

SEASONAL CHARACTERISTICS OF FECAL INDICATORS IN WATER ENVIRONMENT RECEIVING EFFLUENTS OF DECENTRALIZED WASTEWATER TREATMENT FACILITIES

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Decentralized domestic wastewater treatment systems are generally used in rural areas for preventing pollution of local water environment. Treated waters of decentralized systems potentially contain fecal contaminants and the degree of contamination can vary seasonally. To identify the seasonal characteristics of fecal indicators in the area of decentralized system, seasonal monitoring on the characteristics of fecal indicators (total coliforms, *Escherichia coli*, and F-specific coliphages) was performed along an open channel receiving *johkasou* effluents over 3-year period. Fecal indicators were detected higher than environmental quality standard throughout the season, particularly for total coliforms, and the concentrations of *Escherichia coli* downstream seemed to be higher than upstream in winter. The low water quality was recorded during cold-low flow season and the effluent of *johkasou* was an important factor reflecting water environment quality. In contrast with most physicochemical parameters, significant seasonal differences of total coliforms and *Escherichia coli* were not observed both in water and sediment. The principal component analysis results show that four dominant factors related to chemical contaminants, environmental conditions, byproducts, and fecal contaminants were responsible for the water quality data structure and significant seasonal differences were observed for chemical contaminants, environmental conditions, and byproducts, accounting for 59 % of the total variance in the data set. Seasonal maintenance of discharged water quality and controlling downstream network capacities are necessary to reduce the potential impact of *johkasou* effluents into local water environment.

Key Words: Fecal indicators, *johkasou*, principal component analysis, seasons, water environment

1. INTRODUCTION

Decentralized domestic wastewater treatment systems in rural areas are important for preventing pollution of aquatic environments and sanitation of the local environment¹⁾. Small-scale onsite domestic wastewater treatment system called *johkasou* systems have been developed and become popular in Japan and continue to expand into other countries^{1,2)}. The installation number of *johkasou* in Japan at the end of fiscal 2012 was 7.76 million *johkasou* facilities, which include the *tandoku johkasou* (58%, 4.53 million units) and *gappei*

johkasou (42%, 3.23 million units)³⁾. *Tandoku johkasou* is the type of *johkasou* system which can treat only black water and *gappei johkasou* is an improvement over the *tandoku johkasou* which treats both black and grey water⁴⁾. Most of *johkasou* systems are household-sized units with a capacity of 1–2 m³/day¹⁾. Unlike European septic tanks, *johkasou* even the smallest unit (5 – 10 population equivalents) undergo an aerobic process⁵⁾. *Johkasou* system is generally composed of a primary treatment unit, an aerobic biological treatment unit, and a disinfection chamber¹⁾. *Johkasou* systems are

considered to be an effective means for wastewater treatment in rural areas, which are designed, located, operated, and maintained satisfactorily.

Treated water of decentralized wastewater treatment systems, including *johkasou* system, is generally discharged directly into water environment through open channels, ditches, or drains built within residential areas. The treated water is essential for maintaining sufficient water in channels, enhancing the water circulation in the local areas, and contributing to the life of the aquatic organisms⁶⁾. However, it has been recorded that the treated waters of decentralized wastewater treatment systems contained several contaminants such as organics, nutrients, and microorganisms^{7,8)}. Assessment of water quality has relied on the detection of fecal indicator organisms such as total coliforms and *Escherichia coli*⁹⁾. Those bacteria are often associated with fecal contamination and possible presence of waterborne pathogens. On the other hand, human pathogenic viruses are generally present in fecal-contaminated waters and could persist longer than enteric bacteria¹⁰⁾. It is thus unsafe to rely on bacteriological standards to assess the virological water safety in any kind of water. Besides the technical difficulties, it is impracticable to monitor the presence of all viral pathogens. F-specific coliphages have been proposed as viral indicators because of their similarity in size, structure, transport, and persistency in the environment with many human enteric viruses¹¹⁾. F-specific coliphages are generally associated either with humans or animals faeces¹²⁾. The presence of bacterial indicators along with viral indicators both in water and sediment can obviously indicate fecal contamination in the aquatic environment.

