

**Evaluation of water and sediment quality  
in open channels receiving effluent from  
small-scale onsite wastewater treatment  
facilities**

(小規模汚水処理施設の処理水放流先水路  
における水質および底質の評価)

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# Abstract

In regard to water environment protection, the Ministry of Environment Japan strictly regulates the wastewater treatment systems in all of areas even though in rural areas. The rural areas that have fewer inhabitants generally use onsite domestic wastewater treatment systems. An alternative onsite domestic wastewater treatment system, named johkasou, has widely been used in rural areas and also the areas that are not covered by centralized wastewater treatment system. This system has a function to protect a local water environment by treating household wastewater before discharging into the stream water. The effluent of johkasou is generally discharged into stream channel through the drainages or ditches. However, johkasou effluent may transmit several contaminants (including organic and inorganic matter, nutrient, chemical, fecal indicators, and pathogenic bacteria) that cannot be completely removed by johkasou system. Thus, these contaminants may possible change the water quality, and some contaminants can also impair the sediment quality by disposition and sedimentation onto the sediment of local water environment.

Monitoring of water and sediment quality in stream channel where johkasou facilities are installed is necessary to know the environmental condition and the effect of johkasou effluent. Therefore, the goal of this study is to reveal the impact of johkasou effluent in both water and sediment of open channels in a residential area. An area using johkasou facilities in Gifu prefecture, Japan, was investigated in this study. Samples of water and sediment were collected from several sites in both the open channels and the johkasou drainage channel through 3-year study period. Several parameters were measured in both samples of water (20 indices) and sediment (6 indices) in order to evaluate the characteristics of physicochemical and microbial parameters in the open channels after receiving johkasou.

Physicochemical parameters were used to evaluate their characteristics along open channels after receiving johkasou effluent. Concentrations of organic matter (BOD and COD) and nutrient (TN and TP) in the johkasou effluent were significantly different compared to those at sampling sites in open channels. These contaminants were generally detected two orders magnitude higher. However, the concentrations of physicochemical parameters among the sampling sites in the open channels were not significantly different during spring and summer in which the flow rate in the channel are relatively high mainly due to irrigation from surrounding paddy field. This indicated that impact of johkasou effluent was not significant in terms of physicochemical parameters in the period. The concentrations of organic matter and nutrients in the open channels were high in winter. This high concentration of those parameters in winter was coincided with the lower flow rate in the open channel, suggesting that the dilution ratio of the johkasou effluent to water in the open channel can affect the level of impact of johkasou effluent on water quality downstream. This result indicates that seasonal water quality management should be considered for the open channels receiving johkasou effluent.

Concentrations of microbial indicators related to VB, HPC, TC, *E. coli*, and DNA-total bacteria were evaluated to know their levels in the open channels after receiving johkasou effluent. The concentrations of the microbial indicators in water were not significantly different among sampling sites in the open channels and those concentrations were generally two orders magnitude of lower compared to their concentrations in the johkasou effluent. Significant differences of microbial indicators in seasons were only found for *E. coli* concentrations both in downstream channel and johkasou effluent. These results indicate that the johkasou effluent may contribute the fecal contamination for local downstream channel especially during winter. This is also suggests the improvement of johkasou performance on disinfection process to enhance the removal capacity of the microbes.

The contents of HPC, TC, and *E. coli* in sediments were relatively high in the open channels compared to those in some urban rivers. High contents of *E. coli* in downstream sediment were found during the period of low flow rate in the open channel

(autumn and winter), indicating the disposition or sedimentation of the microbes on sediment bed especially from the johkasou effluents. Whereas, during the period of high flow (spring and summer), the low contents of microbial indicators were observed in downstream sediment, indicating flushing or re-suspending of the microbes associated sediment particles. These results suggest that the flow condition in the open channels receiving johkasou effluents can also vary the microbial contents in sediment. Positive relationships between the content of *E. coli* and organic content in the sediment were found. The *E. coli* content were also relatively high in the smaller size fraction of the sediment particulates, suggesting that *E. coli* can be attached with fine organic particulates in the sediment.

Statistical multivariate analyses were conducted to extract valuable information from data set of water and sediment quality, and to classify the indices into groups of similar quality. Spatial variations of water and sediment quality in the open channels were grouped within three clusters, indicating that the sites receiving johkasou effluent are different in water quality from the other sites. Principal component analysis (PCA) enables to group the indices in water and sediment quality into several factors that can reflect the local water environment after receiving johkasou effluent. The loadings of principal components of water quality indicate that the water quality in the channel can be mainly affected by the flow rate in the channel and polluting effect by johkasou effluents. The distribution of factor scores from PCA revealed that significant seasonal differences in water quality of the channel. The loadings of principal components of sediment quality indicate that the sediment quality in the channel can be mainly affected by the contents of microorganisms and the amount of sediment.

# Table of Contents

<b>Abstract</b>	i
<b>List of tables</b>	viii
<b>List of figures</b>	ix
<b>List of appendices</b>	xii
<b>Acknowledgments</b>	xiii
 <b>Chapter 1</b>	
<b>Introduction</b>	1
1.1. Background	1
1.2. Research goal and objectives	3
1.3. Synopsys of study	4
1.4. Structure of the dissertation	5
 <b>Chapter 2</b>	
<b>Literature review</b>	6
2.1 Onsite wastewater treatment system	6
2.2 Johkasou as an alternative onsite wastewater treatment system in Japan	8
2.3 Reviews on onsite wastewater treatment systems	11
2.3.1 Development on performance of onsite domestic systems	11
2.3.2 Environmental issues in areas of onsite wastewater treatment facilities	13

## **Chapter 3**

<b>Methodology</b>	18
3.1 Study site	18
3.2 Sample collection	18
3.3 Analytical method	23
3.4 Statistical analysis	26

## **Chapter 4**

<b>Evaluation of physicochemical parameters in open channels receiving johkasou effluent</b>	28
4.1. Background	28
4.2. Spatial variation of physicochemical parameters	29
4.3. Temporal variation of physicochemical parameters in the open channels	33
4.4. Temporal variation of physicochemical parameters in the johkasou effluent	37
4.5. Summary	38

## **CHAPTER 5**

<b>Evaluation of microbial indicators in the open channels receiving johkasou effluent</b>	39
5.1. Background	39
5.2. Spatial variation in microbial indicators	42
5.3. Temporal variation of microbial indicators in the open channels	46

5.4.	Temporal variation of microbial indicators in the johkasou drainage channel	47
5.5.	Relation between microbial indicators in water and sediments.	52
5.6.	Summary	53

## **Chapter 6**

	<b>A statistical approach for evaluation of local environmental quality receiving johkasou effluent</b>	<b>55</b>
6.1	Introduction	55
6.2	Statistical procedures	56
6.2.1	Selection water quality data	56
6.2.2	Data treatment and multivariate statistical methods	56
6.2.3	Principal component analysis/factor analysis (PCA/FA)	57
6.2.4	Cluster Analysis (CA)	57
6.2.5	Correlation coefficient and analysis of variance	58
6.3	Results and discussion	58
6.3.1	Classification of sampling sites	58
6.3.2	Water quality evaluation in open channel using PCA	60
6.3.3	Evaluation of sediment quality in open channel using PCA	62
6.4	Summary	64

## **Chapter 7**

	<b>Distribution and survival of microbial indicators in the sediment open channels receiving johkasou effluent</b>	<b>65</b>
7.1	Background	65

7.2	Material and methods	67
7.2.1	Site description	67
7.2.2	Sediment characterization	67
7.2.3	Microbial enumeration	69
7.3	Results and discussion	69
7.3.1	Characteristic of sediment particles.	69
7.3.2	Distribution of microbial indicator in sediments	74
7.3.3	The source of fecal indicators in sediment of decentralized area	75
7.3.4	Relation of microbial indicator between water and sediment	76
7.3.5	Relations between microbial indicators and sediment contents	79
7.4	Summary	79
 <b>Chapter 8</b>		
	<b>Conclusions</b>	80
	<b>References</b>	83
	<b>Appendix A</b>	91
	<b>Appendix B</b>	94
	<b>Appendix C</b>	97
	<b>Appendix D</b>	102



# List of tables

Table 2. 1	Table hazards and contributing factors related to onsite wastewater treatments systems (Carroll <i>et al.</i> , 2006).	14
Table 3. 1	Parameters indices, units, and analytical methods	24
Table 4. 1	Description results of physico-chemical analyses in the water at six sampling points during November 2010 – January 2013.	30
Table 4. 2	Summary results of one-way ANOVA for all sampling sites and seasons	34
Table 5.1	Summaries of microbial indicators analysis in the water and sediments at six sampling points during study periods.	41
Table 5. 2	Summary results of one-way ANOVA for microbial indicators in sampling sites and seasons	52
Table 5. 3	Spearman rank correlation coefficients between microorganisms in the water and sediment.	53
Table 7. 1	Classification of sediment particles in sediments using wet sieve method	70

# List of figures

<b>Fig. 2. 1</b>	Conventional septic tank system	7
<b>Fig. 3. 1</b>	Sampling site map in a residential area using johkasou facilities in Gifu prefecture, Japan. Notes: black dots represent the open channel sampling sites (SP.1, 2, 3, 5, and 6) and a yellow square indicates the johkasou effluent collected from the johkasou drainage channel (SP.JO).	19
<b>Fig. 3. 2</b>	SP. 1 of this study area	20
<b>Fig. 3. 3</b>	SP. 2 of this study area	20
<b>Fig. 3. 4</b>	SP. 3 of this study area	21
<b>Fig. 3. 5</b>	SP. JO of this study area	21
<b>Fig. 3. 6</b>	SP. 5 of this study area	22
<b>Fig. 3. 7</b>	SP. 6 of this study area	22
<b>Fig. 4. 1</b>	Seasonal variation in physico-chemical parameters for (a) flow rate, (b) WT, (c) BOD, and (d) COD, along the open channel and in the johkasou drainage channel. Data in the graphs are means and standard deviations pooled from the study periods. The bars without standard deviation showed raw result.	35
<b>Fig. 5. 1</b>	Spatial variation of microbial indicators in water for a) VB, b) HPC, c) DNA, d) TC and e) <i>E. coli</i> at six sampling points during study periods.	43
<b>Fig. 5. 2</b>	Spatial variation of microbial indicators in sediment for a) VB, b) HPC, c) DNA-based total bacteria, TC, and <i>E. coli</i> at six sampling points during study periods.	45
<b>Fig. 5. 3</b>	Seasonal variation of microbial parameters for a) VB, b) HPC, and c) DNA in the water of the upstream channel, the johkasou drainage, and the downstream channel. Data in the graphs are geometric means and standard deviations pooled from the study	

periods, and n and asterisk marks indicate number of samples and raw results, respectively.	48
<b>Fig. 5. 4</b> Seasonal variation of microbial parameters for a) VB, b) HPC, and e) DNA in the sediment of the upstream channel, the johkasou drainage, and the downstream channel. Data in the graphs are geometric means and standard deviations pooled from the study period, and n and asterisk marks indicate number of samples and raw results, respectively	50
<b>Fig. 6. 1</b> Sites clustering for water (a) and sediment (b) in the open channel receiving johkasou effluent.	59
<b>Fig. 6. 2</b> Factor score plots for water quality in open channels between VF 1 against VF 2(a) and VF (b)	61
<b>Fig. 6. 3</b> Factor score plots of sediment quality in open channels between VF 1 against VF 2 and VF 3.	64
<b>Fig. 7. 1</b> Distribution of sediment sites along open channel receiving johkasou effluents in a residential area.	68
<b>Fig. 7. 2</b> Microbial indicators associated with particle fractions. ND means not detected.	71
<b>Fig. 7. 3</b> Distribution of microbial indicators related to a) HPC, TC, and E. coli in sediments of the open channels and johkasou drainage channel for samples collection in January 2013 (winter) and June 2014 (spring).	72
<b>Fig. 7. 5</b> Flow rate level (a) and sediment contents (solid sediments (b) and organic content (c)) distribution along the open channels and johkasou drainage channel for samples collection in January 2013 (winter) and June 2014 (spring).	73

<b>Fig. 7.5</b> Microbial contents at channels of johkasou (J1-J4), river, and paddy field for one time sampling on December 9, 2014.	76
<b>Fig. 7. 6</b> Relationships of HPC, TC, and E. coli between water and sediment.	77
<b>Fig. 7. 8</b> Correlations of fecal indicators with solid contents in upstream sediment that received inputs from agricultural and tandoku johkasou (St. 1 ~ St. 3).	78
<b>Fig. 7. 1</b> Correlations of fecal indicators with solid contents in downstream sediment that mostly received johkasou effluent (St. 4 ~ St. 7).	78

## List of appendices

<b>Appendix A</b>	: Spatial variation of physico-chemical parameters at six sampling points during study periods.	78
<b>Appendix B</b>	: Temporal variation of physico-chemical parameters in the open channels receiving johkasou effluent during study period.	80
<b>Appendix C</b>	: Values of significant multiple comparisons in sampling sites for physico-chemical and microbial concentrations in the johkasou effluent calculated by one-way ANOVA with Tukey's post hoc analysis.	83
<b>Appendix D</b>	: Values of significant multiple comparison in seasons for physico-chemical and microbial concentrations in both the open channels and the johkasou drainage channels calculated by one-way ANOVA with Tukey's post hoc analysis.	88

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# Chapter 1

## Introduction

### 1.1. Background

Decentralized systems as an adequate treatment of household wastewater have the importance to ensure the protection of water quality and to reduce requirements for treatment of potable water prior the product discharged into water environment. The onsite domestic wastewater treatment systems are an alternative facility in decentralized areas to treat household wastewater that is necessary to minimize the pollution into the local water environment.

Since decades ago, onsite domestic wastewater systems, called johkasou, have been widely applied in Japan. It is being famous to be employed in rural areas and the areas that were not covered by centralized wastewater treatment plant. The increasing numbers of population in rural areas are linearly to the use of onsite domestic wastewater systems. According to the Ministry of The Environment Japan, a total number of johkasou facilities in FY 2012 (end of March 2013) were 7.76 million (MILT, 2013). A former type of johkasou that treats only black water called tandoku johkasou made up the greater proportion of this installation at 58% (4.53 million), while the remaining 42% (3.23 million) was the combined type that treats both black and grey water, known as gappei johkasou.

The treated water from onsite domestic systems including johkasou facilities is generally discharged into the water environment through the open channels, ditches or drain built within residential areas. So, that treated water can deteriorate the water quality and changes the sediment contents in stream channels since it contains many pollutants such as organic substances, chemical, nutrient and microorganisms



(Savichtcheva *et al.*, 2007; Wihters *et al.*, 2011) and especially for grey water, that it contains untreated domestic wastewater except toilet (Eriksson *et al.*, 2002).

Onsite domestic wastewater systems including johkasou have been an issue of water pollution in the water environment of decentralized areas. Inappropriate treatment and less performance in efficiency of onsite domestic wastewater systems can impair the receiving natural waters, such as rivers, lakes and estuaries. For instance, tandoku johkasou was banned by Japanese Government in 2001 because it was reported as a major pollution source by disposing untreated grey water into the natural stream water (Gaulke, 2006). Moreover, the large installation number of tandoku johkasou than the gappei johkasou nowadays may continuously contribute the sources of domestic pollution in many receiving water bodies.

Furthermore, analysis of water quality alone may underestimate the function of sediment as a reservoir of contaminations into the water environment. Sediment constitute is an important phase to track and find the evident of environmental contamination because sediment acts as a bank that can receive, pretend, and keep the contaminations such as organic matter, nutrient contents and microbial indicators in longer time. A previous study documented at high density of bacteria in the sediments of johkasou drainage channel (Helard *et al.*, 2012). The number of fecal coliforms in sediment may contain 100 – 1000 times greater than in the overlying water (Bai & Lung, 2005). The survival of microorganism particularly fecal indicators is longer in the sediments than in water because sediments contain organic substances and optimal nutrient conditions for microbes to multiply (Garzio-Hadzick *et al.*, 2010), and shielding from exposure to UV sunlight (Koirala *et al.*, 2008).

The importance of sediment as a reservoir of fecal indicators has been documented during high flow events in many studies. The microbes associated sediment particles have an important role for transporting and resuspension of microbial indicators through the settleable particles (Jamieson *et al.*, 2004; Characklis *et al.*, 2005). Thus, high level of fecal indicators in sediment can contaminate the downstream water network by disposition with settleable particles during growing seasons such as heavy rainfall and

storm runoff. The high concentrations of fecal indicator in flocculated suspended sediment and bed sediment conclude the understanding of interaction between fecal indicators and sediment must be improved so that risk to public health can be properly evaluated.

Besides, seasonal variation can also influence the concentrations of physicochemical and microbial indicators in both of the onsite domestic systems and receiving natural stream water. Most of the quality of physicochemical parameters in surface water showed moderate variations in their concentration of all seasons. 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.

Therefore, the studies on monitoring of water and sediments quality and including effect of seasons in the open channels receiving johkasou effluents were conducted in decentralized area in Mizuho-shi, Gifu Prefecture, Japan. Most all of houses in this study area use johkasou system for treating household wastewater and the effluent is flowing through the small open channel surrounding the area before entering the receiving water bodies. The sampling sites were selected as representative of a typical residential area where the water environment quality could be closely related to the household activities.

## **1.2. Research goal and objectives**

The goal of this study was to reveal the impact of johkasou effluent in both water and sediments of open channel in a residential area. In order to reach the goal this study, several objectives were set as follows;

- To evaluate contribution of johkasou effluent on varying the quality of physicochemical and microbial parameters in the water and sediments of open channels.

- To identify the influenced of seasonal variation in concentrations physicochemical and microbial parameters in both the water and sediments of open channels receiving johkasou effluent.
- Evaluation of water and sediment quality data sets using statistical multivariate analyses to obtain valuable information and to classify the parameters into similar water quality.
- To evaluate the distribution of fecal indicators in sediment of open channels in the local water environment receiving johkasou effluents.

### 1.3. Synopsys of study

Evaluation of water quality along with sediment content was conducted in the johkasou drainage and in the open channels receiving johkasou effluent during 3-year study periods. Here, the physical parameters related to flow rate, water temperature (WT), pH, dissolved oxygen (DO), suspended solid (SS) and electrical conductivity (EC); chemical parameters related to dissolved organic carbon (DOC), biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorous (TP); dissolved nitrogen forms related to ammonia nitrogen ( $\text{NH}_4\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ), and phosphate phosphorus ( $\text{PO}_4\text{-P}$ ); total chlorine and microbial quality related to viable bacteria (VB), heterotrophic plate count bacteria (HPC), total coliform (TC), *Escherichia coli* (*E. coli*), and DNA-based total bacteria (DNA-total bacteria) were comprehensively examined in both water and sediments of johkasou drainage channel and open channels. The measured results of these parameters were then used to evaluate their significant variation among sampling sites and seasons by applying statistical analysis using One-way analysis of variance (ANOVA) together with Tukey's post hoc analysis. Furthermore, the distribution and survival of microbial indicators in sediments open channels were also evaluated. Principal component analysis was applied for evaluation and interpretation of a large water quality data set in a decentralized area of johkasou facilities.

#### **1.4. Structure of the dissertation**

In this dissertation, several chapters were designed with different discussions in each of chapter which is follows;

- Chapter 1. This chapter presents the overall structure and aim of this study. A synopsis of the study and the structure of the dissertation were also provided.
- Chapter 2. This chapter provides a literature review onsite domestic wastewater treatment systems, structure of johkasou and current situation of receiving water in decentralized areas.
- Chapter 3. This chapter provides a detail methodology use for samples collection and analyses in physicochemical and microbial indicators measured both in the water and sediments. Here also explained the seasonality data decision.
- Chapter 4. This chapter discusses the variations of physicochemical concentrations in spatial and temporal for both the johkasou drainage channel and the open channels.
- Chapter 5. This chapter discusses the variations of microbial concentrations in spatial and temporal for both the johkasou drainage channel and the open channels.
- Chapter 6. This chapter discuss the statistical multivariate analysis approach to evaluate the local environmental quality in open channel receiving johkasou effluent.
- Chapter 7. This chapter focuses on the distribution and survival of microbial indicators in sediments of stream channel receiving effluents of decentralized treatment systems.
- Chapter 8. This chapter presents the conclusion of the study

## Chapter 2

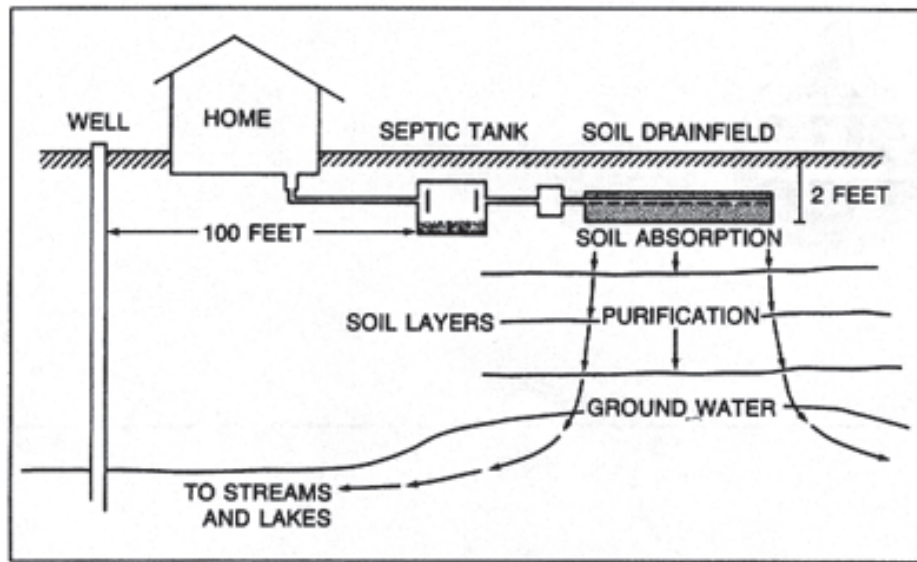
### Literature review

#### 2.1 Onsite wastewater treatment system

Domestic wastewater treatment systems in rural areas are essential to prevent the pollution of aquatic environment, which has been of increasing concern for both researchers and governments (Ichinari *et al.*, 2008). Households in rural areas that do not equipped by public sewers must depend on onsite treatment systems to treatment their wastewater. Onsite system can be designed, located, operated, and maintained to meet required effluent standards and promotes better watershed management by avoiding the potentially large transfer of water from one watershed to another watershed by using centralized system. The U.S. EPA states that adequately managed decentralized wastewater systems are a cost-effective and long-term option for meeting public health and water quality goals, particularly in less densely populated areas (EPA, 1997).

Household wastewater consists of two types, black water which is the water from the toilet containing most of the solid wastes, and grey water which is the water from the kitchen, shower, bath and laundry. Black and grey water not only contain high levels of bacteria and other micro-organisms but they also contain nutrients such as nitrogen and phosphorus, which in excess concentration can harm the environment as well as other things such as sodium (from salt). Therefore, effective removal of contaminants during onsite treatment can be critical to protecting ecosystem and human health. Onsite wastewater treatment systems differ with the conventional septic tank systems (STS). STS applied a soil absorption field known as a subsurface wastewater infiltration system which serves three purposes: sedimentation of solids in the wastewater, storage of solids, and anaerobic breakdown of organic materials. This system consists of three main parts: the septic tank, the drainfield and the soil beneath the drainfield (Fig. 2.1).

Conventional systems work well if they are installed in areas with appropriate soils and hydraulic capacities; designed to treat the incoming waste load to meet public health, ground water, and surface water performance standards; installed properly; and maintained to ensure long-term performance.



**Fig. 2. 1** Conventional septic tank system (Carroll *et al.*, 2006)

Improper maintenance by the homeowner may result conventional STS failure such not pump out on a regular basis, sludge builds up inside the septic tank and clogged absorption fields and decreased oxygen supply within biomat. Thus, the conventional STS might not be adequate for minimizing nitrate contamination of ground water, removing phosphorus compounds and attenuating pathogenic organisms (e.g., bacteria, viruses). Recent catchment-based studies indicated that the nitrates and phosphorus discharged into surface waters or through subsurface flows can spur algal growth and lead to eutrophication and low dissolved oxygen in lakes, rivers, and coastal areas (Withers *et al.*, 2011; May *et al.*, 2010). In addition, pathogens reaching ground water or surface waters can cause human disease through direct consumption, recreational contact, or ingestion of contaminated shellfish.

Newer or “alternative” onsite treatment technologies are more complex than conventional systems and incorporate pumps, recirculation piping, aeration, and other features (e.g., hydraulic flows; retain oils, grease, and settled solids; and provide some minimal anaerobic digestion of settleable organic matter). Current alternative onsite treatment systems widely vary in sophistication from simpler filter systems, to constructed wetlands, multi-stage biological treatment systems, and membrane bioreactors (Revitt *et al.*, 2011; Winward *et al.*, 2008). Nevertheless, all systems are based on a combination of chemical, physical and biological processes such as adsorption, coagulation, precipitation, filtration, aeration, biodegradation, and disinfection. Alternative onsite treatment systems require specialized design, ongoing or periodic monitoring and maintenance, and enhanced management oversight. Therefore, the performance of these systems results under this approach can vary significantly, with operation and maintenance functions driven mostly by complaints or failures.

## **2.2 Johkasou as an alternative onsite wastewater treatment system in Japan**

In Japan, an onsite domestic wastewater treatment system, known as johkasou has been applied in rural areas and in other areas that is not covered by wastewater treatment plants (Yang *et al.*, 2010). Johkasou literally means purification tank in Japanese language. Johkasou system became an effective means as an onsite wastewater treatment unit and an important role to protect the local water environment in decentralized areas. This system has remarkable advantages, such as a) high treatment performance with low initial investment cost; b) a short period of time for installation; and c) less topographic influence. The johkasou has well management, maintenance and periodically legal inspection to kept effluent quality under the standard, as biological oxygen demand less than 20 mg/L, before discharge to the local water environment. (Nakajima *et al.*, 1999).

The johkasou facility uses wastewater treatment plant systems to treat the household wastewater, which the capacity is  $1.2 \text{ m}^3 \text{ day}^{-1}$  and the main purposes are to decrease organic matters and biochemical oxygen demand (BOD). A johkasou system consists of a primary unit; anaerobic and aerobic biological treatment unit, a sedimentation tank, and a disinfection chamber using chlorine tablets (Ichinari *et al.*, 2008). Johkasou is

majorly divided into two types; former type called tandoku johkasou that only treat black water and untreated grey water being discharge to the stream water, and gappei johkasou is an improvement over tandoku johkasou which treats both black water and grey water.

According to the Ministry of Environment Japan, the total number of johkasou facilities in FY 2012 (end of March 2013) was 7.76 million (MILT, 2013). Tandoku johkasou, made up the greater proportion of this installation at 58% (4.53 million), while the remaining 42% (3.23 million) was gappei johkasou. In order to meet Japan's regulation standards, tandoku johkasou was banned for new installation by the Japanese government in 2001 (Gaulke, 2006). Therefore, this tandoku johkasou system needs an improvement or the conversion to the advanced type in order to protect the local receiving water bodies since the untreated grey water of this effluent may lead to eutrophication occurrence.

There are still many difficulties to control johkasou facilities since their operation and maintenance are depend on the owners usage (Gaulke, 2006). Therefore, in recent years, many studies have been developed the technologies to increase the performance and effluent quality. Several types of johkasou system that have been developed are explained as follows;

1. Tandoku johkasou. This is the first type of johkasou intended for the treatment of only black water while grey water is discharged directly into the environment. The removal efficiency of tandoku johkasou are 65% BOD from black water, which together with untreated grey water resulted in an effluent value of 31.5 g BOD per capita per day (Watanabe *et al.*, 1993). Around 30–50 million people in Japan are currently still using tandoku johkasou (Magara, 2003; Yang *et al.*, 2001). In order to meet Japan's regulation standards, tandoku johkasou was banned for new installation by Japanese Government in 2001 (JECES).
2. Gappei johkasou. This is an improvement of tandoku johkasou that treats all wastewater from the house both black and grey water (gappei means combined or merged). There are also several types of gappei johkasou:



- a. Anaerobic filter – contact aeration process. This treatment process is most widely used in small-scale gappei johkasou systems. The required effluent BOD concentrations of this process are less than 20 mg/l. The johkasou consists of an anaerobic filter tank, a contact aeration tank, a sedimentation tank and a disinfection tank. In the anaerobic filter tank and the contact aeration tank, filter media or contact media are filled.
- b. Denitrification type anaerobic filter–contact aeration process. This treatment process is designed for both BOD and nitrogen removal with effluent BOD and TN concentrations less than 20 mg/l. To ensure a smooth nitrification, the volume of the contact aeration tank is bigger than standard type and the aeration intensity is higher than that in the anaerobic filter–contact aeration process, respectively. The denitrification is realized by recirculating aerobically treated wastewater from the contact aeration tank to the anaerobic filter tank.
- c. Membrane johkasou. This newly developed membrane in johkasou is based on the application of the intermittent activated sludge process and using plate and frame membrane (PFM) modules. Membranes have been used to upgrade tandoku johkasou to gappei johkasou for on-site wastewater treatment including reclamation-quality effluent and the treatment of night soil. The membrane johkasou consists of a sedimentation/separation tank, an intermittent aeration tank with plate and frame membrane (PFM) modules and a disinfection tank. Treated wastewater was sucked from the system using a siphon system (Yang *et al.*, 2001). Membranes can be submerged in the activated sludge chamber to separate the liquid stream from solids (Ohmori *et al.*, 2000). Ohmori *et al.* (2000) and Yang *et al.* (2001) found that membrane *johkasou* performed well (TN: 8 mg/l, BOD: 2.3 mg/l, SS<5 mg/l and total coliform <100 cells/ml), but needed maintenance every three months, sludge withdrawal and membrane cleaning with sodium hypochlorite every six months to prevent fouling of the membrane.

## 2.3 Reviews on onsite wastewater treatment systems

### 2.3.1 Development on performance of onsite domestic systems

Numerous studies have been reported the development and an adequate performance of onsite domestic systems to reduce the environmental contamination within these systems. Generally, the performance of onsite wastewater systems focuses on the removal of organic and nutrient compound, and other literatures emphasis on fecal indicators and pathogenic bacteria removal. The reviews of performance in onsite wastewater systems including johkasou systems were provided as follows.

Inchinari *et al* has developed the onsite domestic systems, which combining an aerobic sludge digester (ASD) within wastewater treatment process to reduce the sludge production and the total volume number of the systems. As results, this developed system could reduce 30 % of the sludge production compared to the johkasou. In addition, the hydraulic retention time (HRT) in the unit of an aerobic fluidized bed in this system was shorter than that of the johkasou. Although, the averages of BOD and SS in the final effluent of the developed system were slightly higher than those forms of johkasou. However, both systems were observed did not significantly affect wastewater treatment performance during low water temperature, which indicates low effluent quality discharged during low-cold season.

Study on removal of viruses using onsite domestic wastewater treatment system named johkasou was reported by Kaneko (1997). A small pilot model was investigated under standard BOD loading of 0.076 BOD Kg/m<sup>3</sup>/day. Around 97% of *E. coli* phage T2, 98% of poliovirus 1 and 93% of coxsackievirus B3 were removed from inlet wastewater by the system. About 80% of the viruses in the influent were removed in the first and second anaerobic zones under the standard conditions. When the loading was increased to double the standard loading (0.152 Kg/m<sup>3</sup>/day) the removal rate decreases to 64%. It was found that the higher the BOD loading rates showed the low the concentration of the variables.

Another study from the same research group above reported the behavior of pathogenic *E. coli* 0157 and *Salmonella enteritidis* in small domestic sewage treatment system

(johkasou) (Kaneko et al., 2001). The performance rate of johkasou was significantly different in the water temperature on removing the pathogenic bacteria. The maximum removal rate up to 4 log was obtained at temperature of 20 °C and 30 °C. The minimum removal rate of pathogenic bacteria around 1 log was observed at 10 °C or low. This study indicated that the cold water temperature could influence the reduction rate of pathogenic removal. The results suggest that the disinfection process in the johkasou should satisfactorily be operated to keep receiving waters safe and external factor of temperature would vary the level of some pathogen in the effluents particularly during winter season.

Development of johkasou systems using adsorption and desorption process for recovery and recycling oriented on phosphorus removal was studied by Ebie *et al.* (2008). Adsorbent particles made of zirconium were set in a column, and adsorption was installed as subsequent stage of BOD and nitrogen removal type in johkasou. The effluent quality from adsorption column in a number of experimental sites was monitored. The effluent phosphorus concentration was kept below 1 mg/L during 90 days at all the sites. Furthermore, over 80% of the sites achieved 1 mg/L of TP during 200 days. This adsorbent was durable, and deterioration of the particles was not observed over a long duration. The adsorbent collected from each site was immersed in alkali solution to desorb phosphorus. Then the adsorbent was reactivated by soaking in acid solution. The reactivated adsorbent in this system could be reused for several times and it showed the same adsorption capacity as a new one. Meanwhile, the desorbed phosphorus was recovered with high purity as trisodium phosphate by crystallization. It is proposed as a new decentralized system for recycling phosphorus that paves the way to high-purity recovery of finite phosphorus.

In order to verify the treatment performance of newly developed johkasou facilities with membrane separation, Ohmori *et al.* (2000) used three different johkasou types for experimental study. It was found that each of johkasou facilities has a high treatment performance for removing BOD, nitrogen. These types could be operated steadily by monitoring the function and maintaining the devices at every three months and by withdrawing accumulated sludge at every six months. It was also found that periodical cleaning of the membrane by sodium hypochlorite solution and neutralizing cleaning

wastewater by sodium thiosulfate solution at every six months is important to maintain a steady permeability of the membranes. No adverse effects on treatment performance were observed by leached sodium hypochlorite solution membrane cleaning.

