to be important [13]. The exchangeable fraction of Cd as determined by 1 M MgCl₂ in the sequential extraction procedure increased, whereas the Fe-Mn oxide bound fraction decreased, with increasing levels of organic matter addition [14]. As mentioned above, some soil properties could influence the solubility and mobility of soils Cd, and the degree of the influence is thought to be different between contaminated and uncontaminated soils. Thus, there is a need to compare the relation between Cd fractions and soil characteristics in both contaminated and uncontaminated soil. Furthermore, any changes in the proportion of ex-Cd lead to changes in the proportions of inorganically bound, organically bound, and oxide-occluded Cd. However, few articles have compared the relationships between Cd fractions in soil and soil properties in uncontaminated and contaminated soils. Furthermore, support the assessment of potential Cd risk in arable soils, it is important to determine the proportions of each soil Cd fraction by sequential extraction of soils.

The aim of this study was to evaluate the relationships between Cd fractions and soil properties in uncontaminated and contaminated soils.

2. Materials and Methods

2.1. Soils. Samples of Cd-contaminated soil were collected from 10 paddy fields in Hokuriku district that were contaminated with Cd by mining waste water and sediments deposited by flooding from the 1930s to 1960s [15]. It is probable that fields in this area produce rice grains with Cd concentrations above 0.4 mg Cd kg⁻¹, the new international threshold established by the Codex Alimentarius Commission [16] for Cd concentrations in brown rice. Uncontaminated soil samples were collected from 10 other paddy fields. All soils were collected from the plow layer from five points in each field and were bulked before analysis.

2.2. Analysis of Soil Physicochemical Properties. All samples were air-dried and passed through a 2-mm-mesh sieve before analysis. Soil pH was determined using a glass electrode (TOA pH meter HM-30S, Japan) with a 1:2.5 w/w ratio of soil to either water or 1 mol L⁻¹ KCl. The total carbon and total nitrogen concentrations were measured using an elemental analyzer (Perkin Elmer 2400 II, USA). There is no replication of the soil chemical analysis and the sequential extraction, because we applied the regression analysis for the data analysis.

2.3. Sequential Extraction of Soil Cd. We extracted the soil Cd by means of a single-extraction method with 0.1 mol L⁻¹ HCl solution (1:5 w/v) by shaking side-to-side for 1 h [17]. The sequential extraction of Cd from soil was performed as follows (modified from [18]) using 5 g of dry soil and 30 mL of extraction solution. First, the ex-Cd fraction was obtained by extraction with 0.05 mol L⁻¹ Ca(NO₃)₂ (1:10 w/v, 24 h shaking), and the extract was separated by centrifugation. Next, to obtain the inorganically bound Cd (in-Cd), the residue of the ex-Cd fraction was reextracted with 2.5% acetic acid (1:10 w/v, 24 h shaking). The organically bound Cd (or-Cd) was obtained by extraction with 2.5% acetic acid (1:10 w/v, 24 h shaking) after the decomposition of organic matter from the residue of the in-Cd fraction with 6% H₂O₂. Finally, the oxide-occluded Cd (ox-Cd) was extracted from the residue of the or-Cd fraction with a mixture of 0.1 mol L⁻¹ oxalate and 0.175 mol L⁻¹ ammonium oxalate (1:30 w/v) in a boiling water bath for 1 h with occasional stirring. Total soil Cd was quantified by digestion with concentrated HNO₃ and 60% HClO₄. The difference between total Cd and the summed extracted fractions gave the residual fraction (re-Cd). The particle size distribution was determined using the pipette method [19]. The cation exchange capacity (CEC) and exchangeable cations were analyzed after extraction with 1 mol L⁻¹ CH₃COONH₄ (pH 7.0) [20]. Other analyses were performed based on the methods described by [21].

2.4. Statistical Analysis. The relationship between soil Cd fraction and soil properties was investigated using Pearson’s correlation coefficient test at P = 0.05, 0.01, and 0.001 in Statcel 2 software [22]. Soil properties identified as statistically significant at P < 0.001 in contaminated soil were compared with those in uncontaminated soil.

3. Results

3.1. Soil Properties. The chemical property values for the uncontaminated and contaminated soils were mentioned in Table 1. The soil textures ranged from sandy loam to light clay. None of the soil chemical and physical properties differed significantly between uncontaminated and contaminated soils (P > 0.05).

3.2. Chemical Forms of Cd in Uncontaminated and Contaminated Soils. Table 2 shows the concentration of each form of Cd and its proportion of the total. The average total Cd concentration was 0.26 mg kg⁻¹ with a range from 0.21 to 0.36 mg kg⁻¹ in uncontaminated soils. In contaminated soils, the average Cd concentration was 0.65 mg kg⁻¹ ranging from 0.43 to 0.88 mg kg⁻¹. The average concentration of each form of Cd (mg kg⁻¹) in uncontaminated and contaminated soils was exchangeable (0.07, 0.16); inorganically bound (0.07, 0.17); organically bound (0.08, 0.19); oxide occluded (0.03, 0.11); and residue (0.02, 0.03). The concentration of each form
Table 1: Soil properties.

| Soil                | Soil typea | pH (H₂O) | pH (KCl) | T-C (g kg⁻¹) | T-N (cmol kg⁻¹) | CEC (cmol kg⁻¹) | Exchangeable cations | Base saturation (%) | Available phosphate (mg kg⁻¹) | PAC (g kg⁻¹) | Particle size distr. | Free iron oxide (g kg⁻¹) | Oxalate extractable (g kg⁻¹) | Ako | Feo | Ako + 1/2 Feo |
|---------------------|------------|----------|----------|--------------|----------------|----------------|----------------------|-------------------|--------------------------|---------------|------------------|--------------------------|-----------------------------|-----|-----|------------|
| Uncontaminated-1    | Fluvaquents| 5.5      | 4.0      | 25.4 2.7     | 18.7          | 5.2 1.7 0.3   | 38.0                | 272               | 9.2                      | 28.2 34.1 37.7 | 8.9              | 3.2 5.5 6.0            |                             |     |     |           |
| Uncontaminated-2    | Fluvaquents| 6.5      | 5.5      | 20.7 1.6     | 9.1           | 6.0 1.5 0.3   | 86.7                | 912               | 4.7                      | 4.4 18.2 77.4 | 9.4              | 3.1 2.8 4.4            |                             |     |     |           |
| Uncontaminated-3    | Endoaquands| 5.8      | 4.8      | 62.1 4.4     | 25.8          | 9.8 2.7 0.8   | 51.7                | 434               | 10.5                     | 38.4 31.5 30.0 | 8.5              | 4.7 3.8 6.6            |                             |     |     |           |
| Uncontaminated-4    | Fluvaquents| 5.8      | 4.2      | 15.8 0.9     | 8.7           | 2.7 0.7 0.2   | 41.7                | 188               | 3.8                      | 8.6 12.3 79.1 | 5.5              | 2.0 1.8 3.8            |                             |     |     |           |
| Uncontaminated-5    | Fluvaquents| 5.9      | 4.3      | 24.4 1.8     | 16.1          | 6.3 1.4 0.3   | 49.6                | 327               | 5.8                      | 16.4 19.7 63.9 | 5.0              | 2.7 1.6 4.3            |                             |     |     |           |
| Uncontaminated-6    | Fluvaquents| 5.5      | 4.0      | 22.9 1.8     | 13.3          | 3.6 1.1 0.4   | 38.5                | 148               | 6.0                      | 13.8 17.8 68.5 | 3.1              | 2.2 2.2 4.4            |                             |     |     |           |
| Uncontaminated-7    | Epiaquepts | 5.5      | 4.1      | 33.6 2.2     | 20.6          | 5.4 2.1 0.2   | 38.1                | 145               | 9.8                      | 22.8 23.7 53.5 | 10.9             | 3.4 4.7 8.1            |                             |     |     |           |
| Uncontaminated-8    | Fluvaquents| 5.5      | 4.0      | 25.5 1.6     | 17.6          | 5.4 2.0 0.5   | 45.4                | 160               | 7.7                      | 17.5 30.4 52.1 | 11.0             | 2.4 5.4 7.9            |                             |     |     |           |
| Uncontaminated-9    | Fluvaquents| 6.1      | 4.6      | 19.6 1.7     | 9.9           | 3.8 1.3 0.3   | 54.8                | 236               | 5.6                      | 11.6 19.1 69.3 | 9.2              | 3.9 1.9 5.8            |                             |     |     |           |
| Uncontaminated-10   | Fluvaquents| 6.0      | 4.4      | 18.8 2.1     | 15.2          | 7.2 2.6 0.3   | 66.2                | 224               | 7.2                      | 18.8 23.7 57.6 | 9.6              | 2.4 4.9 7.3            |                             |     |     |           |
| Contaminated-1      | Fluvaquents| 5.7      | 4.3      | 32.0 2.6     | 14.3          | 4.3 0.9 0.1   | 37.0                | 570               | 6.6                      | 14.1 13.7 68.6 | 7.7              | 3.1 2.9 4.6            |                             |     |     |           |
| Contaminated-2      | Fluvaquents| 5.5      | 4.3      | 32.6 2.5     | 10.6          | 3.1 0.8 0.2   | 38.4                | 277               | 4.3                      | 10.1 15.0 74.9 | 4.7              | 2.2 2.0 3.2            |                             |     |     |           |
| Contaminated-3      | Fluvaquents| 5.6      | 4.7      | 46.3 3.7     | 17.6          | 6.0 1.9 0.6   | 47.9                | 708               | 7.6                      | 14.8 22.1 63.1 | 9.1              | 4.1 4.7 6.5            |                             |     |     |           |
| Contaminated-4      | Fluvaquents| 6.1      | 4.5      | 32.6 2.6     | 12.1          | 3.4 1.0 0.3   | 38.8                | 401               | 4.2                      | 14.4 14.7 70.9 | 4.5              | 2.7 2.9 4.2            |                             |     |     |           |
| Contaminated-5      | Fluvaquents| 5.9      | 4.9      | 37.8 2.8     | 15.4          | 6.2 1.3 0.3   | 50.7                | 514               | 6.6                      | 10.2 23.5 66.3 | 6.3              | 3.7 2.2 4.8            |                             |     |     |           |
| Contaminated-6      | Endoaquands| 6.2      | 5.1      | 43.5 2.9     | 20.5          | 6.9 2.7 0.4   | 48.8                | 880               | 10.4                     | 16.5 17.1 66.4 | 9.6              | 5.8 2.7 7.1            |                             |     |     |           |
| Contaminated-7      | Endoaquands| 5.6      | 4.5      | 48.2 3.7     | 17.4          | 5.2 1.2 0.5   | 39.6                | 349               | 9.0                      | 23.7 31.6 44.7 | 11.4             | 4.6 3.4 6.3            |                             |     |     |           |
| Contaminated-8      | Fluvaquents| 5.7      | 4.5      | 12.7 1.0     | 8.2           | 2.0 0.6 0.2   | 34.0                | 143               | 2.9                      | 5.6 7.8 86.6 | 12.3             | 0.9 3.4 2.6            |                             |     |     |           |
| Contaminated-9      | Fluvaquents| 5.9      | 4.7      | 15.2 1.2     | 8.3           | 2.9 1.1 0.3   | 51.8                | 175               | 3.6                      | 7.0 11.7 81.5 | 9.7              | 1.0 3.1 2.5            |                             |     |     |           |
| Contaminated-10     | Fluvaquents| 5.9      | 4.9      | 33.5 2.6     | 18.4          | 7.9 1.9 0.2   | 54.7                | 528               | 8.5                      | 22.3 36.2 41.5 | 13.9             | 4.2 5.4 6.9            |                             |     |     |           |
| Uncontaminated-AV   |            | 5.8      | 4.4      | 26.9 2.1     | 13.5          | 5.5 1.0 0.3   | 51.0                | 305               | 7.0                      | 18.1 23.1 38.9 | 8.1              | 3.0 3.5 5.9            |                             |     |     |           |
| Contaminated-AV     |            | 5.8      | 4.7      | 33.4 2.6     | 14.3          | 4.8 0.8 0.3   | 44.2                | 455               | 6.4                      | 13.9 19.7 66.4 | 8.9              | 3.2 3.3 4.9            |                             |     |     |           |