The degree of contamination can vary seasonally. Most of the physicochemical parameters of surface water quality showed moderate variations in their concentration for all season^{13,14)}. Several studies have also highlighted the seasonal differences in the microbiological quality of surface water quality due to numerous factors such as the unequal loading of wastewater, solar irradiation, temperature, water flow, dilution, rainfall, organic matter, and the origin of the microorganisms^{15,16)}. In the previous study, fecal indicators have been detected in the channels of *johkasou* systems¹⁷⁾. However, the seasonal characteristics of fecal indicators in the local water environment receiving *johkasou* effluents are little known. Therefore, the objective

of this study was to assess the seasonal characteristics of fecal indicators in local water environment receiving *johkasou* effluents. To achieve the objective, evaluation on the seasonal characteristics of fecal indicators both in water and sediment along an open channel receiving *johkasou* effluents was performed and the extraction of important water quality parameters and their seasonal variability were evaluated using the principal component analysis.

2. MATERIALS AND METHODS

(1) Site description

The study site is located in Gifu, Japan, around a residential area where 39 households used the standard *gappei johkasou* and 13 households used the standard *tandoku johkasou* (Fig. 1). The investigations were carried out at five basis stations (St. 1 – St. 5) in a 1-m wide and 200-m long open channel surrounding the residential area. In addition, a summary effluent of 16 *gappei johkasou* facilities (between St. 3 and St. 4) was also examined before discharging to the downstream network. St. 1 is located upstream, which is surrounded along its

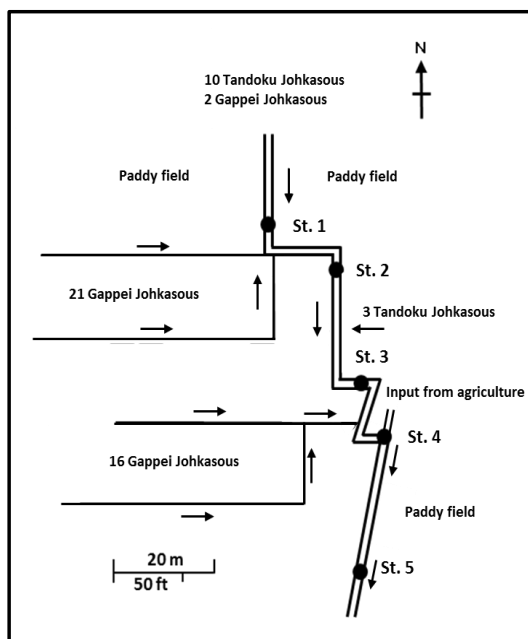


Fig. 1 A model of decentralized area of this study (Gifu Prefecture, Japan).

path by paddy fields and receives the effluents from 10 *tandoku johkasou* and 2 *gappei johkasou* facilities in its upper reach. St. 2 is located 25 m away from St. 1 and receives effluents from St. 1 and 21 *gappei johkasou* facilities. At the head of St. 1, the groundwater is consistently supplied into the open channel. St. 3 is located 30 m downstream of St. 2 and receives effluents from 3 houses that use *tandoku johkasou*. St. 4 is located downstream of the confluence of St. 3 and receives effluents from 16 *gappei johkasou* facilities. St. 5 is located 30 m away from St. 4 and it is surrounded by paddy fields along the path from St. 4. Along this 30-m path, there are no additional *johkasou* discharged sources and paddy fields runoff was recognized in spring and summer.

(2) Sample collection

a) Water sample collection

Water samples were collected at all sampling stations including a summary effluent of 16 *gappei johkasou* facilities. The water samples were collected fifteen times on December 10, 2009; November 17, December 20, 2010; March 15, August 8, September 15, October 14 and 26, November 16, and December 15, 2011; March 8, May 24, August 28, and November 22, 2012; and January 28, 2013. All samples were stored in new 1-L polypropylene sampling bottles, put inside cooler boxes, and delivered to the laboratory on the day of collection for analysis.

b) Sediment collection

Sediment samples were collected at all sampling stations including a summary effluent of 16 *gappei johkasou* facilities. The sediment samples were collected ten times on November 17, 2010; March 15, September 15, October 26, November 16, and December 15, 2011; March 8, May 24, and November 22, 2012; and January 28, 2013. Sediment samples were collected as sediment/water mixed liquors. The mixed liquor was collected by placing the sampling tube into the sediment bed, mixing the sediment enclosed within the tube with the overlaying water using a brush, and finally collecting the sediment as a mixture of sediment and water. The collected sediment samples were stored in new 250-mL polypropylene sampling bottles, put inside cooler boxes, and delivered to the laboratory on the day of collection for subsequent analysis.

(3) Analytical methods

For water samples, the pH, water temperature (Temp), electrical conductivity (EC), oxidation-reduction potential (ORP), dissolved oxygen (DO), ultraviolet absorbance at 260 nm (UV₂₆₀), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorus (TP), suspended solids (SS), chloride ion (Cl⁻), total chlorine, total coliform (TC), *Escherichia coli* (*E.coli*), and F-specific coliphages (F-phages) were analyzed. For sediment samples, total sediment (TS), TC, *E.coli*, and F-phages were analyzed.