The performances of onsite domestic facilities in different capacity of johkasou such as small-household, restaurant and hotels were surveyed by Nakajima *et al.* (1999). In small scale of gappei johkasou, the performance of BOD removal was satisfactory with concentration below 20 mg/L. The nitrification took place in the aerobic filter tanks could reduce the BOD, N-BOD, TN, NO<sub>3</sub>-N and DKN in with recycle liquor operation by using organic substances in the influent. On the other hand, the BOD removal in facilities treating wastewater from restaurants and hotels was largely influenced by the influent of n-Hex (oil) concentration. It was likely to have negative effect on failing the performance if the influent n-Hex was over 30 mg/L. In addition, the performance of TN removal seems low in the facilities of which influent BOD was extremely low, like resort condominiums, even though they were operated in intermittent aeration mode.

The removal characteristics of coliform bacteria from certified structure type small-scale johkasou was studied by Takahasi *et al.* (2012). The effluent quality from 25 johkasou units was investigated. 24 out of 25 units could meet the effluent quality standard of coliform bacteria count that should be less than 3,000 cfu/mL before chlorination process. All units could remove coliform bacteria count less than 1,000 cfu/mL after chlorination. However, about 200 cfu/mL of coliform bacteria count was detected, in spite of residual chlorine in effluent over 2 mg/L could be detected. Coliform bacteria counted in effluent before chlorination was negatively correlated with nitrifying ratio and positively correlated with SS. It was considered that highly removal of coliform bacteria was possible by advanced johkasou which could remove nitrogen and SS.

### **2.3.2 Environmental issues in areas of onsite wastewater treatment facilities**

The impact associated with onsite wastewater treatment systems had become to the fore in recent years in order to protect public health and environment from the consequences of poorly performing systems. A report to the U.S congress in 1997 note that failing

onsite wastewater treatment systems, mostly septic tank-soil absorption systems, were the second leading cause of contamination of natural water sources in the country (US EPA, 1997). Many research literatures have described the consequences of failing onsite treatment systems in natural water sources.

Failure of many onsite domestic systems is generally not due to inappropriate siting and design issues or their operation management (Otis and Anderson.,1994). The overall definition of failure relates to several key scenarios that result in hazards as list in Table 2.1. The various hazards associated with the onsite wastewater treatment systems and the environmental and public health risk. The subsequent phase to know should be performed to know the degree of contaminations in actual condition.

**Table 2. 1** Table hazards and contributing factors related to onsite wastewater treatments systems (Carroll *et al.*, 2006).

Item	Key Hazard	Contributing Factors
OWTS (treatment system and disposal area)	Release of contaminants due to failure of onsite wastewater treatment system	<ol style="list-style-type: none"> <li>1. Soil</li> <li>2. Planning (lot size)</li> <li>3. Environmental sensitivity</li> <li>4. Flooding</li> <li>5. Topography</li> <li>6. Loading rates</li> <li>7. Operation and maintenance practices</li> </ol>
Surrounding soil	Inability to renovate effluent and prevent contaminations from reaching groundwater and/or surface water	<ol style="list-style-type: none"> <li>1. Soil type</li> <li>2. Depth of soil horizons</li> <li>3. Physical characteristics</li> <li>4. Chemical characteristics</li> <li>5. Water table depth</li> </ol>
Public health	Contamination of water/surrounding environment such that a considerable health risk is evident due to the release of contaminations (namely pathogen, which have an impact on human health	<ol style="list-style-type: none"> <li>1. Surface exposure</li> <li>2. Water supply (ground water)</li> <li>3. Aerosol</li> <li>4. Pests (mosquitoes, etc)</li> </ol>
Environmental	Release of contaminants into the receiving environment (ground/surface water) causing environmental degradation (such as eutrophication and causing the environment to be unsuitable	<ol style="list-style-type: none"> <li>1. Surface runoff</li> <li>2. Groundwater discharge</li> <li>3. Flooding</li> <li>4. Water table</li> </ol>

Yates (1985) reported that the approximately 50% of waterborne disease outbreak in the United States were as result of consumption of contaminated groundwater by septic tanks the most frequent cause of contamination. Another case in Australia relating to public health attributed to failing onsite systems was that of a viral Hepatitis A outbreak at Willis Lakes in the State of New South Wales (NSW) (Ryan, 1999). Four hundred and forty-four local residents fell ill with Hepatitis A after consuming shellfish from the lake which was contaminated by sewage effluent. Poorly maintained and failing septic tank-soil absorption systems within the lake's vicinity were found to contribute the contamination. Underground contaminated by failing onsite systems was worse in pathogenic and viral bacteria than any chemical content because the pathogens are capable to remaining viable in groundwater longer than previously surface water and viruses have survival rates in groundwater that minimum rate are equal to bacteria (Harris., 1995).

Disposal of onsite wastewater treatment systems contributes to disperse contamination in receiving environment natural sources. Eutrophication in rivers is most prevalent under low-flow conditions when the number of resident times is greatest. The high concentrations of nutrient are a major point leading the eutrophication in receiving natural waters (Jarvie *et al.*, 2006; Neal *et al.*, 2010). Withers *et al.* (2011) documented that the septic tank systems exhibited high values of nutrient contamination up to 10 orders magnitude greater in upstream section of rural habitation than in stream site. The largest nutrient concentrations were recorded under low flow and the stream discharge was the most important factor determining the eutrophication impact from septic tank systems.

The residential area of onsite treatment systems would potentially be the primary source of the fecal contamination (Jamieson *et al.*, 2003). The loading of fecal contaminants from onsite domestic system would represent a steady state of source pollution that would less influence by hydrological event. Fecal indicator bacteria (FIB) including total fecal coliform and enterococci have been used in many countries as monitoring tool for microbiological impairment in water and for prediction of presence bacterial, viral and protozoan pathogens. A study by Habteselassie *et al.* (2011) reported that onsite wastewater treatment systems are most likely to contribute fecal contamination to

the surrounding water bodies under wet condition when the ground water tables rises, decreasing the height of saturated zone between the leach lines and water table where pathogen treatment can occur. Increasing numbers of fecal coliforms and *E. coli* were also detected in surface water samples that affected by onsite wastewater treatment systems after a high rainfall event (Carrol *et al.*, 2005; Whitlock *et al.*, 2002; Ackerman and Weisbreg, 2003).

In Japan, contribution of onsite wastewater treatment systems including johkasou in stream natural waters has also been reported by many studies. The pollutant loads discharged from the johkasou systems over 500 effluents samples collected in Gunma prefecture were noted by Tanaka *et al.* (2007). In the basis of the pollutant load factor, tandoku johkasou showed the highest level compared to gappei johkasou. This suggests changing the tandoku johkasou to recommended type for preventing the eutrophication of a lake in the unsewered area.

Evaluation of a regional domestic wastewater treatment system using johkasou facilities in Osaka was studied by Okumura *et al* (2009). Most facilities were appropriately operated: 79.7% of them showed the effluent of BOD less than 20 mg/L, 72.7% of them TN less than 20 mg/L. For several facilities, careful adjustment of the operational conditions was required. The discharged loading of BOD and TN were effectively reduced: each reduction ratio was 59% and 3.3%, respectively. On the other hand, the discharged loading of TP increased at 6.8%. From these results, only a little effect on water quality of downstream river was evaluated and the significant effects were not observed by the monitoring of the water quality downstream.

Helard *et al.* (2012) studied the formation and correlation of sediment bed bacterial density in an open channel receiving johkasou effluent to obtain information that can be used as reference for improving the environment inside and surrounding the open channels receiving *johkasou* effluent. The PCA/FA results showed that 3 dominant factors were responsible for water quality data structure. Hierarchical cluster analysis grouped 6 study sites into 3 statistically significant clusters, reflecting different characteristics and pollution levels of the sites. Correlation analysis revealed significant

relationships of the sediment bed bacterial density with BOD, total nitrogen and total phosphorus in the water of the channel receiving *johkasou* effluent.

The occurrence of fecal indicators in the channels of *johkasou* systems have been noted by Setiyawan *et al.* (2009). Fecal indicators were always be detected at high levels both in water and sediment in the channels of three *johkasou* systems, not only total coliforms and *E.coli* but also F-RNA bacteriophages and GIII F-RNA bacteriophages, thus indicates possible fecal contamination in the channels of the *johkasou* systems. The concentrations of fecal indicators were fluctuated in a day mainly due to domestic activities therefore the stable time in the field survey is necessary to provide reliable information of fecal contamination. Significant positive correlation of total coliforms and *E. coli* in water with the total coliforms and *E. coli* in sediment indicated the existence of interaction microbes and sediment particles that potentially important on microbial dynamic in the channels of the *johkasou* systems. However, no significant correlation of F-RNA bacteriophages with total coliforms and *E.coli* indicates a different distribution of mechanism for F-RNA bacteriophages.



## Chapter 3

### Methodology

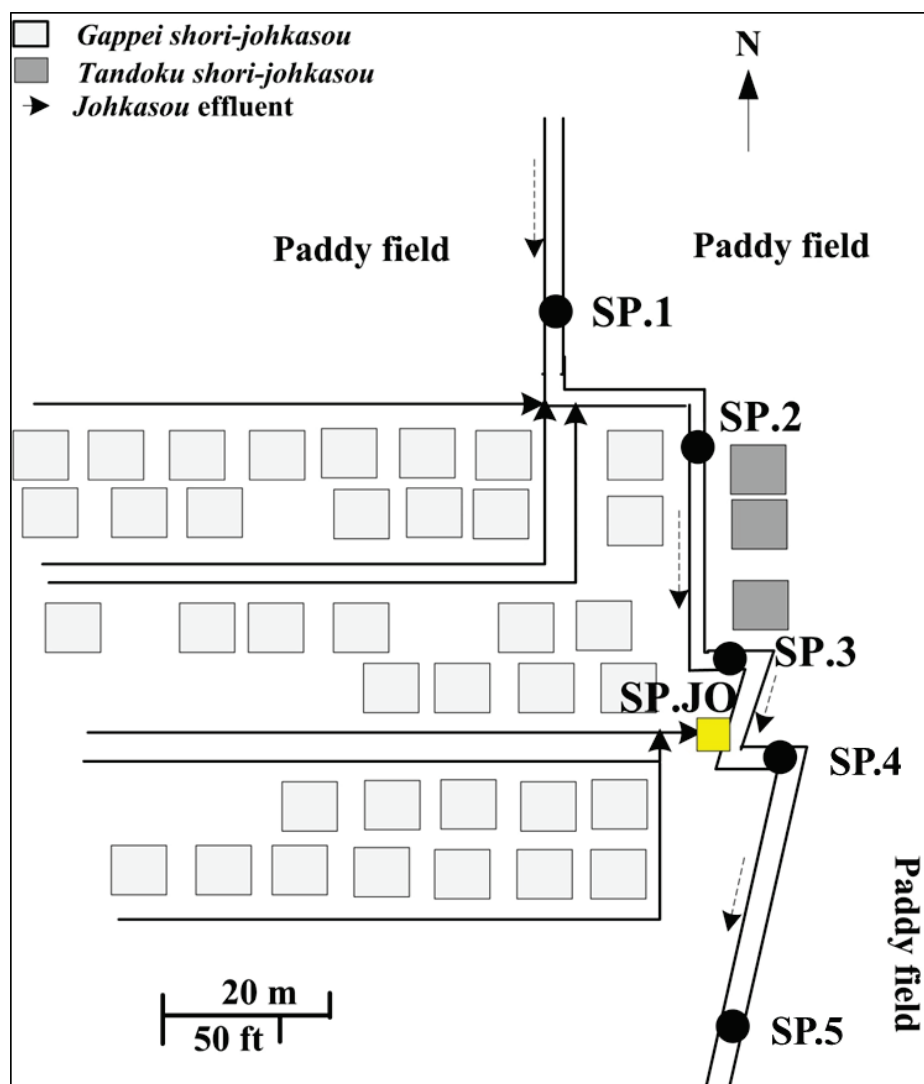
#### 3.1 Study site

The study site is located in Gifu, Japan, near a residential area that has a population of around 250 inhabitants (**Fig. 3.1**). A total of 52 households uses the johkasou facility in this area (39 households [75%] use gappei johkasou and 13 households [25%] use tandoku johkasou). Water samples were collected at six sampling points (SP) along 1-m-wide open channels surrounding the residential area. The open channels consisted of five sites and a small johkasou drainage channel, an outlet of 35-cm width, which was the core sampling site (SP.JO) that received effluent from 16 gappei johkasou facilities. The open channels were divided into upstream and downstream channels of SP.JO, of which three sampling sites were located upstream (SP.1, SP.2, and SP.3) and two sampling sites were located downstream (SP.4 and SP.5). SP.1 was located in the outlet of another open channel, surrounded by a paddy field, which received effluent from 10 tandoku johkasou facilities and two gappei johkasou facilities from another residential area. SP.2, which was located 25 m downstream of SP.1, was located in the channel that connected the SP.1 open channel with another open channel that received ground water mixed with effluent from 23 gappei johkasou facilities. SP.3 was located 30 m after SP.2 and received effluent from three tandoku johkasou facilities. SP.4 was located in downstream of SP.JO. SP.5 was located 30 m after SP.4, which was surrounded by a paddy field.

#### 3.2 Sample collection

Water samples were always collected in new 2 L polypropylene bottles that were confirmed to have no microbial contamination. The samples were then placed in an ice box before being transported to the laboratory. All water samples were placed in the

refrigerator at 5 °C and were immediately analyzed. The water samples were collected on 14 occasions from November 2010 to January 2013. Sampling was conducted on November 17 and December 20, 2010; March 15, August 8, September 15, October 14, October 26, November 16, and December 15, 2011; March 8, May 24, August 28, and November 23, 2012; and January 18, 2013.



**Fig. 3. 1** Sampling site map in a residential area using johkasou facilities in Gifu prefecture, Japan. Notes: black dots represent the open channel sampling sites (SP.1, 2, 3, 4, and 5) and a yellow square indicates the johkasou effluent collected from the johkasou drainage channel (SP.JO).



**Fig. 3. 2** SP. 1 of this study area



**Fig. 3. 3** SP. 2 of this study area



**Fig. 3. 4** SP. 3 of this study area



**Fig. 3. 5** SP. JO of this study area





**Fig. 3. 6** SP. 4 of this study area



**Fig. 3. 7** SP. 4 of this study area

Sediment samples were collected in new 1 L polypropylene bottles that were confirmed to have no microbial contamination. The samples were placed in an ice box before being transported to the laboratory. Sediment samples were collected on 10 occasions from all sampling sites. Sampling was conducted on November 17, 2010; March 15, September 15, October 26, November 16, and December 15, 2011; March 8, May 24, and November 23, 2012; and January 18, 2013. Sediment samples were collected as sediment/water mixed liquor using a tube with an inner diameter of 30 cm for SP.1, 2, 3, 5, and 6, and a small tube with an inner diameter of 10 cm for SP.4. The mixed liquor from each site was then collected by placing the sampling tube on the sediment bed, mixing the sediment with the overlying water, and collecting the sediment mixed with the overlying water in the tube.

### 3.3 Analytical method

The following parameters were analyzed in the water samples; the flow rate, water temperature (WT), dissolved oxygen (DO), dissolved organic carbon (DOC), suspended solids (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), ammonia nitrogen ( $\text{NH}_4\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ), phosphate phosphorous ( $\text{PO}_4\text{-P}$ ), total chlorine, viable bacteria (VB), heterotrophic plate count (HPC) bacteria, total coliform (TC), *E. coli*, and DNA-based total bacteria number (DNA). The following parameters were analyzed in the sediment samples; organic content, HPC, TC, *E. coli*, and DNA. In-situ measurements were conducted for flow rate, WT, DO, and total chlorine. **Table 3. 1** summarizes the methods of analysis for all sediment and water samples.

**Physicochemical analysis.** The flow rate was calculated by multiplying the flow velocity measured using an electro-magnetic velocity meter (AEM1-D, JFE Advantech Co. Ltd, Japan) in addition to water depth and channel width. The WT and DO were measured directly at each site using the corresponding portable meters (DKK-TOA, Japan). Total chlorine was also measured using the corresponding pocket colorimeter (HACH, Germany). Measurements of SS, DOC, BOD, COD, TN, TP, and total chlorine were conducted according to the standard method (APHA, 2005).

**Table 3. 1** Parameters indices, units, and analytical methods

Variables	Abbreviations	Analytical methods	Units
<b><u>Water</u></b>			
Flow rate	Flow rate	Electrical device	L/s
pH	pH	Potentialametry/pH probe	pH units
Water temperature	WT	Potentialametry/temperature probe	°C
Electrical conductivity	EC	Conductometry	mS/m
Dissolved oxygen	DO	Potentialametry/O <sub>2</sub> probe	mg/L
Suspended solids	SS	Drying at 105°C/weighing	mg/L
Dissolved organic carbon	DOC	High temperature combustion	mg/L
Biochemical oxygen demand	BOD	Winkler Azide method	mg/L
Chemical oxygen demand	COD	Permanganate method	mg/L
Total nitrogen	TN	Kjeldhal Method	mg/L
Total phosphorus	TP	Asorbic Acid Method	mg/L
Ammonia-nitrogen	NH <sub>4</sub> -N	Spectrophotometry	mg/L
Nitrite-nitrogen	NO <sub>2</sub> -N	Spectrophotometry	mg/L
Nitrate-nitrogen	NO <sub>3</sub> -N	Spectrophotometry	mg/L
Phosphate-phosphorous	PO <sub>4</sub> -P	Ion cromathography	mg/L
Total chlorine	Total chlorine	Colorimeter	mg/L
Viable bacteria	VB	Plate count method	CFU/ml
Heterotrophic plate count	HPC	Plate count method	CFU/ml
Total coliform	TC	Multiple tube fermentation	MPN/100ml
Escherichia coli	<i>E. coli</i>	Multiple tube fermentation - fluorescence	MPN/100ml
DNA-based bacterial density	DNA-total bacteria	Real-time PCR	Cell/ml
<b><u>Sediment</u></b>			
Total solid	TS	Drying at 105 °C/weighing	g/cm <sup>2</sup>
Volatile solid	VS	Drying at 600 °C/weighing	g/cm <sup>2</sup>
Organic content	Organic content	Votile sediment/total sediment	%
Heterotrophic plate count	HPC	Plate count method	CFU/g-dry weight
Total coliform	TC	Multiple tube fermentation	MPN/g-dry wight
Escherichia coli	<i>E. coli</i>	Multiple tube fermentation - fluorescence	MPN/g-dry wight
DNA-based bacterial density	DNA-total bacteria	Real-time PCR	Cell/g-dry weight

**Microbiological analysis.** Direct measurement of water and sediment samples was conducted based on the standard method (APHA, 2005) for each microbial indicator related to VB, HPC, TC, and *E. coli*.

*Viable bacteria.* VB were analysed based on standard plate count method (9215A) using tryptose glucose yeast extract (TGYE) as the culture medium. The compositions of one litre media for viable bacteria were tryptone/bacto pepton 1 gr, yeast extract 0.5 gr, glucose 0.25 gr, and agar 15 gr. The culture medium was then sterilized by autoclave at 121 °C for 30 min. One millilitre of sample diluted into a 10-fold dilution series water. One millilitre from each dilution water placed on triplicated petridish and added 10 ml of culture medium and then incubated for 20 ± 4 h at 37 °C (APHA, AWWA and WEF,

2005). Plates with countable colonies between 20 and 300 were selected for counting with the unit is colony form unit (CFU).

*Heterotrophic bacteria.* HPC were analysed based on standard plate count method (9215A) using tryptose glucose yeast extract (TGYE) as the culture medium. The compositions of one litre media for heterotrophic bacteria were tryptone/bacto pepton 2 gr, yeast extract 1 gr, glucose 0.5 gr, and agar 15 gr. Sterile the media by autoclave at 121 °C for 30 min. One millilitre sample is diluted into a 10-fold dilution series. One millilitre of each dilution water was then placed on triplicated petridish and added 10 ml of culture medium and then incubated for 7 days at 20 °C. . The incubation time was 7 days and the temperature was 20 °C (APHA, AWWA and WEF, 2005). Plates with countable colonies between 20 and 300 were selected for counting with the unit is colony form unit (CFU).

*Fecal indicator bacteria.* TC and *E. coli* were enumerated based on multiple tube fermentation method using colicatch reagents (ES Colicatch 1000, Eiken Chemical, Japan) and incubated at 37 °C for 24 h. TC and *E. coli* were analyzed using multiple tube fermentation technique as Most Probable Number (MPN) index. Procedures for analysis total coliform and *E.coli* were 10 milliliters of sample diluted into a 10-fold dilution series contained 90 ml of NaCl. Nine milliliters from each series of dilution were placed into three tubes with one milliliter colicath reagent (ES コリキャッチ 1000, Eiken chemical) and incubated them for  $\pm$  24 h at 37 °C. Detection of *E.coli* was recognized by the change color from yellow (indicated total coliform) to blue using ultraviolet ray.

*Total number of bacteria.* DNA-based bacterial density (DNA-total bacteria) was quantitatively measured based on the measurement of DNA bacteria using Thermal Cycler Dice™ Real Time System TP800 (TaKaRa Bio Inc.). The determination involved the following processes: DNA extraction using PowerSoil® DNA Isolation Kit (MO BIO Lab. Inc., CA), amplification with the universal 16S rDNA primer set (SYBR® Premix Ex Taq™,Takara Bio Inc.), and detection by measuring the increase in



fluorescence caused by binding SYBR Green dye to double stranded DNA in a real-time PCR (Zhou *et al.*, 2007; Helard *et al.*, 2012).

Quantification of extracted DNA was carried out using Thermal Cycler Dice™ Real Time System TP800 (Takara Bio Inc.) and SYBR® Premix Ex Taq™ (Takara Bio Inc.), following the instruction manual from the manufacturer. Universal primers, com1 (5'–CAG CAG CCG CGG TAA TAC–3') and com2 (5'–CCG TCA ATT CCT TTG AGT TT–3'), were used for PCR amplification of 16S rDNA (Stach *et al.*, 2001; Zhou *et al.*, 2007). The extracted DNA from samples and a series dilution of *E. coli* DNA were prepared as templates. The solution of PCR reaction (25 µL) contained 0.5 µL of each primer (10 µM), 2 µL of template and 12.5 µL of SYBR® Premix Ex Taq™ and 9.5 µL of pure water.

Amplification of the 16S rDNA followed the 2 step PCR protocol: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 30 s. All samples were performed in triplicates. Specificity of the assay was assessed by the analysis of the melting curve (Fey *et al.*, 2004). The melting was performed from 60 to 95 °C at increments of 0.2 °C/s. The concentration of DNA was then converted to the bacterial number concentration using *E. coli* as the surrogate. The calibration curve for total bacteria in real-time PCR was obtained from extracted *E. coli* DNA according to the extraction method of *Escherichia coli* 12F<sup>+</sup> (No. 13965, NITE Biological Resource Center, Japan) (Zhou *et al.*, 2007). The DNA of *E. coli* was quantified with a NanoVue UV/Visible Spectrophotometer (GE Company), and was then used to generate a standard curve using a 10-fold dilution series in the range 10<sup>1</sup> to 10<sup>-6</sup> ng/µL. The range of melting temperature ( $T_m$ ) for the standard DNA was 86.5 – 87.5 °C.

### 3.4 Statistical analysis

Significant differences in sampling sites and seasons for the physicochemical and microbial parameters were calculated using one-way analysis of variance with Tukey's post-hoc test. The log-transformed was used as preparation data for all microbial concentrations to avoid incorrect statistical analysis. The seasons were tabulated and

categorized from all data, and were defined using a solar calendar for Gifu City area (i.e., spring begins in mid-March, summer begins in mid-June, autumn begins in mid-September, and winter begins in mid-December). The Spearman rank correlation test was used to evaluate the correlations between microbial contamination in the water and sediment. The statistical analyses were performed using IBM® SPSS® Statistic version 21 and excel 2010 with significance at the 95% confidence level.

## Chapter 4

### Evaluation of physicochemical parameters in open channels receiving johkasou effluent

#### 4.1. Background

The continuing increase of populations in rural areas has led water quality deterioration in the local environment. Environmental protection and development of surface water quality assessments are importance for effective sanitary management in rural areas. In Japan, an onsite domestic wastewater system known as johkasou has been widely applied since decades ago in decentralized areas (Yang *et al.*, 2010). According to the Ministry of Environment Japan, the total number of johkasou facilities in FY 2012 (end of March 2013) was 7.76 million (MILT, 2013). A former type of johkasou that treats only black water called tandoku johkasou made up the greater proportion of this installation at 58% (4.53 million), while the remaining 42% (3.23 million) was the combined type that treats both black and grey water, known as gappei johkasou. The contribution of effluents from the johkasou systems to surface water requires water quality monitoring in order to understand the level of contamination due to the inadequate performance.

Johkasou facilities which used anaerobic and aerobic treatment processes with periodic maintenance are dedicated to preserve the local water safety when the treated water is introduced to stream water (JECES, 2003). However, several studies have been reported water quality alteration in the local environment of decentralized areas where johkasou facilities are installed. Gaulke (2006) documented that tandoku johkasou had been reported as a major water impairment via disposal of grey water into the local receiving water body. The decentralized areas that mainly used tandoku johkasou need more effective means of wastewater management than areas using gappei johkasou in term of pollutant load factor (Tanaka *et al.*, 2007). High pollutant load discharged from tandoku

johkasou facilities may cause the eutrophication in the receiving natural water. In addition, seasonal factor such as temperature were recorded to have an influenced on performance of the onsite domestic treatment systems. The removal of BOD and COD were reported to be decrease during winter season (water temperature  $< 15^{\circ}\text{C}$ ) in the johkasou systems (Inchinari *et al.*, 2008). The combined systems in gappei johkasou that required to meet the effluent standard of johkasou (biochemical oxygen demand  $< 20\text{ mg/L}$ ), cannot guarantee the protection of the local aquatic environment (Yang *et al.*, 2010).

Inadequate performance and difficulties in maintenance can be the factors to produce low quality of treated water under regulation effluent standard of onsite domestic wastewater treatment system. This treated water can contaminate the stream water if it is continually introduced and accumulated. The protection of water in the local environment paired with water quality monitoring is of fundamental importance, and this to call for evaluation of effluent effects by onsite domestic systems. Therefore the evaluation of physicochemical parameters in the open channels receiving johkasou effluent was focused in this study to know the characteristics of water quality.

## 4.2. Spatial variation of physicochemical parameters

A summary of physicochemical measurement results at six sampling points is shown in **Table 3.1**. Furthermore, box plot figures were used to describe the data at six sampling points from November 2010 to December 2013. For all box plot figures, a few values were considered explicitly outliers where “whiskers” represent the 95<sup>th</sup> and 5<sup>th</sup> percentiles observed concentration. The measure central tendency was the median, and the upper and lower bars of the “box” represent 75<sup>th</sup> and 25<sup>th</sup> percentiles, respectively.

Flow rate in the johkasou drainage channel (SP.JO) was the lowest (0.41 L/s) than flow rate in the open channels (ranged from 11.7 to 22.6 L/s). This different flow rate level is due to difference water input. The johkasou drainage channel received only effluent from 16 gappei johkasou facilities, whereas the open channels received johkasou effluent from another residential area mixed with ground water. Moreover, additional water input from paddy field runoff can also be seen in the open channels during the

cultivation period from May to August. The pH is an indicator to reflect water condition in environment. The pH level in both the open channels and the johkasou effluent was observed at neutral value (around pH 7). These pH values did not show significant difference within six sampling points in the open channel (**Table 4. 2**). The pH ranged from 6.5 to 8.5 recommends values for water conservation that could support the aquatic life development and reproduction such as, fish and also bacteria.

Ions related to electric conductivity was detected at high level at SP.JO among sampling points in open channels. The mean value of EC at SP.JO was 31.4 mS/m. Johkasou systems may elevate the EC level in its effluent by several possibilities: saturated water in aerobic tank increased the concentration of dissolve oxygen and the presence of chlorine in order to kill the bacteria. The value of EC in johkasou effluent was different compared to its level in the open channels that ranged from 13 to 15 mS/m.

Table 4. 1 Description results of physicochemical analyses in the water at six sampling points during November 2010 – January 2013.

Parameters	Upstream			<i>Johkasou</i> *	Downstream	
	SP.1	SP.2	SP.3	SP. JO	SP.4	SP.5
Flowrate (L/s)	11.7 ± 14.4 (14)	13.7 ± 15.7 (11)	14.5 ± 15.7 (13)	0.41 ± 0.5 (14)	19.2 ± 20.5 (13)	22.6 ± 23.8 (14)
pH	7.4 ± 0.28 (14)	7.6 ± 0.23 (11)	7.5 ± 0.24 (13)	7.4 ± 0.2 (14)	7.5 ± 0.2 (13)	7.5 ± 0.24 (14)
WT (°C)	16.3 ± 6.5 (14)	15.9 ± 6.1 (11)	16.3 ± 6.2 (13)	17.7 ± 6.9 (14)	16.5 ± 6.1 (13)	16.4 ± 6.2 (14)
EC (mS/m)	21.1 ± 9.4 (14)	13.9 ± 1.4 (11)	14.3 ± 2.0 (13)	31.3 ± 12.1 (14)	15.0 ± 2.3 (13)	15.0 ± 3.3 (14)
DO (mg/L)	6.5 ± 0.5 (14)	6.5 ± 0.5 (12)	6.5 ± 0.5 (13)	4.4 ± 1.1 (14)	6.6 ± 0.5 (13)	6.5 ± 0.6 (14)
SS (mg/L)	8.0 ± 6.9 (14)	5.3 ± 6.9 (11)	6.2 ± 6.8 (13)	6.4 ± 7.6 (14)	8.7 ± 15.0 (13)	4.9 ± 4.0 (14)
DOC (mg/L)	3.5 ± 2.6 (14)	2.6 ± 2.3 (10)	2.4 ± 2.2 (13)	6.4 ± 6.8 (14)	2.1 ± 1.3 (12)	2.1 ± 1.1 (13)
BOD (mg/L)	2.5 ± 1.9 (7)	1.2 ± 0.5 (5)	1.9 ± 1.6 (7)	8.6 ± 3.2 (7)	1.8 ± 1.0 (7)	2.2 ± 2.1 (7)
COD (mg/L)	4.6 ± 3.9 (7)	1.4 ± 0.4 (5)	1.9 ± 1.4 (7)	10.5 ± 2.7 (7)	2.2 ± 1.5 (7)	2.8 ± 2.1 (7)
TN (mg/L)	2.6 ± 1.8 (12)	2.0 ± 2.1 (10)	1.4 ± 0.7 (12)	12.2 ± 7.2 (12)	1.9 ± 1.1 (12)	2.1 ± 1.3 (12)
TP (mg/L)	0.26 ± 0.20 (6)	0.16 ± 0.05 (4)	0.27 ± 0.23 (7)	1.68 ± 0.48 (7)	0.33 ± 0.29 (7)	0.25 ± 0.18 (6)
NH <sub>4</sub> -N (mg/L)	0.59 ± 0.6 (6)	0.25 ± 0.18 (4)	0.55 ± 0.65 (7)	6.09 ± 3.36 (7)	0.82 ± 0.96 (7)	0.57 ± 0.58 (6)
NO <sub>2</sub> -N (mg/L)	0.14 ± 0.17 (6)	0.03 ± 0.0 (4)	0.15 ± 0.29 (7)	0.78 ± 1.31 (7)	0.26 ± 0.55 (7)	0.06 ± 0.06 (6)
NO <sub>3</sub> -N (mg/L)	0.89 ± 0.5 (6)	0.81 ± 0.24 (4)	0.71 ± 0.36 (7)	4.72 ± 3.58 (7)	0.81 ± 0.46 (7)	0.99 ± 0.44 (6)
PO <sub>4</sub> -P (mg/L)	0.18 ± 0.09 (6)	0.17 ± 0.05 (4)	0.17 ± 0.06 (7)	1.30 ± 0.41 (7)	0.2 ± 0.06 (7)	0.19 ± 0.06 (6)
Total chlorine (mg/L)	0.05 ± 0.06 (9)	0.04 ± 0.03 (9)	0.03 ± 0.02 (9)	0.06 ± 0.04 (9)	0.06 ± 0.05 (9)	0.08 ± 0.1 (9)

The data shown are arithmetic means with standard deviations and number of sample

Asterisk mark indicates the *johkasou* effluent collected in the *johkasou* drainage channel.

The concentrations of organic matter (DOC, BOD, and COD) and nutrient contents (TN and TP) were measured to know the chemical contaminations in the open channels after receiving johkasou effluent. The concentrations of organic matter and nutrient in the johkasou effluent significantly varied compared to those concentrations in the open channel sampling sites (**Table 4. 2**). The mean concentrations of DOC, BOD, COD, TN and TP in the johkasou effluent were 6.36, 8.37, 10.5, 12.0, and 1.58 mg/L, respectively. Onsite wastewater treatment systems including johkasou use sludge and microorganisms for nitrifying and degrading total solid and organic matters from household wastewater (Ichinari *et al.*, 2008; Ebie *et al.*, 2002). These systems contributed lower concentration of dissolved oxygen in johkasou effluent and relatively high concentrations of organic matter and nutrients compared to natural water. High level of BOD (averagely 50 mg/L) has been noted in the effluents of tandoku johkasou that is highly affected by grey water products from sink, washing machine, kitchen, and bath (Yahashi *et al.*, 2000; Tanaka *et al.*, 2007). Based on the johkasou standard, the concentrations of BOD and TN at SP.JO were below the guideline limit (with less than 20 mg/L being recorded) whereas the TP concentrations relatively exceeded the johkasou standard (TP < 1 mg/L) (JECES, 2009). These results indicate that the primary treatment of anaerobic and aerobic unit could remove the organic matter; however, this system could not properly remove the nutrient contaminant in term of TP.