T-C, total carbon; T-N, total nitrogen; CEC, cation exchange capacity; PAC, phosphate absorption coefficient.

aClassified by Soil Taxonomy [23].
Table 2: Soil Cd content and fractions of total Cd.

| Soil          | Total (mg kg⁻¹) | 0.1 M HCla (mg kg⁻¹) | Cd content by sequential extraction | Residue | 0.1 M HCl Exchangeable (mg kg⁻¹) | Residue | 0.1 M HCl Exchangeable (mg kg⁻¹) | Residue | Oxide occluded (%) | Oxide occluded (%) | Oxide occluded (%) | Oxide occluded (%) |
|---------------|-----------------|----------------------|-------------------------------------|---------|---------------------------------|---------|---------------------------------|---------|-------------------|-------------------|-------------------|-------------------|
| Uncontaminated-1 | 0.22            | 0.11                 | 0.069                               | 0.037   | 0.046                           | 0.045   | 0.022                           | 50.3    | 31.5              | 16.7              | 21.1              | 20.6              | 10.2              |
| Uncontaminated-2 | 0.36            | 0.21                 | 0.043                               | 0.131   | 0.105                           | 0.036   | 0.042                           | 58.7    | 12.0              | 36.8              | 29.5              | 10.0              | 11.7              |
| Uncontaminated-3 | 0.33            | 0.18                 | 0.051                               | 0.119   | 0.108                           | 0.024   | 0.024                           | 56.4    | 15.7              | 36.5              | 33.0              | 7.4               | 7.4               |
| Uncontaminated-4 | 0.27            | 0.18                 | 0.105                               | 0.053   | 0.073                           | 0.021   | 0.017                           | 65.2    | 39.0              | 19.5              | 27.2              | 7.9               | 6.3               |
| Uncontaminated-5 | 0.29            | 0.23                 | 0.079                               | 0.085   | 0.091                           | 0.017   | 0.022                           | 78.0    | 27.0              | 28.8              | 30.9              | 5.8               | 7.5               |
| Uncontaminated-6 | 0.23            | 0.16                 | 0.092                               | 0.058   | 0.059                           | 0.012   | 0.013                           | 68.0    | 39.3              | 24.9              | 25.0              | 5.1               | 5.7               |
| Uncontaminated-7 | 0.21            | 0.17                 | 0.047                               | 0.052   | 0.079                           | 0.012   | 0.019                           | 79.6    | 22.5              | 24.7              | 38.0              | 5.9               | 8.9               |
| Uncontaminated-8 | 0.21            | 0.15                 | 0.064                               | 0.050   | 0.063                           | 0.015   | 0.018                           | 73.4    | 30.4              | 23.9              | 30.0              | 7.3               | 8.5               |
| Uncontaminated-9 | 0.22            | 0.10                 | 0.035                               | 0.048   | 0.061                           | 0.039   | 0.036                           | 44.1    | 15.9              | 21.7              | 28.0              | 17.8              | 16.6              |
| Uncontaminated-10 | 0.28           | 0.18                 | 0.073                               | 0.057   | 0.070                           | 0.052   | 0.027                           | 65.3    | 26.2              | 20.3              | 25.0              | 18.6              | 9.8               |
| Contaminated-1    | 0.43           | 0.33                 | 0.096                               | 0.113   | 0.140                           | 0.063   | 0.020                           | 76.1    | 22.2              | 26.2              | 32.4              | 14.5              | 4.6               |
| Contaminated-2    | 0.55           | 0.31                 | 0.166                               | 0.118   | 0.163                           | 0.083   | 0.019                           | 56.9    | 30.2              | 21.5              | 29.6              | 15.1              | 3.5               |
| Contaminated-3    | 0.58           | 0.43                 | 0.113                               | 0.167   | 0.200                           | 0.079   | 0.025                           | 73.3    | 19.4              | 28.6              | 34.2              | 13.4              | 4.3               |
| Contaminated-4    | 0.61           | 0.39                 | 0.224                               | 0.126   | 0.164                           | 0.070   | 0.023                           | 63.7    | 36.8              | 20.8              | 27.1              | 11.5              | 3.7               |
| Contaminated-5    | 0.62           | 0.43                 | 0.107                               | 0.187   | 0.184                           | 0.104   | 0.038                           | 69.2    | 17.2              | 30.2              | 29.7              | 16.7              | 6.1               |
| Contaminated-6    | 0.67           | 0.50                 | 0.048                               | 0.192   | 0.258                           | 0.141   | 0.031                           | 73.8    | 7.2               | 28.6              | 38.4              | 21.0              | 4.7               |
| Contaminated-7    | 0.67           | 0.60                 | 0.137                               | 0.193   | 0.218                           | 0.077   | 0.049                           | 89.2    | 20.3              | 28.6              | 32.3              | 11.5              | 7.3               |
| Contaminated-8    | 0.74           | 0.45                 | 0.288                               | 0.129   | 0.132                           | 0.155   | 0.032                           | 60.4    | 39.1              | 17.5              | 18.0              | 21.1              | 4.3               |
| Contaminated-9    | 0.79           | 0.62                 | 0.272                               | 0.202   | 0.168                           | 0.117   | 0.031                           | 78.9    | 34.4              | 25.6              | 21.3              | 14.8              | 3.9               |
| Contaminated-10   | 0.88           | 0.75                 | 0.142                               | 0.280   | 0.231                           | 0.166   | 0.061                           | 85.5    | 16.1              | 31.8              | 26.2              | 18.9              | 7.0               |
| Uncontaminated-AV | 0.26           | 0.17                 | 0.07                                | 0.07    | 0.08                            | 0.03    | 0.02                            | 63.9    | 25.9              | 25.4              | 28.8              | 10.6              | 9.2               |
| Contaminated-AV   | 0.65           | 0.48                 | 0.16                                | 0.17    | 0.19                            | 0.11    | 0.03                            | 72.7    | 24.3              | 26.0              | 28.9              | 15.9              | 4.9               |

r-test  

|       | P < 0.001 | P < 0.001 | P < 0.01 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.05 | P < 0.001 |
|-------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|

aCd content extracted with 0.1 mol L⁻¹ HCl (1:5 w/v).
Table 3: Correlation between proportions of Cd chemical forms and soil properties.

| Soil properties | Uncontaminated soils | Contaminated soils |
|-----------------|----------------------|-------------------|
|                 | Exchangeable         | Inorganically bound | Organically bound | Oxide occluded | Residue | Exchangeable | Inorganically bound | Organically bound | Oxide occluded | Residue |
| pH(H₂O)         | (-)                 | (+)                |                  |               |         | (-)         | (+)                |                  |               |         |
| pH(KCl)         | (-)**               | (+)*               |                  |               |         | (-)         | (+)**              |                  |               | (+)    |
| T-C             | (-)                 | (+)**              |                  |               |         | (-)         | (+)**              |                  |               | (+)    |
| T-N             | (-)**               | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| CEC             | (-)**               | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Ex-Ca           | (-)**               | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Ex-Mg           | (-)**               | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Ex-K            | (-)*                | (+)**              |                  |               |         | (-)*        | (+)**              |                  |               | (+)    |
| Base saturation | (-)*                | (+)**              |                  |               |         | (-)*        | (+)**              |                  |               | (+)    |
| Available phosphate | (-)*          | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| PAC             | (-)**               | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Free iron oxide |                     |                    |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Sand            | (+)*                | (-)                |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Silt            | (+)*                | (-)                |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Clay            |                     |                    |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |
| Alo + 1/2 Feo^b| (-)**               | (+)**              |                  |               |         | (-)**       | (+)**              |                  |               | (+)    |

^a(+): positive correlation, (−): negative correlation, *(P < 0.05), **(P < 0.01), and ****(P < 0.001).