The concentrations of F-phages were determined by following the PFU method of ISO 10705-1 (2001) using *Salmonella enterica* serovar Typhimurium WG49 as the host strain. For water samples, 1 mL of the sample was directly inoculated into the agar medium. For sediment samples, viral particles were firstly extracted using 3% beef extract (Kyokuto pharmaceutical Industry Co., Ltd, Japan) at pH 9 and centrifuged at 12,000×g for 10 min, and then 1 mL of supernatant was inoculated into the agar medium¹⁸⁾. Plaque count was carried out after incubation at 37°C for 24 h. Simultaneous examination of parallel plates with added RNase was carried out for confirmation by differential counts. Each sample was tested in triplicate.

E.coli and TC were analyzed according to the standard methods¹⁹⁾. In addition, the general water quality indices including, total chlorine (HACH, Japan), Temp, pH, EC, ORP, and DO (DKK-TOA, Japan), BOD, TN, TP, SS, TS, UV₂₆₀, and Cl⁻ were also analyzed according to standard methods²⁰⁾. The flow rate was measured during sampling together with Temp, pH, EC, ORP, DO and total chlorine.

(4) Statistical analysis

A one-way analysis of variance (ANOVA) was used to test significant differences of parameters within seasons and stations. Tukey post tests were used for specific means comparisons. The Kolmogorov-Smirnov (K-S) test was used to evaluate the goodness-of-fit of the data to log-normal distribution. A principal component analysis (PCA) was used to identify the important components of the water quality of the *johkasou* system. All statistical analyses were conducted using Microsoft Office Excel 2010 and IBM® SPSS® Statistic version 21.

3. RESULTS AND DISCUSSION

(1) Seasonal characteristics of general water quality induces in the open channel

Table 1 summarizes the measurement results (mean value and standard deviation) at all seasons during the study period. Seasonal variation was performed in four different seasons defined according to the Japanese solar calendar when winter begins on the middle of December, spring

begins on the middle of March, summer begins on the middle of June, and autumn begins on the middle of September.

The mean value of flow increased from upstream (11 L/s) to downstream (15 L/s) thus indicates the source inputs along the open channel. The open channel receives inputs from agriculture fields at St.1 and *johkasou* effluents at its upper reach. The flow increased approximately 14 % at St.2 expected from *johkasou* effluents and

Table 1 Measurement results for water and sediment quality in the local water environment of *johkasou* system in all season during December 2009 to January 2013

Variable	Season				Diff*
	Winter	Spring	Summer	Autumn	
<u>Water</u>					
Temp (degree C)	10.3 ± 1.5 (14)	16.5 ± 4.0 (12)	26.4 ± 1.4 (14)	14.3 ± 1.6 (25)	a
Flow rate (L/S)	4.9 ± 1.8 (19)	34 ± 33 (12)	36 ± 23 (14)	7.0 ± 6.3 (25)	a
pH	7.32 ± 0.11 (19)	7.75 ± 0.13 (12)	7.52 ± 0.09 (14)	7.55 ± 0.28 (25)	a
ORP (mV)	53 ± 22 (11)	128 ± 57 (12)	200 ± 45 (14)	139 ± 42 (25)	a
EC (mS/m)	17.8 ± 6.1 (17)	13.8 ± 3.5 (12)	12.4 ± 0.5 (14)	16.9 ± 5.7 (25)	a
DO (mg/L)	6.6 ± 0.5 (10)	6.0 ± 0.4 (10)	6.5 ± 0.7 (9)	6.9 ± 0.4 (10)	a
SS (mg/L)	2.4 ± 2.1 (18)	4.2 ± 2.8 (12)	17.1 ± 13.0 (14)	3.8 ± 3.6 (23)	a
UV ₂₆₀ (1/m)	4.21 ± 1.96 (14)	2.11 ± 1.56 (12)	2.17 ± 0.79 (14)	3.73 ± 2.83 (23)	c
BOD (mg/L)	2.2 ± 1.5 (14)	1.4 ± 0.2 (10)	1.2 ± 0.1 (4)	1.4 ± 1.4 (10)	c
TP (mg/L)	0.22 ± 0.09 (11)	0.19 ± 0.03 (10)	0.12 ± 0.01 (4)	0.22 ± 0.16 (10)	c
TN (mg/L)	2.36 ± 1.04 (16)	2.22 ± 2.03 (10)	0.68 ± 0.20 (14)	2.02 ± 1.05 (20)	a
Cl (mg/L)	8.9 ± 6.15 (11)	4.7 ± 1.1 (10)	3.1 ± 0.2 (4)	7.7 ± 4.8 (10)	c
Total chlorine (mg/L)	0.03 ± 0.02 (10)	0.04 ± 0.02 (10)	0.12 ± 0.09 (5)	0.01 ± 0.01 (15)	a
TC (MPN/100mL)	(5.1 ± 6.2)×10 ⁵ (14)	(1.8 ± 1.5)×10 ⁵ (12)	(1.9 ± 1.1)×10 ⁵ (14)	(7.4 ± 9.3)×10 ⁵ (23)	c
<i>E.coli</i> (MPN/100mL)	(1.9 ± 3.0)×10 ⁴ (14)	(6.8 ± 7.4)×10 ³ (12)	(2.6 ± 6.1)×10 ⁴ (14)	(0.9 ± 2.5)×10 ⁴ (23)	c
F-phages (PFU/mL)	6.9 ± 8.7 (5)	0.1 ± 0.1 (10)	No data	12.6 ± 24.9 (15)	c
<u>Sediment</u>					
TS (mg/cm ²)	24.6 ± 17.8 (10)	8.2 ± 9.6 (10)	No data	13.3 ± 9.6 (10)	a
TC (MPN/mg)	(1.5 ± 2.6)×10 ⁷ (10)	(1.7 ± 3.0)×10 ⁶ (10)	No data	(5.1 ± 7.4)×10 ⁶ (10)	c
<i>E.coli</i> (MPN/mg)	(1.8 ± 3.1)×10 ⁴ (10)	(4.0 ± 5.5)×10 ⁴ (10)	No data	(2.0 ± 2.2)×10 ⁴ (10)	c
F-phages (PFU/mg)	32.8 ± 44.4 (5)	2.1 ± 2.5 (10)	No data	0.7 ± 1.8 (10)	b