Furthermore, various forms of nutrient or dissolved nutrients such as  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{PO}_4\text{-P}$  in the johkasou effluent were also examined in order to know the performance of johkasou facility. The concentrations of those dissolved nutrient forms at SP.JO significantly varied compared to other open channel sampling sites (**Table 4. 2**). The concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{PO}_4\text{-P}$  at this site ranged from 0.58 to 10.1 mg/L, 0.03 to 1.65 mg/L, 1.65 to 11.0 mg/L, and 0.7 to 1.9 mg/L, respectively. Those concentrations were under the reported values by another study on the pilot of johkasou facility (mean values were 24.3, 0.2, 2.2, and 1.2 mg/L, respectively) (Ichinari *et al.*, 2008). These results indicate good performance of johkasou on removing the dissolved nutrient.

In the open channel, concentrations of organic matter and nutrient contents showed not significantly different among open channel sampling sites. The concentrations of DOC, BOD, and COD along open channels ranged from 0.65 to 8.72, 0.6 to 6.95, and 0.9 to 9.4 mg/L, respectively while TN and TP ranged from 0.24 to 7.89 and 0.001 to 1.00 mg/L, respectively. As shown in Table 4.1, the concentrations of organic matter and nutrient were relatively higher at SP.1 rather than other sites in the open channels. This slightly high of organic and nutrient concentrations may be due to the effect of effluent from tandoku johkasou by discharging untreated grey water. According to the previous study, tandoku johkasou was reported as a major source of water pollution in local water environment due to disposal of grey water (Tanaka *et al.*, 2007; Gaulke, 2006). Since, the grey water originated from household wastewater except toilet comprised high levels of organic matter, chemical, nutrient and microbial contamination (Eriksson *et al.*, 2002; Ottoson & Stenstrom, 2003; Benami *et al.*, 2013). Compared to the environmental quality standard for conservation of the living environment (Ministry of Environment Japan), the concentrations of TN and TP along open channels were slightly higher than environmental quality standard ( $TN \leq 1$  mg/L and  $TP \leq 0.1$  mg/L) which may suggest the possibility of eutrophication in downstream receiving water.

The forms of dissolved nutrient in the natural water have function as denitrification process that can be used to identify the occurrence of eutrophication. Significant differences of  $NH_4-N$ ,  $NO_2-N$ ,  $NO_3-N$ , and  $PO_4-P$  concentrations were not observed in the open channel sampling sites (Table 4. 2). In addition, the concentrations of those dissolved nutrient forms in the open channels were lower than several environmental standards, indicating the source affect was not significant in the open channel receiving johkasou effluent. The significant difference of total chlorine was not observed among the sampling site. The concentrations of total chlorine in the johkasou drainage channel ranged from 0.01 to 0.11 mg/L and along the open channels were from not detected to 0.25 mg/L. The low residual chlorine concentrations in the johkasou effluent can lead the high number of fecal contamination in the downstream water.

### 4.3. Temporal variation of physicochemical parameters in the open channels

Seasonal variation in physicochemical parameters along the open channel is shown in **Fig. 4. 1**. Seasonal variation was performed in four different seasons defined according to the Japanese solar calendar when winter begins in the middle of December, spring begins in the middle of March, summer begins in the middle of June, and autumn begins in the middle of September.

The flow rate levels increased from upstream (13 L/s) to downstream (23 L/s), indicating that there is source inputs along the open channel. The open channel receives the inputs from agriculture fields surrounding SP.1 and effluent from johkasou facilities at upper side. The flow rate level increased approximately 14 % at SP.2 expected from effluent of 21 johkasou facilities mixed with groundwater supplied between SP.1 and SP.2. Houses between SP. 2 and SP.3 contributed approximately 4 % of the total flow rate level in the open channel. The highest water input around 17 % was at SP. 4 in which the effluents from 16 johkasou facilities and input of agriculture from another area. The flow rate level increased approximately 1 % at SP. 5 that contributed from agriculture fields along the path from SP. 4. The different conditions were observed throughout the season. The levels of flow rate were high in spring and summer (47 and 36 L/s) and low levels in autumn and winter (6.9 and 4.8 L/s). The significant differences of flow rate were recorded in spring and summer compared to other seasons (**Table 4. 2**) that because the flow rate levels during these seasons increased ten times higher than other seasons due to water loads from agriculture activities. The low flow rate levels during winter and autumn exhibited an important factor to reflect water quality because of low mixing ratio with water in the open channel.

The significant differences in seasons were observed for water temperature (WT) along the open channels (Tukey's test  $P < 0.001$ ). The maximum and minimum range values of water temperature in the open channel were found in summer (23 – 28°C) and in winter (7.2 – 15°C), respectively. The mean values of WT increased slightly from upstream (16.1 °C) to downstream (16.7 °C). The decrease of water temperature was observed at SP.2 because this site receives input from groundwater that had always lower WT than surface water in the open channel.

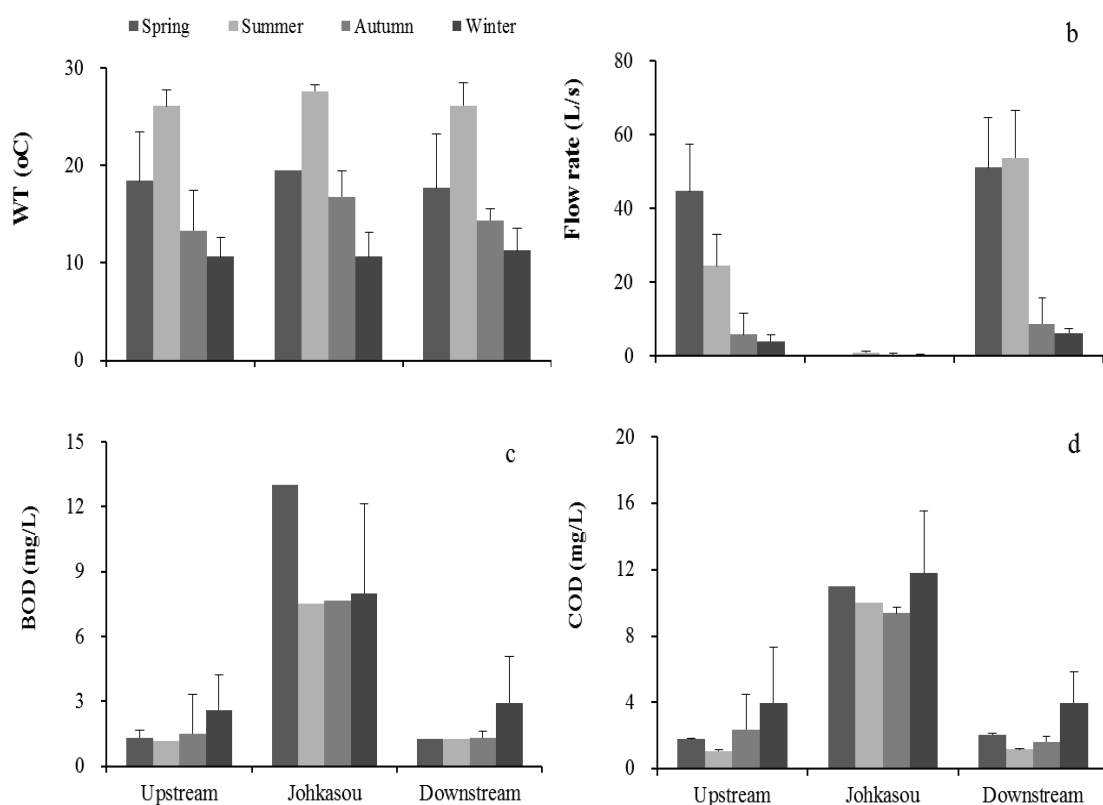


**Table 4. 2** Summary results of one-way ANOVA for all sampling sites and seasons

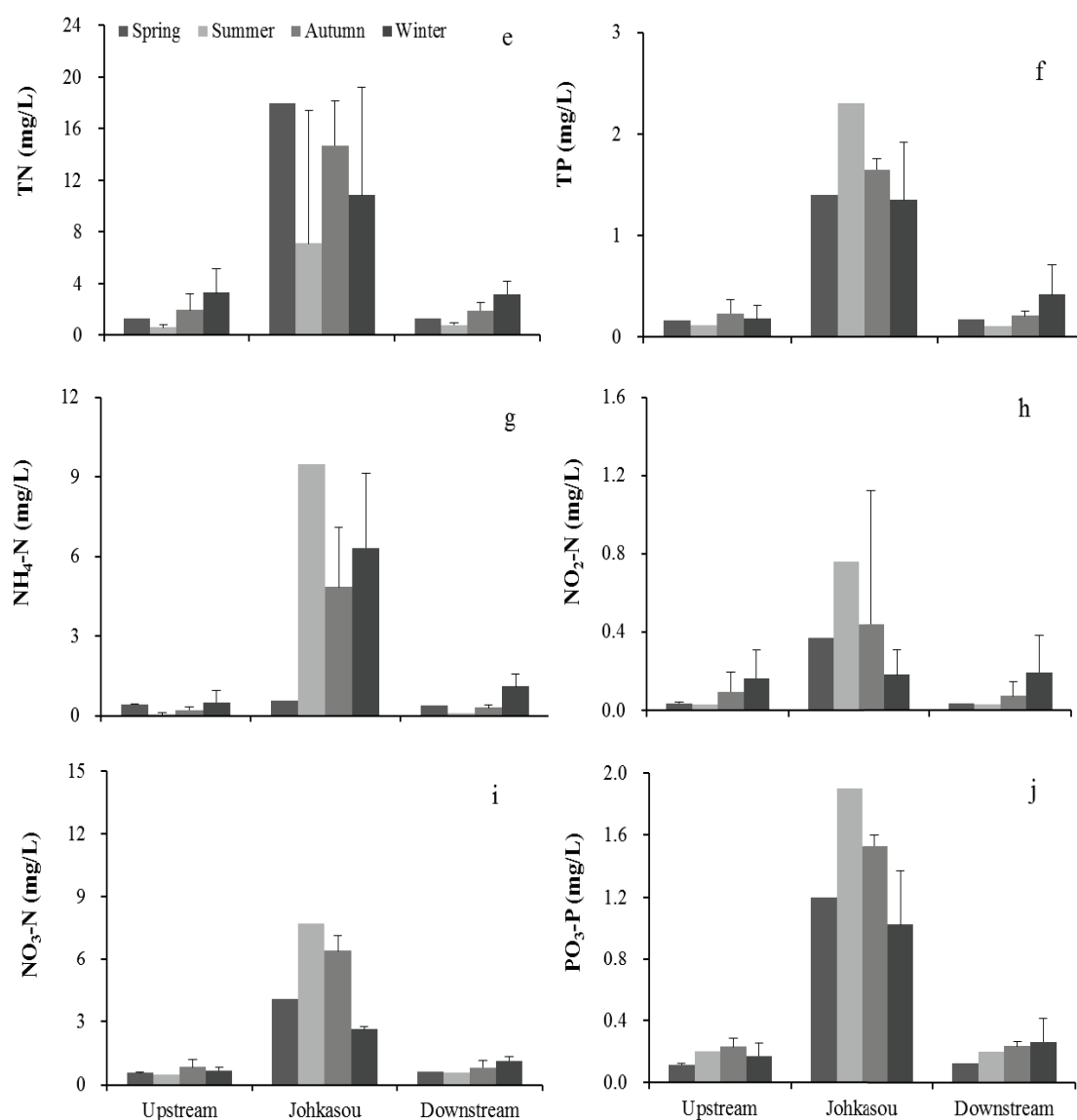
Variable	Sampling site			Season		
	df	<i>F</i>	<i>P</i> -value	df	<i>F</i>	<i>P</i> -value
Flowrate	5	1.98	0.09	3	41.04	0.00
pH	5	1.00	0.42	3	0.49	0.69
WT	5	0.14	0.98	3	127.60	0.00
EC	5	13.88	0.00	3	7.29	0.00
DO	5	23.46	0.00	3	25.96	0.00
SS	5	0.43	0.83	3	16.76	0.00
DOC	5	3.79	0.00	3	6.87	0.00
BOD	5	29.83	0.00	3	6.51	0.00
COD	5	32.79	0.00	3	4.32	0.01
TN	5	20.73	0.00	3	16.45	0.00
TP	5	63.52	0.00	3	1.51	0.22
NH <sub>4</sub> -N	5	28.49	0.00	3	10.05	0.00
NO <sub>2</sub> -N	5	3.03	0.02	3	3.84	0.02
NO <sub>3</sub> -N	5	18.11	0.00	3	1.48	0.23
PO <sub>4</sub> -P	5	85.74	0.00	3	2.76	0.05
Total chlorine	5	1.01	0.42	3	18.90	0.00

The mean concentrations of DOC, BOD, COD, TN, and TP in the upstream were 2.8, 1.9, 2.8, 2.0, and 0.23 mg/L, respectively. The concentrations of those parameters in the downstream channel were 1.8, 2.0, 2.5, 2.0, and 0.27 mg/L, respectively. The concentrations of most chemical parameters significantly varied within seasons with the Tukey's post-hoc test showed significant concentrations in winter rather in other seasons (**Appendix D**). This indicates that the source effect of johkasou effluent can vary the water quality in the open channel during winter that is coincided with the low flow rate levels. 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 (Ministry of The Environment Japan). Mean values of BOD in the open channels meets the environmental quality standard; however concentrations of TN and TP were under of

the environmental quality standard. This high levels of nutrients may be due to the influenced of johkasou effluent since the types of johkasou facilities used in this area are mostly BOD removal type and it does not include the function of nitrogen and phosphorus removal. Therefore, the self-purifications and dilution factors along the open channels are supposed to be able reduce the nutrient contaminants in local water environment of decentralized area especially in winter.



**Fig. 4. 1** Seasonal variation in physicochemical parameters related to (a) flow rate, (b) WT, (c) BOD, and (d) COD along the open channel and in the *johkasou* drainage channel. Data in the graphs are means and standard deviations pooled from the study periods. The bars without standard deviation showed raw results.



**Fig. 4. 1 (Continued)** Seasonal variation in physico-chemical parameters for (e) TN, and (f) TP, (g)  $\text{NH}_4\text{-N}$ , (h)  $\text{NO}_2\text{-N}$ , (i)  $\text{NO}_3\text{-N}$ , and (j)  $\text{PO}_4\text{-P}$  along the open channel and in the *johkasou* drainage channel. Data in the graphs are means and standard deviations pooled from the study period. The bars without standard deviation showed raw results.

The seasonal variations in the dissolved nitrogen forms related to  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{PO}_4\text{-P}$  in the open channels were also investigated. The concentrations those dissolved nitrogen forms were relatively same between upstream channel (0.33,

0.11, 0.74, and 0.2 mg/L, respectively) and downstream channel (0.58, 0.11, 0.9, and 0.23, respectively). Those concentrations were further determined the significance in seasons (**Table 4. 2**). However, Tukey's post-hoc test showed only the  $\text{NH}_4\text{-N}$  significantly varied in winter compared to other seasons (**Appendix D**). The significant levels of  $\text{NH}_4\text{-N}$  in the open channels may be due to the contribution of anthropogenic source and the low flow rate level during winter suggested low dilution function. Ammonia nitrogen in concentration can also undergo the nitrification in the presence of certain organisms that are *Nitrosomonas* as the primary bacterial genus to convert ammonia to nitrite. The increasing levels of ammonia in the stream water lead to extend the certain organism and also increase the levels of nitrite.

#### 4.4. Temporal variation of physicochemical parameters in the johkasou effluent

In order to know the seasonal impact on effluents from johkasou facilities before discharging into the downstream water environment, seasonal characteristics of johkasou effluents at the end point of channel that received effluents from 16 gappei johkasou facilities were evaluated. Seasonal variation in physicochemical parameters is displayed in **Fig. 4. 1**. There were no significant differences in WT between upstream and downstream throughout the season. This suggests that the WT in johkasou drainage channel was not affected by seasonal factor, whereas the discharge of johkasou effluent may strongly affect the WT.

The different flow rate between johkasou effluent and open channels showed dilution factor that fluctuated in season. The dilution/mixing ratio between johkasou effluent and flow water in the open channel was calculated using mass balance equation (Eq. 1).

$$Q_1 + Q_2 = Q_3 \quad \text{Eq. 1}$$

$$Q_1 \cdot X_1 + Q_2 \cdot X_2 = Q_3 \cdot X_3 \quad \text{Eq. 2}$$

Where, Q is flow rate level,  $Q_1$  is flow rate from upstream,  $Q_2$  is flow rate from johkasou effluent, and  $Q_3$  is flow rate at downstream. And, X is representative conservative concentration of parameter, which the EC data was used in this calculation. Based on this calculation, the mixing ratio of flow rate in spring, summer, autumn, and winter was 56 : 1000 : 8 : 4, respectively. This result that during winter is the lowest

mixing ratio that can affect the downstream water quality, while summer shows the high mixing ratio that can decrease the effect of johkasou effluent.

The concentrations of organic matter, nutrient contents, dissolved nutrient forms in the johkasou effluent were not significantly difference in seasons (**Table 4. 2**). This suggests that seasonal variance may not affect the johkasou performance for treating domestic wastewater. As shown in **Fig. 4. 1**, the high concentrations of BOD, COD, TN, and TP in johkasou effluent were observed in spring (13, 11, 18, and 1.4 mg/L) while the high concentrations of BOD, COD, TN, and TP in downstream channel were observed in winter. This indicates that high dilution capacity in downstream during spring (~1:56) could be enough to reduce the impact of johkasou effluents, while low dilution capacity in winter (~1: 4) might cause degradation in downstream water quality. It suggests that the seasonal flow rate level could contribute to vary the water quality after receiving johkasou effluent.

#### 4.5. Summary

Evaluation of physicochemical parameters in the open channel receiving johkasou effluent through 3-year study period was conducted. The concentrations of organic matter and nutrients in the johkasou effluent significantly varied compared to other sampling site in the open channel. However, concentrations of TN and TP in the open channel were slightly higher compared to Japanese environmental quality standard that might suggest possible impairment of water quality in the downstream receiving water. The significant variations of organic matter and nutrient in seasons were recorded along the open channel receiving johkasou effluent. Those parameters were significantly different during winter compared to other seasons and the lowest mixing ratio was observed in this season. A real concern of water quality degradation in a decentralized area using johkasou facility is considered during cold-low flow seasons in which the effluent from johkasou can be a pollution source to reflect the local water environment.

## CHAPTER 5

### Evaluation of microbial indicators in the open channels receiving johkasou effluent

#### 5.1. Background

Johkasou has been applied as an alternative wastewater treatment system in the rural areas of Japan, in addition to other areas that do not have wastewater treatment plants (Yang *et al.*, 2010). Johkasou differs to septic tank and wetland systems because it consists of a primary anaerobic and aerobic biological treatment unit, a sedimentation tank, and a disinfection using chlorine tablets (Ichinari *et al.*, 2008). The effluent quality standard of johkasou was set that should be less than 20 mg/L biological oxygen demand (BOD) (Nakajima *et al.*, 1999). This system is considered an effective way in treating household wastewater before the effluent is discharged into a drainage or stream channel in residential areas.

The treated water of onsite domestic systems, including johkasou, may contain several pollutants, such as organic substances, chemicals, nutrients, and microorganisms (Savichtcheva *et al.*, 2007; Wihters *et al.*, 2011). Several studies have documented unsatisfactory quality of treated water from johkasou systems. Tandoku johkasou has been reported as a major pollution source (e.g., organic matter, and nutrients) in natural stream water of residential areas by discharging grey water (Gaulke, 2006). Insufficient removal of fecal indicators and pathogenic bacteria by gappei johkasou has been recorded at low temperatures (i.e., during winter) (Kaneko *et al.*, 2001). Setiyawan *et al.* (2014) reported high levels of fecal indicators in the drainage channels of various types of johkasou systems. Therefore, water quality monitoring in residential areas plays an important role in evaluating the influence of johkasou effluent in the local environmental water.

Analysis of water quality alone may underestimate the risk of human exposure to potential pathogenic microorganisms in sediments. This is because sediments constitute an important reservoir of microbial indicators in the water environment, including stream channels in decentralized areas. A previous study documented a high density of bacteria in the sediments of channels containing johkasou effluent (Helard *et al.*, 2012). Sediment may contain 100 – 1000 times more fecal coliforms than the overlying water (Bai and Lung, 2005). Compare to water, sediments contain organic substances and optimal nutrient conditions for development of microorganisms, particularly fecal indicators, resulting in their long survival (Garzio-Hadzick *et al.*, 2010).

The degree of contamination in surface water can vary seasonally. Most of the physicochemical parameters of surface water quality showed moderate variations in their concentration for all seasons (Mandal *et al.*, 2011; Adeyemo *et al.*, 2008). 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 (Kolerevic *et al.*, 2012; Selvakumar *et al.*, 2006). In the previous study, fecal indicators have been detected in the channels of johkasou systems (Setiyawan *et al.*, 2014). However, the seasonal characteristics of fecal indicators in the local water environment receiving johkasou effluents are little known.

Despite the importance of onsite domestic wastewater treatment systems in the water cycle of local communities is to protect public health. The physicochemical and microbial quality in water and sediments of open channels receiving johkasou effluent are rarely measured because most investigations to date have mainly focused on the treatment performance of johkasou. Therefore, the aims of this study were (i) to evaluate how johkasou effluent contributes to microbial indicators in the water and sediments of open channels, and (ii) to identify whether these parameters are influenced by seasonal variation in the water flow of the open channels.

**Table 5. 1** Summaries of microbial indicators analysis in the water and sediments at six sampling points during study periods.

Parameters	Upstream			<i>Johkasou</i>		Downstream	
	SP.1	SP.2	SP.3	SP.JO	SP.4	SP.5	
<b><u>Water</u></b>							
VB (CFU/ml)	(2.6 ± 7.7)× 10 <sup>4</sup> (14)	(1.9 ± 1.8)× 10 <sup>4</sup> (11)	(2.9 ± 2.1)× 10 <sup>4</sup> (13)	(2.9 ± 28.3)× 10 <sup>5</sup> (14)	(6.2 ± 8.3)× 10 <sup>4</sup> (12)	(4.9 ± 61.8)× 10 <sup>4</sup> (13)	
HPC (CFU/ml)	(8.0 ± 10.9)× 10 <sup>4</sup> (14)	(8.1 ± 65.1)× 10 <sup>4</sup> (11)	(1.0 ± 2.4)× 10 <sup>5</sup> (13)	(4.8 ± 64.3)× 10 <sup>5</sup> (14)	(1.2 ± 1.8)× 10 <sup>5</sup> (12)	(1.1 ± 6.3)× 10 <sup>5</sup> (13)	
TC (MPN/100ml)	(2.1 ± 4.1)× 10 <sup>5</sup> (14)	(2.3 ± 10.0)× 10 <sup>5</sup> (11)	(4.6 ± 9.4)× 10 <sup>5</sup> (13)	(1.7 ± 3.7)× 10 <sup>6</sup> (14)	(2.4 ± 6.6)× 10 <sup>5</sup> (12)	(2.2 ± 5.4)× 10 <sup>5</sup> (13)	
<i>E. coli</i> (MPN/100ml)	(3.3 ± 11.1)× 10 <sup>3</sup> (14)	(3.3 ± 35.5)× 10 <sup>3</sup> (11)	(1.9 ± 12.9)× 10 <sup>3</sup> (13)	(6.7 ± 62.5)× 10 <sup>5</sup> (14)	(5.0 ± 30.7)× 10 <sup>3</sup> (12)	(4.7 ± 64.8)× 10 <sup>3</sup> (12)	
DNA (cell/ml)	(2.5 ± 19.3)× 10 <sup>7</sup> (14)	(1.1 ± 32.1)× 10 <sup>7</sup> (11)	(1.8 ± 17.0)× 10 <sup>7</sup> (14)	(0.9 ± 22.1)× 10 <sup>8</sup> (14)	(1.1 ± 4.3)× 10 <sup>7</sup> (14)	(2.3 ± 14.7)× 10 <sup>7</sup> (13)	
<b><u>Sediment</u></b>							
DS (mg/cm <sup>2</sup> )	19.1 ± 8.2 (10)	12.2 ± 14.0 (9)	9.8 ± 5.7 (10)	8.2 ± 9.9 (10)	3.4 ± 2.5 (10)	16.9 ± 16.3 (10)	
VS (mg/cm <sup>2</sup> )	1.97 ± 1.07 (9)	4.68 ± 6.93 (8)	1.15 ± 0.85 (9)	1.5 ± 1.58 (9)	0.46 ± 0.4 (9)	1.92 ± 1.84 (9)	
Organic content	10.2 ± 3.7 (10)	15.3 ± 11.2 (9)	11.4 ± 4.7 (10)	36.8 ± 16.5 (10)	19.8 ± 10.7 (10)	14.2 ± 11.6 (10)	
HPC_S (CFU/g)	(4.0 ± 19.0)× 10 <sup>7</sup> (10)	(8.4 ± 18.2)× 10 <sup>7</sup> (9)	(9.4 ± 9.9)× 10 <sup>7</sup> (10)	(2.2 ± 3.6)× 10 <sup>9</sup> (10)	(3.9 ± 11.3)× 10 <sup>8</sup> (10)	(9.47 ± 29.0)× 10 <sup>7</sup> (10)	
TC_S (MPN/g)	(1.3 ± 3.9)× 10 <sup>6</sup> (9)	(1.3 ± 10.0)× 10 <sup>6</sup> (8)	(1.3 ± 2.3)× 10 <sup>6</sup> (8)	(1.7 ± 29.8)× 10 <sup>7</sup> (9)	(1.2 ± 10.2)× 10 <sup>7</sup> (9)	(2.5 ± 56.8)× 10 <sup>7</sup> (9)	
<i>E. coli</i> _S (MPN/g)	(5.9 ± 5.6)× 10 <sup>3</sup> (9)	(9.9 ± 10.6)× 10 <sup>3</sup> (8)	(9.5 ± 10.1) × 10 <sup>3</sup> (9)	(3.0 ± 64.76)× 10 <sup>5</sup> (9)	(4.8 ± 21.4)× 10 <sup>4</sup> (9)	(0.8 ± 11.2)× 10 <sup>4</sup> (9)	
DNA_S (cell/g)	(2.3 ± 32.1)× 10 <sup>8</sup> (9)	(1.7 ± 5.0)× 10 <sup>8</sup> (7)	(1.2 ± 7.0)× 10 <sup>8</sup> (8)	(0.8 ± 56.6)× 10 <sup>9</sup> (9)	(8.3 ± 39.5)× 10 <sup>7</sup> (8)	(2.6 ± 6.0)× 10 <sup>8</sup> (9)	
Data shown are geometric means (excluded for DS, VS, and organic content are in arithamathic means) with standard deviations and number of samples.							

Data shown are geometric means (excluded for DS, VS, and organic content are in arithmetic means) with standard deviations and number of samples.



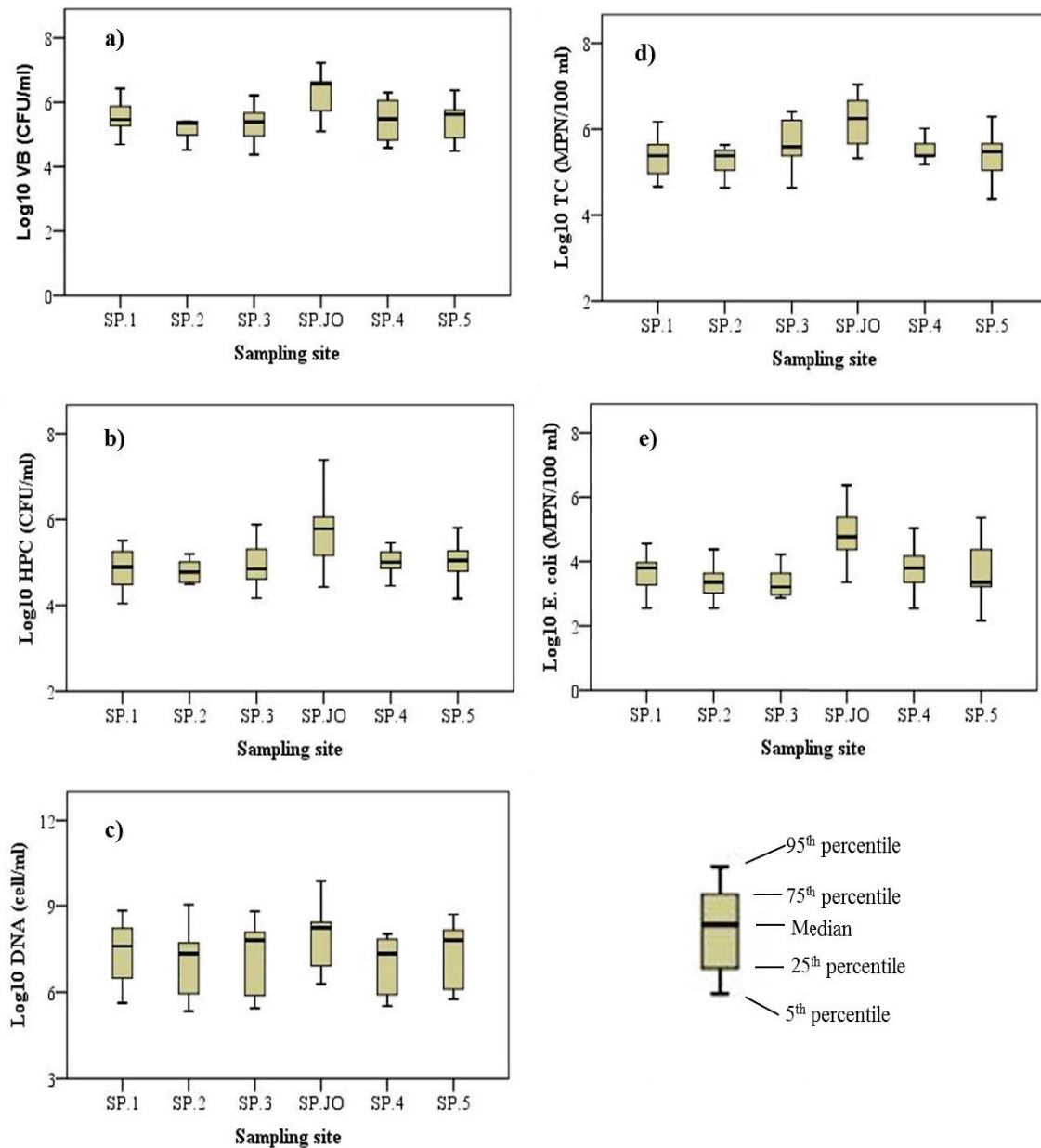
## 5.2. Spatial variation in microbial indicators

A summary of VB, HPC, TC, *E. coli*, and DNA-total bacteri in the water and sediments of the johkasou drainage channel and the open channels is shown in **Table 5. 1**. The box plots were used to describe microbial concentrations in water and sediments at six sampling points from November 2010 to November 2012 (**Fig. 5. 1** and **Fig. 5. 2**, respectively). For all box plot figures, a few values were considered explicitly outliers where “whiskers” represent the 95<sup>th</sup> and 5<sup>th</sup> percentiles observed concentration. The measure central tendency was the median, and the upper and lower bars of the “box” represent 75<sup>th</sup> and 25<sup>th</sup> percentiles, respectively.

The concentrations of VB, HPC, TC, and *E. coli* significantly varied in both water (johkasou effluent) and sediment of the johkasou drainage channel (SP. JO) (Tukey’s test  $P < 0.01$  and  $P < 0.05$ , respectively) compared to those in the open channels. The geometric mean concentrations of microbial indicators were up to two orders higher in the johkasou effluent than in the open channels (**Table 5. 1**). The geometric mean concentrations of VB, HPC, TC, and *E. coli* in the johkasou effluent were  $5.4 \times 10^6$  CFU/ml,  $6.4 \times 10^6$  CFU/ml,  $3.7 \times 10^6$  MPN/100 ml, and  $6.2 \times 10^5$  MPN/100 ml, respectively. Microbial indicators in sediment of johkasou drainage channel were observed two orders magnitude higher compare in the overlying water, which the geometric means concentrations were  $1.7 \times 10^9$  CFU/g,  $3.6 \times 10^9$  CFU/g,  $6.5 \times 10^8$  MPN/g, and  $6.5 \times 10^6$  MPN/g, respectively. The sediments at SP.JO had higher organic content than at any sampling sites in the open channels. High contents of organic matter in the johkasou effluent may affect the organic content in sediment by settlement.