^b Extracted with acid oxalate.

of Cd (except re-Cd) was significantly higher in contaminated soils than in uncontaminated soils *(P < 0.01).* The proportions of ex-Cd, in-Cd, and or-Cd were not significantly different between the soil types, but those of ox-Cd *(P < 0.05)* and re-Cd *(P < 0.001)* were different. The sum of the proportions of ex-Cd, in-Cd, and or-Cd ranged from 65% to 90% in both soils and did not differ significantly.

### 3.3. Proportions of Chemical Forms of Cd and Soil Properties

The proportions of most chemical forms of Cd exhibited correlations with several soil properties (Table 3). In uncontaminated soils, ex-Cd displayed negative correlations with pH (H₂O), pH (KCl), base saturation, and available phosphate *(P < 0.05 or 0.01)*, and in-Cd was positively correlated with pH (KCl) and available phosphate *(P < 0.05).* No other soil properties displayed any correlation with any Cd fractions in uncontaminated soils.

In the contaminated soils, ex-Cd showed strong negative correlations with CEC, ex-Ca, ex-Mg, available phosphate, PAC, and amorphous iron and aluminum oxides *(P < 0.001 or 0.01),* and in-Cd showed strong positive correlations with CEC, ex-Ca, base saturation, PAC, silt content, and amorphous iron and aluminum oxides *(P < 0.001 or 0.01).* Additionally, strong positive correlations were observed between or-Cd and total C, total N, CEC, available phosphate, and PAC *(P < 0.001 or 0.01);* and re-Cd was correlated negatively with sand content *(P < 0.01) and positively with silt content *(P < 0.001).* The remaining properties pH (H₂O), ex-K, and free iron oxide were not correlated with any Cd fractions in either type of soil. Also, there are no correlations between ox-Cd and soil properties.

The soil properties that exhibited strong correlations with the proportions of Cd fractions in contaminated soils are shown in Figure 1. It is suggested that several of the soil properties in Figure 1 affect the proportions of Cd chemical forms and specifically the transformation of ex-Cd into in-Cd or or-Cd.

### 4. Discussion

#### 4.1. Comparison of the Relationships between Soil Properties and Proportion of Exchangeable Cd between Uncontaminated and Contaminated Soils

Few articles have compared the relationships between Cd fractions and soil properties in uncontaminated and contaminated soils in detail. Sadamoto et al. [18] reported that the proportion of ex-Cd was approximately 20% in uncontaminated soil but approximately 30 to 40% in contaminated soil. However, we found no statistical difference in this proportion between uncontaminated and contaminated soils. Sadamoto et al. [18] used only three fluvic paddy soils (two contaminated, one uncontaminated), which did not represent a wide variety of properties such as particle size and total C content. Moreover, their contaminated soils contained large amounts of copper (Cu) and a higher organic fraction than ours. The solubility constants of chelates formed between Cd and soil humic acid at pH 5 and 7 are lower.
than those of Cu [24], so we consider that the Cd was distributed primarily in the exchangeable fraction due to its lower covalent bonding to humic acid compared to Cu. Morera et al. [25] studied the competitive adsorption of heavy metals in different soils and reported that Cd was scarcely adsorbed in the presence of Cu. These results may identify the reason why the proportion of ex-Cd in the contaminated soil was not higher than that in the uncontaminated soil.
We suggest that the proportion of ex-Cd depends on the soil type and especially on differences in humic acid and Cu contents.

As the proportion of ex-Cd increased, the proportions of in-Cd and or-Cd decreased in both contaminated and uncontaminated soils (Table 2). Furthermore, there was no correlation between in-Cd and or-Cd. These fractions exist in equilibrium [26], so we consider that changes in soil moisture, soil pH, oxidation-reduction state, and/or the decomposition of organic matter led to changes in the chemical forms of Cd in soil. During the extraction process by using a mixture of oxalate and ascorbic acid, ferrous oxalate dehydrate could be precipitate, which induced coprecipitation of some metals except As and Cu in Andisols [27]. This provides question validity of the extraction method, while it was used even recently. In our experiment, the existence ratios of ox-Cd and re-Cd are relatively low compared to other fractions, suggesting minor role of those fractions to plant availability and dynamics in soils. The effect of changes in soil conditions on the forms of Cd should be analyzed further.

4.2. Relationship between Cd Chemical Forms and Soil Properties. Heavy metals in soils are adsorbed to clay minerals, free oxides, and humic substances. Many methods have been used to fractionate these heavy metals [28–30]. As the proportion of ex-Cd in contaminated soils was not significantly higher than that in uncontaminated soils, we examined the relationships between the proportion of ex-Cd and soil properties in contaminated soil in detail. In general, soil pH plays a major role in the adsorption of heavy metals: with decreasing soil pH, the proportion of ex-Cd increases [31] and the proportion of in-Cd decreases [32]. As the pH of our contaminated soils ranged from 5.5 to 6.2 and there was no correlation between soil pH and any of the Cd fractions under the experimental conditions, the soil pH had no effect on the soil Cd fractions in these soils. Humic substances have many surface functional groups and can bind specific heavy metals. The total C content, which indicates the humic substance content, was strongly correlated with the proportion of or-Cd in the contaminated soils. However, it was only slightly correlated with ex-Cd and
was not correlated with in-Cd. Therefore, the total C content primarily affected the or-Cd fraction. CEC and PAC were very strongly correlated with ex-Cd and strongly positively correlated with in-Cd. We therefore investigated the relationships between the contents of total C, clay, and amorphous iron and aluminum oxides, which are considered to affect CEC and PAC [33, 34] (Figure 2). The total C and clay contents were strongly correlated, and the amorphous iron and aluminum oxides correlated very strongly with CEC and PAC. Thus, the main Cd adsorbents in our soil samples were amorphous iron and aluminum oxides.

The binding of metals to inorganic soil constituents involves a continuum of reactive sites, ranging from those where there are weak physical forces (van der Waals forces) and electrostatic outer-sphere complexes (e.g., ion exchange) to those where precipitation [35] or strong chemical bonds occur (by inner-sphere complexation). The outer-sphere complex is formed on a negative electron charge resulting from isomorphous substitution. Because this complex can release metals easily with changes in the soil solution, its contents are available to plants. In contrast, the inner-sphere complex forms on clay edges or on the surfaces of metal hydroxides, the active surface hydroxide groups of which are able to bind to metals via coordinate bonds [36]. Because these bonds are stronger than the outer-sphere ones, the plant availability of those adsorbed metals is relatively lower. We consider that the reduction in the proportion of ex-Cd with increasing CEC and PAC (Figure 1) was due to the formation of inner-sphere complexes with amorphous iron and aluminum oxides.

Yanagisawa et al. [2] suggested that the influence of soil texture, soil permeability, CEC, soil moisture, and other factors masks the correlation between soil properties and Cd content in brown rice. Because soil properties were correlated with the proportions of various Cd forms in our contaminated soils, we suggest that the soil properties determined the distribution of Cd forms derived from the same source.

Cd uptake by cabbage [10] and paddy rice [37] is affected by the fractions of Cd in the soil. Furthermore, the Cd content of soybean is affected by soil pH, soil Cd content as extracted by 0.1 M HCl, and PAC. [38]. Thus, soil properties are very important for Cd uptake and are major factors to consider in estimating potential Cd risk in soils.

5. Conclusion

The sequential extraction of cadmium from soil revealed the relationship between chemical forms of soil Cd and properties in contaminated and uncontaminated paddy soils. The proportions of soil Cd fractions were almost the same between the two soil groups, although the Cd concentration of several fractions in contaminated soils was statistically higher than those in uncontaminated soils except for residual fraction. The proportion of exchangeable Cd was correlated with the CEC and PAC in contaminated soil but not in uncontaminated soil, suggesting a new finding on the difference of Cd-chemical form between the two soil groups. Soil properties appear to affect the proportions of soil Cd fractions in contaminated soil and should be considered when evaluating soil Cd mobility.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Effect of Volatile Fatty Acid Concentration on Anaerobic Degradation Rate from Field Anaerobic Digestion Facilities Treating Food Waste Leachate in South Korea

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The purpose of this study was to investigate the effect of volatile fatty acid concentration on anaerobic degradation rate of food waste leachate in the anaerobic digestion facilities. The anaerobic digestion facilities treating food waste leachate (FWL), codigestion with food leachate and animal manure (A-MIX), and codigestion with food waste leachate and sewage sludge (S-MIX) were selected for this study. In accordance with the regulation under Wastes Control Act in South Korea, the guideline of volatile solid removal rate for anaerobic digestion facility is set as 65% for anaerobic degradation efficiency. Highest volatile solids removal rates were achieved from FWL (63.5%) than A-MIX (56.4%) and S-MIX (41.2%). Four out of eight FWLs met the guidelines. The concentration of volatile fatty acids, therefore, was analyzed to determine the relationship with volatile solid removal rate. The results showed that, in order to meet the Korean guideline of 65% volatile solid removal rate, volatile fatty acid concentrations should remain below 4,000 mg/L on the field anaerobic digestion facilities treating FWL. Volatile fatty acid concentrations should be used along with others as an operational parameter to control and manage the anaerobic digestion process.