Data are arithmetic mean ± standard deviation (sample number). Limit of detections for all fecal indicators are 0 value and it is used for the negative data. *Statistical significance of mean value differences between seasons (One way ANOVA with Tukey post test). The letter "a" means statistically significant at $p < 0.01$, "b" means statistically significant at $p < 0.05$, and "c" is not statistically significant.

groundwater supplied between St.1 and St.2. Houses between St. 2 and St.3 contributed approximately 4 % of the total flow in the open channel. The highest flow input around 17 % was at St. 4 due to effluents from *johkasou* facilities. The flow increased approximately 1 % at St. 5 contributed from agriculture fields along the path from St. 4. The different conditions were observed throughout the season. The high flows were observed in spring and summer (33 and 36 L/s) and the low flows were observed in winter and autumn (4.9 and 7.0 L/s). These significant differences were because of the high agriculture activities in spring and summer. The low flows during winter and autumn are the most important factor reflecting water environment quality because of low mixing ratio with water environment.

The water temperature in the open channel reflects the air temperature, which was at the highest in summer (23.8 – 28.1 °C) and at the lowest in winter (7.2 – 12.2 °C). The significant differences were observed within seasons. However, there were no significant differences of water temperatures at all stations along the open channel. The mean values of water temperature slightly increased from upstream (16.1 °C) to downstream (16.7 °C). The decrease of water temperature was observed at St. 2 because input from groundwater which always had a lower temperature than surface water of the open channel. The water temperature has been noted as one factor that affects the performance of *johkasou* system²¹⁾.

The mean values of BOD, TN, and TP slightly decreased from upstream (2.3, 2.25, and 0.23 mg/L, respectively) to downstream (2.1, 1.83, and 0.21 mg/L, respectively) possibly due to mixing with other water sources along the open channel. The concentrations of BOD, TN, and TP were highest at St. 1 because this site receives effluents from houses that use *tandoku johkasou* facilities which contain untreated grey water. Grey water corresponds to household wastewater from bathtubs, kitchen sink, washing machine, and hand basin that contain high levels of BOD, nutrients, and microorganisms^{22,23)}. The high concentrations of BOD, TN, and TP were in winter (2.2, 2.36, and 0.22 mg/L, respectively). The significantly seasonal differences were observed for TN between summer and other seasons. Meanwhile significantly seasonal differences were not observed for BOD and TP. The environmental quality standards for

conservation of the living environment are set at 3 mg/L BOD, 1 mg/L TN, and 0.1 mg/L TP. Mean values of BOD always meets the environmental quality standard for conservation of the living environment which is lower than 3 mg/L, but concentrations of TN and TP still do not meet the standard levels because those *johkasou* type are mostly BOD type removal which have no function on removal of nitrogen and phosphorus. The environment self-purifications and dilution with water environment are expected to reduce the concentration of TN and TP in water environment.