The microbial concentrations in the open channel sampling sites were not significantly different in both the water and sediments samples (Tukey’s test  $P > 0.05$ ), although concentrations of microbial indicators in the sediment samples were being relatively higher compared to overlying water (**Table 5. 1**). The geometric mean concentrations of VB, HPC, TC, *E. coli*, and DNA-total bacteria in the water and sediments were similar among the open channel sampling sites. The ranges of those microorganisms were  $1.4 \times 10^4 - 2.7 \times 10^6$  CFU/ml,  $1.1 \times 10^4 - 2.4 \times 10^6$  CFU/ml,  $9.3 \times 10^3 - 3.5 \times 10^6$  MPN/100

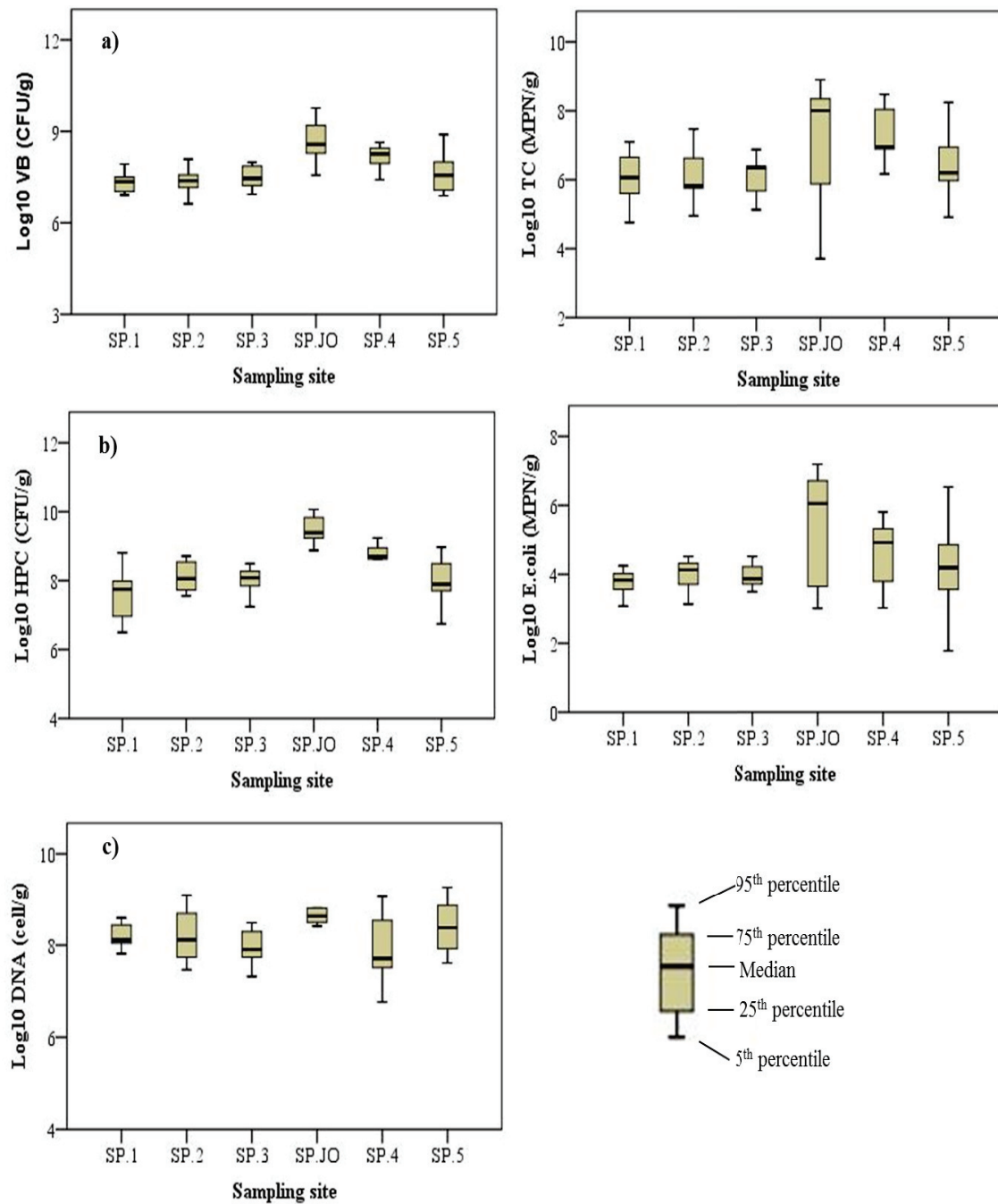
ml,  $0 - 2.3 \times 10^5$  MPN/100 ml, and  $2.5 \times 10^5 - 1.1 \times 10^9$  cell/ml, respectively in the water and were  $4.2 \times 10^6 - 7.9 \times 10^8$  CFU/g,  $1.9 \times 10^6 - 3.8 \times 10^9$  CFU/g,  $5.7 \times 10^4 - 3.0 \times 10^8$  MPN/g,  $0 - 3.4 \times 10^6$  MPN/100 g, and  $5.8 \times 10^6 - 9.8 \times 10^9$  cell/ml, respectively in the sediments.



**Fig. 5. 1** Spatial variations of microbial indicators in water for a) VB, b) HPC, c) DNA, d) TC and e) *E. coli* at six sampling points during study periods.

The concentrations of microbial indicators (HPC, TC, *E. coli*, and DNA-total bacteria) were consistently detected at high levels in the johkasou effluent through study period. Those microbes could be detected even though the total chlorine were measured with the maximum concentration around 0.11 mg/L at the accumulation point of johkasou drainage channel (SP. JO). This result indicates that total chlorine had insufficient contact time with johkasou effluent, resulting in high microbial concentrations in the johkasou drainage channel, supporting a previous report (Nambu *et al.*, 1996). In addition, TC concentrations at the johkasou drainage channel exceeded the guideline for johkasou effluent quality which should be less than 3000 CFU/ml (JECES, 2009). This result indicates that johkasou effluent may be a potential source of fecal contamination in local surface waters. High microbial content was also detected in the sediment of the johkasou drainage channel, which might be caused by the settling and growth of microbes due to the presence of organic matter and nutrients in the sediments, combined with appropriate temperature (Pote *et al.*, 2009; Garzio-Hadzick *et al.*, 2010).

Ministry of Environment Japan established the environmental quality standard for water conservation for rivers and lakes that TC concentrations should be less than 5000 MPN/100 ml. Whereas, concentrations of TC along the open channels receiving johkasou effluent is over the environmental quality standard. Concentrations of *E. coli* were slightly increased in the downstream channel compared to upstream channel (**Fig. 5. 1**). This result indicates that johkasou effluent can potentially be a source impact of fecal contamination. Moreover, *E. coli* concentrations in this area were one order of magnitude higher than in the major rivers of Gifu (Sasajima *et al.*, 2007). Furthermore, microbial contents in sediment of open channels were detected slightly higher in the downstream channel than in those the upstream channel. The high concentrations of microbes in the water may affect the microbial content in the sediments by settlement of certain microbes with settleable particles. Thus, these high concentrations of microbes in the sediment receiving open channels might be transported by hydrological events, which are coincided with possible health risk in the downstream water network.



**Fig. 5. 2** Spatial variation of microbial indicators in sediment for a) VB, b) HPC, c) DNA-total bacteria, TC, and *E. coli* at six sampling points during study periods.

### 5.3. Temporal variation of microbial indicators in the open channels

Seasonal variation was evaluated to identify the significance of natural effect on microbial concentration along the open channels. Seasonal variations in microbial parameters for VB, HPC, TC, *E. coli*, and DNA-total bacteria in the johkasou drainage channel and the open channels are showed in **Fig. 5. 3** The seasonal variation of microbes in the upstream channel was pooled from data of SP.1, SP.2, and SP.3, while seasonal variation in the downstream channel was pooled from upstream channel (data of SP.4 and SP.5) through the study period.

As shown in **Fig. 5. 3**, the geometric mean concentrations of VB, HPC, TC, and *E. coli* in the downstream channel increased in winter ( $2.3 \times 10^5$  CFU/ml,  $1.6 \times 10^5$  CFU/ml,  $3.6 \times 10^5$  MPN/100 ml, and  $7.8 \times 10^3$  MPN/100 ml, respectively). A significant difference for HPC and *E. coli* concentrations along the open channels was obtained among seasons. Tukey's post-hoc test showed that these microbes were significantly different in winter compared to the other seasons ( $P = 0.042$  and  $P = 0.018$ , respectively). These results indicate that johkasou effluent influenced the microbial quality in the downstream channel, particularly for *E. coli* during winter when the low mixing ratio occurred. Inactivation rate of fecal indicators in natural water was reported low during low temperature, which enhanced the survival of fecal indicators (Flint., 1987). Several investigators have been reported that the levels of fecal coliform vary with seasonal changes in rainfall and rivers discharge (Lipp *et al.*, 2001; Chigbu *et al.*, 2004). A study by Selvakumar *et al.* (2006) reported that microorganisms in urban area were significantly during warmer months compared to cooler months. However, the information in about the effect of climatic or inter-annual variations of environmental factors such as salinity and temperature on fecal coliform levels in the coastal water is limited (Lipp *et al.*, 2001; Rose *et al.*, 2001).

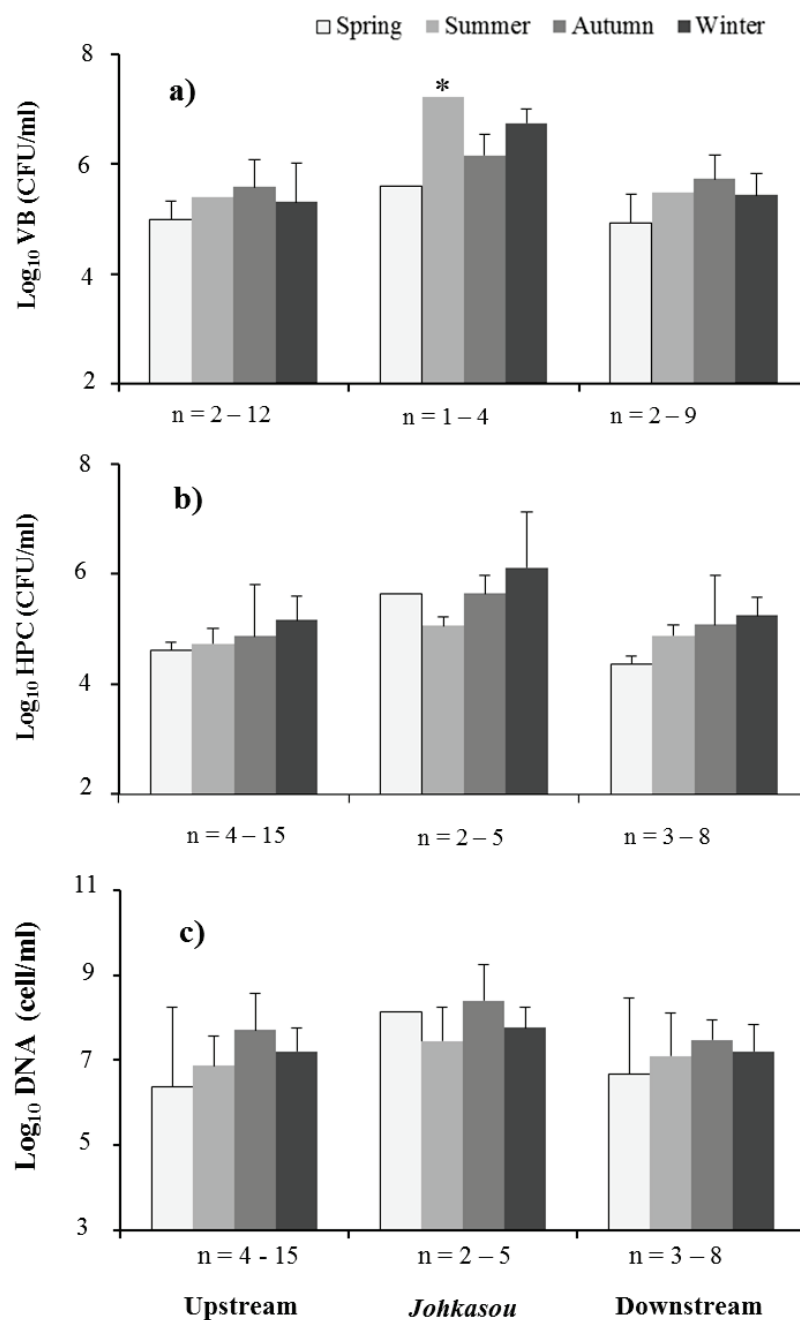
The contents of VB, HPC, TC, *E. coli*, and DNA-total bacteria in the sediments of open channels are presented in **Fig. 5. 2**. The minimum and maximum concentrations of these microbes were recorded in spring (in geometric means =  $2.4 \times 10^7$  CFU/g,  $2.1 \times 10^7$  CFU/g,  $4.2 \times 10^5$  MPN/g,  $5.0 \times 10^3$  MPN/g, and  $5.4 \times 10^8$  cell/g) and autumn in geometric means =  $4.2 \times 10^7$  CFU/g,  $1.9 \times 10^8$  CFU/g,  $5.2 \times 10^6$  MPN/g,  $1.9 \times 10^4$  MPN/g, and  $1.9 \times 10^8$

cell/g), respectively. Seasonal variation of the microbial content in the sediment was statistically significant for HPC and TC levels. Tukey's post-hoc analysis showed that HPC and TC levels varied in spring compared to other seasons ( $P = 0.022$  and  $P = 0.014$ , respectively). These significant typically peaked in spring, which was coincided with the high flow rate in the open channels. The transportation and resuspension of microbes associated with sediment particles can be caused by high flow rate during spring (**Fig. 4. 1**), and this could also be the possible reason to remove the microbial content in sediment. In the other seasons, however, the sediment contained high and stable index of microbial indicator due to the accumulation and settlement of microbes associated settleable particles. This high content of TC in sediments may provide a reservoir of TC in the water especially during period of stormwater runoff.

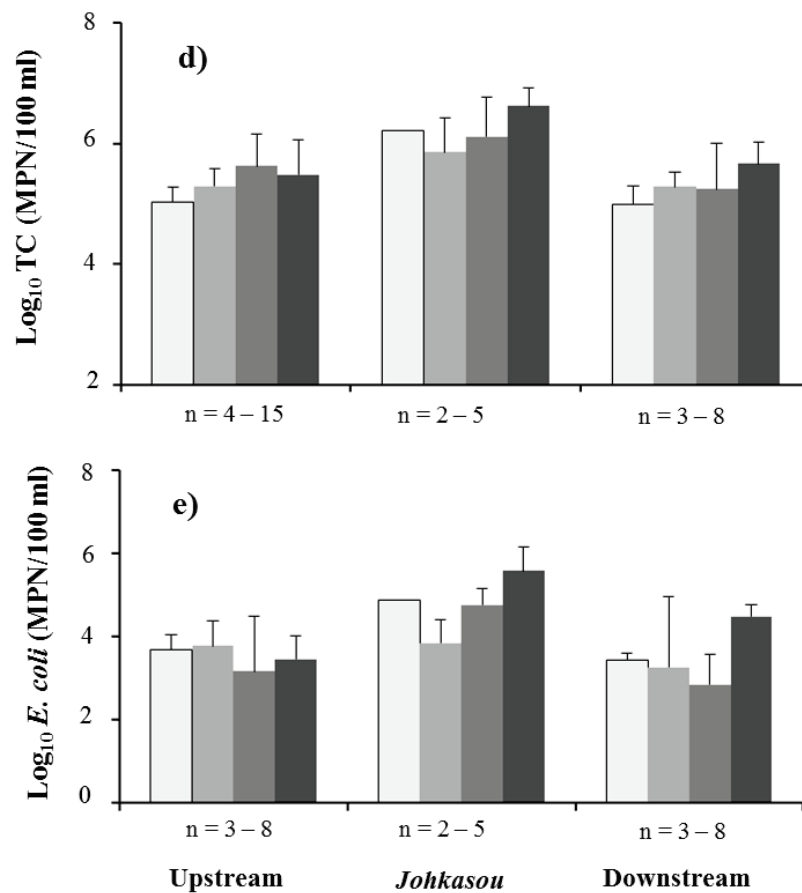
#### 5.4. Temporal variation of microbial indicators in the johkasou drainage channel

Seasonal variations of microbial concentrations in the johkasou drainage channel were also evaluated to reveal the effect of johkasou effluent and to find the seasonal influenced of effluent from this facility to downstream water. Concentrations of VB, HPC, TC, *E. coli* and DNA- total bacteria in seasons are shown in **Fig. 5. 3** (in the water) and in **Fig. 5. 4** (in the sediment).

As shown in **Fig. 5. 3**, the minimum and maximum contents of microbial indicators in *johkasou* effluent were accounted in spring and winter except for DNA-total bacteria that showed similar levels in all seasons. In spring, geometric mean concentrations of VB, HPC, TC, and *E. coli* were  $3.9 \times 10^5$  CFU/ml,  $4.3 \times 10^5$  CFU/ml,  $1.6 \times 10^6$  MPN/100 ml, and  $7.7 \times 10^4$  MPN/100 ml, respectively. In winter, the geometric means concentrations of those microbes were  $5.5 \times 10^6$  CFU/ml,  $1.3 \times 10^6$  CFU/ml,  $4.2 \times 10^6$  CFU/100 ml, and  $3.9 \times 10^5$  MPN/100 ml. As well as in the johkasou effluent, the contents of VB, HPC, TC, and *E. coli* in sediment johkasou drainage channel were increased in winter than other seasons (**Fig. 5. 4**). The contents of those microbes were  $1.6 \times 10^9$  CFU/g,  $4.1 \times 10^9$  CFU/g,  $5.1 \times 10^7$  MPN/g, and  $6.3 \times 10^5$  MPN/g, respectively.



**Fig. 5. 3** Seasonal variation of microbial parameters for a) VB, b) HPC, and c) DNA-total bacteria in the water of the upstream channel, the johkasou drainage, and the downstream channel. Data in the graphs are geometric means and standard deviations pooled from the study period, and n and asterisk marks indicate number of samples and raw results, respectively.

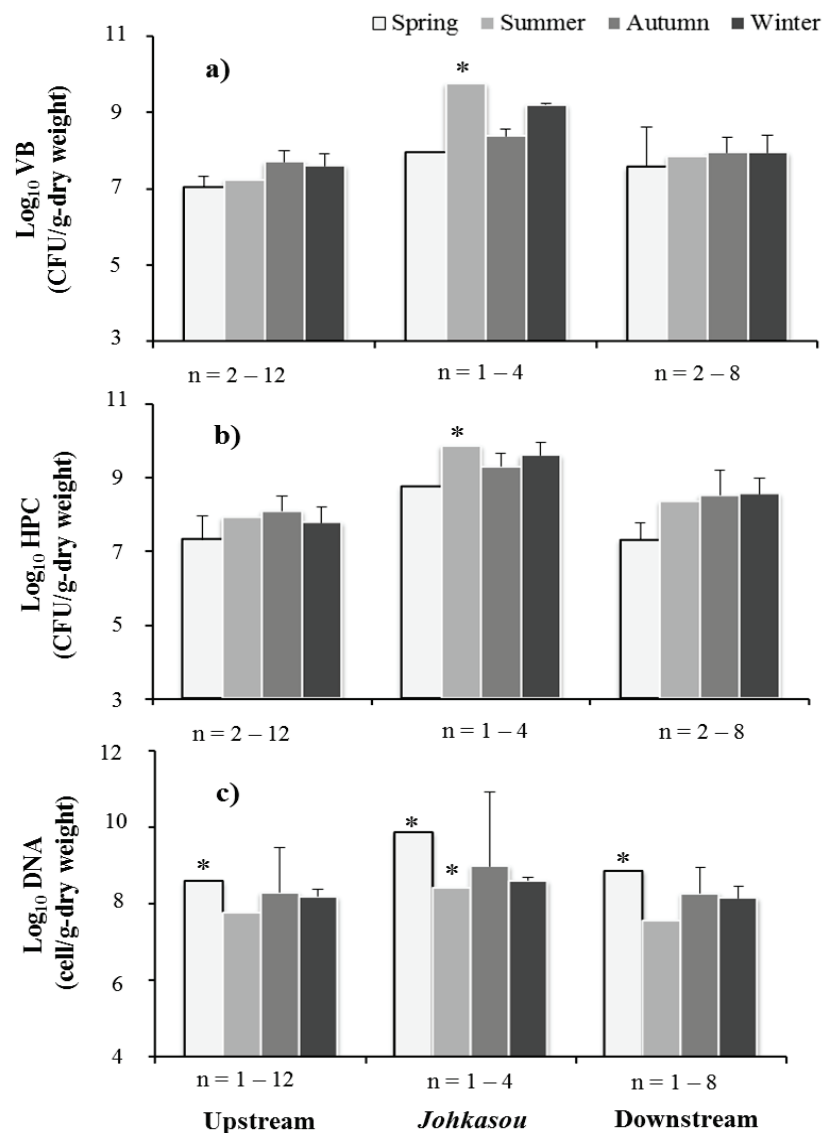


**Fig. 5. 3 (Continued)** Seasonal variation of microbial parameters for d) TC and e) *E. coli* in the water of the upstream channel, the johkasou drainage, and the downstream channel. Data in the graphs are geometric means and standard deviations pooled from the study period, and n and asterisk marks indicate number of samples and raw results, respectively

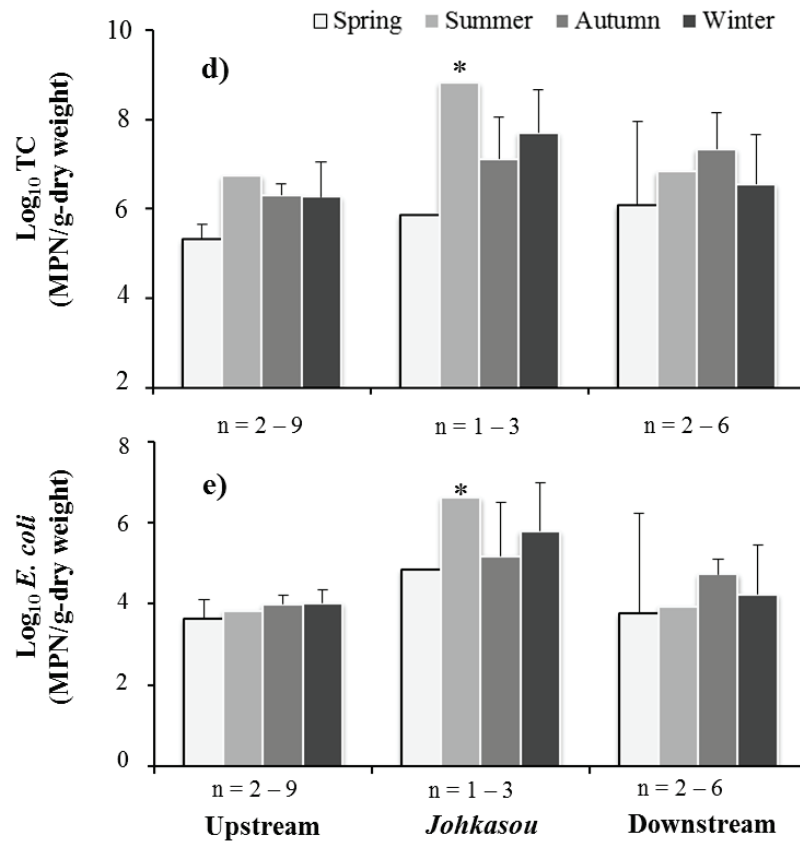
Statistically, seasonal variation of microbial parameters in the johkasou effluent was only significant for *E. coli* concentrations, which Tukey's post hoc test showed a significant difference between winter and summer ( $P = 0.007$ ). The concentration of *E. coli* in the johkasou effluent was recorded up to two orders higher in winter compared in other seasons. This indicates that low cold temperature in winter may affect the removal of fecal indicator in the johkasou systems. This finding is consistent with others, who also reported that the low temperature as an external factor inhibits the removal of fecal



indicators in onsite domestic systems (Reinoso *et al.*, 2008; Papadopoulos *et al.*, 2011). High concentrations of *E. coli* in the johkasou effluent and in the downstream water may suggest the influenced of johkasou effluent when winter is possess low dilution factor.



**Fig. 5. 4** Seasonal variation of microbial parameters for a) VB, b) HPC, and c) DNA-total bacteria in the sediment of the upstream channel, the johkasou drainage, and the downstream channel. Data in the graphs are geometric means and standard deviations pooled from the study period, and n and asterisk marks indicate number of samples and raw results, respectively



**Fig. 5.4 (Continued)** Seasonal variation of microbial parameters for d) TC and e) *E. coli* in the sediment of the upstream channel, the johkasou drainage, and the downstream channel. Data in the graphs are geometric means and standard deviations pooled from the study period, and n and asterisk marks indicate number of samples and raw results, respectively

In addition, VB contents in sediment of johkasou drainage channel showed significant in seasons among the microbial indicators. However, due to lack of data, furthermore analysis of microbial contents in sediments is needed to grasp more appropriate information of effect johkasou effluent in sediments and their changes in seasons. In addition, the high concentrations of fecal indicators in the johkasou effluent may cause the high content of microbes in the johkasou drainage channel that possible due to settlement and growth.

**Table 5. 2** Summary of one-way ANOVA results for microbial indicators in sampling sites and seasons

Variable	Sampling site			Seasonal		
	df	<i>F</i>	<i>P</i> -value	df	<i>F</i>	<i>P</i> -value
<b>Water</b>						
VB	5	3.76	0.006	3	3.14	0.034
HPC	5	3.90	0.003	3	3.24	0.028
TC	5	5.95	0.000	3	2.16	0.102
<i>E. coli</i>	5	5.58	0.000	3	3.22	0.028
DNA	5	1.49	0.202	3	3.19	0.029
<b>Sediment</b>						
VB	5	10.34	0.000	3	0.71	0.549
HPC	5	9.35	0.000	3	5.19	0.004
TC	5	2.09	0.083	3	3.82	0.017
<i>E. coli</i>	5	2.99	0.020	3	0.73	0.54
DNA	5	1.81	0.131	3	1.62	0.201

*P*-value is significant at  $\leq 0.05$

### 5.5. Relation between microbial indicators in water and sediments.

The relationship among the log-transformed concentrations of microbial indicators in both the water and sediments was examined using Spearman rank correlation (**Table 5. 3**). Positive correlations were found for the HPC number with TC and *E. coli* in both the water ( $r = 0.31$  and  $0.28$ , respectively;  $p < 0.05$ ), and the sediment ( $r = 0.55$  and  $0.56$ , respectively;  $p < 0.05$ ). These indicate that high concentrations of heterotroph bacteria may also be presence the fecal coliform group in the water environment. Positive correlations were also observed between TC and *E. coli* in both the water ( $r = 0.47$ ;  $p < 0.05$ ) and the sediment ( $r = 0.61$ ;  $p < 0.05$ ). However, there was no correlation between HPC number and DNA-total bacteria in either water or sediments, which indicated that other types of bacteria may contribute to DNA amount rather than HPC bacteria.

HPC in the sediments was positively correlated with HPC, TC, and *E. coli* in the water ( $r = 0.33, 0.40, \text{ and } 0.38$ , respectively;  $p < 0.05$ ). Positive correlations were also observed for HPC, *E.coli*, and DNA-total bacteria between water and sediments ( $r = 0.33, 0.27, \text{ and } 0.43$ , respectively;  $p < 0.05$ ). These positive correlations indicate that bacteria may interact with both the sediment and overlying water. The dynamic existence of fecal indicators in the sediment may be due to the association of fecal indicators with the settleable particles under normal flow condition (Characklis *et al.*, 2005). Thus, the fecal indicators in the sediment may reflect the overlying water by resuspension of fecal indicators from the sediments during storm runoff (Droppo *et al.*, 2009). Therefore, it is recommended that the fecal indicator content of the sediments is monitored to determine microbial behavior during storm-water runoff.

**Table 5. 3** - Spearman rank correlation coefficients between microorganisms in the water and sediment.

	Microorganisms in water				Microorganisms in sediment			
	HPC	TC	<i>E.coli</i>	DNA	HPC	TC	<i>E.coli</i>	DNA
<b>Water</b>								
HPC								
TC	<b>0.31</b> (77)							
<i>E.coli</i>	<b>0.28</b> (76)	<b>0.47</b> (76)						
DNA	0.22 (77)	<b>0.34</b> (77)	0.09 (76)					
<b>Sediment</b>								
HPC_S	<b>0.33</b> (54)	<b>0.40</b> (54)	<b>0.38</b> (54)	0.15 (54)				
TC_S	0.21 (50)	0.15 (50)	-0.05 (50)	0.16 (50)	<b>0.55</b> (53)			
<i>E.coli</i> _S	0.07 (50)	0.12 (50)	<b>0.27</b> (50)	0.26 (50)	<b>0.56</b> (52)	<b>0.61</b> (53)		
DNA_S	0.24 (48)	-0.03 (50)	0.19 (48)	<b>0.43</b> (48)	0.13 (50)	-0.06 (44)	-0.25 (44)	

Data shown is coefficient correlations and number of samples

Highlighted values are significant correlation at  $p < 0.05$

## 5.6. Summary

This study comprehensively monitored the microbial indicators in the water and sediments of open channels receiving johkasou for 3-year study period. High concentrations of microbial parameters (VB, HPC, TC, *E. coli*, and DNA-total bacteria)

in the water and sediments were detected in the johkasou effluent, with the microbial concentrations in open channels being an order magnitude lower than those in the johkasou effluent. Significant different of *E. coli* concentrations both in johkasou effluent and in the open channel during winter indicates the influenced of johkasou effluent more visible during low temperature and low dilution factor. This result suggests further advance of johkasou performance particularly disinfection process in order to remove the microbial contamination particularly in cold season. Positive correlations of HPC bacteria and *E. coli* between the water and sediments indicated that the microbes may interact both the sediments and water. In conclusion, it is advised that the fecal indicators in the sediments are monitored to determine microbial behavior and its transport during period of storm-water runoff.

## Chapter 6

### A statistical approach for evaluation of local environmental quality receiving johkasou effluent

#### 6.1 Introduction

Environmental protection and development in assessment of surface water quality are importance for effective sanitary management in rural areas. Assessment of water quality involves many parameters that generally based on the conventional measurement of physicochemical parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), and total phosphorous (TP). The analyses of physicochemical parameters only are insufficient to do the comprehensive evaluation of the aquatic environment. Assessment of fecal indicators (e.g., total coliform and *E.coli*) associated with physicochemical parameters is necessary for water quality security on preventing health human risks. The intensive water quality monitoring usually generates a large data set with complications on understanding the necessary information. The use of conventional techniques on description of analyses has limitations and poor delineation to interpret the water quality

Based on the results in the previous chapters, the concentrations of physicochemical and microbial parameters were not significant among sampling sites in the open channels. However, some parameters in both physicochemical and microbial parameters significantly varied in seasons. This exhibited the difficulty in decision the factors (sites or seasons) that can affect the water quality in open channels receiving johkasou effluent. A large data set with many parameters caused confusedness to extract the most valuable parameters that can characterize the impairment of water quality. Therefore, approach other than conventional results such as statistical multivariate analysis is required to comprehensively evaluate and extract the environmental quality.

Multivariate statistical techniques, such as principal component analysis/factor analysis (PCA/PFA) and cluster analysis (CA) have been addressed for better characterizing and evaluating surface and freshwater quality. These techniques are useful in verifying temporal and spatial variation caused by natural and anthropogenic factors linked to seasonality. To date, most of the previous studies using PCA methods were focused on rivers, lakes, coastal areas, and irrigation water while less attention has been paid to the monitoring of the local water environment monitoring. Therefore, interpretation of water quality using cluster and PCA/PFA was conducted to (1) to evaluate the variation of water and sediment quality in open channels receiving johkasou effluents and 2) to determine the important factors that can reflect water quality in environment of decentralized systems.

## **6.2 Statistical procedures**

### **6.2.1 Selection water quality data**

A total of twenty-one variables measured in water and seven variables measured in sediment was conducted at five sites along the open channels receiving johkasou effluents (**Table 3.1**). The samples of water and sediment were collected through 3-year sampling period from November 2010 to January 2013 with 14 times sampling collections in total. In regard to extract the important information from PCA results, exclude cases listwise was set in the PCA analysis (Ouyang, 2005).

### **6.2.2 Data treatment and multivariate statistical methods**

The Kolmogorov-Smirnov (K-S) statistics were used to test the goodness-of-fit of the data to log-normal distribution. According to the K-S test, all the variables are log-normal distributed with 95 % or higher confidence. To examine the suitability of the data for principle component analysis, Kaiser-Meyer-Olkin (KMO) and Barlett's test were performed. KMO measures the sampling adequacy that indicates the proportion of variance. High value (close to 1) of KMO generally indicates that principal component/factor analysis may be useful. Barlett's test of sphericity indicates whether correlation matrix is an identity matrix, which would indicate that variables are unrelated. The significance level which is 0.00 in this study (less than 0.05) indicated that there are significant relationships among the variables.

### 6.2.3 Principal component analysis/factor analysis (PCA/FA)

Principal component analysis (PCA) is designed to transform the original variables into new, uncorrelated variables (axes), called the principal components (PC), which are a linear combination of the original variables. PC provides information on the most-meaningful parameters, which describes a whole data set affording data reduction with minimum loss of original information. PCA of normalized variables (a water quality data set) was performed to extract significant PCs and further to reduce the contribution of variables with minor significance; these PCs were subjected to varimax rotation for generating varifactors (VF). A VF can include unobservable, hypothetical, latent variables while a PC is a linear combination of observable water-quality variables.