1. Introduction

Before ban of direct landfill of food waste was imposed in South Korea, more than 90% of food waste was landfilled and the rest was treated by composting, incineration, feeding livestock, and anaerobic digestion [1, 2]. Landfilling of food waste has been banned in Korea since 2005 because of problems of leaching and odour from landfilling of food waste [1, 3]. Ocean dumping of food wastes has also been banned since 2012 in compliance with the London Convention and Protocol [4]. Effective treatment option for organic waste has been sought thereafter.

Anaerobic digestion treatment has been one of the effective treatment options for biodegradable organic waste including food waste/food waste leachate, animal manure, and sewage sludge as it effectively reduces the amount of organic waste and produces biogas as a renewable energy [3, 5].

Food waste is a good resource for anaerobic digestion treatment because it contains high organic matter with appropriate moisture content [2] and it is easily biodegradable [3, 6]. Animal manure provides high buffering capacity [7]; therefore, it has been often treated by anaerobic codigestion with sewage sludge and/or food waste [8–10]. Because of its low concentration of organic matters, sewage sludge has been known to produce low amount of biogas compared to anaerobic digestion of food waste and animal manure [2]. It often has been treated by anaerobic codigestion with food waste to improve anaerobic degradation efficiency [2]. There have been many studies on improving its degradation efficiency by
anaerobic codigestion of animal manure and sewage sludge with food waste. Some of them are on anaerobic codigestion system with food waste, animal manure, and sewage sludge [10], anaerobic digestion system with food waste and sewage sludge [11–13], and anaerobic codigestion system with food waste and animal manure [14, 15]. These studies were based on bench- or pilot-scales. More analysis of the process in the actual facilities is required to understand and monitor the efficiency.

There currently are 57 anaerobic digestion/codigestion facilities nationwide, and they are either at conventional wastewater treatment plants or at separate anaerobic digestion/codigestion plants for the organic fraction of municipal solid waste (OFMSW), mainly food waste/food waste leachate in South Korea [16].

Anaerobic digestion involves a series of metabolic reactions (hydrolysis, acidogenesis, and methanogenesis) [17, 18]. Among these intermediate products of anaerobic digestion, two volatile fatty acids (acetic acid and butyric acid) are among the most favored for methane formation while acetic acid contributes more than 70% to the methane formation [19]. Namely, acetic acid, butyric acid, isobutyric acid, isovaleric acid, and propionic acid have been known as good indicators for monitoring performance of anaerobic digestion process, especially in the activity of acetogenic and methanogenic bacteria [17, 19–22]. Additionally, various physicochemical parameters (pH, temperature, alkalinity, volatile fatty acid, retention time, biogas, etc.) influence these reactions [23–25]. The complexity of the process made the interpretation of the performance of the process difficult; therefore a combination of those parameters was suggested as a better method for monitoring the performance of the process [26].

In South Korea, the anaerobic digestion facilities treating food waste and food waste leachate have been regulated by the Wastes Control Act and volatile solid removal rate for anaerobic digestion facility is set as 65% in accordance with the guidelines for anaerobic degradation efficiency [16].

Therefore, the objective of this paper was not only to identify the parameters that can be used to determine the performance of the anaerobic digestion process in terms of anaerobic degradation efficiency in South Korea but also specifically to investigate the effect of the volatile fatty acid concentration on anaerobic degradation rate of food waste leachate in these anaerobic digestion facilities.

2. Materials and Methods

2.1. Selection of Facilities and Sampling. Seventeen anaerobic digestion/codigestion facilities at conventional wastewater treatment plants and at separate anaerobic digestion/codigestion plant for OFMSW were selected for this investigation. These facilities were treating more than 50 t/d of feedstock rate. They include 8 facilities treating food waste leachate (FWL 1–FWL 7), 3 anaerobic codigestion facilities with a mixture of animal manure and food waste leachate (A-MIX 1–A-MIX 3), and 6 anaerobic codigestion facilities with a mixture of sewage sludge and food waste leachate (S-MIX 1–S-MIX 6). The types of feed waste and the digestion system were presented in Table 1.

Samples for analysis were collected from the inlet and outlet valves of anaerobic digester at each facility. And they were kept refrigerated until they were analyzed.

2.2. Analytical Methods. Total solids, moisture content, and volatile solids were determined according to Standard Methods 1684 [26]. CODcr was analyzed according to closed reflux, titrimetric method (5220 C) and NH4+-N and T-N were analyzed according to Standard Methods 4500 [26] and standard methods for testing water [35].

Volatile fatty acids were analyzed according to Standard Methods (5560 D-Gas chromatographic method) [26, 36, 37]. The concentration of each volatile fatty acid, namely, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid, was conducted by gas chromatography (Agilent 6890, USA; column: DB-FFAP, 25 m × 0.32 mm × 0.5 µm; oven temperature program: 2 min, 95 °C, 2 min, 140 °C at 10 °C/min, and 5 min, 240 °C at 40 °C/min; injection temperature: 240 °C; injection mode: split (10:1); flow: 1.0 mL/min) equipped with FID detector. Helium was used as a carrier gas. Samples were acidified to pH 2 with phosphoric acid and centrifuged at 3500 rpm for 5 min. The supernatant was extracted with 1 g of NaCl and diethyl ether after vortexing for 5 min before being analyzed with GC-FID.

3. Results and Discussion

3.1. Characteristics of Feed Waste. Table 2 shows the physiochemical characteristics of feed wastes. The moisture content, fixed solids, and volatile solids of individual feed waste were presented in Figure 1. Total solids content in FWL from 8 anaerobic digestion facilities varied from 3.6% to 12.2% with the average total solids content of 7.2%. Volatile solid content in FWL from 8 anaerobic digestion facilities varied from 1.8% to 10.4% with the average volatile solid content of 5.5%. Total solid content in A-MIX from 3 anaerobic digestion facilities varied from 4.3% to 5.3% with the average total solid content of 4.6%, and volatile solid content in A-MIX from 3 anaerobic digestion facilities varied from 3.0% to 3.7% with the average volatile solid content of 3.3%. Total solid content in S-MIX from 6 anaerobic digestion facilities varied from 3.1% to 9.4% with the average total solid content of 5.0%, and volatile solid content in S-MIX from 6 anaerobic digestion facilities varied from 2.0% to 7.4% with the average volatile solid content of 3.5%. The highest total solid and volatile solid content were from FWL as expected. The volatile solid content in total solids (volatile solid/total solids) from all types of feed wastes was 71.0, 72.0, and 71.5% for FWL, A-MIX, and S-MIX, respectively. Volatile solid/total solid presents the amount that is biodegradable in total solid. The physicochemical characteristics of three types of feed waste from the literature are presented in Table 3. Borowski and Weatherley [38] also observed similar percentage of volatile solid in total solid (volatile solid/total solids) for poultry manure and sewage sludge, even though animal manure contained higher total solid and volatile solid than those in sewage sludge. Volatile
Table 1: Operating conditions of selected anaerobic digestion facilities.

| Types of feed waste | Individual anaerobic digestion facility | Mixing ratio* | T (°C) | Types of system** |
|---------------------|----------------------------------------|---------------|--------|------------------|
| Food waste leachate (FWL) | FWL1 | 1 | 55 | 2 |
| | FWL2 | 1 | 35 | 2 |
| | FWL3 | 1 | 35 | —*** |
| | FWL4 | 1 | 35 | 1 |
| | FWL5 | 1 | 35 | — |
| | FWL6 | 1 | 55 | 1 |
| | FWL7 | 1 | 55 | — |
| | FWL8 | 1 | — | 2 |
| Animal manure + food waste leachate (A-MIX) | A-MIX1 | 3:1† | 35 | 1 |
| | A-MIX2 | 7:3 | 55 | 1 |
| | A-MIX3 | — | 35 | 2 |
| Sewage sludge + food waste leachate (S-MIX) | S-MIX1 | 3:2‡ | 55 | 1 |
| | S-MIX2 | 13:1 | 35 | 1 |
| | S-MIX3 | 4.5:1 | 35 | 2 |
| | S-MIX4 | 4:1 | 35 | 1 |
| | S-MIX5 | 50:1 | 55,35 | 1 |
| | S-MIX6 | 6.7:1 | 35 | 1 |

* Ratio = feed ratio into anaerobic digester. † Animal manure: food waste leachate for A-MIX, ‡ sewage sludge: food waste leachate for S-MIX. ** 1: single stage system; 2: two-stage system. *** —: unknown data.

Table 2: Physicochemical characteristics of feed waste.

| Parameters | Unit | FWL (mean, SD) | A-MIX (mean, SD) | S-MIX (mean, SD) |
|------------|------|----------------|------------------|------------------|
| Moisture content | % | 92.8 (2.9) | 95.4 (0.5) | 95.0 (2.1) |
| Volatile solid | % | 5.5 (3.0) | 3.3 (0.3) | 3.5 (1.8) |
| Total solids | % | 7.2 (2.9) | 4.6 (0.5) | 5.0 (2.1) |
| Volatile solid/total solids* | % | 71.0 (15.1) | 72.0 (11.1) | 71.5 (9.5) |
| T-N | mg/L | 3,190.5 (953.0) | 4,744.0 (5,260.0) | 4,914.4 (2,406.0) |
| NH3-N | mg/L | 686.7 (749.3) | 777.1 (979.9) | 98.8 (372.0) |
| Total volatile fatty acid | mg/L | 12,420.0 (9,878.9) | 9,115.0 (2,937.5) | 3,679.5 (4.6) |

Mean values of 17 selected facilities and standard deviation in parentheses.
(n = 8 for FWL, n = 3 for A-MIX, and n = 6 for S-MIX.)
* Volatile solid/total solids = [Volatile solid/(Volatile solid + Fixed solid)] × 100.