(2) Seasonal characteristics of fecal indicators in the open channel

Bacterial indicators (*E.coli* and TC) and viral indicators (F-phages) were used to identify possible fecal contamination both in water and sediment in the water environment receiving effluents of *johkasou* systems.

As shown in Table 1, fecal indicators were detected throughout the season. In winter, TC, *E.coli*, and F-phages were always detected both in water and sediment. In spring, TC and *E.coli* were always detected both in water and sediment, while the detection frequencies of F-phages were 50% in water and 100% in sediment. In summer, TC and *E.coli* were always detected both in water and sediment. In autumn, TC and F-phages were always detected both in water and sediment, while the detection frequencies of *E.coli* were 96% in water and 100% in sediment. The simultaneous occurrences of fecal indicators throughout the season and their high detection frequencies both in water and sediment indicate the possible fecal contamination in the water environment receiving *johkasou* effluents.

The fecal indicators were detected at high concentrations throughout the season, particularly for TC. As shown in Table 1, the mean concentrations of TC in winter, spring, summer, and autumn were 5.1×10^5 , 1.8×10^5 , 1.9×10^5 , and 7.4×10^5 MPN/100mL, respectively. The Japanese standard value for TC in rivers is set at 5,000 MPN/100mL²⁴⁾. The average values of TC were always greater than standard value throughout the season. As suggested by Nambu (1996), insufficient disinfection process and low contact time have been recorded as a cause of increasing concentrations of fecal coliforms in *johkasou* effluents²⁵⁾. Significant seasonal differences in concentrations of TC and

E.coli were not observed both in water and sediment. However, concentrations of F-phages in sediment show seasonal differences. More investigation on the behavior of F-phages is needed to determine precisely seasonal characteristics of F-phages in the open channel.

The mean concentration of TC both in water and sediment increased from upstream (3.3×10^5 MPN/100mL and 2.2×10^6 MPN/mg) to downstream (8.0×10^4 MPN/100mL and 3.9×10^6 MPN/mg) possibly due to contribution of *johkasou* effluents along the open channel. The mean concentration of *E.coli* in sediment increased from upstream (1×10^4 MPN/mg) to downstream (5.4×10^4 MPN/mg), while the mean concentration in water decreased from upstream (8.0×10^4 MPN/100mL) to downstream (2.8×10^4 MPN/100mL). This indicates that possible interaction between *E.coli* in water and sediment, likely settled onto sediment bed, may increase the concentrations of *E.coli* in sediment channel. Several studies suggest that the association of microbes and settleable particles has important implication for microbial transport in receiving water through sedimentation and resuspension^{26,27}. The mean concentration of F-phages both in water and sediment decreased from upstream (28.2 PFU/mL and 23.2 PFU/mg) to downstream (1.1 PFU/mL and 4.6 PFU/mg) possibly due to the distance from a hot spot St.1. The high concentration of F-phages both in water and sediment at St 1 (28 PFU/mL and 23 PFU/mg) is because this site receives mostly effluents from houses using *tandoku johkasou* that contain untreated grey water. Several studies reported that F-specific coliphages were detected not only in the faeces of humans and animals but also in various wastewater and grey water^{28,29}.

(3) Seasonal characteristics of *johkasou* effluents

In order to know the seasonal impact of effluents from *johkasou* system into water environment downstream, seasonal characteristics of *johkasou* effluents at an end point of channel that collects effluents from 16 *gappei johkasou* facilities were evaluated. Fig. 2 shows seasonal characteristics of effluents from *johkasou* system (between St. 3 and St. 4) and receiving water environment upstream (St. 3) and downstream (St. 4) for flow, temperature, BOD, TN, TC, *E.coli*, and F-phages both in water and sediment during the study period.

As shown in Fig. 2, the flow ratio between *johkasou* effluents and downstream network varied in seasons with the lowest flow ratio was in winter (~1:32). There were no significant differences in temperatures between upstream and downstream throughout the season. This suggests that discharge of *johkasou* effluents had little impact on the main channel temperatures due to the low flow rate. The mean concentrations of BOD, TN, TC and *E.coli* at *johkasou* effluents both in water and sediment were always higher than downstream network that potentially affect the water quality downstream. The highest concentrations of BOD and TN at *johkasou* effluents were observed in spring, while the highest concentrations of BOD and TN at downstream were observed in winter. This indicates that high dilution capacity of downstream in spring (~1:1000) could reduce the impact of *johkasou* effluents, while low dilution capacity in winter (~1:32) might cause degradation in downstream water quality. The concentrations of TC in water were not significant difference in all seasons thus indicates the consistent contribution of *johkasou* effluents on the concentrations of TC into water environment. The high concentrations of TC in sediment both in *johkasou* effluents and downstream network were observed in winter and autumn. The same trend with TC in sediment, the high concentrations of *E.coli* in *johkasou* effluents both in water and sediment were in winter and autumn but the concentration after discharging into water environment were not significant different. However, concentrations of *E.coli* downstream seem to be higher than that upstream in winter. As for FRNA bacteriophages, the mean concentrations at *johkasou* effluents both in water and sediment were lower than downstream network. However, low concentration of F-phages could still be detected in the effluents possibly due to association with the solid particles may protect them from the inactivation by disinfection³⁰.