PCA and principal factor analysis (PFA) processes can be calculated in five major steps as described by Ouyang (2005): start by transforming the data into a standardized matrix  $\chi_1, \chi_2, \dots, \chi_p$  to have zero mean and unit variance to ensure that they all have equal weights in analysis; 2) calculate the covariance matrix  $C$  (i.e., the correlation coefficient matrix of  $X_i$ ); 3) find the eigenvalues  $\lambda_1, \lambda_2, \dots, \lambda_p$  and the corresponding eigenvectors  $a_1, a_2, \dots, a_p$ ; 4) identifying the principal components and discarding any components that only account for a small proportion of the variation in data set; 5) the last step is for PFA by developing the factor loading matrix and perform a varimax rotation on the factor loading to infer the varifactors. The cumulative percentage variance of greater than 70% was used as a criterion in this study to select the principal components.

### 6.2.4 Cluster Analysis (CA)

Cluster analysis is a group of multivariate technique whose primary purpose is to assemble the objects based on the characteristics they possess. Cluster analysis classifies objects, so that each object is similar to the others in cluster with respect to a predetermined selection criterion. The result of cluster should then exhibit high internal (within-cluster) homogeneity and high external (between clusters) heterogeneity. In this study, hierarchical agglomerative CA was performed on the normalized data set by means of the ward's method using Euclidean distance as a measure of similarity to



obtain dendrograms, and sampling sites in the same category have the similar source of pollution. The spatial variability of microbial indicator and water quality in the whole river basin was determined from CA, using the linkage distance, reported as rescaled distance cluster combine, which preserving the ration of the distance between steps. Cluster analysis was used in the present study because a visual summary of intra-relationship amongst variations parameters can lead to a better understanding of governing factors.

### **6.2.5 Correlation coefficient and analysis of variance**

The correlation coefficient measures how well the variance of each constituent can be explained by relationship between each parameter related to the microbial water quality. As preparation data, the log transforms were used for all microbial data to avoid incorrect statistical analysis. The Spearman rank correlation test, a non-normal distribution test, was performed and the mean values were used to evaluate the correlation between the microbial contamination and the physicochemical parameters. The terms “strong”, “moderate”, and “weak” were applied to factor loading and absolute loading values  $> 0.75$ ,  $0.75 - 0.50$ , and  $0.50 - 0.30$ , respectively. The statistical analyses were performed using IBM® SPSS® Statistic version 21 and excel 2010. All statistical analyses used a significance level of  $p < 0.05$ .

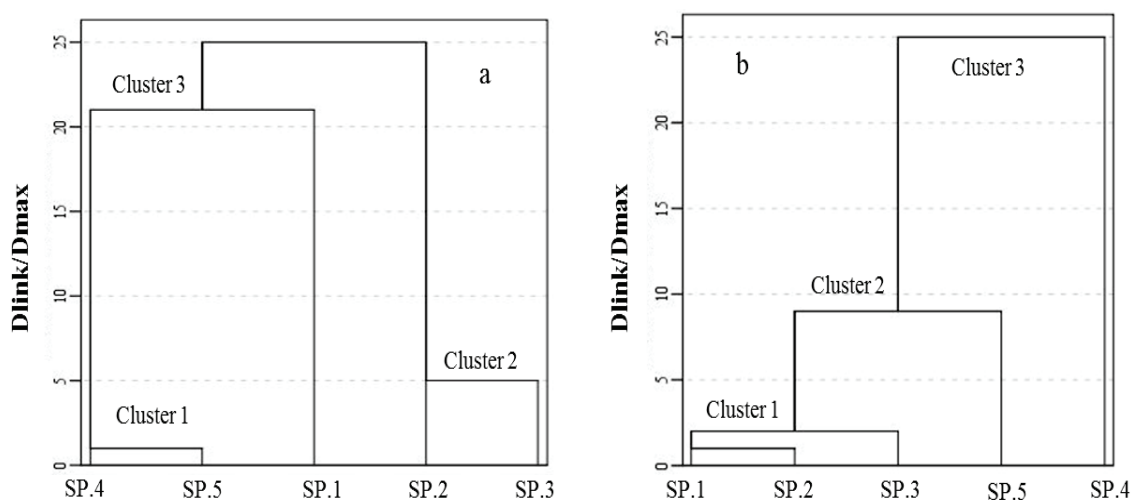
## **6.3 Results and discussion**

### **6.3.1 Classification of sampling sites**

The quality of water and sediment along the open channels was classified based on their similarity using hierarchical cluster analysis. Hierarchical cluster analysis was performed on the water and sediment quality data set to evaluate spatial variation among the sampling sites. This analysis classified the sampling sites into three clusters/groups (**Fig. 6.1**).

The CA classified the water quality among sampling sites of the open channel into three groups of cluster. Cluster 1 represents a group of downstream channel consisting of SP.4 and SP.5. Cluster 2 represents a group of upstream channel consisting of SP. 2 and SP.3 and the water quality in this cluster showed slightly different than cluster 1. Cluster 3

consists only SP.1 that showed different water quality compared to cluster 1 and cluster 2. The discharge of untreated grey water from tandoku johkasou in the upper area of SP.1 may suppose a negative contribution on receiving local water. Since, grey water contained high organic, inorganic, chemical, fecal bacteria and etc.



**Fig. 6. 1** Sites clustering for water (a) and sediment (b) in the open channel receiving johkasou effluent.

Sediment quality in the open channels was classified into three groups of cluster. Cluster 1 represents the sites in upstream channel (SP.1, 2, and 3), which had relatively different sediment quality compared to cluster 2 that consists of SP. 5. This slightly different may be due to the input of johkasou effluent in which the contaminants deposited during transport within 30 m straight open channel. Cluster 3 represents SP.4 located in the downstream channel that just after johkasou effluent discharged. This cluster exhibited different sediment quality among sampling site in the open channels, which suggested that the effluent of johkasou could also influence the sediment quality of receiving local water by settlement and/or disposition of its contaminants. These results imply for rapid assessment of water and sediment quality in the open channels using CA technique. The clustering procedure generated three groups/clusters in a very convincing way, as the sites in these groups have similar characteristics and natural backgrounds.

### 6.3.2 Water quality evaluation in open channel using PCA

A data set of 21 water quality indices was evaluated using PCA to interpret the necessary information along open channel sampling sites that received effluent from johkasou facilities. Prior to PCA analysis, the suitability data were performed by using Keiser-Meyer-Olkin (KMO) and Bartlett's test. KMO values close to 1 indicate the sampling adequacy for principal component analysis, and a value of KMO was 0.64 in this study. Bartlett's test, a value of 1049 showing the Bartlett chi-square statistic was calculated (degrees of freedom is 210 at the 95% significance level), indicating that the variables are not orthogonal but correlated. The PCA revealed five important principal components (PC) that sufficient to explain about 72.5 % of the total variance from the 21 variables data set.

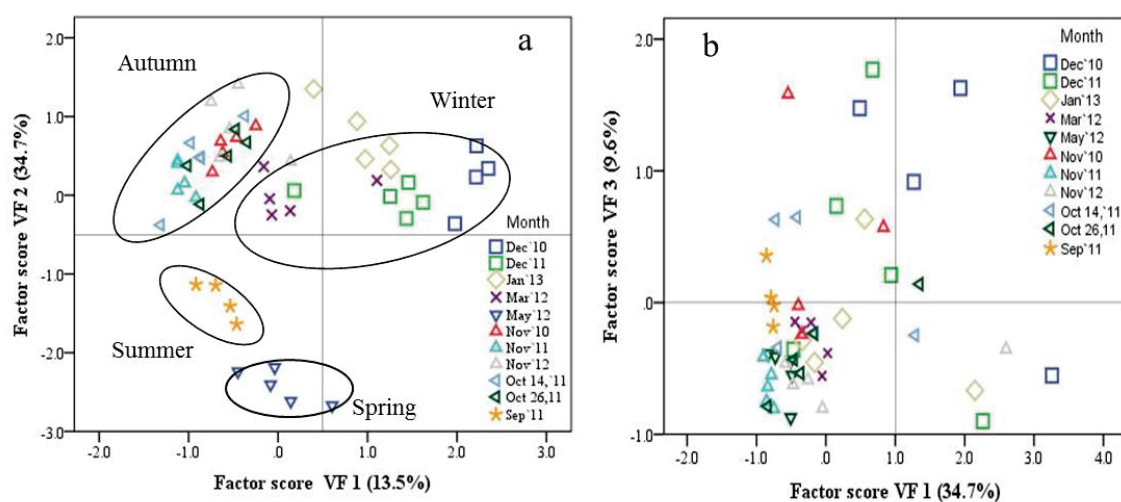
Table 6. 1 Loadings of environmental variables on the varimax-rotated  
PCs for water quality in the open channels.

Parameter	Verifactor				
	1	2	3	4	5
Flow rate	-.391	<b>-.724</b>	-.203	.311	-.067
pH	<b>-.415</b>	-.182	<b>-.419</b>	-.122	.161
WT	-.352	-.384	-.100	<b>.668</b>	.088
EC	<b>.864</b>	.219	-.086	.142	.012
DO	.058	<b>.850</b>	-.060	-.011	.029
SS	.031	.063	.107	<b>.810</b>	-.008
DOC	.352	<b>-.515</b>	-.218	.146	.033
BOD	<b>.853</b>	-.175	.200	-.169	.022
COD	<b>.954</b>	-.091	.042	.033	-.023
TN	<b>.725</b>	-.013	.050	-.192	.128
NH <sub>4</sub> -N	<b>.743</b>	-.195	.106	-.341	-.036
NO <sub>2</sub> -N	<b>.938</b>	-.060	.002	.070	-.099
NO <sub>3</sub> -N	<b>.596</b>	.326	.215	-.409	.230
TP	<b>.819</b>	.050	.221	-.257	.131
PO <sub>4</sub> -P	-.116	<b>.742</b>	.020	.150	.190
Cl	<b>.929</b>	.152	-.009	.041	-.040
VB	.074	.110	-.006	.072	<b>.885</b>
HPC	-.020	.076	-.084	-.053	<b>.878</b>
TC	.033	.061	<b>.771</b>	-.038	-.068
<i>E. coli</i>	.173	-.133	<b>.750</b>	-.194	.017
DNA	-.048	.225	<b>.744</b>	.323	.020
% of variance	34.7	13.5	9.6	7.7	6.9
% of cumulative	34.7	48.2	57.8	65.5	72.4

Extraction Method: Principal Component Analysis  
Rotation Method: Varimax with Kaiser Normalization.  
a. Rotation converged in 6 iterations.

A PFA was conducted To further identify the important variables for revealing water quality variations in the open channels receiving johkasou effluents. The eigenvalues

and loadings of these varifactors (VF), which were obtained using varimax rotation in the PFA, are displayed in Table 6.1. The PFA results showed that VF 1 explained 34.7% of total variance, and had highly positive loadings on parameters related to organic matter (BOD, COD), nutrients (TN, TP,  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ , and  $\text{NO}_3\text{-N}$ ), inorganic chloride ion ( $\text{Cl}^-$ ), and mineral EC, and negative participant of pH. The variables included in VF 1 can indicate as the anthropogenic pollution from onsite domestic systems. VF 2, accounted of 13.5% of total variance, was positive loadings on DO and  $\text{PO}_4\text{-P}$  and negative participant of flow rate, and DOC. This factor represents the environmental factor that can vary the water quality in the open channels particular for flow level that can indicate as dilution function.



**Fig. 6. 2** Factor score plots for water quality in open channels between VF 1 against VF 2(a) and VF (b)

The VF 3 (9.6% of variance) had positive loading on total coliform, *E. coli*, and DNA, and negatively correlated with pH. VF 4 (7.7% of variance) had positive participants of physical factor for water temperature and SS. The VF 3 and VF 4 can represent as fecal contaminants and physical factors of water quality along the open channels. Finally, VF 5 (6.7 % of total variance) is participated by a highly positive contribution of viable bacteria and HPC which indicates the bacterial number. The first three varifactors, accounted 57.8% of the total variance, showed the essential parameters to evaluate the water quality variation in the decentralized areas using onsite domestic wastewater treatment systems including johkasou.

The factor score from each varifactor was then used further analysis to classify the water quality based on seasonality characteristics. The scores of VF 1 against VF 2 showed the water quality in the open channels were different in seasons (**Fig. 6. 2**). In this graph, factor score clearly grouped the water quality based on seasonal characteristics. (**Fig. 6.2-a**). The water quality in spring and summer exhibited significantly different compared to other seasons that may be due to the high flow levels. And, winter also showed relatively different of water quality in the open channel, which can be explained by contribution of organic matter and nutrients from effluent of johkasou facilities. The scores of VF 1 against VF 2 showed no significance in seasons except some data in downstream channel during winter (**Fig. 6.2-b**). Based on these graphs, it suggested that johkasou effluent might deteriorate water quality in the open channels during winter when low flow rate level was observed.

### **6.3.3 Evaluation of sediment quality in open channel using PCA**

A data set of nine sediment quality indices was evaluated using PCA to interpret the necessary information along open channel sampling sites that received effluent from johkasou facilities. Prior to principal component analysis, the suitability data were performed by using Keiser-Meyer-Olkin (KMO) and Barlett's test. A value of KMO was 0.52 in this study and Barlett's test, a value of 137 showing the Bartlett chi-square statistic was calculated (degrees of freedom is 36 at the 95% significance level), indicating that the variables are not orthogonal but correlated. The Barlett's test showed low degree of freedom due to the small number of parameters and sampling collection. The PCA revealed three important principal components (PC) were sufficient to explain about 67.3 % of the total variance from the nine variables data set.

The eigenvalues and loadings of varifactors (VF) in sediment quality, which were obtained using varimax rotation in the PFA, are displayed in Table 6.2. The VF 1, explained 28.2 of total variance, had strong positive loading related to HPC, TC, and DNA. This varifactor indicates the microbial contents in sediment of the stream channel that received johkasou effluent. VF 2 (24.5% of total variance) had positive loading related to total solid, depth of sediment, VB, and DNA that represent the sediment

quantity. The fecal contaminant that represents as VF 3 had moderate positive loading with correlated to flow rate and *E. coli*, and negative correlated to volatile solid. The VF 3 can explain by dependent variable of *E. coli* as fecal contamination in the sediment quality from effluent of johkasou system.

Table 6. 2 Loadings of sediment quality in the open channels  
based on rotated-varimax method

Parameter	Verifctor		
	1	2	3
Flow rate	-.313	-.080	<b>.821</b>
Solid sediment	-.161	<b>.709</b>	-.411
Volatile sediment	-.099	.050	<b>-.529</b>
High of sediment	-.160	<b>.839</b>	.145
VB	.435	<b>.782</b>	-.186
HPC	<b>.882</b>	-.070	-.008
TC	<b>.819</b>	-.082	.348
<i>E.coli</i>	.213	-.067	<b>.698</b>
DNA	<b>.579</b>	<b>.483</b>	-.107
% of variance	28.2	24.5	14.6
% of cumulative	28.2	52.7	67.3

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 5 iterations.

The factor scores produce from PCA were then used to reveal the sediment quality data within seasonal difference (**Fig. 6.3**). The significant of sediment contents in open channel were identify in downstream channel during winter. While, the microbial indicator showed significant quality in along the open channels in spring when flow levels increased.

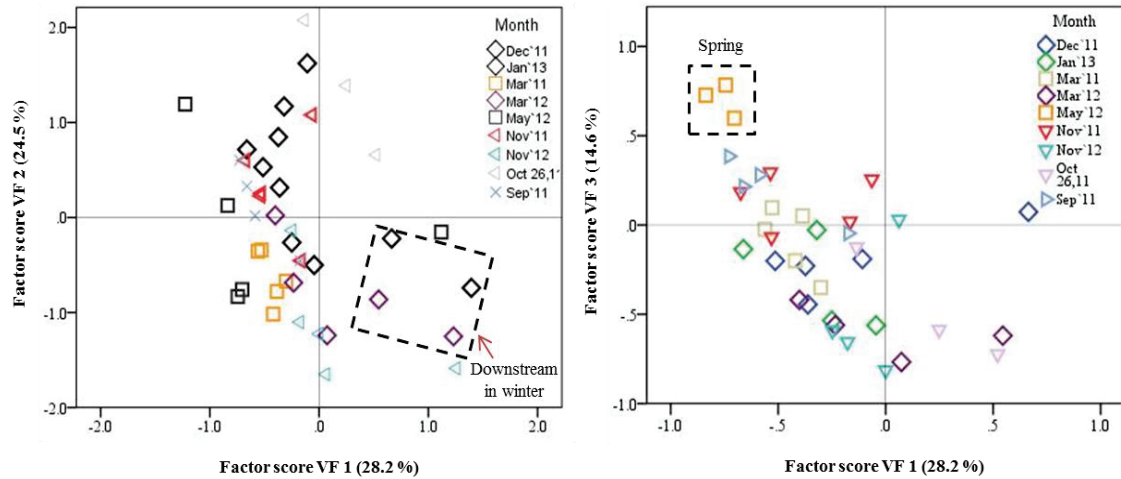


Fig. 6. 3 Factor score plots of sediment quality in open channels between VF 1 against VF 2 and VF 3.

#### 6.4 Summary

Statistical multivariate analyses were conducted to extract valuable information from data set of water and sediment quality and to classify the indices into groups of similar quality. Spatial variations of water and sediment quality in the open channels were grouped within three clusters, which the sites receiving effluent from johkasou possess different quality. The principle component analysis enables to group the indices of 21 water quality and nine sediment quality which can reflect the environmental condition. In the water, the first three dominant factors (anthropogenic contaminants, environmental factor, and fecal contaminants) indicate the influence of domestic wastewater that may change the water quality along the open channels. Sediment quality can be characterized by the factors of bacterial contents, the sediment quantity, and fecal contamination. The score factors of each component classified the water and sediment quality of open channels within seasons, which spring showed different water quality due to high flow levels, while different sediment quality was exhibited during winter.

## Chapter 7

### **Distribution and survival of microbial indicators in the sediment open channels receiving johkasou effluent**

#### **7.1 Background**

Onsite domestic systems have been stated as a potential source of fecal pollution in water environment of decentralized areas (Geary and Gardner, 1998, Ahmed *et al.*, 2005, Griffin *et al.*, 2003). Inappropriate performance of onsite domestic systems releases the nutrients, organic matter, fecal indicators, and pathogens in its treated effluents into the natural water bodies (Withers *et al.*, 2011). These contaminants can deposit on the sediment bed of stream water by associated with settleable particles. These conditions can increase the microbial number in sediment especially fecal contaminants, e.g., total coliform and *Escherichia coli*. The high number of fecal indicators in the water environment would often be linked with the presence of waterborne pathogen that has a significant impact on human health and quality life.

Besides effluents of onsite domestic systems, living organisms including fecal indicators in sediment bed can be an alternative source of microbial contaminants in decentralized environment. Sediment has been identified as a reservoir for *E. coli* based on the comparisons of bacterial concentration in sediment with concentration in the water column directly above the sediment layer. Several studies reported that sediments contained higher population of fecal coliform and *E. coli* than the overlying water. The survival rate of fecal indicators is increased when attached to the sediment particles (Davies *et al.*, 1995; Rahman and Soupir., 2009). Several factors are reported to be able influenced the survival of fecal indicators in sediment such as soluble organic matter



and nutrient (Jamieson *et al.*, 2005a,b), protection from the predators such as protozoa (Jamieson *et al.*, 2005a,b; Decamp and Warren, 2000), and shielding from exposure to UV sunlight (Koirala *et al.*, 2008).

The continuous discharge of effluents from onsite domestic treatment systems would potentially be the primary source of fecal contamination in the environment of decentralized area (Jamieson *et al.*, 2003). Previous study reported that high concentrations of total coliform and *E. coli* were recorded in both water and sediment of drainage channels receiving effluents from johkasou (Setiyawan *et al.*, 2014). This high concentration of fecal indicators could be observed although during high flow rate condition. The microbes associated sediment particles have an important role to transport and resuspend the microbial indicators associated the settleable particles (Jamieson *et al.*, 2004; Characklis *et al.*, 2005). Thus, high level of fecal indicators in sediment can contaminate the downstream water network by their disposition with settleable particles during growing seasons such as heavy rainfall and storm runoff.

Several studies had documented the distribution and transport of microbes in sewage systems of urban areas (Selvakumar and Borst, 2006; Bayram *et al.*, 2013) and in big natural water such as rivers and estuaries (Obiri-Danso and Jones, 1999; Halle *et al.*, 2009). Little is known, however, about the distribution of fecal indicators in sediment of stream channels of decentralized areas, which its effluents are constantly discharged. Stream channels of decentralized areas have different characteristics compared to natural rivers since the channels are artificial made by concrete material and low flow condition due to the main inputs of effluents from decentralized systems and underground water. Therefore, the objective of this study was to evaluate the distribution and survival of microbial indicators in sediment open channels of the local water environment receiving johkasou effluents. To achieve this goal, the assessments of microbial indicators (heterotrophic bacteria, total coliform, and *E. coli*) in several sediment spots along open channels were performed and their affinities with sediment particles were also measured.

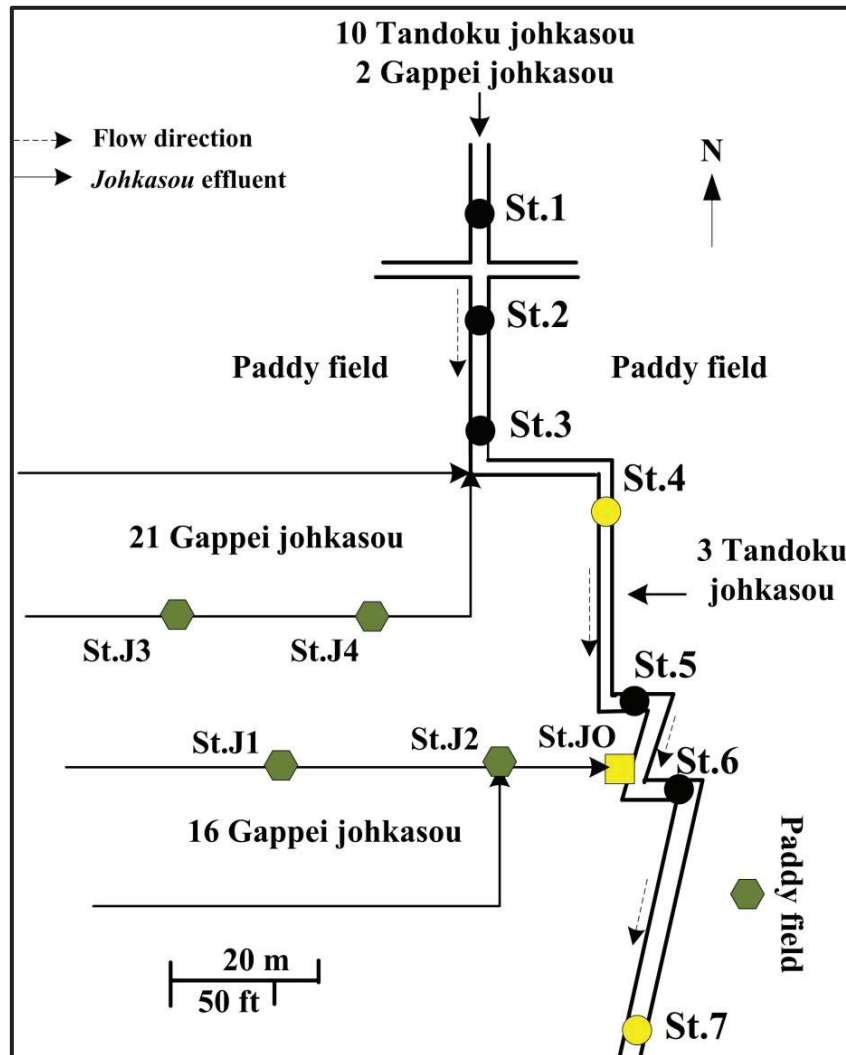
## 7.2 Material and methods

### 7.2.1 Site description

The study site is located in Gifu, Japan, near a residential area with the population around 250 inhabitants. A total of 52 household uses johkasou facility in this area (39 houses (75%) using gappei-shori johkasou and 13 households (25%) using tandoku-shori johkasou). Sediment and water samples were collected from seven sites (SP) along 1-m-wide open channels surrounding the residential area (St.1 – St.7). And, samples from johkasou drainage channel (St. JO) were also collected in which the effluents from 16 gappei johkasou facilities consistently discharged into open channel. Along open channels, several water inputs were recognized; effluent from 10 tandoku johkasou and 2 gappei johkasou facilities in upstream channel before St. 3; effluents of 21 gappei johkasou facilities and underground water between St.3 and St.4; and paddy field runoff during agricultural period from May to August; effluent from 16 gappei johkasou from St. JO. And, there are no other inputs along downstream channel (St.6 – St. 7) except a paddy field runoff. Additional soil/sediment samples from a paddy field, johkasou channels, and small river were also collected

### 7.2.2 Sediment characterization

*Samples collection.* Samples of water and sediments were collected in three times during fine weather: January 28<sup>th</sup>, 2013 and December 9, 2014 (winter), and June 11<sup>th</sup>, 2014 (spring) at several points along 200-m open channels that received effluents from johkasou facilities. Sediments were also collected from johkasou drainage channel and agricultural soils surrounding the open channels. The sediment mixed water was collected using a tube with an inner diameter of 30 cm for sites in the open channels, St.1 to St.7; a small tube with an inner diameter of 10 cm for site in johkasou drainage channel (St.JO); and syringe opened mouth for collecting agricultural soils. The mixed liquor from each site was then collected by placing the sampling tube on the sediment bed, mixing the sediment with the overlying water, and collecting the sediment mixed with overlying water in the tube. The sediment sample was then placed in new 250-ml polypropylene bottles and was then placed into cooler boxes prior transport to the laboratory. Sediment samples were store at 5 °C and analyzed on the day of collection.



**Fig. 7. 1** Distribution of sediment sites along open channel receiving johkasou effluents in a residential area.

*Particle size analysis.* Particle size distributions of sediment were classified using sieving method referred to the standard of The Japanese Geotechnical Society (JGS 0051-2000) with the fraction scales as follow; silt/clay : < 0.063 mm; fine sand : 0.063 – 0.250 mm; medium sand : 0.250 – 0.850 mm; and coarse sand : 0.850 – 2 mm. The representative of sediment samples from St. 4, St. JO, and St.7 were selected to know the sediment characteristics using the collected sediment sample of December 9, 2014. Briefly, 250 mL mixture of sediment and water placed on to sieving scales. The samples were stirred on each of sieve and the residue through the sieve was washed by using the

deionized water until the wash water runs clear. And prior the dry weight analysis, the residue was then placed on a large evaporation dish by carefully back-wash the sieve.

*Dry weight determination.* To determine the dry weight of sediment, a known weight of residues was placed in an oven at 105 °C for 24 h and weights the proportion of gravel, sand, and mud from the total weight. Then, dry residues were further used to determine the dry volatile solid content by placed in an oven at 600 °C for 30 min. The percent dry weight was then calculated (by difference) from these results.

### **7.2.3 Microbial enumeration**

*Sediment extraction.* Approximately 1 g well-mixed wet sediment was placed in 15 ml centrifuge tube and centrifuged at 2,500×g, 15 °C for 10 min to separate between remaining water and sediment. The bulk wet-sediment was then used to analyze the microbial content. For microbial analysis, the centrifuged sediment was mixed by adding 20 ml deionized sterile PBS water for 30 min, and then centrifuge at 4,000×g, 15 °C for 15 min (Davies *et al.*, 1995). The supernatant was then subjected to further microbial analyses listed below.

*Microbiological analysis.* The HPC bacteria, TC, and *E. coli* in sediment and water samples were enumerated based on the standards method (APHA, 2005). The HPC bacteria were determined by the plate count method using tryptone glucose yeast extract (TGYE) agar. Fecal indicator bacteria such as TC and *E. coli* were conducted by the multiple tube fermentation technique using colicatch reagents (ES Colicatch 1000, Eiken Chemical, Japan) within a series of 10-fold dilution, with three tubes per dilution.

## **7.3 Results and discussion**

### **7.3.1 Characteristic of sediment particles.**

Classifications of particles size in the sediments of open channels and johkasou drainage channel were summarized in Table 7.1. The particle size was relatively different between channels. Medium sand particles were the large fraction at St. 4 with the proportions of 40.7% among the particle sizes. Sediment particles at St. JO were categorized as almost same percentage for each fraction with the proportion of 21.3%

coarse sand, 23.3% medium sand, 31.3% fine sand, and 2.0% silt/clay. Sediment particles at St. 7 mainly consist of medium sand with the proportion of 28.5%. The proportion of mud was increased from upstream to downstream channel. This indicates the possible transfer of settleable particles to downstream, which might also transport the microbial indicator associated sediment particles.

**Table 7. 1** Classification of sediment particles in sediments using wet sieve method

	Coarse sand <sup>a</sup>	Medium sand <sup>b</sup>	Fine sand <sup>c</sup>	silt/clay <sup>d</sup>
<b>St. 4</b>				
Fraction (%)	7.5	40.7	29.8	10.5
Organic content (%)	7.0	6.2	5.2	4.4
Particle density (g/cm <sup>3</sup> )	0.006	0.012	0.010	0.015
<b>St. JO</b>				
Fraction (%)	21.3	23.3	31.3	2
Organic content (%)	4	4.7	9.2	7.3
Particle density (g/cm <sup>3</sup> )	0.006	0.015	0.002	0.005
<b>St. 7</b>				
Fraction (%)	19.2	28.5	12.7	13.3
Organic content (%)	9.5	8.3	7.5	5
Particle density (g/cm <sup>3</sup> )	0.003	0.010	0.009	0.009

a = 2 mm - 850  $\mu$ m, b = 850 - 250  $\mu$ m, c = 250 - 63  $\mu$ m, d = 63  $\mu$ m <

The each fraction of particles size was then extracted to measure the contents of HPC, TC and *E. coli* (Fig. 7.2). High contents of TC were found at fraction of silt/clay for most sites, whereas the *E. coli* contents were detected high level at fraction of clay/silt for all sites as well. However, significant difference in particles fraction for *E. coli* is not observed. The contents of HPC bacteria were observed at all of particle size fractions with the high content found at fractions of medium sand and silt/clay. The high contents of microbial indicators at fraction of silt/clay for most of sites may indicate that the microbial indicators can associate with very fine sediment particle that easy to be resuspended and transported into downstream areas. The high content of volatile solid at silt/clay may introduce as the organic sources for bacteria to growth.

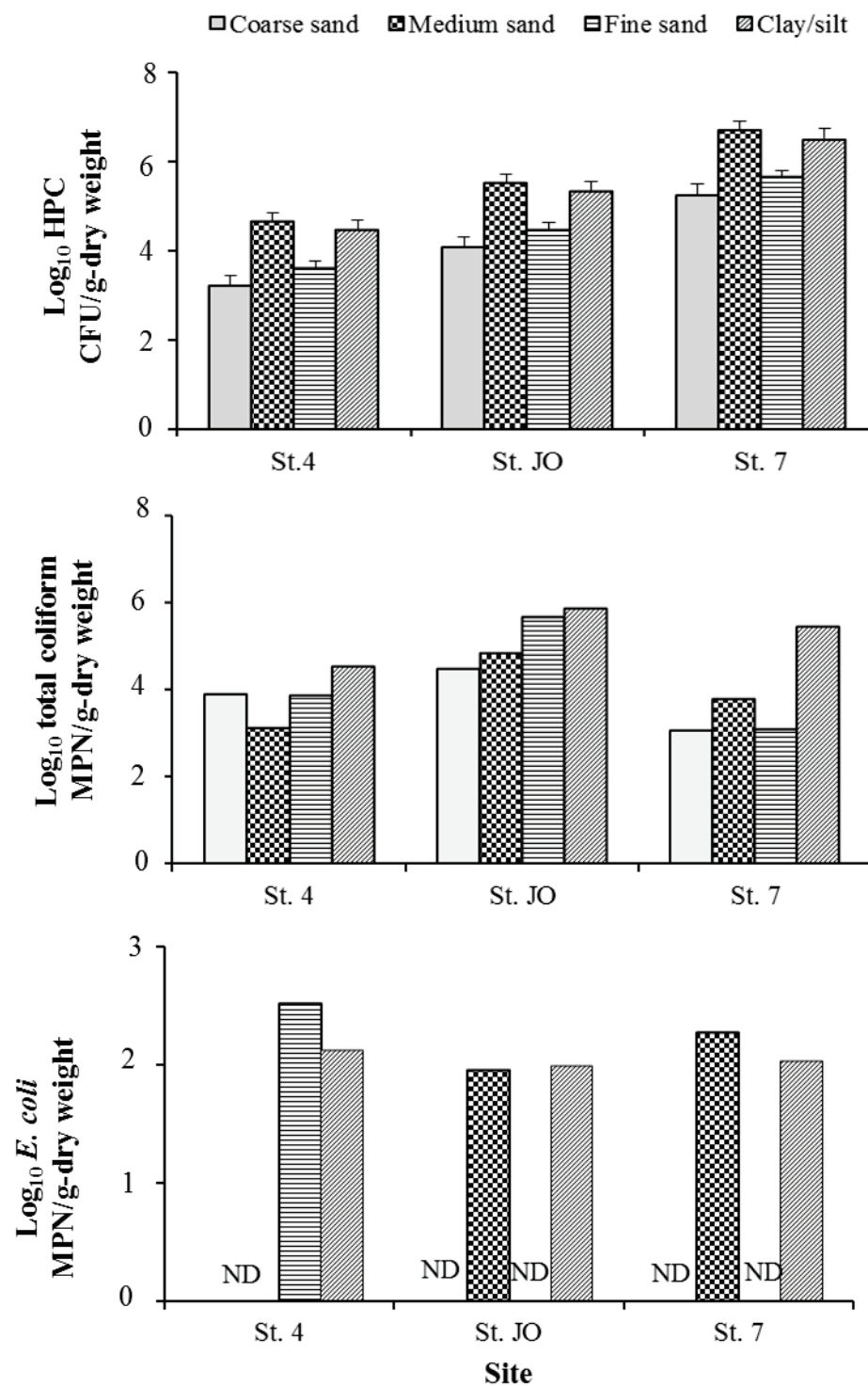


Fig. 7. 2 Microbial indicators associated with particle fractions. ND means not detected.

