JOURNAL OF ENVIRONMENTAL HYDROLOGY

Open Access Online Journal of the International Association for Environmental Hydrology

VOLUME 24 2016

SPATIOTEMPORAL VARIATIONS IN STREAM AND GROUNDWATER CHEMISTRY AND MICROBIAL COMMUNITY IN AN INTENSIVE AGRICULTURAL AREA

Heejung Kim[1]
Jin-Yong Lee[2]

[1]School of Earth and Environmental Sciences
Seoul National University, Seoul, Korea
[2]Department of Geology
Kangwon National University, Chuncheon, Korea

Multivariate analyses including factor analysis (FA) and cluster analysis (CA) were used to evaluate the hydrogeochemical variations in stream and groundwater in an intensive agricultural area in the Haean basin of Korea. In total, 143 stream and groundwater samples were collected from sites with different land uses in August and November 2011 and April, June, and September 2012 during the farming and agricultural off seasons, and analysed for their physicochemical constituents and isotopic compositions. From the FA, four latent factors were identified as being responsible for the physicochemical weathering and anthropogenic influences in the sampled waters. The results of CA revealed four groups of similarity between the sampling sites reflecting the different physicochemical characteristics and pollution levels of the study area. The hyporheic exchange was also measured in August 2011 to investigate hydroecological processes and the flux indicated a strong loss of stream water. The contaminants in stream water derived from agricultural activities were loaded to groundwater through the hyporheic exchange. In addition, pyrosequencing of 16S rRNA analysis were used to evaluate the microbial ecosystem in the hyporheic zone. The bacterial communities acted as a sink for contaminated stream water depending on the flux and microbial community. The study results provide useful information regarding variation in stream and groundwater quality and can aid in the development of effective pollution control and management systems in an intensive agricultural area.

INTRODUCTION

The quality and quantity of groundwater and stream water are serious concerns in agricultural areas today (Hooda et al., 2000; Peters and Meybeck, 2000; Simeonov et al., 2003; Li et al., 2011; Varol et al., 2012; Kim et al., 2013, 2014). The quality of groundwater and stream water is a major factor affecting human health and ecological systems, especially in intensive agricultural areas where waters receive contaminants released from agricultural effluents (Chae et al., 2004, 2009; Zhang et al., 2012). Anthropogenic influences (e.g. agricultural activities) and natural processes (e.g. changes in precipitation, weathering of crustal materials, and erosion of soil) can degrade water quality (Lee et al., 2001; Kaown et al., 2009; Esmaeili and Moore, 2012; Jin et al., 2012).

The quantities of available groundwater and stream water are also important to consider for sustainable agricultural use (Scanlon et al., 2007). However, it is difficult to quantify the water resources in hydrological systems. Previous studies have considered various aspects of water quality, quantity, and assessment approaches (Chapman, 1996; Shrestha and Kazama, 2007; Kazi et al., 2009; Otero et al., 2009; Olsen et al., 2012). The groundwater and stream water interaction zone is a particularly interesting area for hydrogeologists, hydrologists, and ecologists. Investigations of the exchange of groundwater and stream water are crucial for understanding the quantity of water in a hydro-system (Hayashi and Rosenberry, 2002; Pretty et al., 2006; Smith et al., 2009). Furthermore, the exchange region can be an ecological hotspot (Boulton et al., 1998). This region could potentially have a vast capability to act as a biogeochemical filter for contaminated groundwater or stream water.

Assessments of groundwater and stream water quality and quantity are mostly based on hydrochemical, hydrological, or biological analyses. The present study focused on the Haean basin, a main base of agricultural production (vegetables) in Gangwon Province, Korea. The groundwater and stream water resources in the Haean area are mainly used to irrigate vegetables and grain crops, but are also used for drinking water. Management of water quality and quantity for these purposes is thus crucial in the study area.

The use of multivariate statistical techniques, such as factor analysis (FA) and cluster analysis (CA), allows for interpretation of a complex data matrix, such as data related to groundwater and stream water quality, and identification of possible factors that influence the groundwater and stream water system. Such analysis techniques are also useful for managing water contamination caused by agricultural activities in rural areas (Omo-Irabor et al., 2008; Krishna et al., 2009; Wang et al., 2013). Multivariate statistical techniques have been widely used to evaluate groundwater and stream water quality, and to identify the latent sources that influence groundwater and stream water (Singh et al., 2004; Huang et al., 2010). The present study used these techniques to investigate aspects of water quality and quantity. Bacterial communities in the interaction zone of groundwater and stream water were also examined.