In Japan, the most stringent effluent standards of *gappei johkasou* are set at 20 mg/L BOD, 10 mg/L TN, and 3,000 MPN/100mL TC^{1,31}. The mean concentration of BOD, TN, and TC were 7.1 mg/L, 9.3 mg/L, and 2.1×10^6 MPN/100mL in winter, 7.5 mg/L, 19 mg/L, and 1.8×10^6 MPN/100mL in summer, 13 mg/L, 18 mg/L, and 4.6×10^6 MPN/100mL in spring, and 8.1 mg/L, 15.4 mg/L, and 4.4×10^6 MPN/100mL in autumn. Compared to the designated standard values, the average values

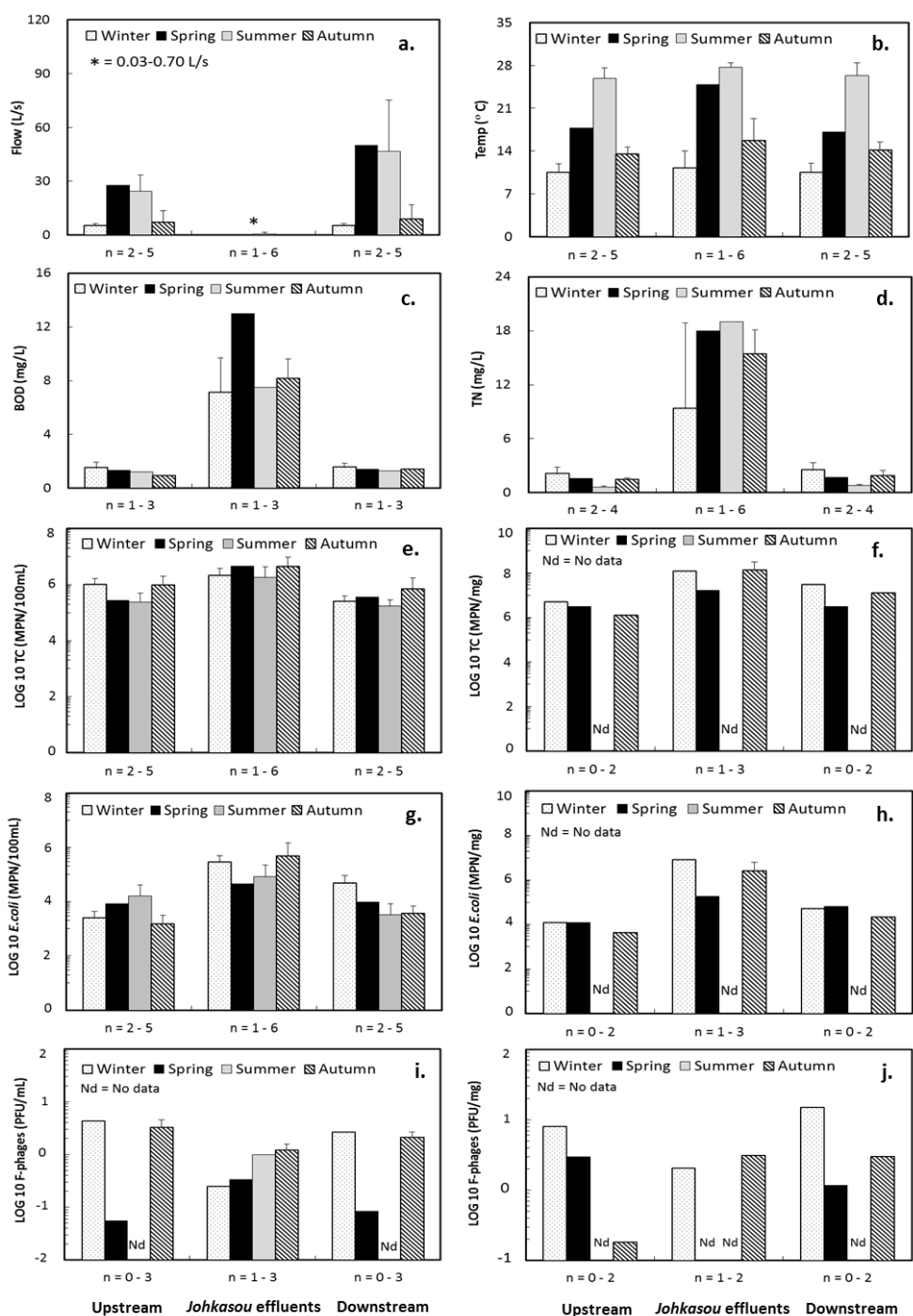


Fig. 2 Seasonal characteristics of effluents from *johkasou* system and receiving water environment for a. flow, b. temperature, c. BOD, d. TN, e. TC in water, f. TC in sediment, g. *E. coli* in water, h. *E. coli* in sediment, i. F-phages in water, and j. F-phages in sediment. The data shown are arithmetic mean with standard deviation. The data without standard deviations are because the sample numbers less than three.

of TC are higher in all seasons and the average values of TN are higher in spring and autumn. Seasonal maintenance of discharged water quality including disinfection process and controlling downstream network capacities are required to reduce the potential impact of *johkasou* effluents into local water environment.

(4) Seasonal characteristics of water quality using principal component analysis

PCA was performed to identify the important components that explain most of the variances of water quality variables. A rotation of principal components was used to achieve a simpler and more meaningful representation of the underlying factors. A planar plot of the 15 variables is displayed in **Fig. 3**.

In this study, four PCs were retained, which could explain 68 % of the variance of information contained in the original data set. The first factor (PC 1) accounted for 36 % of the total variance and was strongly contributed by nutrients (TN and TP), organic matters (BOD and UV₂₆₀), inorganic chloride ion (Cl⁻), and mineral (EC). The PC 1 can be interpreted as a component of chemical contaminants. The second factor (PC 2), which accounted for 14 % of the total variance, was

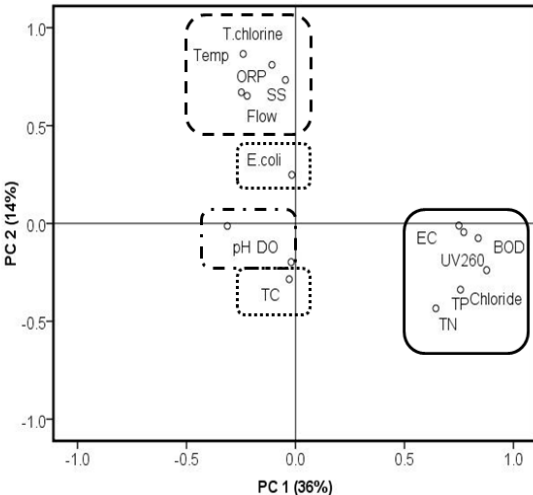