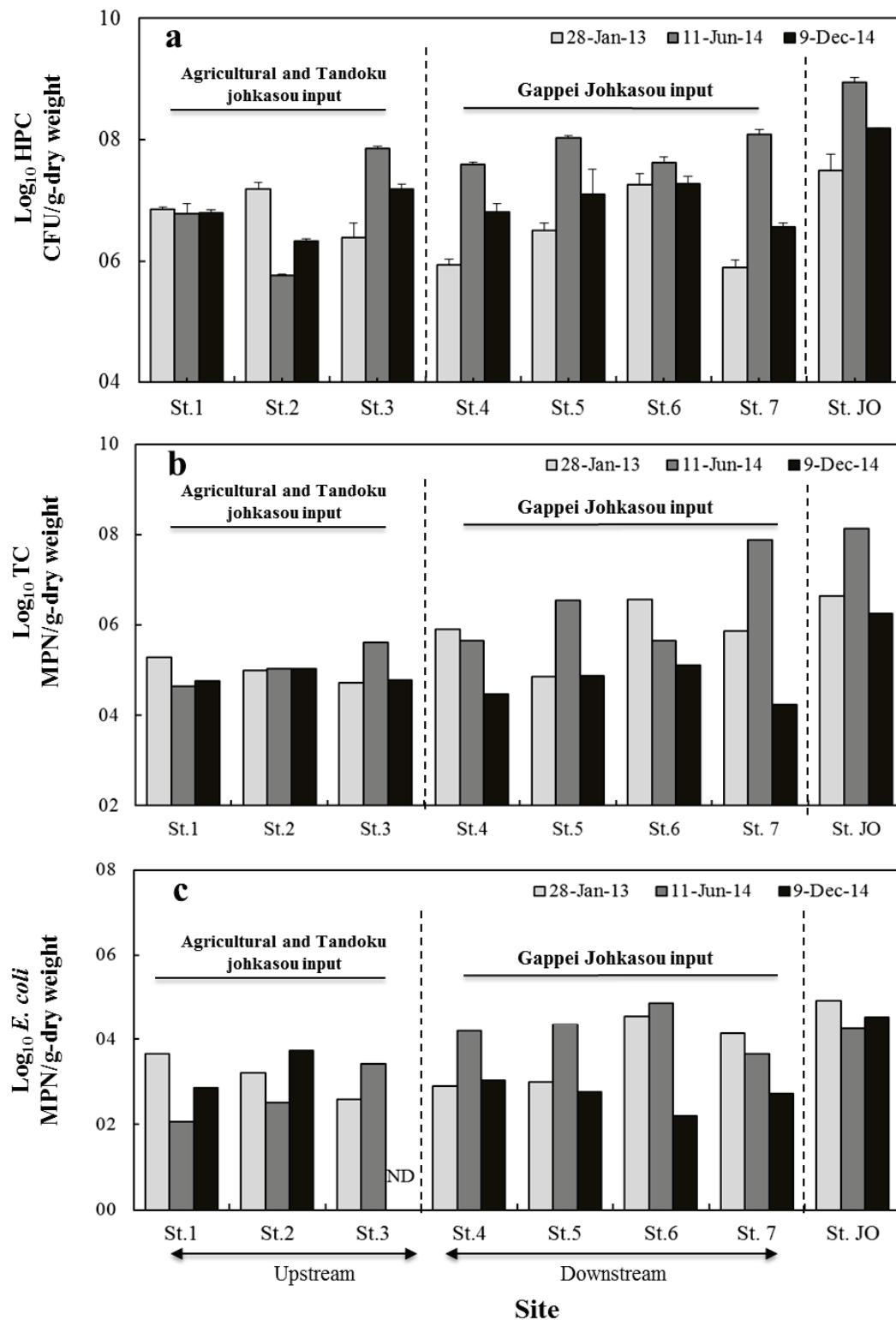


Fig. 7. 3 Distribution of microbial indicators: a) HPC, b) TC, and c) *E. coli* in sediments along open channels and johkasou drainage channel for sampling in January 28<sup>th</sup>, 2013 (winter), June 11<sup>th</sup>, 2014 (spring), and December 9<sup>th</sup>, 2014 (autumn).

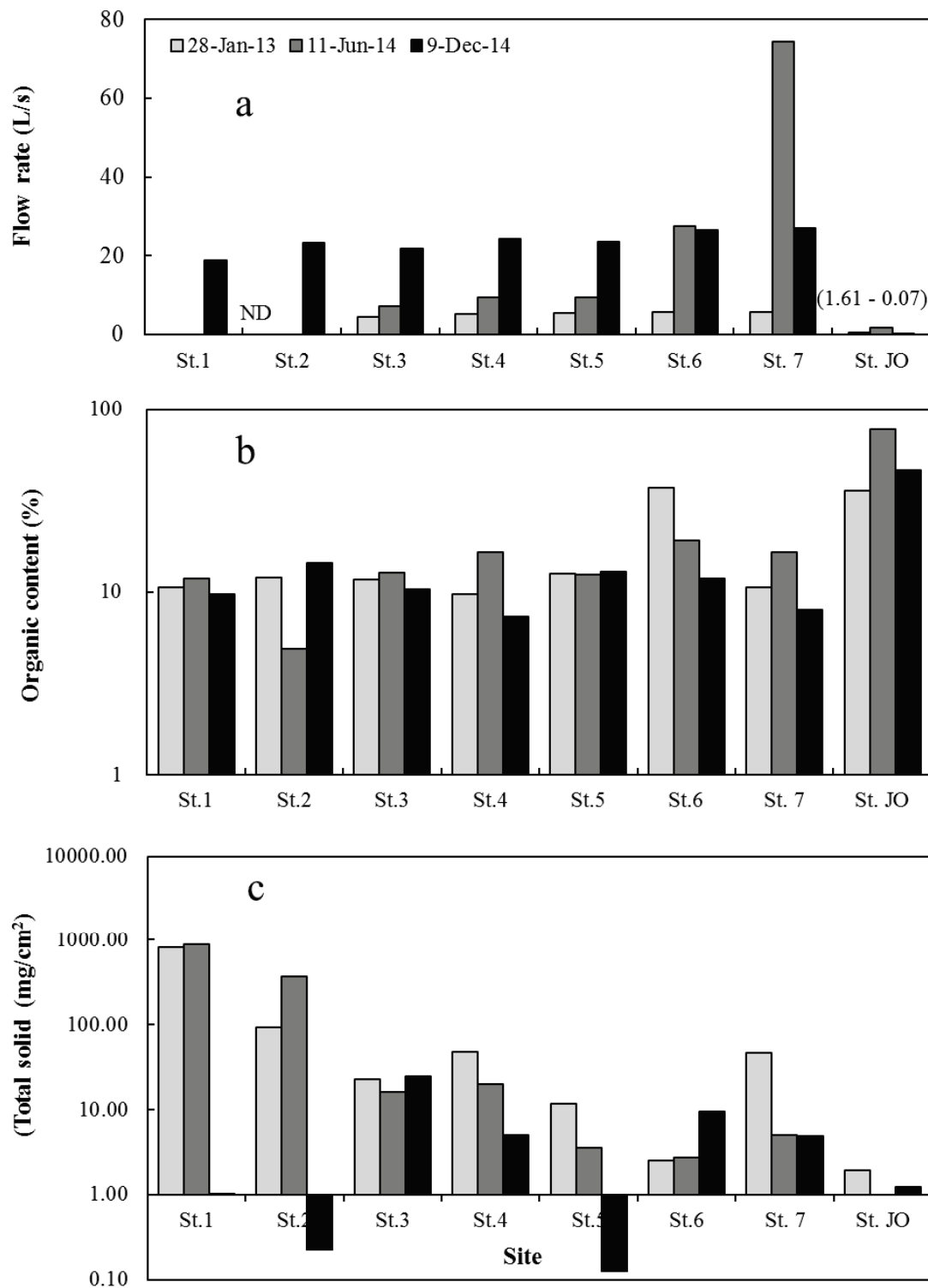


Fig. 7. 4 Flow rate (a), organic content (b) and total solid (c) along the open channels and the johkasou drainage channel of three sampling collections.



### 7.3.2 Distribution of microbial indicator in sediments

As explained in Chapter 5, the concentrations of microbial indicators not significantly varied along the open channel. Therefore, in this chapter, further analysis on distributions of microbial indicators in sediment of open channels was conducted. Distributions of microbial indicators related to HPC, TC, and *E. coli* in sediments of open channels and *johkasou* drainage channel (St. JO) are shown in **Fig. 7.3**. In this figures, the microbial distributions were measured during winter (January 28<sup>th</sup>, 2013), spring (June 11<sup>th</sup>, 2014), and autumn (December 9<sup>th</sup>, 2014) with significantly different in flow rate.

The contents of microbial indicators in sediments were higher in the *johkasou* drainage channel compared to its contents at other sampling sites in the open channels. Statistically significant difference among the sites in the open channels was not observed for microbial contents in the sediment. The mean contents of HPC, TC, and *E. coli* in the *johkasou* drainage channel were  $3.5 \times 10^8$  CFU/g,  $4.8 \times 10^7$  MPN/g, and  $4.6 \times 10^4$  MPN/g, respectively. The mean contents of HPC, TC, and *E. coli* in sediment of open channels were around  $2.3 \times 10^7$  CFU/g,  $4.2 \times 10^6$  MPN/g, and  $9.1 \times 10^3$  MPN/g, respectively. The high microbial content in sediments of both *johkasou* drainage channel and open channels might be sources of the microbial contaminant to the overlying water. By high organic content in sediment of *johkasou* drainage channel rather than other open channel sampling sites (Fig. 7. 4-b), this could be utilized for bacteria to grow and survive in this channel.

Along the open channels, the sediment sampling sites could be divided into two areas. The upstream area is an area that received effluent from 10 tandoku *johkasou* facilities and agriculture input for St.1 to St.3, and downstream area is an area that received mostly effluent from 37 gappei *johkasou* facilities mixed with ground water from St.4 to St.7 (Fig. 7.3). The water in downstream area is the continuing flow water from upstream area. The sediment downstream area contained higher microbial indicators rather than in the upstream area. This indicated that the number of *johkasou* facilities could also contribute to affect the content of environmental sediment of stream water. The increasing number of *johkasou* user will raise the load of *johkasou* effluent that

carried several contaminants. These contaminants can settle down and deposit on downstream sediment by hydrological function.

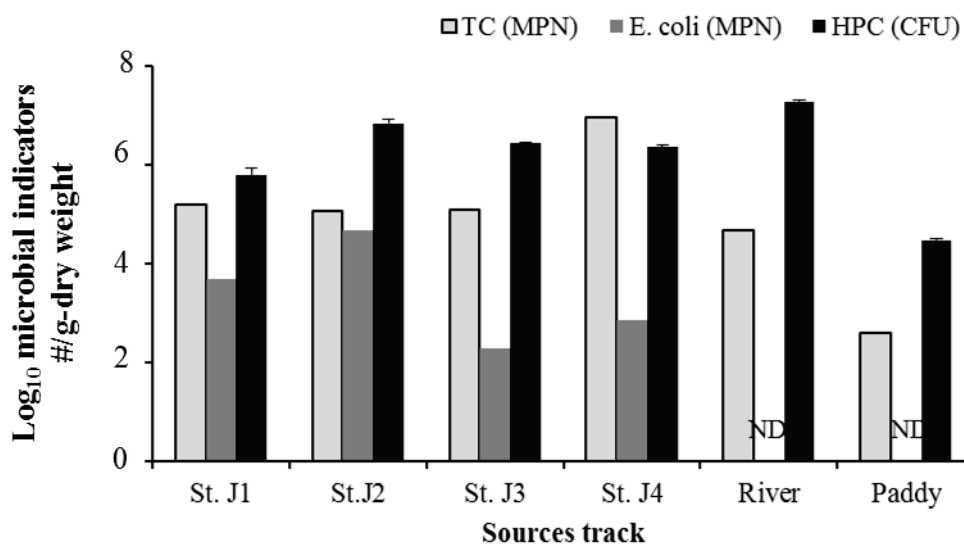
Distributions of HPC, TC, and *E. coli* in sediments were fluctuated in seasons along the open channels. The content of microbial indicator was relatively high during spring compared to winter and autumn. The mean contents of HPC, TC, and *E. coli* in sediments along the open channels ranged from  $9.9 \times 10^6$  CFU/g,  $1.2 \times 10^6$  MPN/g, and  $1.8 \times 10^4$  MPN/g, respectively in winter; from  $2.5 \times 10^7$  CFU/g,  $2.8 \times 10^5$  MPN/g, and  $5.4 \times 10^3$  MPN/g, respectively in autumn; and, from  $1.6 \times 10^8$  CFU/g,  $2.8 \times 10^7$  MPN/g, and  $1.8 \times 10^4$  MPN/g, respectively in spring.

The high content of TC and *E. coli* downstream during spring could be explained by the seasonal flow water and the proportion of organic content in the open channels. The flow rate during spring was observed higher than in other seasons (**Fig. 7.4a**) and the proportion of organic content in downstream channel that related to fine sediment particles was identified higher rather in upstream channel (**Fig. 7.4b**). This indicates that the sediment particles associated microbial indicators were transported into downstream channel through hydrological events. Several studies reported that high flow event is an important role to carry microbial associated settleable particles and resuspended downstream receiving water. Bacterial indicator organisms exhibited relatively same behavior, with an average of 20 – 30 % of organisms associated with the particles in dry weather and 30 – 55 % in stormweather (Characklis *et al.*, 2005). Krometis *et al.* (2007) documented that higher concentrations of both settleable particles and microbes entering the water column soon after the onset of a storm led to higher loading rates of settleable microbes. This higher removal of particles-associated microbes relative to the total microbial concentration suggests that sedimentation may be an important removal mechanism of microbes in sediment.

### **7.3.3 The source of fecal indicators in sediment of decentralized area**

The contents of microbial indicators at different areas were also measured to know the possible source of microorganisms in the open channels receiving johkasou effluent and also to compare with the microbial contents from other areas. The sediment samples

from johkasou drainage channels (St. J1-J4), a small river that flow to main open channel (river) and paddy field were collected and the microbial contents from each source were displayed in **Fig. 7.5**. The microbial indicators from paddy field was significantly different by the lower contents compared to other sources. The contents of HPC and TC showed similar levels in johkasou drainage channels and river. However, *E. coli* contents were only detected in the johkasou drainage channels. This indicates that the effluent from the johkasou might possible be a source for fecal contaminant compared to other sources in this area. Therefore, the improvement of johkasou performance plays an important key to protect the environmental water quality before the effluent discharged into the local environmental water.

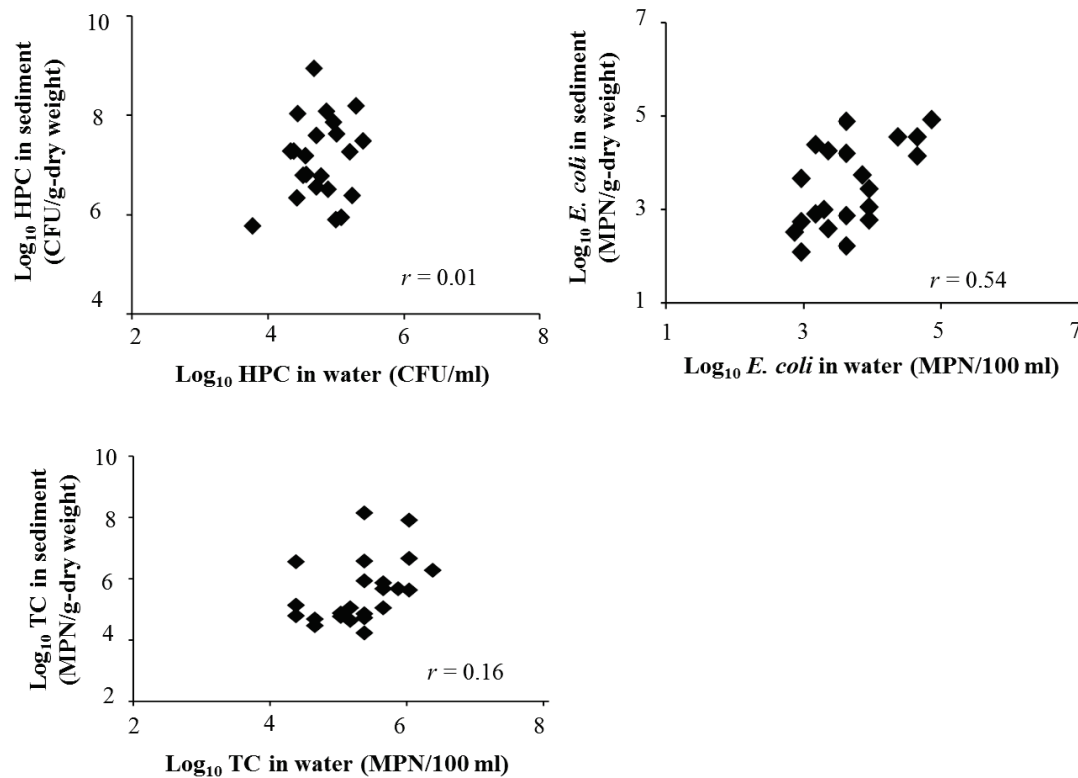


**Fig. 7.5** Microbial contents at channels of johkasou (J1-J4), river, and paddy field for one time sampling on December 9, 2014.

#### 7.3.4 Relation of microbial indicator between water and sediment

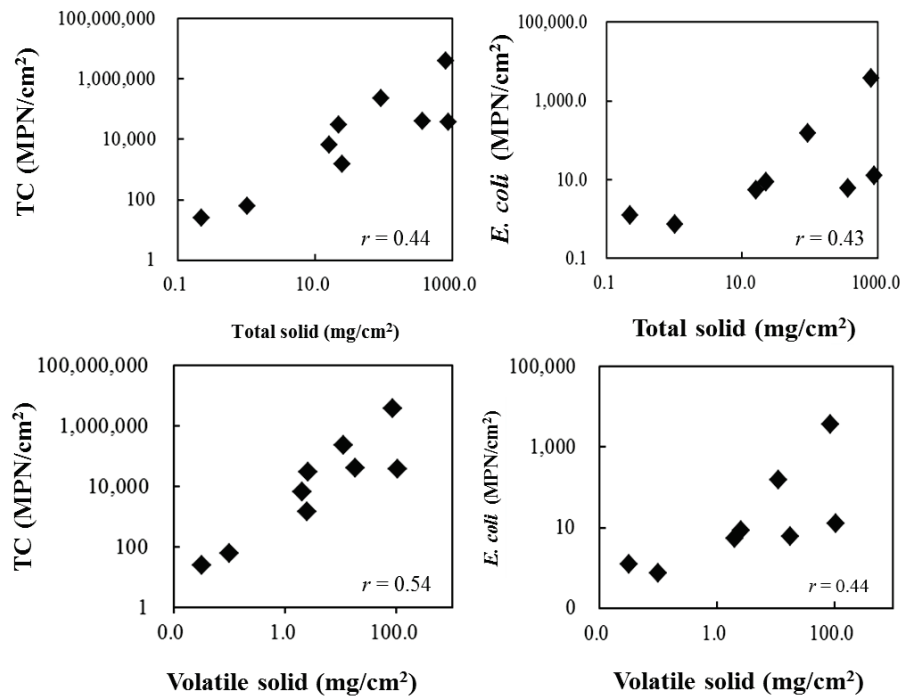
The association of microorganisms between water and sediment was evaluated to determine the possible interaction of microorganisms in water with microorganisms in sediments along open channels receiving johkasou effluent. **Figure 7.6** shows the correlations of HPC, TC, and *E. coli* in water with those in sediments. A weak and

positive correlation was obtained between concentrations of HPC and TC in water with HPC and TC in sediments ( $r = 0.01$  and  $r = 0.16$ ), respectively. This indicates that the concentrations of HPC and TC were not interacted between water and sediments in the open channels receiving *johkasou* effluent.

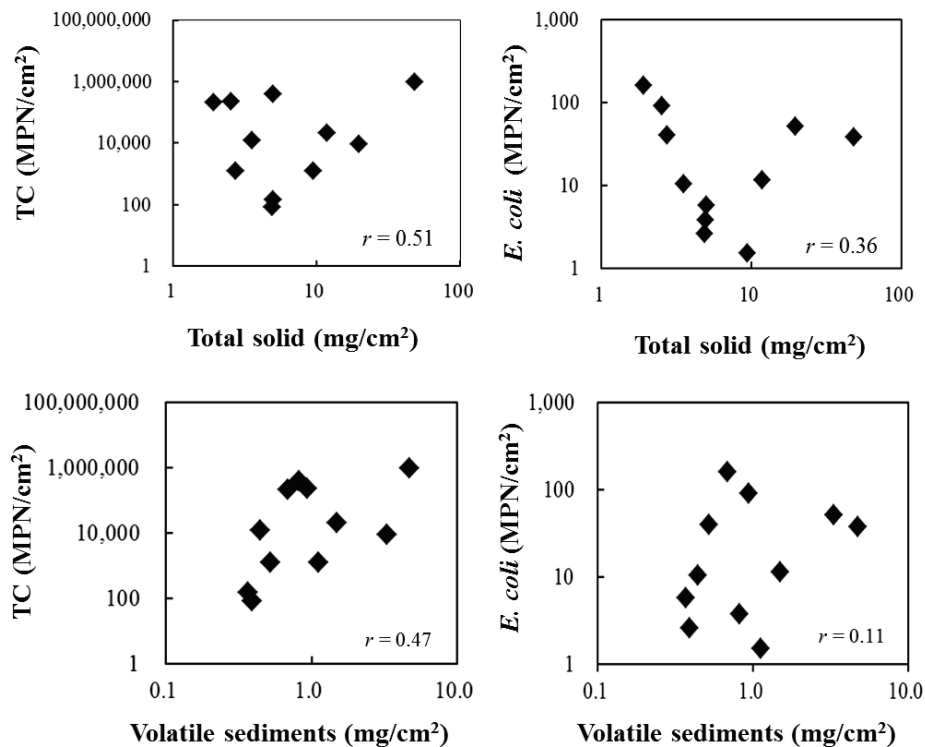


**Fig. 7. 6** Relationships of HPC, TC, and *E. coli* between water and sediment

*E. coli* concentrations in water were positively moderate correlated with content of *E. coli* in sediments ( $r = 0.54$ ) in the open channels. Although in moderate correlation, this suggest that the *E. coli* content in sediment may interact with that in the overlying water. The high *E. coli* contents in sediment may reflect the concentrations of *E. coli* in water by the resuspension during hydrological events.



**Fig. 7. 7** Correlations of fecal indicators with solid contents in upstream sediment that received inputs from agricultural and tandoku johkasou (St. 1 ~ St. 3).



**Fig. 7. 8** Correlations of fecal indicators with solid contents in downstream sediment that mostly received johkasou effluent (St. 4 ~ St. 7).

### 7.3.5 Relations between microbial indicators and sediment contents

Correlations of microbial indicators and solid contents were evaluated to identify the behavior of microbes associated sediment particles. The relations TC and *E. coli* with total solid (TS) and volatile solid (VS) in sediment of upstream channel and downstream channel are displayed in **Fig. 7.7** and **Fig. 7.8**, respectively. The contents of TC and *E. coli* in upstream channel have positive and moderate correlations with TS and VS.

HPC densities were strongly correlated to solid sediments and volatile sediments ( $r = 0.64$  and  $r = 0.67$ , respectively). While, the fecal indicators, TC and *E. coli*, were moderately correlated with solid sediments ( $r = 0.45$  and  $r = 0.47$ , respectively) and volatile sediments ( $r = 0.47$  and  $r = 0.52$ ), respectively. These positive correlations indicates that the microbial indicators were associated with sediment particles that could be carrier, especially for volatile sediments that part of fine particles, to export the microbes to downstream water network. Another study noted that sediment particles of around 20 – 30 % associated to bacterial indicator organisms during dry weather (Characklis *et al.*, 2005). Therefore, furthermore sediment analysis is required to reveal the behavior microbial indicators associated particles sediments that might be a key role on microbial sedimentation during storm events.

## 7.4 Summary

Distribution and survival of microbial indicators in sediments of open channels and johkasou drainage channel were evaluated during three different weathers. Distribution of microbial indicators in sediment of downstream channel showed at higher contents compared to upstream channel. This indicated that the contents of microbial indicators were affected by contribution of source impact in which low flow rate condition can settle down the microbes attaching to settleable particles. TC and *E. coli* contents downstream exhibited increasing trends during spring, which is affected by increasing flow rate level that can carry the fine particles associated microbes. Positive correlations of most microbial indicators with total solid and volatile solid could suggest the essential of sediments as carrier to transport sediment particles associated microbes to downstream receiving waters.

## Chapter 8

### Conclusions

Comprehensive evaluations of physicochemical and microbial parameters were conducted to determine the influence of johkasou effluent in the water and sediment of open channels through 3-year study period. The physicochemical parameters (16 indices) and microbial indicators (HPC, TC, *E. coli*, and DNA-total bacteria) were measured in water sample. The sediment contents including microbial indicators, total solid, volatile solid were also evaluated in this study.

Evaluation of physicochemical parameters in water along the open channels and in the johkasou effluent was performed. The concentrations of organic matter (DOC, BOD, and COD) and nutrient contents (TN, TP, NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, and PO<sub>4</sub>-P) in the johkasou effluents were significantly different compared to those in the open channels. However, the physicochemical concentrations were similar among the open channel sampling sites. Significant variations of DOC, BOD, COD, TN, TP, NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, and PO<sub>4</sub>-P in seasons were recorded along the open channel receiving johkasou effluent. Those parameters were significantly different during winter compared to other seasons, which is coincided with the low flow rate levels. This indicates that low dilution ratio during winter may cause water degradation after receiving johkasou effluent. Based on this investigation, the local environmental water quality in downstream areas requires different treatment in each season. The flow rate level in the open channels has a key role to maintain the water quality after receiving johkasou effluent particularly in winter.

Compared to physicochemical parameters, microbial indicators in water and sediment of open channels receiving johkasou effluents were recorded at high concentrations especially for total coliform that exceeded the environmental quality standard. The microbial indicators in johkasou effluents were up to two orders magnitude higher

compared to the open channels for both water and sediments. Seasonal variation revealed significant difference in *E. coli* concentrations during winter in both johkasou effluent and open channel. These results indicates that johkasou effluent could be a source of microbial contamination in local water environment, which its influenced was more visible during low temperature and low dilution factor.

Based on the results of microbial contents in sediment in the previous work, furthermore distribution and survival of microbial indicators along open channel receiving johkasou effluent were investigated. The distribution of microbial indicators in downstream sediment that received mostly johkasou effluent was higher level compared to upstream channel that has less johkasou facilities. This indicated that the contents of microbial indicators in sediment could be affected by contribution of source impact. In addition, the contents of microbial indicators were moderately positive correlations to the volatile solid. These results indicate that the high flow level in the open channels may carry the microbes associated with fine organic particles to the downstream channel. Regarding to results in this investigation, further study on re-suspension or transport mechanisms of microbial contents in sediment during storm weather will be focused.

Statistical multivariate analyses were conducted to extract valuable information from data set of water and sediment quality, and to classify the indices into groups of similar quality. Spatial variations of water and sediment quality in the open channels were grouped within three clusters, which the sites receiving effluent from johkasou possess different quality. Principal component analysis (PCA) enables to group the indices in water and sediment quality into several factors that can reflect the local water environment after receiving johkasou effluent. The score factors of each component revealed that the water quality along the open channels could be affected by seasonal variation but less effect of season was observed for sediment quality.

The findings obtained from these studies exhibited that johkasou effluents have contribution on microbial contamination rather than the physicochemical parameters in the open channels. The high contents of microbial indicators that distributed in sediments along the open channels could suggest as a reservoir of microbes in overlying



water and might possible be a non-point source of microbial pollution in downstream water during hydrological events. Therefore, the water quality control in local environment should be considered in each of seasons in which seasonal flow rate level has an important factor as dilution function to reduce the effect of johkasou. Moreover, the maintenance and improvement on johkasou performance can lead less contribution of contamination into local water environment.

## References

- Adeyemo, O.K., Adedokun, O.A., Yusuf, R.K., Adeleye, E.A., 2008. Seasonal Changes in Physico-Chemical Parameters and Nutrient Load of River Sediments in Ibadan City, Nigeria. *Global Nest J.* **10**, 326-336.
- Ahmed, A. U., and D. L. Sorensen.1995. Kinetics of pathogen destruction during storage of dewatered biosolids. *Water Environ Res* **67**, 143–150.
- APHA-AWWA-WEF, 2005. Standard methods for the examination of water and wastewater, 21st ed. APHA, New York, USA.
- Badgley, B. D., Thomas, F. I. M., & Harwood, V. J. (2011). Quantifying environmental reservoirs of fecal indicator bacteria associated with sediment and submerged aquatic vegetation. *Environmental Microbiology*, *13*(4), 932-942.
- Bai, S., & Lung, W. S. (2005). Modeling sediment impact on the transport of fecal bacteria. *Water Research*, *39*(20), 5232-5240.
- Benami, M., Gross, A., Herzberg, M., Orlofsky, E., Vonshak, A., & Gillor, O. (2013). Assessment of pathogenic bacteria in treated graywater and irrigated soils. *Science of the Total Environment*, *458*, 298-302.
- Blott, S. J., & Pye, K. (2012). Particle size scales and classification of sediment types based on particle size distributions: Review and recommended procedures. *Sedimentology*, *59*(7), 2071-2096.
- Carroll, S., Hargreaves, M., & Goonetilleke, A. (2005). Sourcing faecal pollution from onsite wastewater treatment systems in surface waters using antibiotic resistance analysis. *Journal of Applied Microbiology*, *99*(3), 471-482.
- Carroll, S., Thomas, E., Hargreaves, M., Frost, R., and Dawes, L. (2006). Integrated risk framework for onsite wastewater treatment systems. *Environmental Management*, *38*(2), 286-303.
- Characklis, G. W., Dilts, M. J., Simmons, O. D., Likirdopulos, C. A., Krometis, L. A. H., & Sobsey, M. D. (2005). Microbial partitioning to settleable particles in stormwater. *Water Research*, *39*(9), 1773-1782.

- Davies, C. M., Long, J. A. H., Donald, M., & Ashbolt, N. J. (1995). Survival of Fecal Microorganisms in Marine and Fresh-Water Sediments. *Applied and Environmental Microbiology*, 61(5), 1888-1896.
- Droppo, I. G., Liss, S. N., Williams, D., Nelson, T., Jaskot, C., & Trapp, B. (2009). Dynamic Existence of Waterborne Pathogens within River Sediment Compartments. Implications for Water Quality Regulatory Affairs. *Environmental Science & Technology*, 43(6), 1737-1743.
- Ebie, Y., et al. (2008). Recovery oriented phosphorus adsorption process in decentralized advanced Johkasou. *Water Science and Technology*, 57(12), 1977-1981.
- Ebie, Y., Matsumura, M., Noda, N., Tsuneda, S., Hirata, A., & Inamori, Y. (2002). Community analysis of nitrifying bacteria in an advanced and compact Gappel-Johkasou by FISH and PCR-DGGE. *Water Science and Technology*, 46(11-12), 105-111.
- Eriksson, Eva., Auffarth, K., Henze, M., & Ledin, A. (2002). Characteristics of gray water. *Urban Water*, 4, 85-104.
- Fey, A., Eichler, S., Flavier, S., Christen, R., Hofle, M. G., & Guzman, C. A. (2004). Establishment of a real-time PCR-based approach for accurate quantification of bacterial RNA targets in water, using Salmonella as a model organism. *Applied and Environmental Microbiology*, 70(6), 3618-3623.
- Flint, K. P. (1987). The Long-Term Survival of Escherichia-Coli in River Water. *Journal of Applied Bacteriology*, 63(3), 261-270.
- Fries, J. S., Characklis, G. W., & Noble, R. T. (2006). Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, NC. *Journal of Environmental Engineering-Asce*, 132(10), 1338-1345.
- Fujimura, Y., and Nakajima, J. (1998). Effluent water quality of small scale on-site treatment facilities for household waste-water and nitrogen removal performance with recycle operation. *Japan Society on Water Environment*, 21, 157.
- Garzio-Hadzick, A., Shelton, D. R., Hill, R. L., Pachepsky, Y. A., Guber, A. K., & Rowland, R. (2010). Survival of manure-borne E. coli in streambed sediment: Effects of temperature and sediment properties. *Water Research*, 44(9), 2753-2762.