3.2. Removal Rates of Volatile Solid and COD. The volatile solid removal rate from each anaerobic digestion facility with individual feed waste is presented in Figure 2. The guideline of anaerobic degradation efficiency in South Korea is stipulated to monitor the efficiency of the process. Figure 2(a) shows volatile solid removal rates of 17 anaerobic digestion facilities and the bold horizontal dashed line denotes the volatile solid removal rate of 65% as set forth in Wastes Control Act. Considering the types of feed waste, the average volatile solid removal rate of FWL was 63.4%, and it was higher than volatile solid removal rate of A-MIX (56.4%) and S-MIX (41.2%). Regarding FWL, 4 out of 8 anaerobic digestion facilities with food waste leachate achieved volatile solid removal rates greater than 65% and they were ranged from 78 to 88%. Two out of 8 facilities with FWL achieved volatile solid removal rates greater than 65% and they were ranged from 78 to 88%. Two out of 8 facilities with FWL achieved volatile solid removal rates greater than 65% and they were ranged from 78 to 88%. Two out of 8 facilities with FWL achieved volatile solid removal rates greater than 65% and they were ranged from 78 to 88%. Two out of 8 facilities with FWL achieved volatile solid removal rates greater than 65% and they were ranged from 78 to 88%.
solid removal rate just below 65% (59 and 60%). Regarding A-MIX, 2 out of 3 facilities reached volatile solid removal rate just below 65% (63 and 64%). Regarding S-MIX, 1 out of 6 facilities with S-MIX reached volatile solid removal rate over 65% (74%) and 3 out of 6 facilities with S-MIX operated at the volatile solid removal rate of 51–57%.

Lee et al. [2] obtained a similar result of volatile solid removal rate ranging between 46.6 and 61.7% from a bench-scale anaerobic codigestion reactor with food waste and sewage sludge. Reasonably high volatile solid removal rates with the range of 39.5–86.1% were observed from food waste in the literature (Table 3) and they are comparable...
Table 3: Total and volatile solid of food waste, animal (poultry, pig, and cow) manure, and sewage sludge and their VS removal rates in the literature.

| Waste                        | Total solids (%) | Volatile solid (%) | Volatile solid/total solids (%) | Volatile solid removal rate (%) | References                        |
|------------------------------|------------------|--------------------|---------------------------------|--------------------------------|-----------------------------------|
| Poultry manure               | 28.9             | 21.5               | 72.7                            | 43.1–49.4                      | KMOE [16], McCarty and Smith [27] |
|                              | 27.7             | 20.5               | 74.0                            | —                              | Borowski et al. [28]              |
| Pig manure                   | 9.2              | 7.0                | 76.1                            | 23.9–32.5                      | Anjum et al. [29]                 |
|                              | 12.4             | 9.0                | 72.6                            | —                              | Scano et al. [30]                 |
| Cow/dairy manure             | 13.8             | 11.0               | 79.9                            | —                              | Zhang et al. [15]                 |
|                              | 17.1             | 16.3               | 84.3                            | —                              |                                   |
|                              | 9.2              | 13.2               | 81                              | —                              |                                   |
| Fruit and vegetable wastes   | 12.7             | 11.0               | 86.6                            | —                              | Anjum et al. [29]                 |
|                              | 3.4–21.8         | 2.7–20.4           | —                               | 53 (no pH control)             | Borowski et al. [28]              |
|                              | 4.4–4.5          | 3.9–4.0            | —                               | 70 (with pH control)           | Ganesh et al. [31]                |
|                              | 7.3–10.0         | 9.7                | —                               | —                              | Lee et al. [2]                    |
|                              | 27.5             | 22.7               | 82.5                            | —                              | Ganesh et al. [31]                |
|                              | 3.0–4.5          | 2.9–4.3            | —                               | 39.5 (with activated waste sludge and mesophilic) | El-Mashad and Zhang [32] |
| Composite food waste         | 22.4             | 18.9               | 84.4                            | —                              |                                   |
| (grain, fish, meat, fruit, and vegetable) | 18.0–23.0       | 16.4–21.9          | —                               | 74.1–86.1                      | Kim et al. [12]                   |
|                              | 7.0–20.0         | 6.6–19.0           | —                               | —                              | Cabbai et al. [33]                |
|                              | 28.0             | 24.1               | 85.0                            | —                              | Scano et al. [30]                 |
|                              | 30.9             | 26.4               | 92.0                            | —                              | Cabbai et al. [33]                |
|                              | 18.5             | 17.0               | 92.9                            | —                              | Zhang et al. [15]                 |
|                              | 24.8             | 23.0               | —                               | 68.3–80.6                      | Cavinato et al. [34]              |
| Sewage sludge                | 4.7–4.9          | 3.5–3.7            | 74.1                            | 33.9–36.3                      | KMOE [16], McCarty and Smith [27] |
|                              | 3.5              | 2.3                | 65.7                            | —                              | Ganesh et al. [31]                |
|                              | 3.8              | 2.3                | 60.5                            | —                              | El-Mashad and Zhang [32]           |

Only those types of waste relevant to the Korean dietary habit were considered and those green wastes (grass, wood, etc.) were not included.
* Composite food wastes were mainly collected from household, restaurant, canteen, and cafeteria.
** Pilot-scale and lab-scale; —: data unavailable.

with volatile solid removal rate of FWL (avg. 63.5%) in this study. The second highest volatile solid removal rates were achieved with A-MIX (avg. 56.4%) in this study and the result was higher than the volatile solid removal rate with animal manure in the literature (23.9–49.4%). The lowest volatile solid removal rate was observed from S-MIX (avg. 41.2%) and the result was also higher than the result in the literature (26.8–38.2%). The reason for higher volatile solid removal rates observed from A-MIX and S-MIX in this study was due to the codigestion with food waste leachate. The result surely could not be directly compared with the results in the literature due to different operational systems and conditions; however the trend in volatile solid removal rate achieved from food waste leachates in this study was comparable with the results in the literature (Table 3).

Higher volatile solid generally means higher amount of organic materials that are convertible to biogas. Also higher volatile solid/total solids increased the amount of biodegradable materials and it would cause the increase of the microbial activities, thereby increasing volatile solid removal rate [38]. Figure 2(b) indicated a reasonable trend of increasing volatile solid removal rates with increasing volatile solid/total solids. In the current study, volatile solid/total solids of three types of feed waste, such as FWL, A-MIX, and S-MIX, were analyzed within the average range of 71.0–72.0% and they agreed with volatile solid/total solids in the literature (Table 3). Therefore the performance of anaerobic digestion was related to the volatile solid/total solids.

COD concentrations of input and output of each anaerobic digestion facility are presented in Figure 3. Average COD concentration of FWL in inlet ("FWL-In") was 85,169 mg/L, average COD concentration of A-MIX in inlet ("A-MIX-In") was 80,267 mg/L, and average COD concentration of S-MIX in inlet ("S-MIX-In") was 64,033 mg/L. After anaerobic
Table 4: Results of COD and VS removal rate.

| Types of feed waste | Individual anaerobic digestion facility | CODcr removal rate (%) | VS removal rate (%) |
|---------------------|----------------------------------------|------------------------|---------------------|
| FWL                 | FWL1 79 ± 5                           | 81 ± 6                 |
|                     | FWL2 58 ± 2                           | 36 ± 3                 |
|                     | FWL3 14 ± 1                           | 20 ± 3                 |
|                     | FWL4 72 ± 3                           | 60 ± 5                 |
|                     | FWL5 92 ± 6                           | 88 ± 3                 |
|                     | FWL6 53 ± 4                           | 59 ± 4                 |
|                     | FWL7 83 ± 3                           | 85 ± 4                 |
|                     | FWL8 78 ± 4                           | 78 ± 3                 |
| Mean ± SD           | 66 ± 13                               | 63 ± 23                |

| A-MIX               | A-MIX1 35 ± 2                         | 63 ± 3                 |
|                     | A-MIX2 83 ± 6                         | 64 ± 4                 |
|                     | A-MIX3 71 ± 5                         | 42 ± 4                 |
| Mean ± SD           | 63 ± 20                               | 56 ± 10                |

| S-MIX               | S-MIX1 83 ± 3                         | 74 ± 5                 |
|                     | S-MIX2 65 ± 5                         | 57 ± 2                 |
|                     | S-MIX3 51 ± 5                         | 51 ± 4                 |
|                     | S-MIX4 85 ± 6                         | 8 ± 1                  |
|                     | S-MIX5 39 ± 4                         | 46 ± 3                 |
|                     | S-MIX6 24 ± 3                         | 11 ± 1                 |
| Mean ± SD           | 58 ± 18                               | 41 ± 24                |

Note: Mean ± standard deviation.