Fig. 3 Scores of 15 variables on the bi-dimensional plane for the principal components PC 1 and PC 2. Four rotated principal components were extracted by principal component analysis. The Varimax was used for rotation method.

Table 2 Result of One-way ANOVA for four principal components of water quality between group and within group of seasons

Component	Group of seasons	p - value	Diff. within group	Diff. between group
PC1	Winter and spring	0.26	c	
	Winter and summer	0.03	b	
	Winter and autumn	0.29	c	b
	Spring and summer	0.83	c	
	Spring and autumn	0.98	c	
	Summer and autumn	0.48	c	
PC2	Winter and spring	0.45	c	
	Winter and summer	0.00	a	
	Winter and autumn	0.95	c	a
	Spring and summer	0.00	a	
	Spring and autumn	0.69	c	
	Summer and autumn	0.00	a	
PC3	Winter and spring	0.00	a	
	Winter and summer	0.96	c	
	Winter and autumn	1.00	c	a
	Spring and summer	0.00	a	
	Spring and autumn	0.00	a	
	Summer and autumn	0.98	c	
PC4	Winter and spring	0.08	c	
	Winter and summer	0.73	c	
	Winter and autumn	0.30	c	
	Spring and summer	0.54	c	c
	Spring and autumn	0.74	c	
	Summer and autumn	0.95	c	

Data are values of significance differences (One-way ANOVA) between group and within group of seasons. The letter "a" means statistically significant at $p < 0.01$, "b" means statistically significant at $p < 0.05$, and "c" is not statistically significant.

contributed by flow, temperature, ORP, SS, and total chlorine. The PC 2 is related to the environmental conditions. The third factor (PC 3) accounted for 9 % of the total variance was strongly contributed by pH and negatively contributed by DO. The PC 3 can be interpreted as a component of byproducts. The forth factor (PC 4), accounting for 8 % of the total variance, were strongly contributed by *E.coli* and TC. The PC 4 can be interpreted as a component of fecal contaminants excluding of F-phages. F-phages were not included in the statistical analysis because of lack of adequate data set for all seasons. By using PCA, the 15 parameters were successfully reduced to four key independent factors. Each factor significantly related to specific parameters representing a different dimension of the water.