- Gaulke, L. S. (2006). On-site wastewater treatment and reuses in Japan. *Proceedings of the Institution of Civil Engineers-Water Management*, 159(2), 103-109.
- Griffin, D. W., Gibson III, C.J., Lipp, E.K., Riley, K., Poul, J.H., & Rose, J.B. (1999). Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of Florida Keys. *Applied and Environmental Microbiology*, 65, 4118-4125.
- Habteselassie, M.Y. , Kirs, M., Conn, K.E. , Blackwood, K.E., Kelly, G., & Noble, R.T. (2011). Tracking microbial transport through four onsite wastewater treatment systems to receiving waters in eastern North California. *Applied Microbiology*, 111, 835-847.
- Harris, P. J. (1995). Water quality impacts from on-site waste disposal systems to coastal areas through groundwater discharge. *Environmental Geology*, 26(4), 262-268.
- Helard, D., Fajri, J.A., Setiyawan, A.S., Li, F., Yamada, T., & Horio, A. (2012). Formation and role of microbial community in the sediment bed of open channel receiving Johkasous effluent; Multivariate statistical analysis interpretation. *Japan Society of Civil Engineering*.
- Helard, D., Fajri, J.A., Setiyawan, A.S., Li, F., Yamada, T., & Horio, A. (2012). Formation and role of microbial community in the sediment bed of open channel receiving Johkasous effluent; Multivariate statistical analysis interpretation. *Japan Society of Civil Engineering*.
- Ichinari, T., et al. (2008). Wastewater treatment performance and sludge reduction properties of a household wastewater treatment system combined with an aerobic sludge digestion unit. *Process Biochemistry*, 43(7), 722-728.
- Ishii, S., & Sadowsky, M. J. (2008). *Escherichia coli* in the environment: Implications for water quality and human health. *Microbes and Environments*, 23(2), 101-108.
- Jamieson, R. C., Gordon, R. J., Tattrie, S. C., & Stratton, G. W. (2003). Sources and persistence of fecal coliform bacteria in a rural watershed. *Water Quality Research Journal of Canada*, 38(1), 33-47.
- Jamieson, R. C., Joy, D. M., Lee, H., Kostaschuk, R., & Gordon, R. J. (2005). Resuspension of sediment-associated *Escherichia coli* in a natural stream. *Journal of Environmental Quality*, 34(2), 581-589.

- Jamieson, R., Gordon, R., Joy, D., & Lee, H. (2004). Assessing microbial pollution of rural surface waters - A review of current watershed scale modeling approaches. *Agricultural Water Management*, 70(1), 1-17.
- Jamieson, R., Joy, D. M., Lee, H., Kostaschuk, R., & Gordon, R. (2005). Transport and deposition of sediment-associated *Escherichia coli* in natural streams. *Water Research*, 39(12), 2665-2675.
- Jarvie, H. P., Neal, C., & Withers, P. J. A. (2006). Sewage-effluent phosphorus: A greater risk to river eutrophication than agricultural phosphorus? *Science of the Total Environment*, 360(1-3), 246-253.
- JECES. (2009). Japan Education Center of Environment Sanitation. <http://www.jeces.or.jp/en/database/index.html>,
- Kaneko, M. (1997). Virus removal by the domestic wastewater treatment system named Johkasou. *Water Science and Technology*, 35(11-12), 187-191.
- Kaneko, M., Nambu, T., & Tokoro, M. (2001). Behaviour of pathogenic E-coli and *Salmonella enteritidis* in small domestic sewage treatment apparatus ("Johkasou"). *Water Science and Technology*, 43(12), 191-193.
- Kim, G., & Hur, J. (2010). Mortality rates of pathogen indicator microorganisms discharged from point and non-point sources in an urban area. *Journal of Environmental Sciences-China*, 22(6), 929-933.
- Koirala, S. R., Gentry, R. W., Perfect, E., Schwartz, J. S., & Sayler, G. S. (2008). Temporal variation and persistence of bacteria in streams. *Journal of Environmental Quality*, 37(4), 1559-1566.
- Kolarevic, S., Knezevic-Vukcevic, J., Paunovic, M., Vasiljevic, B., Kracun, M., Gacic, Z., Vukovic-Gacic, B., 2012. Seasonal Variations of Microbiological Parameters of Water Quality of the Velika Morava River Serbia. *Arch Biol Sci* **64**, 1017-1027.
- Krometis, L. A. H., Characklis, G. W., Simmons, O. D., Dilts, M. J., Likirdopulos, C. A., & Sobsey, M. D. (2007). Intra-storm variability in microbial partitioning and microbial loading rates. *Water Research*, 41(2), 506-516.
- Lipp, E. K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. R., & Rose, J. B. (2001). The effects of seasonal variability and weather on microbial fecal

- pollution and enteric pathogens in a subtropical estuary. *Estuaries*, 24(2), 266-276.
- Magara, Y. (2003). Status of onsite-treatment of domestic wastewater management in Japan. *3rd World Water Forum- Proceedings of Johkassou Session, JECES(Kyoto)*, 1-6.
- Mandal, U. K., et al. (2008). Evaluating impact of irrigation water quality on a calcareous clay soil using principal component analysis. *Geoderma*, 144(1-2), 189-197.
- Ministry of the Environment Japan. Environmental Quality Standards for the conservation of the living environment.  
<http://www.env.go.jp/en/water/wq/wp.pdf>
- Ministry of the Environment, About the status of johkasou installation at the end of fiscal 2012, Accessed on April 20, 2014: <https://www.env.go.jp/en/headline>
- Nakajima, J., Fujimura, Y., & Inamori, Y. (1999). Performance evaluation of on-site treatment facilities for wastewater from households, hotels and restaurants. *Water Science and Technology*, 39(8), 85-92.
- Nambu, T., A., Horio., Asano, H., & Aoki, T. (1996). Disinfection performance of effluent from small wastewater treatment plant. *Japanese society of water treatment biology*, 32(3), 173-178.
- Neal, C., Jarvie, H. P., Withers, P. J. A., Whitton, B. A., & Neal, M. (2010). The strategic significance of wastewater sources to pollutant phosphorus levels in English rivers and to environmental management for rural, agricultural and urban catchments. *Science of the Total Environment*, 408(7), 1485-1500.
- Ohmori, H., Yahashi, T., Furukawa, Y., Kawamura, K., Yamamoto, Y., 2000. Treatment performance of newly developed johkasous with membrane separation. *Water Sci Technol* **41**, 197-207.
- Ottoson, J., & Stenstrom, T. A. (2003). Faecal contamination of greywater and associated microbial risks. *Water Research*, 37(3), 645-655.
- Ouyang, Y. (2005). Evaluation of river water quality monitoring stations by principal component analysis. *Water Research*, 39(12), 2621-2635.

- Papadopoulos, F. H., Tsihrintzis, V. A., & Zdragas, A. G. (2011). Removal of faecal bacteria from septage by treating it in a full-scale duckweed-covered pond system. *Journal of Environmental Management*, 92(12), 3130-3135.
- Pote, J., Haller, L., Kottelat, R., Sastre, V., Arpagaus, P., & Wildi, W. (2009). Persistence and growth of faecal culturable bacterial indicators in water column and sediments of Vidy Bay, Lake Geneva, Switzerland. *Journal of Environmental Sciences-China*, 21(1), 62-69.
- Pundsack, J., Axler, R., Hicks, R., Henneck, J., Nordman, D., & McCarthy, B. (2001). Seasonal pathogen removal by alternative on-site wastewater treatment systems. *Water Environment Research*, 73(2), 204-212.
- Ryan (199). Ryan versus Great Lakes Council, Federal Court of Australia, 177.
- Rehmann, C. R., & Soupir, M. L. (2009). Importance of interactions between the water column and the sediment for microbial concentrations in streams. *Water Research*, 43(18), 4579-4589.
- Reinoso, R., Torres, L. A., & Becares, E. (2008). Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater. *Science of the Total Environment*, 395(2-3), 80-86.
- Revitt, D. M., Eriksson, E., & Donner, E. (2011). The implications of household greywater treatment and reuse for municipal wastewater flows and micropollutant loads. *Water Research*, 45(4), 1549-1560.
- Savichtcheva, O., & Okabe, S. (2006). Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, 40(13), 2463-2476.
- Savichtcheva, O., Okayama, N., & Okabe, S. (2007). Relationship between Bacteroides 16s-rRNA genetic markers and presence of bacterial enteric pathogens and conventional fecal indicators. *Water Research* 41, 3615-3628.
- Sasajima, Y., Yoshimura, C., & Li, F. (2008). Relationship between land used and coliphage concentration in Rivers- A case study in Gifu City. *Japan Society of Civil Engineering*, 45, 379-387.
- Selvakumar, A., Borst, M., 2006. Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *J Water Health* 4, 109-124.

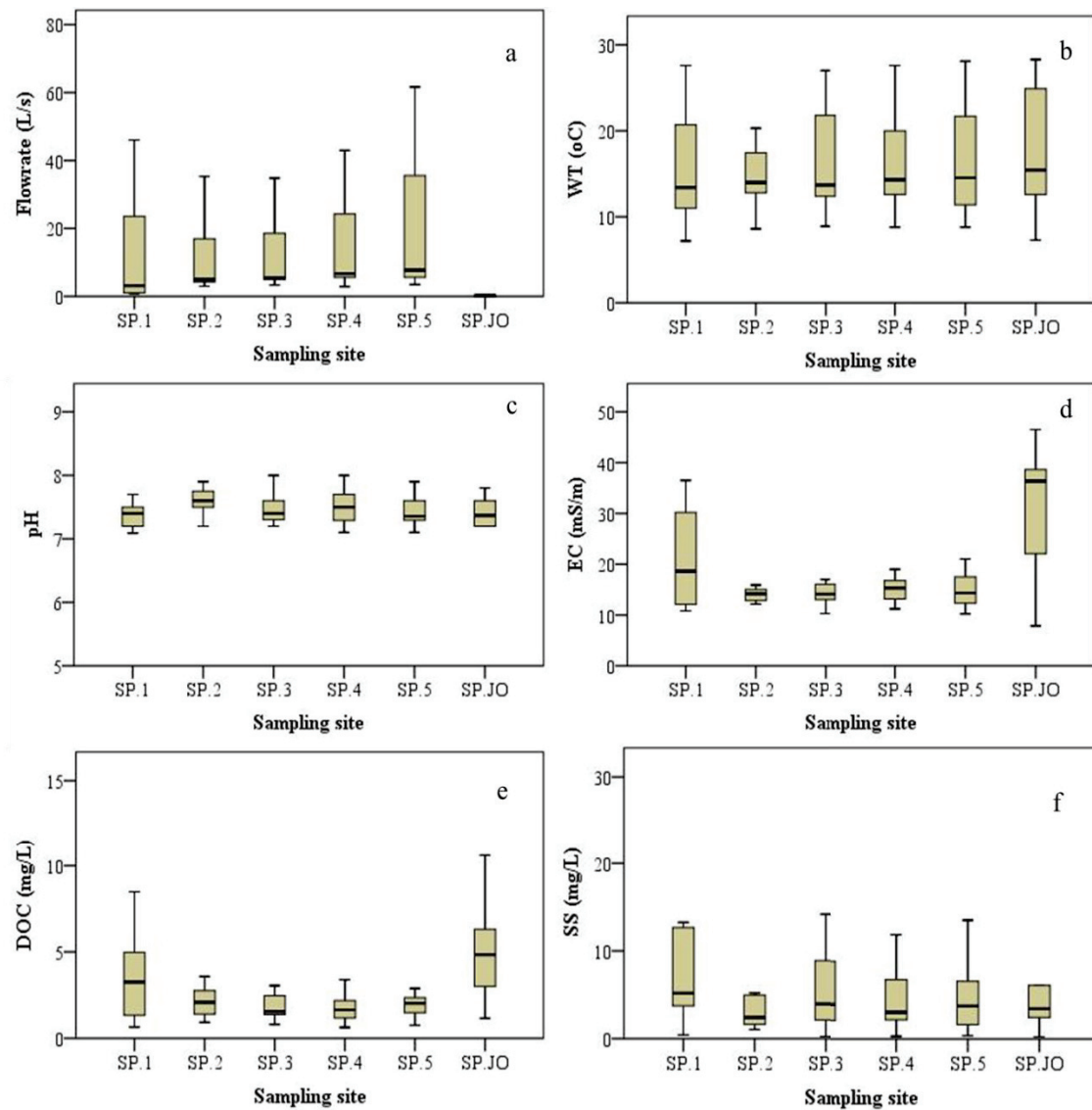
- Setiyawan, A.S, Yamada, T, Fajri, J.A, & Li, F. (2014). Characteristics of fecal indicators in channels of *johkasou* systems. *Japan of Water and Environmental Technology*.
- USEPA. (1997). "Response to Congress on Use of Decentralized Wastewater Treatment Systems". EPA 832-R-97-001b. U.S. Environmental Protection Agency, Washington, DC. USEPA, 2002. Onsite wastewater treatment systems manual.
- Takahashi, N., Matuhashi, H., Nishimura, O., & Sudo, R. (2012). Removal characteristics of fecal coliform by certificated structure type small-scale *Johkasou*. *Japan Society of Civil Engineering*, 68(7), III\_429-III\_434.
- Tanaka, T., Ogiwara, T., Kobayashi, Y., Kinoshita, E., Sugiyama, Hideyuki. (2007). Pollutant load discharged from *johkasou* systems and its impact on water quality of river and lake. *Journal of Japan Society on Water Environment*, 30(4), 219-225.
- Whitlock, J. E., Jones, D. T., & Harwood, V. J. (2002). Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. *Water Research*, 36(17), 4273-4282.
- Wihthers, P.JA., Jarvie, H.P., & Stoate, C. (2011). Quantifying the impact of septic tank systems on eutrophication risk in rural headwaters. *Environment International*, 37 644-653.
- Winward, G. P., et al. (2008). A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse. *Ecological Engineering*, 32(2), 187-197.
- Yahashi, T., Furukawa, Y., Watanabe, T., Ohmori, H., Inoue, Y. (2000). Analysis of water quality characterics in domestic wastewater treatment facilities. *Japan Society on Water Environment*, 23, 6.
- Yang, X.M., Morita, A., Nakano, I., Kushida, Y., & Ogawa, H. (2010). History and current situtation of night soill treatment systems and decentrilized wastewater treatment systems in Japan. *water Practice and Technolgy*.
- Yahashi, T., Furukawa, Y., Watanabe, T., Ohmori, H., Inoue, Y. (2000). Analysis of water quality characterics in domestic wastewater treatment facilities. *Japan Society on Water Environment*, 23, 6.



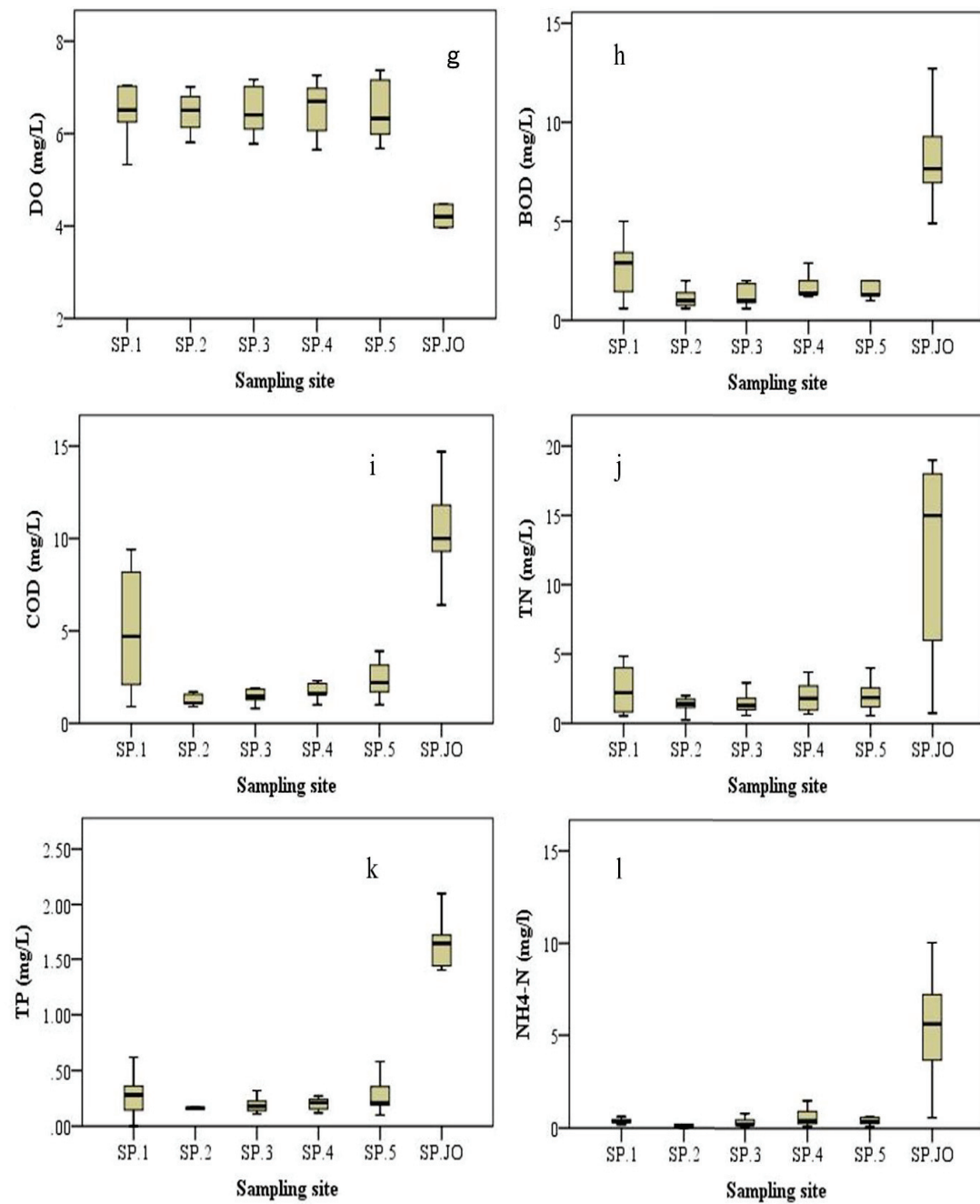
- Yates, M.V. (1985). Septic tank density and groundwater contamination. *Ground Water* 31 (6): 884-889.
- Zhou, W., Kageyama, K. Li, F., Yuasa, S. (2007). Monitoring of microbiological water quality by real-time PCR. *Environmental Technonology*, 28, 545-553.

# Appendix A

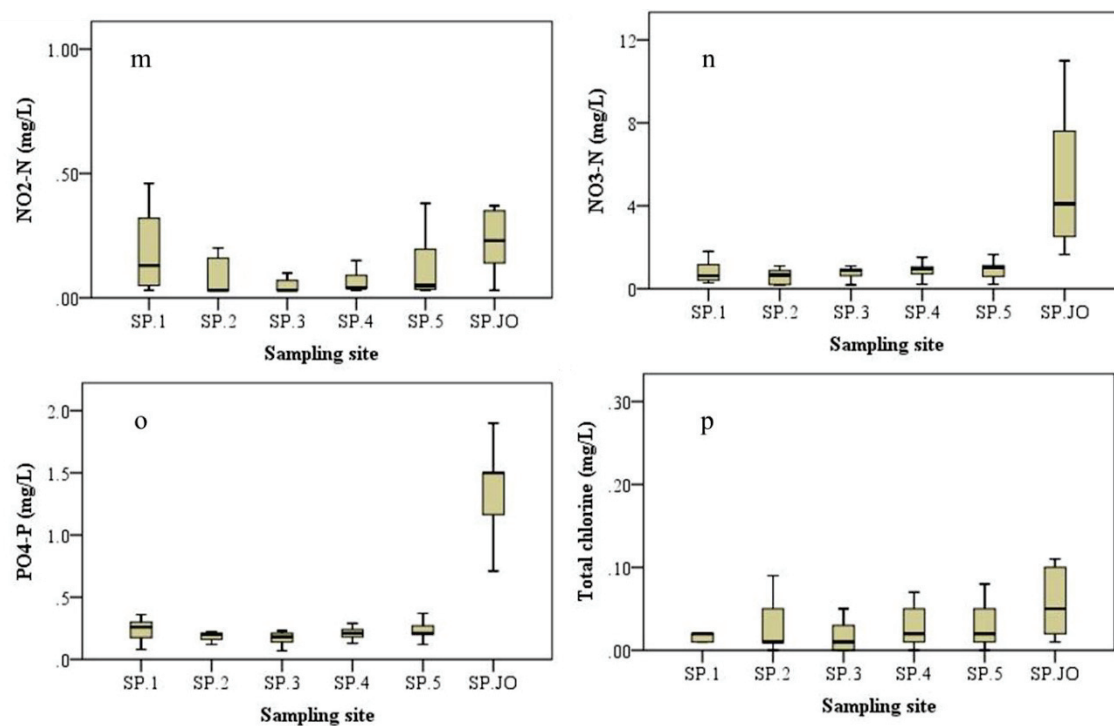
Spatial variation of physico-chemical parameters at six sampling points during study periods.



**Fig. A. 1** Box plots showing variation of physico-chemical parameters for a) flow rate, b) WT, c) pH, d) EC, e) DOC, and f) SS in local water environment receiving *johkasou* effluent during study period



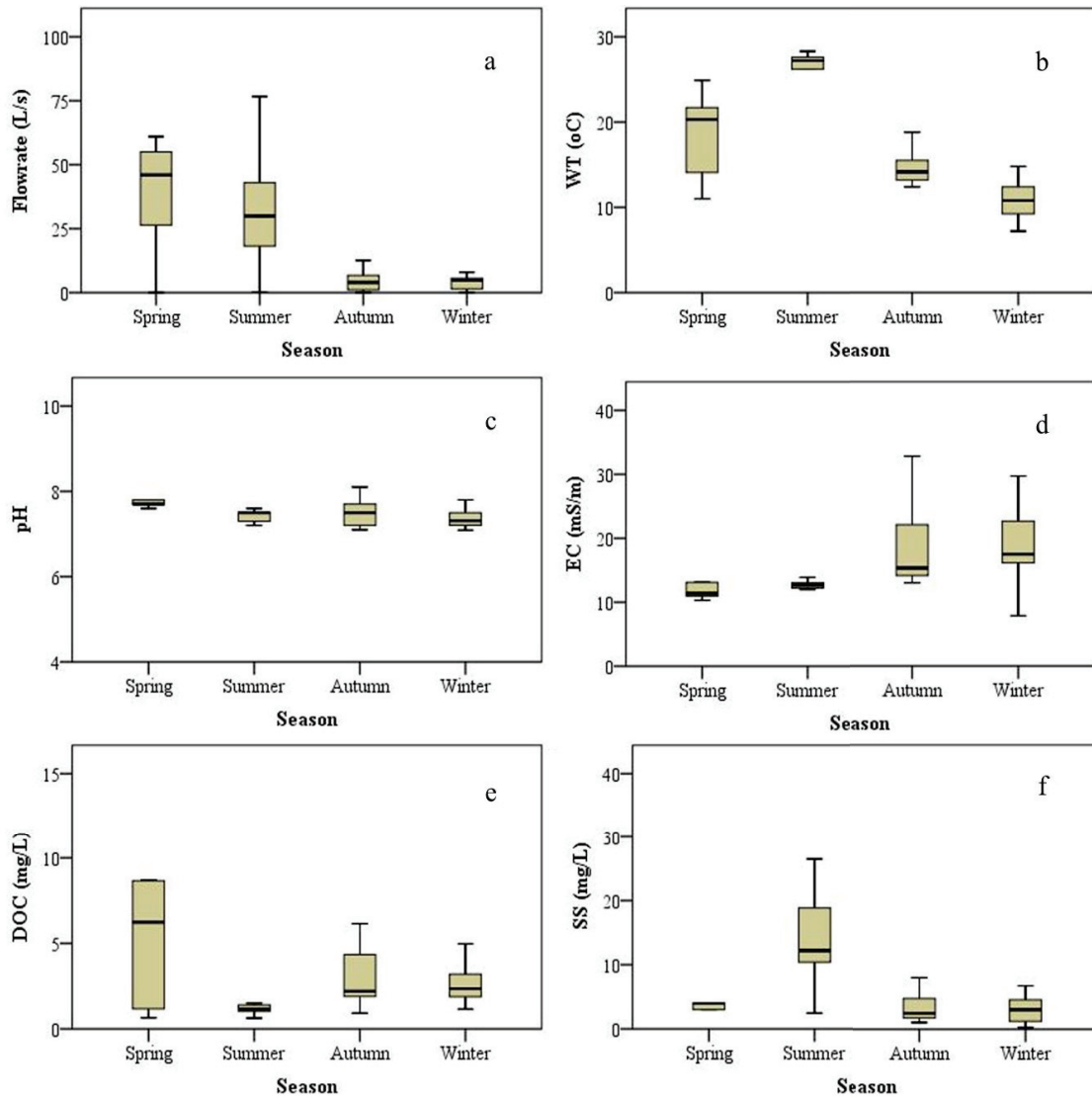
**Fig. A. 2 (Continued)** Box plots showing variation of physico-chemical parameters for g) DO, h) BOD, i) COD, j) TN, k) TP, and l) NH<sub>4</sub>-N in local water environment receiving *johkasou* effluent during study period.



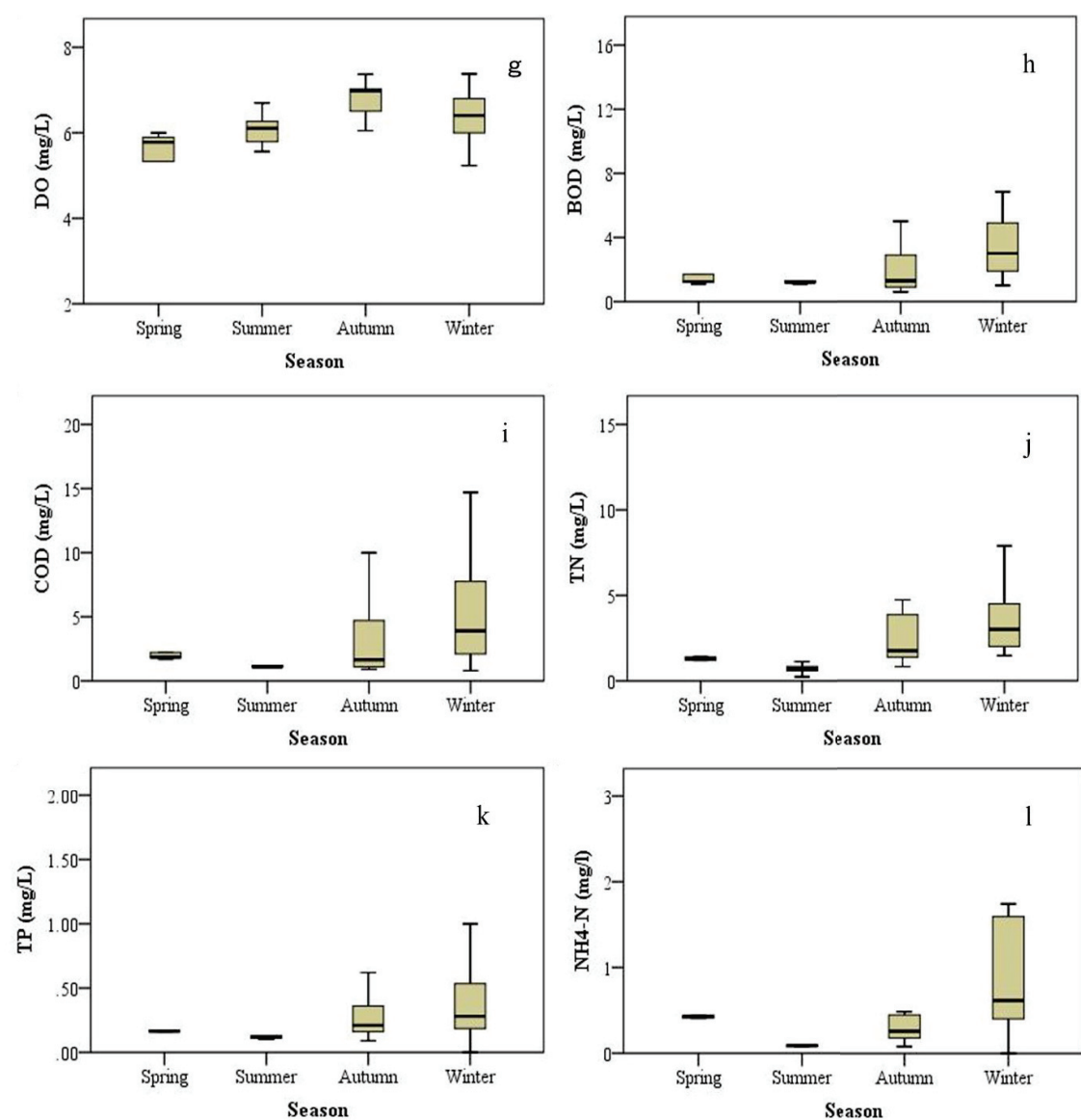
**Fig. A. 3 (Continued)** Box plots showing variation of physico-chemical parameters for m) NO<sub>2</sub>-N, n) NO<sub>3</sub>-N, o) PO<sub>4</sub>-P, and p) total chlorine in local water environment receiving *johkasou* effluent during study period.

## Appendix B

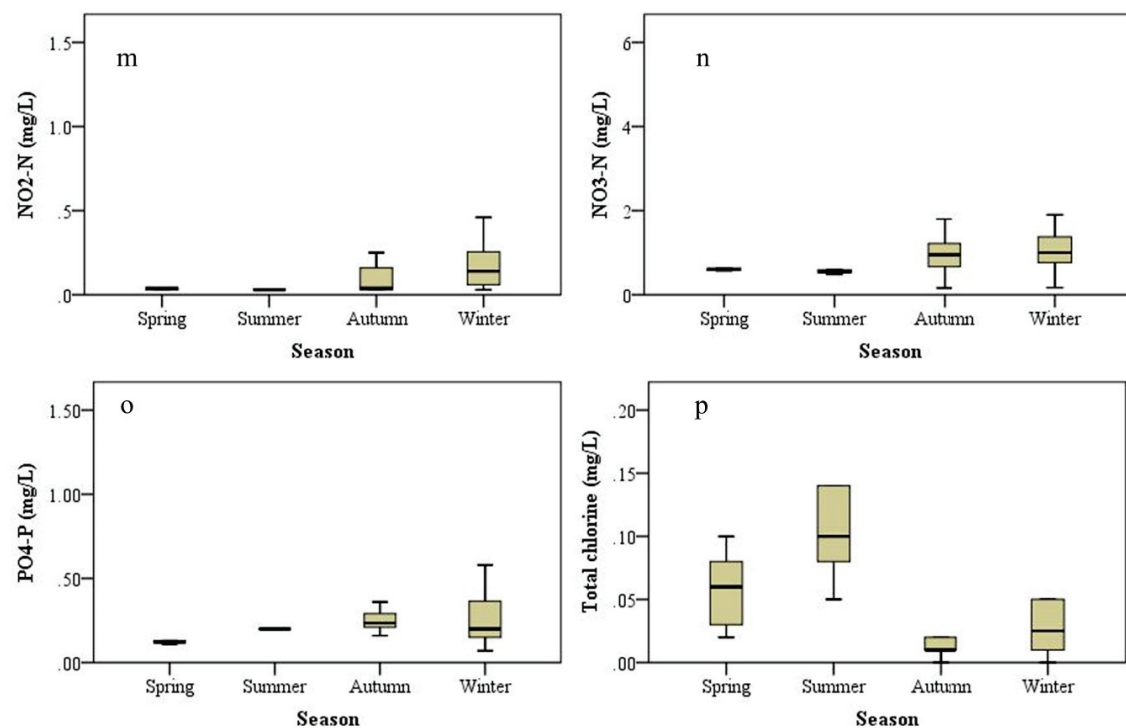
Temporal variation of physico-chemical parameters in the open channels receiving *johkasou* effluent during study periods.



**Fig. B. 1** Box plots of seasonal variation in physico-chemical parameters for a) flow rate, b) WT, c) pH, d) EC, e) DOC, and f) SS in the open channels receiving *johkasou* effluent during study period.



**Fig. B. 1 (Continued)** Box plots of seasonal variation in physico-chemical parameters for g) DO, h) BOD, i) COD, j) TN, k) TP, and l) NH<sub>4</sub>-N in the open channels receiving *johkasou* effluent during study period.



**Fig. B. 4 (Continued)** Box plots of seasonal variation in physico-chemical parameters for m) NO<sub>2</sub>-N, n) NO<sub>3</sub>-N, o) PO<sub>4</sub>-P, and p) total chlorine in the open channels receiving *johkasou* effluent during study period.

## Appendix C

Values of significant multiple comparisons in sampling sites for physico-chemical and microbial concentrations in the *johkasou* effluent calculated by one-way ANOVA with Tukey's post hoc analysis.