3.3. Relationship between Volatile Fatty Acid Concentration and Anaerobic Degradation Rate.

Figure 3 shows the concentration of 6 individual volatile fatty acids, namely, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid, found in inlet and outlet of each anaerobic digestion process. For FWL, the concentration of acetic acid was highest in feed waste, and the average concentration of acetic acid was 5,431 mg/L. The high concentration of butyric acid was observed from feed waste of FWL2 and FWL3 (6,014 and 9,049 mg/L, resp.) and high concentration of propionic acid was also observed from feed waste of FWL2 and FWL4 (2,065 and 2,248 mg/L, resp.). This result agreed with Wijekoon et al. [19] who observed acetic acid and butyric acid as the predominant volatile fatty acid. High concentrations of propionic acid were relatively found in outlet from FWL2, FWL3, FWL4, and FWL6 (569, 1,282, 1,795, and 847 mg/L, resp.). For A-MIX, the concentration of acetic acid was dominantly high in feed waste with the average concentration of 3,925 mg/L. Concentration of propionic acid was high (average concentration of 1,751 mg/L) in outlet of A-MIX3. For S-MIX, the concentration of acetic acid was observed to be the highest in feed (average of 2,218 mg/L). The highest concentration of acetic acid was observed from one of the inlets (denoted as “S-MIX3-In1” in Figure 4) of S-MIX3 (1,213 mg/L) where food waste leachate was fed into the anaerobic digester (S-MIX3-In1) separately and sewage sludge was fed through other inlet (denoted as “S-MIX3-In2” in Figure 4) of S-MIX3. Acetic acid has been known as an important intermediate for overall anaerobic digestion process as it is directly related to the end product, methane, and carbon dioxide [17, 19], and propionic acid was important for supplying electron flow [17]. Gorris et al. [17] noticed that complete degradation of propionic acid was observed when low concentration of acetic acid (less than 100 mg/L) existed and high concentration of acetic acid (4,700 mg/L) blocked the degradation of propionic acid. Many agreed that higher concentration of acetic acid inhibited the degradation of propionic acid [17, 21] and inhibited the acetate-utilizing digestion treatment, COD removal rates for FWL, A-MIX, and S-MIX were found to be 66.1%, 62.6%, and 57.8% (Table 4), respectively.

Although there is still a debate about whether the volatile solid removal rate of 65% set forth in Wastes Control Act is reasonable, the results from 17 anaerobic digestion facilities in South Korea agreed with those of the literature that the highest removal rate was observed with food waste while the lowest removal rate was observed with sewage sludge (Table 4).
methanogenic bacteria [39]. The accumulation of propionic acid might indicate the sign of disturbance of the process [17, 23, 40]. Björnsson et al. [23] reported that accumulation of propionic acid is closely related to the concentration of hydrogen; therefore hydrogen concentration could be a possible parameter to monitor the accumulation of volatile fatty acid [23, 26]. Some studies have found that propionic acid should be treated as a toxic volatile fatty acid in anaerobic digester and the methanogenic bacteria have been shown vulnerable to propionic acid concentration greater than 1,000∼2,000 mg/L [19]. Although Gourdon and Vermande [41] observed no inhibitory effect of propionic acid even at 6,000 mg/L they agreed that the accumulation of propionic acid should be seen as the warning sign and should take the attention of the process before it would cause a disturbance. Also Ahring et al. [22] suggested that volatile fatty acid should be used as indicators of imbalance of the process rather than an inhibitor. Therefore the volatile fatty acid should be treated as a monitoring parameter rather than an inhibitor.

Direct comparison with the literature was impossible in this study due to different system and operational conditions; however the effect of acetic acid on degradation of propionic acid and resulting production of methane as the end product has been reported. McCarty and Smith [27] suggested that the propionic acid accumulation appeared to predominate in the complex waste and the high concentration of propionic acid in FWL and A-MIX might be related to this finding. Several studies have found that OFMSW tended to produce long volatile fatty acids due to the presence of high level of protein and fat contents and they can lead to operational problems and instability of the digestion performance; therefore codigestion is recommended to alleviate this adverse effect and improve the efficiency of the process [15, 27]. Volatile solid removal rate of FWL3 was lowest, and this might indicate instability of the digestion performance and the methanogenic bacterial activity. Further study is required to conclude the effect; however better degradation of propionic acid has been noticed when lower concentration of acetic acid was found in S-MIX.

Figure 5 shows the relationship between volatile fatty acid concentration and volatile solids removal rate on the FWLs. It showed a linear relationship between volatile fatty acid concentration and volatile solid removal rate. According to this linear relationship, volatile fatty acid concentration should be below 4,000 mg/L in order to meet the Korean guideline of 65% volatile solid removal rate on the FWL. As A-MIX and S-MIX were without the guideline of volatile solid removal rate as well as with very low volatile solid removal rate, this relationship was analyzed except for them. In addition, the average volatile fatty acids of even the inlets of A-MIX and...
The performance of anaerobic digestion process and, volatile acid/alkalinity ratio has been used to monitor degradation rate of the anaerobic digestion facilities. In addition, the effect of the volatile fatty acid concentration on anaerobic volatile fatty acid component is necessary to underpin the relationship was observed from this specific study and it might not be applicable to all. More detailed study of each fraction (FWL) with volatile solid removal rate that were higher than 70%, below 4,000 mg/L should be recommended. This relationship was observed from this specific study and it might not be applicable to all. More detailed study of each volatile fatty acid component is necessary to underpin the effect of the volatile fatty acid concentration on anaerobic degradation rate of the anaerobic digestion facilities. In addition, volatile acid/alkalinity ratio has been used to monitor the performance of anaerobic digestion process. However considering 4 facilities (FWL) with volatile solid removal rate that were higher than 70%, below 4,000 mg/L should be recommended.

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4. Conclusions

In Korea, there is lack of information on the field data for operation of anaerobic digestion facilities treating food waste leachate, especially for operational parameter for checklist of troubleshooting. This study evaluated the effect of volatile fatty acid concentration on volatile solid removal rate and investigated the relationship between them. The volatile solid removal rates of field anaerobic digestion facilities with food waste leachate were evaluated and the average volatile solid removal rates were below the Korean guideline of 65%. In order to meet the Korean guideline of 65% volatile solid removal rate, volatile fatty acid concentrations should remain below 4,000 mg/L on the field anaerobic digestion facilities treating FWL. Volatile fatty acid concentrations should be used as an important operational parameter to control and manage the anaerobic digestion process.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

Figure 5: Relationship between volatile solid removal rate and volatile fatty acid concentration. * denotes significance at 5.0% level.
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Enhancement of Nutrient Removal in a Hybrid Constructed Wetland Utilizing an Electric Fan Air Blower with Renewable Energy of Solar and Wind Power

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The sewage treatment efficiency of hybrid constructed wetlands (CWs) was evaluated under different ventilation methods. The removal efficiencies of biochemical oxygen demand (BOD), total nitrogen (TN), and total phosphorus (TP) in the vertical flow- (VF-) horizontal flow (HF) CWs using an electric fan air blower by the renewable energy of solar and wind power were higher than those by natural ventilation, excluding only suspended solids (SS). The TN treatment efficiency in the CW using the air blower especially increased rapidly by 16.6% in comparison with the CW employing natural ventilation, since the VF bed provided suitable conditions (aerobic) for nitrification to occur. The average removal efficiencies of BOD, SS, TN, and TP in the effluent were 98.8, 97.4, 58.0, and 48.3% in the CW using an electric fan air blower, respectively. The treatment performance of the CWs under different ventilation methods was assessed, showing TN in the CW using an electric fan air blower to be reduced by 57.5–58.6% for inlet TN loading, whereas reduction by 19.0–53.3% was observed in the CW with natural ventilation. Therefore, to increase the removal of nutrients in CWs, an improved ventilation system, providing ventilation via an electric fan air blower with the renewable energy, is recommended.

1. Introduction

Constructed wetlands (CWs) are considered as low-cost alternatives for the treatment of municipal, industrial, domestic, and agricultural wastewater [1]. Removal of nitrogen compounds in CWs is governed mainly by microbial nitrification and denitrification, while other mechanisms such as plant uptake and ammonia volatilization are generally of less importance [2]. While the efficiency of constructed wetlands for the removal of biochemical oxygen demand (BOD) and suspended solids (SS) is very high, nitrogen removal in most of the currently operating wetland systems (predominantly horizontal flow beds) is deficient, mainly due to insufficient supply of oxygen [3–5]. Higher nitrification efficiency was noted in vertical flow beds based on the Seidel model [6]. In the nitrification process, ammonia is oxidized mainly to nitrate. Nitrate is subsequently reduced to gaseous nitrogen by denitrification, where biomass or other organic residues are utilized as carbon and electron sources [2].

The most common CW systems are designed as horizontal flow (HF) systems in Europe [7]. Various types of CWs may be combined to achieve higher treatment effect (especially for nitrogen). However, hybrid systems comprise most frequently vertical flow (VF) and HF systems arranged [8]. HF CW systems for secondary treatment proved to be very satisfactory where the standard required only BOD, chemical oxygen demand (COD), and SS removal. However, there has been a growing interest in achieving fully nitrified
Table 1: Chemical characteristics of raw sewage and treated water in 1st and 2nd treatments.

| Stage                                      | Ventilation method | BOD (mg L$^{-1}$) | SS (mg L$^{-1}$) | TN (mg L$^{-1}$) | TP (mg L$^{-1}$) | DO (mg L$^{-1}$) |
|--------------------------------------------|-------------------|-------------------|------------------|------------------|-----------------|-----------------|
| Raw sewage                                 |                   | 58.5 ± 25.5       | 38.3 ± 21.0      | 11.8 ± 3.4       | 1.30 ± 0.38     | 0.82 ± 0.13     |
| Treated water from the 1st bed (VF bed)    | Natural ventilation | 7.0 ± 5.5        | 5.0 ± 6.5        | 9.8 ± 3.2        | 1.02 ± 0.28     | 5.42 ± 1.5      |
|                                           | Electric ventilation | 5.5 ± 1.1       | 6.3 ± 1.7        | 8.1 ± 1.8        | 0.84 ± 0.08     | 6.97 ± 1.3      |
| Treated water in the 1st (VF) and 2nd (HF) beds (effluent) | Natural ventilation | 1.2 ± 0.7        | 0.7 ± 0.4        | 6.5 ± 2.0        | 0.66 ± 0.19     | 1.57 ± 0.41     |
|                                           | Electric ventilation | 0.7 ± 0.1        | 1.4 ± 0.3        | 5.7 ± 0.8        | 0.64 ± 0.15     | 1.61 ± 0.23     |

Table 2: Physicochemical characteristics of filter media used.

| Bed             | Porosity (%) | Bulk density (g cm$^{-3}$) | $d_{10}$ (mm) | $d_{60}$ (mm) | Uniformity coefficient ($d_{60}/d_{10}$) | pH (1:5H$_2$O) | EC (dS m$^{-1}$) | O.M. (%) |
|-----------------|--------------|----------------------------|---------------|--------------|------------------------------------------|----------------|----------------|---------|
| VF bed          | 44.8         | 1.48                       | 2.0           | 2.9          | 1.45                                     | 7.9            | 0.05           | 0.54    |
| HF bed          | 37.0         | 1.59                       | 0.13          | 1.4          | 10.7                                     | 7.5            | 0.05           | 0.42    |

effluents. HF CW systems cannot do this due to their limited oxygen transfer capacity. On the other hand, VF CW systems do provide a suitable condition for nitrification (but no denitrification occurs in these systems). In the 1990s and early 2000s, VF-HF systems were built in many European countries and now this type is getting more attention in most European countries [1, 8]. In hybrid systems, the advantages of the HF and VF systems can be combined to complement each other [8]. It is possible to produce an effluent low in BOD, COD, SS, and TN (nitrification and denitrification occur in VF-HF CWs) [1, 9].