The aim of this study was to investigate the spatial and temporal distributions of chemical and isotopic compositions of dissolved components including contaminants in groundwater and stream water, as well as the hydroecological features of the groundwater and stream water mixing area. A data matrix generated from a 2-year monitoring period (2011–2012) was subjected to multivariate statistical approaches (FA and CA) to identify factors that potentially explain the variations in water quality parameters in the Haean basin. The results of this study are helpful for achieving sustainable use of water in this agricultural area. Furthermore, identification of microorganisms in the groundwater and stream water interaction area may aid in understanding hydroecological processes.

STUDY AREA

Geology

The study area was Haean basin located in Yanggu County, Gangwon Province, Korea (Figure 1). The area is 64 km^2 in altitude from 339 m to 1,320 m and has a 'punch bowl' shape (Lee et al., 2013). The geographical features of the area have been formed through prolonged differential erosion (Yun et al., 2009). Haean basin is located in northeast of the Gyeonggi gneiss complex. The bed rocks of Haean basin mainly compose of Pre-Cambrian metamorphic rocks and Jurassic igneous rocks. The metamorphic rocks are distributed in the outer-rim region and are mainly made up of alternating meta-sedimentary rocks of mica schist, biotite-feldspar gneiss and quartzite. The igneous rocks intruding into the composite metamorphic rocks are distributed in the central region (Kwon et al., 1990). The igneous rocks are relatively weaker to weathering than the metamorphic rocks.

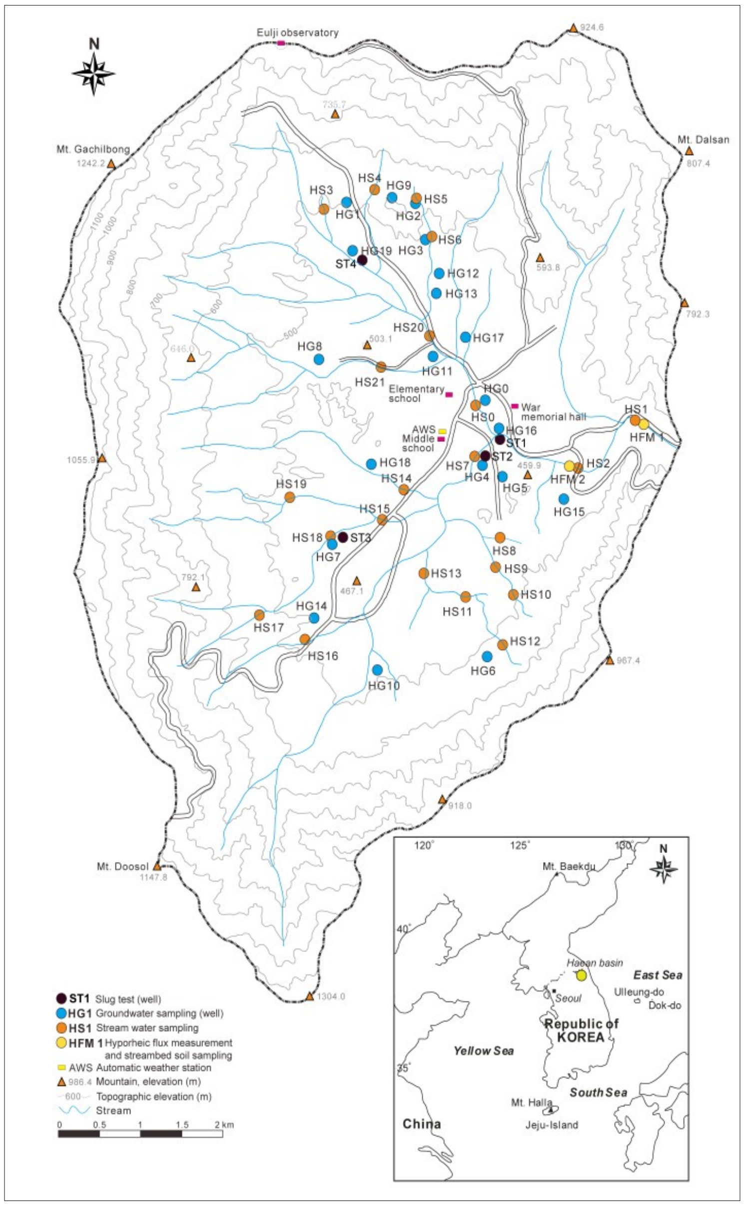

Figure 1. Location of the study area and distribution of the monitoring points.

Hydrology

The area has a very simple water drainage system. It has three main streams (Seonghwang, Dosol, and Mandae streams) with several branches. The streams converge in the flat area of eastern region of the basin and the stream water leaves the basin at the eastern border of the study area, where it eventually converges with the Soyang River. Most surface water exists as the type of stream water. The total length of the stream is 63 km. Therefore, the hydrogeological system of the area is relatively simple and can be understood easily when it comes to comparing it with other areas (Choi and Lee, 2010, Lee et al., 2013). In addition, the groundwater is converged with the stream. Grain size of sediment at streambed ranges from medium sand to fine gravel. Especially, sediment is composed of gravel at upstream and fine sand at downstream, respectively (Kim et al., 2014).