**Table C. 1** – Multiple comparisons values of Flow rate, pH, WT, EC, DO, and SS

Variable	I (Site)	J (Site comparison)	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Flow rate	SP. JO	SP. 1	-7.99348	7.21	.876	-29.10	13.12
		SP. 2	-9.91944	7.69	.789	-32.42	12.58
		SP. 3	-10.74478	7.35	.689	-32.26	10.77
		SP. 4	-16.89401	7.35	.208	-38.41	4.62
		SP. 5	-20.68880	7.21	.058	-41.80	0.42
pH	SP. JO	SP. 1	-.00410	0.33	1.000	-0.97	0.96
		SP. 2	-.16558	0.35	.997	-1.20	0.87
		SP. 3	-.08307	0.34	1.000	-1.07	0.90
		SP. 4	-.08897	0.34	1.000	-1.08	0.90
		SP. 5	.49542	0.33	.667	-0.47	1.46
WT	SP. JO	SP. 1	1.54279	2.40	.987	-5.49	8.58
		SP. 2	1.88918	2.56	.977	-5.61	9.39
		SP. 3	1.49866	2.45	.990	-5.67	8.67
		SP. 4	1.27910	2.45	.995	-5.89	8.45
		SP. 5	1.34720	2.40	.993	-5.69	8.38
EC	SP. JO	SP. 1	10.31222*	2.56	.002	2.81	17.81
		SP. 2	17.45021*	2.73	.000	9.45	25.45
		SP. 3	17.06447*	2.61	.000	9.42	24.71
		SP. 4	16.33986*	2.61	.000	8.70	23.98
		SP. 5	16.33937*	2.56	.000	8.84	23.84
DO	SP. JO	SP. 1	-2.09837*	0.25	.000	-2.83	-1.36
		SP. 2	-2.07930*	0.27	.000	-2.86	-1.30
		SP. 3	-2.12014*	0.26	.000	-2.87	-1.37
		SP. 4	-2.16165*	0.26	.000	-2.91	-1.41
		SP. 5	-2.14071*	0.25	.000	-2.88	-1.41
SS	SP. JO	SP. 1	-1.56858	3.21	.996	-10.96	7.83
		SP. 2	1.15558	3.42	.999	-8.86	11.17
		SP. 3	.21978	3.27	1.000	-9.35	9.79
		SP. 4	-2.28484	3.27	.982	-11.86	7.29
		SP. 5	1.69214	3.21	.995	-7.70	11.09

\*, The mean difference is significant at the 0.05 level.



**Table C.1 – (continued)** Multiple comparisons of DOC, BOC, TN, TP NH<sub>4</sub>-N and NO<sub>2</sub>-N

Variable	I (Site)	J (Site comparison)	Mean Difference (I-J)	Std. Error	P -value	95% Confidence Interval	
						Lower Bound	Upper Bound
DOC	SP. JO	SP. 1	2.91300	1.25	.194	-0.74	6.57
		SP. 2	3.70571	1.37	.085	-0.30	7.71
		SP. 3	4.09841*	1.27	.022	0.38	7.82
		SP. 4	4.61533*	1.27	.007	0.89	8.34
		SP. 5	4.48214*	1.25	.008	0.83	8.13
BOD	SP. JO	SP. 1	5.63364*	0.69	.000	3.60	7.66
		SP. 2	7.23768*	0.73	.000	5.10	9.38
		SP. 3	6.63273*	0.69	.000	4.60	8.66
		SP. 4	6.52727*	0.69	.000	4.50	8.56
		SP. 5	6.18636*	0.69	.000	4.16	8.22
COD	SP. JO	SP. 1	5.49091*	0.84	.000	3.00	7.98
		SP. 2	9.22414*	0.89	.000	6.60	11.85
		SP. 3	8.61000*	0.84	.000	6.12	11.10
		SP. 4	8.33091*	0.84	.000	5.84	10.82
		SP. 5	7.67727*	0.84	.000	5.19	10.17
TN	SP. JO	SP. 1	9.40077*	1.27	.000	5.68	13.13
		SP. 2	10.09811*	1.33	.000	6.21	13.99
		SP. 3	10.53923*	1.27	.000	6.81	14.26
		SP. 4	10.11462*	1.27	.000	6.39	13.84
		SP. 5	9.90692*	1.27	.000	6.18	13.63
TP	SP. JO	SP. 1	1.31273*	0.10	.000	1.02	1.60
		SP. 2	1.43374*	0.10	.000	1.13	1.74
		SP. 3	1.40000*	0.10	.000	1.11	1.69
		SP. 4	1.35727*	0.10	.000	1.07	1.65
		SP. 5	1.25364*	0.10	.000	0.96	1.54
NH <sub>4</sub> -N	SP. JO	SP. 1	4.950000*	0.54	.000	3.35	6.55
		SP. 2	5.266364*	0.54	.000	3.67	6.86
		SP. 3	5.070000*	0.54	.000	3.47	6.67
		SP. 4	4.813636*	0.54	.000	3.22	6.41
		SP. 5	4.877273*	0.54	.000	3.28	6.47
NO <sub>2</sub> -N	SP. JO	SP. 1	.178182	0.10	.428	-0.10	0.46
		SP. 2	.291111	0.10	.056	0.00	0.59
		SP. 3	.311818*	0.10	.021	0.03	0.59
		SP. 4	.297273*	0.10	.031	0.02	0.58
		SP. 5	.222727	0.10	.194	-0.06	0.50

\*. The mean difference is significant at the 0.05 level.

**Table C.1 – (continued)** Multiple comparisons of NO<sub>3</sub>-N, PO<sub>4</sub>-P and total Chlorine

Variable	I (Site)	J (Site comparison)	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
						Lower Bound	Upper Bound
NO <sub>3</sub> -N	SP. JO	SP. 1	4.145455*	0.56	.000	2.49	5.80
		SP. 2	4.329192*	0.59	.000	2.59	6.07
		SP. 3	4.171818*	0.56	.000	2.52	5.82
		SP. 4	4.042727*	0.56	.000	2.39	5.70
		SP. 5	4.045455*	0.56	.000	2.39	5.70
PO <sub>4</sub> -P	SP. JO	SP. 1	1.11455*	0.07	.000	0.91	1.32
		SP. 2	1.16909*	0.07	.000	0.95	1.39
		SP. 3	1.17818*	0.07	.000	0.97	1.39
		SP. 4	1.13909*	0.07	.000	0.93	1.35
		SP. 5	1.09273*	0.07	.000	0.88	1.30
Total Chlorine	SP. JO	SP. 1	.027778	0.02	.756	-0.03	0.09
		SP. 2	.024444	0.02	.840	-0.04	0.09
		SP. 3	.033333	0.02	.590	-0.03	0.09
		SP. 4	.012222	0.02	.991	-0.05	0.07
		SP. 5	-.001111	0.02	1.000	-0.06	0.06

\*. The mean difference is significant at the 0.05 level.

**Table C. 2** – Multiple comparisons values of VB, HPC, TC, *E.coli*, and DNA-based total bacteria in water

Variable	I (Site)	J (Site comparison)	Mean Difference (I-J)	Std. Error	P -value	95% Confidence Interval	
						Lower Bound	Upper Bound
VB	SP. JO	SP. 1	.82891	0.28	.054	-0.01	1.67
		SP. 2	1.06469*	0.29	.007	0.20	1.92
		SP. 3	1.00836*	0.28	.010	0.17	1.85
		SP. 4	.89210*	0.28	.030	0.05	1.73
		SP. 5	.85447*	0.28	.043	0.02	1.69
HPC	SP. JO	SP. 1	.77776*	0.21	.004	0.18	1.38
		SP. 2	.74628*	0.21	.010	0.12	1.37
		SP. 3	.68206*	0.21	.017	0.08	1.28
		SP. 4	.64523*	0.21	.029	0.04	1.25
		SP. 5	.59719	0.21	.053	-0.01	1.20
TC	SP. JO	SP. 1	.88792*	0.20	.000	0.30	1.47
		SP. 2	.86179*	0.21	.001	0.25	1.47
		SP. 3	.56568	0.20	.063	-0.02	1.15
		SP. 4	.80763*	0.20	.002	0.22	1.39
		SP. 5	.86113*	0.20	.001	0.28	1.45
<i>E. coli</i>	SP. JO	SP. 1	1.30373*	0.34	.003	0.31	2.30
		SP. 2	1.34671*	0.35	.004	0.31	2.38
		SP. 3	1.54268*	0.34	.000	0.55	2.54
		SP. 4	1.09449*	0.34	.023	0.10	2.09
		SP. 5	1.44544*	0.34	.001	0.45	2.44
DNA	SP. JO	SP. 1	.57441	0.40	.714	-0.61	1.76
		SP. 2	.92354	0.42	.252	-0.31	2.15
		SP. 3	.70858	0.40	.502	-0.47	1.89
		SP. 4	.98650	0.40	.156	-0.20	2.17
		SP. 5	.57238	0.40	.717	-0.61	1.75

\*. The mean difference is significant at the 0.05 level.

**Table C. 3** – Multiple comparisons values of VB, HPC, TC, E.coli, and DNA-based total bacteria in sediment

Variable	I (Site)	J (Site comparison)	Mean Difference (I-J)	Std. Error	P -value	95% Confidence Interval	
						Lower Bound	Upper Bound
VB	SP. JO	SP. 1	1.35521*	0.23	.000	0.68	2.03
		SP. 2	1.25813*	0.23	.000	0.57	1.95
		SP. 3	1.17148*	0.23	.000	0.50	1.84
		SP. 4	.57383	0.23	.136	-0.10	1.25
		SP. 5	1.06025*	0.23	.000	0.39	1.73
HPC	SP. JO	SP. 1	1.74120*	0.29	.000	0.88	2.60
		SP. 2	1.42266*	0.30	.000	0.54	2.31
		SP. 3	1.37423*	0.29	.000	0.51	2.23
		SP. 4	.76065	0.29	.111	-0.10	1.62
		SP. 5	1.37062*	0.29	.000	0.51	2.23
TC	SP. JO	SP. 1	1.12434	0.51	.249	-0.38	2.63
		SP. 2	1.11822	0.52	.285	-0.43	2.67
		SP. 3	1.12105	0.51	.251	-0.38	2.63
		SP. 4	.13132	0.51	1.000	-1.37	1.64
		SP. 5	.82883	0.51	.580	-0.68	2.33
<i>E.coli</i>	SP. JO	SP. 1	1.70691*	0.54	.029	0.11	3.30
		SP. 2	1.47809	0.55	.100	-0.16	3.12
		SP. 3	1.49587	0.54	.077	-0.10	3.09
		SP. 4	.82180	0.54	.646	-0.77	2.41
		SP. 5	1.59284	0.54	.050	0.00	3.19
DNA	SP. JO	SP. 1	.52642	0.35	.660	-0.51	1.57
		SP. 2	.67481	0.37	.470	-0.44	1.79
		SP. 3	.83031	0.36	.212	-0.24	1.90
		SP. 4	.97531	0.36	.093	-0.10	2.05
		SP. 5	.48272	0.35	.736	-0.56	1.52

\*. The mean difference is significant at the 0.05 level.

# Appendix D

Values of significant multiple comparisons in seasons for physico-chemical and microbial concentrations in the open channels and the *johkasou* drainage channels calculated using one-way ANOVA with Tukey's post hoc analysis.

**Table D. 1** – Multiple comparisons of Flow rate, pH, and WT.

Variable	I (Season)	J (Season comparison)	Tukey post hoc analysis			95% Confidence Interval	
			Mean	Std. Error	P -value		
			Difference (I-J)			Lower Bound	Upper Bound
Flowrate	Spring	Summer	10.52	5.49	.233	-3.99	25.03
		Autumn	40.46695*	5.08	.000	27.06	53.87
		Winter	42.57257*	5.25	.000	28.71	56.44
	summer	Summer	-10.52	5.49	.233	-25.03	3.99
		Autumn	29.94782*	3.96	.000	19.48	40.41
		Winter	32.05345*	4.18	.000	21.01	43.10
	Autumn	Summer	-40.46695*	5.08	.000	-53.87	-27.06
		Autumn	-29.94782*	3.96	.000	-40.41	-19.48
		Winter	2.11	3.61	.937	-7.44	11.65
	Winter	Summer	-42.57257*	5.25	.000	-56.44	-28.71
		Autumn	-32.05345*	4.18	.000	-43.10	-21.01
		Winter	-2.11	3.61	.937	-11.65	7.44
pH	Spring	Summer	0.30	0.45	.913	-0.90	1.49
		Autumn	0.49	0.42	.646	-0.61	1.59
		Winter	0.35	0.43	.844	-0.78	1.49
	summer	Summer	-0.30	0.45	.913	-1.49	0.90
		Autumn	0.19	0.32	.933	-0.66	1.05
		Winter	0.06	0.34	.998	-0.85	0.96
	Autumn	Summer	-0.49	0.42	.646	-1.59	0.61
		Autumn	-0.19	0.32	.933	-1.05	0.66
		Winter	-0.14	0.30	.968	-0.92	0.65
	Winter	Summer	-0.35	0.43	.844	-1.49	0.78
		Autumn	-0.06	0.34	.998	-0.96	0.85
		Winter	0.14	0.30	.968	-0.65	0.92
WT	Spring	Summer	-7.98730*	1.06	.000	-10.80	-5.18
		Autumn	3.85257*	0.98	.001	1.26	6.45
		Winter	7.19436*	1.02	.000	4.51	9.88
	summer	Summer	7.98730*	1.06	.000	5.18	10.80
		Autumn	11.83987*	0.77	.000	9.81	13.87
		Winter	15.18166*	0.81	.000	13.04	17.32
	Autumn	Summer	-3.85257*	0.98	.001	-6.45	-1.26
		Autumn	-11.83987*	0.77	.000	-13.87	-9.81
		Winter	3.34179*	0.70	.000	1.49	5.19
	Winter	Summer	-7.19436*	1.02	.000	-9.88	-4.51
		Autumn	-15.18166*	0.81	.000	-17.32	-13.04
		Winter	-3.34179*	0.70	.000	-5.19	-1.49

\*. The mean difference is significant at the 0.05 level.

**Table D.1 - (Continued)** Multiple comparisons of DO, SS, and DOC.

Variable	I (Season)	J (Season comparison)	Tukey post hoc analysis				
			Mean	Std. Error	P -value	95% Confidence Interval	
			Difference (I-J)			Lower Bound	Upper Bound
DO	Spring	Summer	-0.40	0.16	.070	-0.82	0.02
		Autumn	-1.12800*	0.15	.000	-1.52	-0.74
		Winter	-.68553*	0.15	.000	-1.09	-0.28
	summer	Summer	0.40	0.16	.070	-0.02	0.82
		Autumn	-.72904*	0.12	.000	-1.03	-0.43
		Winter	-0.29	0.12	.096	-0.61	0.03
	Autumn	Summer	1.12800*	0.15	.000	0.74	1.52
		Autumn	.72904*	0.12	.000	0.43	1.03
		Winter	.44247*	0.10	.000	0.17	0.72
	Winter	Summer	.68553*	0.15	.000	0.28	1.09
		Autumn	0.29	0.12	.096	-0.03	0.61
		Winter	-.44247*	0.10	.000	-0.72	-0.17
SS	Spring	Summer	-13.53857*	3.00	.000	-21.47	-5.61
		Autumn	0.29	2.77	1.000	-7.04	7.61
		Winter	0.51	2.87	.998	-7.06	8.09
	summer	Summer	13.53857*	3.00	.000	5.61	21.47
		Autumn	13.82634*	2.16	.000	8.11	19.54
		Winter	14.05240*	2.28	.000	8.02	20.09
	Autumn	Summer	-0.29	2.77	1.000	-7.61	7.04
		Autumn	-13.82634*	2.16	.000	-19.54	-8.11
		Winter	0.23	1.97	.999	-4.99	5.44
	Winter	Summer	-0.51	2.87	.998	-8.09	7.06
		Autumn	-14.05240*	2.28	.000	-20.09	-8.02
		Winter	-0.23	1.97	.999	-5.44	4.99
DOC	Spring	Summer	3.31071*	0.74	.000	1.36	5.26
		Autumn	1.94423*	0.68	.030	0.14	3.75
		Winter	1.91935*	0.70	.041	0.06	3.78
	summer	Summer	-3.31071*	0.74	.000	-5.26	-1.36
		Autumn	-1.37	0.54	.062	-2.78	0.05
		Winter	-1.39	0.56	.073	-2.87	0.09
	Autumn	Summer	-1.94423*	0.68	.030	-3.75	-0.14
		Autumn	1.37	0.54	.062	-0.05	2.78
		Winter	-0.02	0.49	1.000	-1.32	1.27
	Winter	Summer	-1.91935*	0.70	.041	-3.78	-0.06
		Autumn	1.39	0.56	.073	-0.09	2.87
		Winter	0.02	0.49	1.000	-1.27	1.32

\*. The mean difference is significant at the 0.05 level.

**Table D.1 - (Continued)** Multiple comparisons of BOD, COD, and TN.

Tukey post hoc analysis							
Variable	I (Season)	J (Season comparison)	Mean	Std. Error	P-value	95% Confidence Interval	
			Difference (I-J)			Lower Bound	Upper Bound
BOD	Spring	Summer	0.10	0.81	.999	-2.05	2.25
		Autumn	-0.16	0.59	.992	-1.73	1.40
		Winter	-1.62368*	0.61	.047	-3.23	-0.01
	summer	Summer	-0.10	0.81	.999	-2.25	2.05
		Autumn	-0.26	0.65	.977	-1.99	1.46
		Winter	-1.72	0.66	.057	-3.48	0.04
	Autumn	Summer	0.16	0.59	.992	-1.40	1.73
		Autumn	0.26	0.65	.977	-1.46	1.99
		Winter	-1.45968*	0.37	.001	-2.43	-0.49
	Winter	Summer	1.62368*	0.61	.047	0.01	3.23
		Autumn	1.72	0.66	.057	-0.04	3.48
		Winter	1.45968*	0.37	.001	0.49	2.43
COD	Spring	Summer	0.78	1.37	.941	-2.87	4.43
		Autumn	-0.19	1.00	.998	-2.85	2.47
		Winter	-2.09	1.03	.189	-4.83	0.64
	summer	Summer	-0.78	1.37	.941	-4.43	2.87
		Autumn	-0.97	1.10	.815	-3.90	1.96
		Winter	-2.87	1.12	.064	-5.86	0.12
	Autumn	Summer	0.19	1.00	.998	-2.47	2.85
		Autumn	0.97	1.10	.815	-1.96	3.90
		Winter	-1.90263*	0.62	.018	-3.56	-0.25
	Winter	Summer	2.09	1.03	.189	-0.64	4.83
		Autumn	2.87	1.12	.064	-0.12	5.86
		Winter	1.90263*	0.62	.018	0.25	3.56
TN	Spring	Summer	0.60	0.55	.700	-0.86	2.07
		Autumn	-0.66	0.52	.586	-2.04	0.72
		Winter	-1.94842*	0.53	.003	-3.36	-0.54
	summer	Summer	-0.60	0.55	.700	-2.07	0.86
		Autumn	-1.26223*	0.35	.004	-2.20	-0.32
		Winter	-2.54985*	0.37	.000	-3.54	-1.56
	Autumn	Summer	0.66	0.52	.586	-0.72	2.04
		Autumn	1.26223*	0.35	.004	0.32	2.20
		Winter	-1.28762*	0.32	.001	-2.14	-0.43
	Winter	Summer	1.94842*	0.53	.003	0.54	3.36
		Autumn	2.54985*	0.37	.000	1.56	3.54
		Winter	1.28762*	0.32	.001	0.43	2.14

\*. The mean difference is significant at the 0.05 level.

**Table D.1 - (Continued)** Multiple comparisons of TN, NH<sub>4</sub>-N, and NO<sub>2</sub>-N.

Tukey post hoc analysis							
Variable	I (Season)	J (Season comparison)	Mean	Std. Error	P -value	95% Confidence Interval	
			Difference (I-J)			Lower Bound	Upper Bound
TP	Spring	Summer	0.05	0.11	.971	-0.25	0.34
		Autumn	-0.06	0.08	.887	-0.27	0.16
		Winter	-0.12	0.08	.507	-0.34	0.11
	summer	Summer	-0.05	0.11	.971	-0.34	0.25
		Autumn	-0.11	0.09	.625	-0.35	0.13
		Winter	-0.17	0.09	.279	-0.41	0.08
	Autumn	Summer	0.06	0.08	.887	-0.16	0.27
		Autumn	0.11	0.09	.625	-0.13	0.35
		Winter	-0.06	0.05	.664	-0.19	0.08
	Winter	Summer	0.12	0.08	.507	-0.11	0.34
		Autumn	0.17	0.09	.279	-0.08	0.41
		Winter	0.06	0.05	.664	-0.08	0.19
NH <sub>4</sub> -N	Spring	Summer	0.34	0.21	.391	-0.23	0.91
		Autumn	0.16	0.17	.770	-0.28	0.60
		Winter	-0.33	0.17	.208	-0.78	0.11
	summer	Summer	-0.34	0.21	.391	-0.91	0.23
		Autumn	-0.18	0.17	.694	-0.62	0.26
		Winter	-.674000*	0.17	.001	-1.12	-0.23
	Autumn	Summer	-0.16	0.17	.770	-0.60	0.28
		Autumn	0.18	0.17	.694	-0.26	0.62
		Winter	-.493200*	0.10	.000	-0.76	-0.22
	Winter	Summer	0.33	0.17	.208	-0.11	0.78
		Autumn	.674000*	0.17	.001	0.23	1.12
		Winter	.493200*	0.10	.000	0.22	0.76
NO <sub>2</sub> -N	Spring	Summer	0.01	0.08	1.000	-0.20	0.21
		Autumn	-0.05	0.06	.800	-0.20	0.10
		Winter	-0.14	0.06	.082	-0.30	0.01
	summer	Summer	-0.01	0.08	1.000	-0.21	0.20
		Autumn	-0.06	0.06	.792	-0.22	0.11
		Winter	-0.15	0.06	.105	-0.32	0.02
	Autumn	Summer	0.05	0.06	.800	-0.10	0.20
		Autumn	0.06	0.06	.792	-0.11	0.22
		Winter	-0.09	0.04	.061	-0.18	0.00
	Winter	Summer	0.14	0.06	.082	-0.01	0.30
		Autumn	0.15	0.06	.105	-0.02	0.32
		Winter	0.09	0.04	.061	0.00	0.18

\*. The mean difference is significant at the 0.05 level.



Table D.1 - (Continued) Multiple comparisons of NO<sub>3</sub>-N, PO<sub>4</sub>-P, and total chlorine.

Tukey post hoc analysis							
Variable	I (Season)	J (Season comparison)	Mean	Std. Error	P -value	95% Confidence Interval	
			Difference (I-J)			Lower Bound	Upper Bound
NO <sub>3</sub> -N	Spring	Summer	0.06	0.25	.996	-0.61	0.72
		Autumn	-0.24	0.18	.549	-0.73	0.24
		Winter	-0.28	0.19	.458	-0.78	0.22
	summer	Summer	-0.06	0.25	.996	-0.72	0.61
		Autumn	-0.30	0.20	.451	-0.83	0.23
		Winter	-0.33	0.21	.374	-0.88	0.21
	Autumn	Summer	0.24	0.18	.549	-0.24	0.73
		Autumn	0.30	0.20	.451	-0.23	0.83
		Winter	-0.03	0.11	.990	-0.34	0.27
	Winter	Summer	0.28	0.19	.458	-0.22	0.78
		Autumn	0.33	0.21	.374	-0.21	0.88
		Winter	0.03	0.11	.990	-0.27	0.34
PO <sub>4</sub> -P	Spring	Summer	-0.08	0.05	.453	-0.22	0.06
		Autumn	-.11240*	0.04	.031	-0.22	-0.01
		Winter	-0.09	0.04	.126	-0.20	0.02
	summer	Summer	0.08	0.05	.453	-0.06	0.22
		Autumn	-0.03	0.04	.876	-0.15	0.08
		Winter	-0.01	0.04	.995	-0.13	0.11
	Autumn	Summer	.11240*	0.04	.031	0.01	0.22
		Autumn	0.03	0.04	.876	-0.08	0.15
		Winter	0.02	0.02	.807	-0.04	0.09
	Winter	Summer	0.09	0.04	.126	-0.02	0.20
		Autumn	0.01	0.04	.995	-0.11	0.13
		Winter	-0.02	0.02	.807	-0.09	0.04
Total Chlorine	Spring	Summer	-.072000*	0.02	.002	-0.12	-0.02
		Autumn	0.04	0.01	.074	0.00	0.08
		Winter	0.02	0.02	.455	-0.02	0.06
	summer	Summer	.072000*	0.02	.002	0.02	0.12
		Autumn	.109000*	0.01	.000	0.07	0.15
		Winter	.094667*	0.02	.000	0.05	0.14
	Autumn	Summer	-0.04	0.01	.074	-0.08	0.00
		Autumn	-.109000*	0.01	.000	-0.15	-0.07
		Winter	-0.01	0.01	.494	-0.04	0.01
	Winter	Summer	-0.02	0.02	.455	-0.06	0.02
		Autumn	-.094667*	0.02	.000	-0.14	-0.05
		Winter	0.01	0.01	.494	-0.01	0.04

\*. The mean difference is significant at the 0.05 level.

**Table D. 2** - Multiple comparisons of VB, HPC, and TC in water.

Tukey HSD							
Variable	I (Season)	J (Season comparison)	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
						Lower Bound	Upper Bound
VB	Spring	Summer	-.72282	0.36	.195	-1.67	0.23
		Autum	-.64987*	0.24	.042	-1.28	-0.02
		Winter	-.51654	0.25	.181	-1.18	0.15
	Summer	Spring	.72282	0.36	.195	-0.23	1.67
		Autum	.07295	0.33	.996	-0.81	0.95
		Winter	.20628	0.34	.930	-0.70	1.11
	Autum	Spring	.64987*	0.24	.042	0.02	1.28
		Summer	-.07295	0.33	.996	-0.95	0.81
		Winter	.13333	0.21	.921	-0.42	0.69
	Winter	Spring	.51654	0.25	.181	-0.15	1.18
		Summer	-.20628	0.34	.930	-1.11	0.70
		Autum	-.13333	0.21	.921	-0.69	0.42
HPC	Spring	Summer	.01461	0.21	1.000	-0.55	0.58
		Autum	-.23721	0.19	.612	-0.75	0.27
		Winter	-.50106	0.20	.070	-1.03	0.03
	Summer	Spring	-.01461	0.21	1.000	-0.58	0.55
		Autum	-.25182	0.17	.464	-0.70	0.20
		Winter	-.51567*	0.18	.028	-0.99	-0.04
	Autum	Spring	.23721	0.19	.612	-0.27	0.75
		Summer	.25182	0.17	.464	-0.20	0.70
		Winter	-.26385	0.16	.341	-0.68	0.15
	Winter	Spring	.50106	0.20	.070	-0.03	1.03
		Summer	.51567*	0.18	.028	0.04	0.99
		Autum	.26385	0.16	.341	-0.15	0.68
TC	Spring	Summer	-.06725	0.22	.990	-0.65	0.52
		Autum	-.29393	0.20	.472	-0.83	0.24
		Winter	-.42689	0.21	.189	-0.98	0.13
	Summer	Spring	.06725	0.22	.990	-0.52	0.65
		Autum	-.22668	0.18	.592	-0.70	0.25
		Winter	-.35964	0.19	.239	-0.86	0.14
	Autum	Spring	.29393	0.20	.472	-0.24	0.83
		Summer	.22668	0.18	.592	-0.25	0.70
		Winter	-.13296	0.16	.850	-0.56	0.30
	Winter	Spring	.42689	0.21	.189	-0.13	0.98
		Summer	.35964	0.19	.239	-0.14	0.86
		Autum	.13296	0.16	.850	-0.30	0.56

\*. The mean difference is significant at the 0.05 level.

**Table D.2 – (continued)** Multiple comparisons of *E.coli* and DNA in water

Tukey HSD							
Variable	I (Season)	J (Season comparison)	Mean	Std. Error	P -value	95% Confidence Interval	
			Difference (I-J)			Lower Bound	Upper Bound
<i>E. coli</i>	Spring	Summer	.16721	0.37	.969	-0.81	1.14
		Autum	.40939	0.34	.616	-0.47	1.29
		Winter	-.41220	0.35	.642	-1.33	0.51
	Summer	Spring	-.16721	0.37	.969	-1.14	0.81
		Autum	.24219	0.30	.849	-0.54	1.03
		Winter	-.57940	0.31	.261	-1.40	0.25
	Autum	Spring	-.40939	0.34	.616	-1.29	0.47
		Summer	-.24219	0.30	.849	-1.03	0.54
		Winter	-.82159*	0.27	.018	-1.54	-0.11
	Winter	Spring	.41220	0.35	.642	-0.51	1.33
		Summer	.57940	0.31	.261	-0.25	1.40
		Autum	.82159*	0.27	.018	0.11	1.54
DNA	Spring	Summer	-.20749	0.40	.953	-1.24	0.83
		Autum	-.92294	0.36	.056	-1.86	0.02
		Winter	-.45544	0.37	.616	-1.43	0.52
	Summer	Spring	.20749	0.40	.953	-0.83	1.24
		Autum	-.71545	0.32	.119	-1.55	0.12
		Winter	-.24795	0.34	.881	-1.13	0.63
	Autum	Spring	.92294	0.36	.056	-0.02	1.86
		Summer	.71545	0.32	.119	-0.12	1.55
		Winter	.46750	0.29	.379	-0.29	1.23
	Winter	Spring	.45544	0.37	.616	-0.52	1.43
		Summer	.24795	0.34	.881	-0.63	1.13
		Autum	-.46750	0.29	.379	-1.23	0.29

\*. The mean difference is significant at the 0.05 level.

**Table D. 3** - Multiple comparisons of VB, HPC, and TC in sediment.

Tukey HSD							
Variable	I (Season)	J (Season comparison)	Mean Difference (I-J)	Std. Error	P -value	95% Confidence Interval	
						Lower Bound	Upper Bound
VB	Spring	Summer	-.62110	0.36	.318	-1.57	0.33
		Autum	-.27236	0.24	.665	-0.90	0.36
		Winter	-.45981	0.25	.271	-1.13	0.21
	Summer	Spring	.62110	0.36	.318	-0.33	1.57
		Autum	.34874	0.33	.720	-0.53	1.23
		Winter	.16129	0.34	.965	-0.74	1.06
	Autum	Spring	.27236	0.24	.665	-0.36	0.90
		Summer	-.34874	0.33	.720	-1.23	0.53
		Winter	-.18744	0.21	.809	-0.74	0.37
	Winter	Spring	.45981	0.25	.271	-0.21	1.13
		Summer	-.16129	0.34	.965	-1.06	0.74
		Autum	.18744	0.21	.809	-0.37	0.74
HPC	Spring	Summer	-.92060	0.43	.147	-2.05	0.21
		Autum	-.87795*	0.28	.016	-1.63	-0.13
		Winter	-.78594	0.30	.052	-1.58	0.00
	Summer	Spring	.92060	0.43	.147	-0.21	2.05
		Autum	.04265	0.39	1.000	-1.00	1.08
		Winter	.13467	0.40	.987	-0.94	1.21
	Autum	Spring	.87795*	0.28	.016	0.13	1.63
		Summer	-.04265	0.39	1.000	-1.08	1.00
		Winter	.09201	0.25	.983	-0.57	0.75
	Winter	Spring	.78594	0.30	.052	0.00	1.58
		Summer	-.13467	0.40	.987	-1.21	0.94
		Autum	-.09201	0.25	.983	-0.75	0.57
TC	Spring	Summer	-1.52598*	0.56	.042	-3.01	-0.04
		Autum	-1.11699*	0.39	.031	-2.16	-0.08
		Winter	-.93768	0.39	.091	-1.98	0.10
	Summer	Spring	1.52598*	0.56	.042	0.04	3.01
		Autum	.40899	0.53	.867	-1.00	1.82
		Winter	.58830	0.53	.686	-0.82	2.00
	Autum	Spring	1.11699*	0.39	.031	0.08	2.16
		Summer	-.40899	0.53	.867	-1.82	1.00
		Winter	.17931	0.35	.956	-0.75	1.11
	Winter	Spring	.93768	0.39	.091	-0.10	1.98
		Summer	-.58830	0.53	.686	-2.00	0.82
		Autum	-.17931	0.35	.956	-1.11	0.75

\*. The mean difference is significant at the 0.05 level.

**Table D.3 – (continued)** Multiple comparisons of *E.coli* and DNA in sediment

Tukey HSD							
Variable	I (Season)	J (Season comparison)	Mean	Std. Error	P -value	95% Confidence Interval	
			Difference (I-J)			Lower Bound	Upper Bound
<i>E.coli</i>	Spring	Summer	-.54402	0.67	.848	-2.32	1.24
		Autum	-.54625	0.47	.651	-1.79	0.70
		Winter	-.50086	0.47	.710	-1.75	0.75
	Summer	Spring	.54402	0.67	.848	-1.24	2.32
		Autum	-.00223	0.64	1.000	-1.69	1.69
		Winter	.04315	0.64	1.000	-1.65	1.73
	Autum	Spring	.54625	0.47	.651	-0.70	1.79
		Summer	.00223	0.64	1.000	-1.69	1.69
		Winter	.04538	0.42	1.000	-1.07	1.16
	Winter	Spring	.50086	0.47	.710	-0.75	1.75
		Summer	-.04315	0.64	1.000	-1.73	1.65
		Autum	-.04538	0.42	1.000	-1.16	1.07
DNA	Spring	Summer	1.29758	0.54	.095	-0.15	2.75
		Autum	.70674	0.46	.418	-0.51	1.92
		Winter	.86430	0.47	.260	-0.38	2.10
	Summer	Spring	-1.29758	0.54	.095	-2.75	0.15
		Autum	-.59084	0.37	.382	-1.57	0.39
		Winter	-.43328	0.38	.661	-1.44	0.57
	Autum	Spring	-.70674	0.46	.418	-1.92	0.51
		Summer	.59084	0.37	.382	-0.39	1.57
		Winter	.15755	0.23	.905	-0.46	0.78
	Winter	Spring	-.86430	0.47	.260	-2.10	0.38
		Summer	.43328	0.38	.661	-0.57	1.44
		Autum	-.15755	0.23	.905	-0.78	0.46

\*. The mean difference is significant at the 0.05 level.