The main goal of this study was to evaluate a VF-HF hybrid constructed wetland system for the treatment of domestic sewage from agricultural villages under different ventilation methods in order to enhance the organics and nutrient (N and P) removal performance through the VF-HF hybrid CWs. The specific objectives were (1) to evaluate the removal efficiency of pollutants in a VF-HF hybrid constructed wetland under different ventilation methods and (2) to obtain the treatment performance of pollutants in VF-HF hybrid wetlands constructed with different ventilation methods.

2. Materials and Methods

2.1. Characterization of Materials. The domestic sewage used in this study was collected from a village located in Boknae-ri, Bongnae-myeon, Boseong-gun, Jeollanam-do, South Korea. Domestic sewage from this village had a BOD, SS, TN, and TP of 58.5, 38.3, 11.8, and 1.30 mg L$^{-1}$, respectively (Table 1). The physicochemical characteristics of the filter media used in the VF-HF CWs are listed in Table 2.

2.2. Hybrid Constructed Wetlands Experiment. The hybrid constructed wetlands (located in Boknae-ri, Bongnae-myeon, Boseong-gun, Jeollanam-do, South Korea, at 34°53'48.34N latitude and 127°07'43.91E longitude) evaluated herein consisted of 2-stage CWs containing coarse sand (Figure 1). The beds consisted of vertical flow (VF; aerobic conditions) and horizontal flow (HF; anaerobic conditions) and are shown in Figure 1. The VF-HF 2-stage CWs were constructed using a 5.0 m (width) × 7.0 m (length) × 1.0 m (height) bed for VF with a total volume of 35 m$^3$ and a 5.0 m (width) × 7.0 m (length) × 1.0 m (height) bed for HF with a total volume of 35 m$^3$, for which a 1.5 mm thick high density polyethylene (HDPE) liner was used (Figure 2). In the VF bed, a ventilation pipe was installed at 50 cm above the bottom in order to maintain natural ventilation during the 11 months from May 2012 to March 2013. From March 2013 to March 2013, an electric fan air blower which used the renewable energy of solar and wind power was installed at the end of the ventilation pipe to enhance the performance of organics and nutrient (N and P) removal in one of the hybrid CWs. The HF bed was also divided into five sections to maximize the hydraulic retention time in the bed. Domestic sewage was added to the VF bed using the vertical flow method, and the water leaving the bed flowed into the HF bed via horizontal flow.

The VF bed was a planted filter bed for 1st treatment of domestic sewage that was drained at the bottom. In the VF bed, the water flowed vertically down through the filter matrix to the bottom of the basin where it was collected in a drainage pipe. The hydraulic retention time in the VF
FIGURE 2: Diagrams of a VF-HF hybrid constructed wetland with ventilation using ventilation pipe and an electric air fan blower by renewable energy of solar and wind power for treating sewage.

3. Results and Discussion

3.1. Removal Efficiencies of Pollutants in VF-HF Hybrid Constructed Wetlands under Different Ventilation Methods

3.1.1. Biochemical Oxygen Demand (BOD). The concentrations and removal efficiencies of BOD, SS, TN, and TP in the raw water, treated water from the 1st bed (VF bed), and water treated in the 1st (VF) and 2nd (HF) beds (effluent) in the VF-HF CWs for 13 months are shown in Figure 3. BOD in the inflow ranged from 14.5 mg L$^{-1}$ to 117.6 mg L$^{-1}$, with
Figure 4: The concentration and removal rate of SS in the water with time in a VF-HF hybrid constructed wetland under different ventilation methods (◻: inflow; ▲: 1st treatment; ■: 1st + 2nd treatment; Treatment 1 (T1): VF-HF CWs with natural ventilation; Treatment 2 (T2): VF-HF CWs with ventilation using an electric air fan blower).

3.1.2. Suspended Solids (SS). The concentration of SS in the inflow ranged from 8.1 mg L\(^{-1}\) to 83.2 mg L\(^{-1}\), with an overall mean of 38.3 ± 21.0 mg L\(^{-1}\) over the experimental period (Figure 4). In the VF-HF CW ventilated naturally using a ventilation pipe, SS in the effluent ranged from 0.2 mg L\(^{-1}\) to 1.6 mg L\(^{-1}\), with an overall mean of 0.7 ± 0.4 mg L\(^{-1}\). In the case of electric ventilation, SS in effluent ranged from 1.1 mg L\(^{-1}\) to 1.7 mg L\(^{-1}\), with an overall mean of 1.4 ± 0.3 mg L\(^{-1}\). The removal of SS in the VF bed was much higher than that in the HF bed. The rate of BOD consumption by microbes was also higher in the VF bed, likely due to the activity of aerobic bacteria, which provided greater oxidation of the organic matter than anaerobic bacteria [9]. In the effluent, the removal efficiency of BOD was 97.6% (95.3–99.0%) in the VF-HF hybrid CW with natural ventilation. On the other hand, in the VF-HF CWs ventilated using an electric fan air blower with the renewable energy of solar and wind power, BOD in the effluent ranged from 0.5 mg L\(^{-1}\) to 0.8 mg L\(^{-1}\), with an overall mean of 0.7 ± 0.1 mg L\(^{-1}\). In the effluent, the removal efficiency of BOD was 98.8% (98.8–98.9%) in the VF-HF CWs utilizing the electric fan air blower. Vymazal [II] reported that VF-HF systems at Colecott exhibited high removal of BOD and suspended solids. Therefore, the removal efficiency of BOD in the VF-HF CWs with electric ventilation was slightly higher than that in the VF-HF CWs utilizing natural ventilation. Öivel et al. [12] used a VF-HF constructed wetland for the treatment of school house wastewater in Estonia and reported the removal rates of BOD, TSS, TN, and TP of 94%, 87%, 70%, and 91%, respectively.

3.1.3. Total Nitrogen (TN). TN concentration in the inflow varied between 4.4 mg L\(^{-1}\) and 18.1 mg L\(^{-1}\), with an overall mean of 11.8 ± 3.4 mg L\(^{-1}\) for the 13-month period (Figure 5). In the VF-HF CW with natural ventilation, TN in the effluent ranged from 2.0 mg L\(^{-1}\) to 10.1 mg L\(^{-1}\), with an overall mean of 6.5 ± 2.0 mg L\(^{-1}\). In that with electric ventilation, it ranged from 4.7 mg L\(^{-1}\) to 6.7 mg L\(^{-1}\), with an overall mean of 5.7 ± 0.8 mg L\(^{-1}\). In effluent, the removal efficiency of TN was 41.4% (19.0–53.3%) and 58.0% (57.5–58.6%) in the VF-HF CW with natural and electric ventilation, respectively. Therefore, the removal efficiency of TN in the VF-HF CW with electric ventilation was higher than that in the VF-HF CW with natural ventilation. A reasonable explanation for these results is that nitrification efficiency in the VF-HF CW with
electric ventilation (DO concentration was 6.97 mg L$^{-1}$ in the VF bed) was higher than that in the VF-HF CW with natural ventilation (DO concentration was 5.42 mg L$^{-1}$ in the VF bed).

Compared to single HF systems, a much higher removal of the total nitrogen was observed, as a result of high nitrification in the VF section. Nitrate produced in the VF section was successfully removed in the HF section [1]. In the VF-HF CWs, the 1st stage provided suitable conditions (aerobic) for nitrification, while the 2nd stage provided suitable conditions (anoxic/anaerobic) for denitrification to occur [9, 14]. Similar results were reported by Vymazal [15], showing that hybrid constructed wetlands were more efficient in total nitrogen removal than single HF or VF constructed wetlands. Thus, the removal efficiency of TN in the VF-HF CW ventilated using an electric fan air blower rapidly increased by 16.6% in comparison with that in the VF-HF CW with natural ventilation.