In order to identify the seasonal differences of four principal components of water quality, One-way ANOVA was performed to the four principal components. **Table 2** shows the results of one-way ANOVA for four principal components of water quality between group and within group of seasons. Significant seasonal differences were obtained for PC 1, PC, 2, and PC3, accounting for 59% of the total variance in the dataset. Seasonal differences for PC 1 were obtained between winter

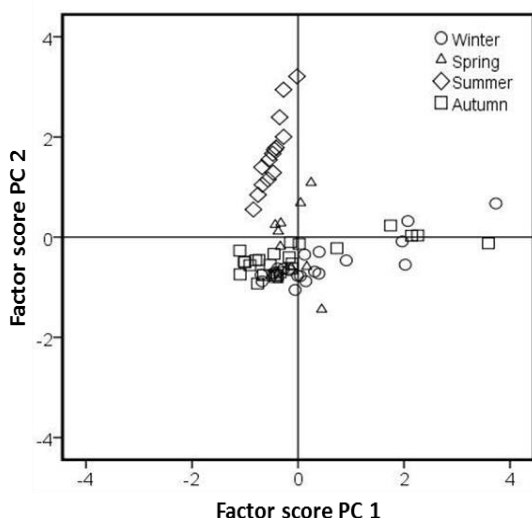


Fig. 4 Factor scores between PC 1 and PC 2 in different season. Factor scores were extracted by principal component analysis using regression method.

and summer. Seasonal differences for PC 2 were obtained between winter and summer, spring and summer, and spring and autumn. Seasonal differences for PC 3 were obtained between winter and spring, spring and summer, and spring and autumn. Meanwhile, there were no significant seasonal differences for PC 4.

Factor scores of four principal components were evaluated to identify the seasonal water quality. **Fig. 4** displays scatter plots of factor scores between PC 1 and PC 2 in different seasons. High and positive scores on PC 1 indicate low water quality, while high and negative scores on PC 1 indicate better water quality. High and positive scores on PC 2 correspond to good environmental condition for reduction of contaminants, while high and negative scores on PC 2 indicate low environment condition for reduction of contaminants. As shown in **Fig. 4**, the high and positive scores of PC 2 can be seen in summer thus indicate good condition for reduction of contaminants. The high and negative scores of PC 1 in summer indicate better water quality than other seasons. The lowest water quality can be seen in winter. It has been noted by Kaneko (2001), the low temperature could decrease the efficiency of *johkasou* facility in reduction of organic matter and pathogenic bacteria²¹⁾.

4. CONCLUSIONS

Our investigation on fecal indicators in water environment receiving effluents from decentralized *johkasou* systems in a typical Japanese rural area showed that fecal indicators of total coliforms, *Escherichia coli*, as well as F-specific coliphages were always detected at high concentrations throughout the season. The concentrations of total coliforms, BOD, and TN were observed from 2 to 30-fold higher at *johkasou* effluent than at downstream network which with low dilution capacity may cause degradation on water quality downstream. A real concern of degradation on water quality in the area of decentralized *johkasou* systems is during cold-low flow seasons and the effluent of *johkasou* is an important factor reflecting water quality in the local water environment. In contrast to most of the physicochemical parameters, significant seasonal differences in concentrations of fecal indicators related to total coliforms and *Escherichia coli* were not observed both in water and sediment through the study period. The principal component analysis results show that four dominant factors related to chemical contaminants, environmental conditions, byproducts, and fecal contaminants, accounting for 68 % of the variance of information contained in the original data set, were responsible for the water quality data structure and significant seasonal differences were observed for chemical contaminants, environmental conditions, and byproducts, accounting for 59 % of the total variance in the dataset. Although, it is not possible in this study to determine the risk of *johkasou* effluents to public health, the high concentrations of fecal indicators throughout the season and deterioration of water quality during cold low flow seasons, there is a need to increase awareness of potential impacts related to *johkasou* system among rural communities, management authorities, and *johkasou* companies in areas where use decentralized *johkasou* systems.

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(Received May 23, 2014)