3.1.4. Total Phosphorus (TP). The concentration of TP in the inflow ranged from 0.55 mg L$^{-1}$ to 2.23 mg L$^{-1}$, with an overall mean of 1.30 ± 0.38 mg L$^{-1}$ over the experimental period (Figure 6). The highest TP values were observed in May 2012. In the VF-HF CW with natural ventilation, TP in the effluent varied between 0.30 mg L$^{-1}$ and 0.99 mg L$^{-1}$, with an overall mean of 0.66 ± 0.19 mg L$^{-1}$. In the case of electric ventilation, TP varied between 0.48 mg L$^{-1}$ and 0.79 mg L$^{-1}$, with an overall mean of 0.64 ± 0.15 mg L$^{-1}$. Removal efficiency of TP in the effluent was 47.0% (28.8–63.2%) in the VF-HF CW with natural ventilation and 48.3% (48.1–48.6%) in that with electric ventilation. According to Sayadi et al. [13], removal of nutrients such as N and P components is dependent on the system properties and operational conditions. Removal efficiency of TP in the VF-HF CW ventilated using an electric fan air blower was slightly higher than that in the VF-HF CW employing natural ventilation. This is because the VF bed in VF-HF systems with electric ventilation provides suitable aerobic conditions for P uptake by polyphosphate accumulating organisms (PAOs) compared to the VF bed in VF-HF systems with natural ventilation. Namely, for P uptake by polyphosphate accumulating organisms (PAOs), aerobic condition in the VF bed with electric ventilation (DO concentration was 6.97 mg L$^{-1}$) was more suitable than that in the VF bed with natural ventilation (DO concentration was 5.42 mg L$^{-1}$). In general, PAOs do release and uptake orthophosphate under anaerobic and aerobic conditions, respectively [16].

Based on the above results, removal efficiencies of BOD, TN, and TP in the VF-HF CW ventilated using an electric fan air blower were higher than those by natural ventilation, excluding only SS.

3.2. Relationship between Pollutant Loading and Removal in VF-HF Hybrid Constructed Wetlands under Different Ventilation Methods. The removal of BOD, SS, TN, and TP was proportional to the influent load in the CWs for the treatment of sewage. The linear relationship between nutrient removal and nutrient loading is illustrated in Figure 7. In the VF-HF CW with natural ventilation, linear regressions for BOD were BOD removal (g day$^{-1}$) = 0.0428 × BOD loading (g day$^{-1}$) + 22.64 ($r = 0.217$) for the VF bed and BOD removal (g day$^{-1}$) = 0.0057 × BOD loading (g day$^{-1}$) + 4.4503
the HF bed. In the VF bed, the SS loading varied between 100 g day$^{-1}$ and 273 g day$^{-1}$ with ventilation using an electric air fan blower). (Figure 6: The concentration and removal rate of TP in the water with time in a VF-HF hybrid constructed wetland under different ventilation methods (□: inflow; ▲: 1st treatment; ○: 1st + 2nd treatment; Treatment1 (T1): VF-HF CWs with natural ventilation; Treatment2 (T2): VF-HF CWs with ventilation using an electric air fan blower). 

$r = 0.226$ for the HF bed. In the case of electric ventilation, linear regressions for BOD were BOD removal (g day$^{-1}$) = 0.2243 × BOD loading (g day$^{-1}$) − 36.196 ($r = 0.819^*, P < 0.05$) for the VF bed and BOD removal (g day$^{-1}$) = −0.0287 × BOD loading (g day$^{-1}$) + 11.421 ($r = 0.871^*, P < 0.05$) for the HF bed. In the VF bed, the organic loading (BOD) varied between 73 and 588 g day$^{-1}$, demonstrating mass removal between 9 and 119 g day$^{-1}$ with natural ventilation, whereas variation between 262 and 309 g day$^{-1}$ with electric ventilation. In the HF bed, the organic loading (BOD) varied between 73 and 588 g day$^{-1}$, demonstrating mass removal between 1 and 19 g day$^{-1}$ in the VF-HF CW with natural ventilation, whereas it varied between 262 and 309 g day$^{-1}$ with electric ventilation.

Linear regressions for SS were SS removal (g day$^{-1}$) = 0.0714 × SS loading (g day$^{-1}$) + 12.59 ($r = 0.245$) for the VF bed and SS removal (g day$^{-1}$) = 0.0049 × SS loading (g day$^{-1}$) + 2.7847 ($r = 0.248$) for the HF bed with natural ventilation. In the case of electric ventilation, linear regressions for SS were SS removal (g day$^{-1}$) = −0.2533 × SS loading (g day$^{-1}$) + 97.229 ($r = 0.353$) for the VF bed and SS removal (g day$^{-1}$) = 0.089 × SS loading (g day$^{-1}$) − 16.267 ($r = 0.764$) for the HF bed. In the VF bed, the SS loading varied between 41 and 416 g day$^{-1}$, showing mass removal between 4 and 100 g day$^{-1}$ with natural ventilation, and varied between 245 and 273 g day$^{-1}$, demonstrating mass removal between 24 and 41 g day$^{-1}$ with electric ventilation. In the HF bed, the SS loading varied between 41 and 416 g day$^{-1}$, presenting mass removal between 1 and 8 g day$^{-1}$ in the VF-HF CW with natural ventilation, whereas variation between 245 and 273 g day$^{-1}$, demonstrating mass removal between 6 and 9 g day$^{-1}$, was observed in the case of electric ventilation.

In the VF-HF CW with natural ventilation, linear regressions for TN were TN removal (g day$^{-1}$) = 0.8408 × TN loading (g day$^{-1}$) + 1.203 ($r = 0.94^*, P < 0.01$) for the VF bed and TN removal (g day$^{-1}$) = 0.4213 × TN loading (g day$^{-1}$) + 8.6059 ($r = 0.757^*, P < 0.01$) for the HF bed. In the case of the electric ventilation, linear regressions for TN were TN removal (g day$^{-1}$) = 1.1524 × TN loading (g day$^{-1}$) − 37.96 ($r = 0.991^*, P < 0.01$) for the VF bed and TN removal (g day$^{-1}$) = 0.509 × TN loading (g day$^{-1}$) − 6.0137 ($r = 0.973^*, P < 0.01$) for the HF bed. In the VF bed, the TN loading varied between 21.9 and 90.6 g day$^{-1}$, presenting mass removal between 12.2 and 83.8 g day$^{-1}$ in the naturally ventilated VF-HF CW, and varied between 60.3 and 78.4 g day$^{-1}$, with mass removal between 30.9 and 52.6 g day$^{-1}$ in the VF-HF CW ventilated with an electric air blower. In the HF bed, the TN loading varied between 21.9 and 90.6 g day$^{-1}$, demonstrating mass removal between 10.2 and 50.5 g day$^{-1}$ in the VF-HF CW with natural ventilation, whereas it varied between 60.3 and 78.4 g day$^{-1}$, with mass removal between 23.7 and 33.6 g day$^{-1}$ under electric ventilation.

In the naturally ventilated VF-HF CW, linear regressions for TP were TP removal (g day$^{-1}$) = 0.4624 × TP loading (g day$^{-1}$) + 2.07 ($r = 0.666^*, P < 0.01$) for the VF bed and TP removal (g day$^{-1}$) = 0.1982 × TP loading (g day$^{-1}$) + 2.0185 ($r = 0.427$) for the HF bed. In the VF-HF CW with
electrically provided ventilation, linear regressions for TP were TP removal (g day\(^{-1}\)) = 0.1916 \times TP loading (g day\(^{-1}\)) + 3.0375 \ (r = 0.689) for the VF bed and TP removal (g day\(^{-1}\)) = 0.4576 \times TP loading (g day\(^{-1}\)) − 0.0041 \ (r = 0.990^{**}, P < 0.01) for the HF bed. In the VF bed, the TP loading varied between 2.8 and 11.2 g day\(^{-1}\), with mass removal between 1.9 and 8.3 g day\(^{-1}\) in the VF-HF CW ventilated naturally, while it varied between 4.9 and 7.6 g day\(^{-1}\), showing mass removal between 3.7 and 4.7 g day\(^{-1}\) under electric ventilation. In the HF bed, the TP loading varied between 2.8 and 11.2 g day\(^{-1}\), with mass removal between 1.5 and 5.0 g day\(^{-1}\) under natural ventilation, and between 4.9 and 7.6 g day\(^{-1}\), with mass removal between 2.4 and 3.9 g day\(^{-1}\) in the VF-HF CW ventilated using an electric air blower. Based on the above results, removal efficiencies of BOD, TN, and TP in the VF-HF CW ventilated using an electric fan air blower were higher than those with natural ventilation.

**4. Conclusion**

To enhance the performance of organics and nutrient (N and P) removal in VF-HF hybrid CWs, the treatment efficiency of VF-HF hybrid CWs was evaluated during the treatment of domestic sewage from agricultural villages under different ventilation methods. The removal efficiencies of BOD, SS, TN, and TP in the effluent were 95.3–99.0, 88.9–99.1, 19.0–53.3, and 28.8–63.2% in the VF-HF CWs with natural ventilation, whereas they were 98.8–98.9, 97.2–97.5, 57.5–58.6, and 48.1–48.6% in the VF-HF CW with a ventilation pipe and an electric fan air blower, providing air by the renewable energy of solar and wind power, respectively. The removal efficiencies of BOD, TN, and TP in the VF-HF CW with the electric ventilation were higher than those ventilated naturally, excluding only SS. The TN treatment efficiency in the VF-HF CW with electric ventilation was especially higher, increasing by 16.6% in comparison with the VF-HF CW with natural ventilation, since the VF bed provided...
suitable conditions (aerobic) for nitrification to occur. The treatment performance of the VF-HF CWs under different ventilation methods was assessed. TN in the VF-HF CW with electric ventilation provided by renewable energy was reduced by 57.5–58.6% for inlet TN loading, whereas TN in the VF-HF CW with natural ventilation was reduced by 19.0–53.3% for inlet TN loading. Therefore, to increase the removal of organics and nutrients (N and P) in VF-HF CWs, an improved ventilation system, providing ventilation via an electric fan air blower with the renewable energy of solar and wind power, is recommended.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Dong Jin Lee, Se Won Kang, and Jong Hwan Park contributed equally to this work.

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