Groundwater levels (depth to water) at topographic elevations of 400 to 450 m (the lowest elevation) ranged from 1 to 3 m, and those at elevations of 500 to 600 m ranged from 5 to 10 m (Yun et al., 2009). The range of groundwater levels in the study area is dependent on the topographic elevation, and the groundwater flow is toward the streams in the centre of the basin. Slug tests at four wells, labelled as ST1, ST2, ST3, and ST4, yielded hydraulic conductivities of 4.94×10^{-5}, 1.0×10^{-3}, 3.07×10^{-5}, and 1.0×10^{-3} cm/sec, respectively. The Haean basin has 111 groundwater wells officially reported in the study area (Lee, 2009), which corresponds to 1.93 wells/km^2 of the total land area but approximately 10 wells/km^2 in the agricultural area. Among the reported wells, most (91%) were developed for agricultural water supply and groundwater pumping mainly occurs from May to August (Lee, 2009). However, there are much more wells, which is not reported, in the agricultural area. In addition, the wells have been poorly managed and neglected.

Climate

The average precipitation from 2008 to 2012 was 1,288.5 mm with 52% falling during the summer season (July and August) (Figure 2) (Korea Meteorological Administration, 2013). The climate is characterized by the East Asian monsoon, which produces two distinct seasons (rainy and dry). From the annual precipitation data for the five years between 2008 and 2012, the dry and rainy seasons were identified to be from October to February and from July to September, respectively.

The annual average air temperature was 9.6 to 10.6ºC for the period (Figure 2). However, it was fairly higher to 26ºC in summer (July) while it was much colder to -15.5ºC in winter (January) (Lee, 2009). Compared with the air temperatures in the summer (30–35ºC) in other inland areas of the country, they are relatively low (cool) in this area, and thus the basin has been one of the main areas for vegetables (cultivated in relatively low temperature) production in this country.

Land Use

Approximately 40% of the total area is used to farming rice and vegetable (Figure 3). The rest is mainly forested (58%) and residential area (2%) (Kim et al., 2013; National Academy of Agricultural Science, 2013). Soils of the agricultural areas can be mainly characterized as terric Cambisols or as Anthrosols (Kettering et al., 2012). The estimated total amount of nitrogen fertilizer applied in the Haean basin is 101 to 179 $kgNha^{-1}yr^{-1}$ (Yanggu County Office, 2013). The main crops of vegetable fields are Chinese cabbage, radish, potato and soybean (Yanggu County Office, 2013).

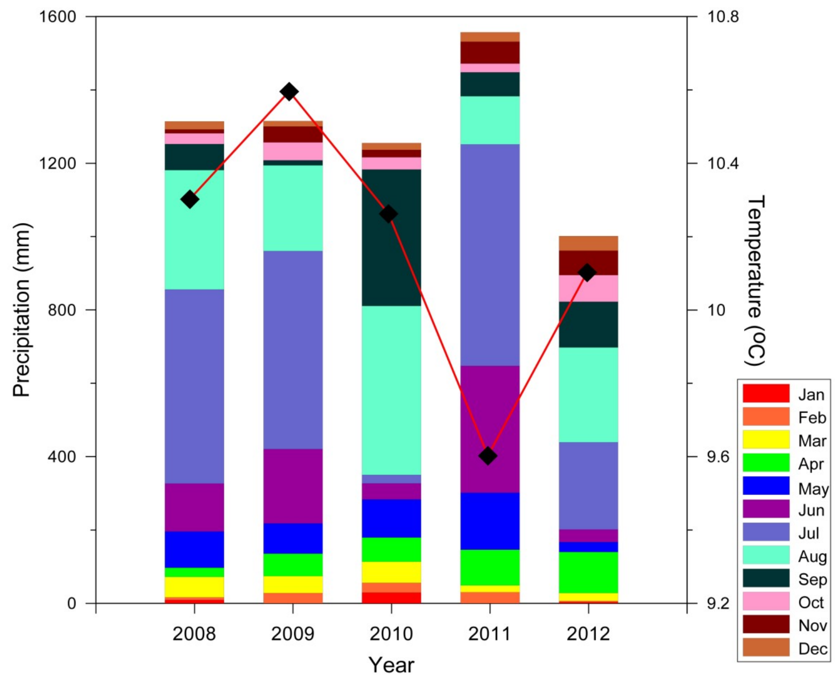

Figure 2. Air temperature and precipitation in the study area.

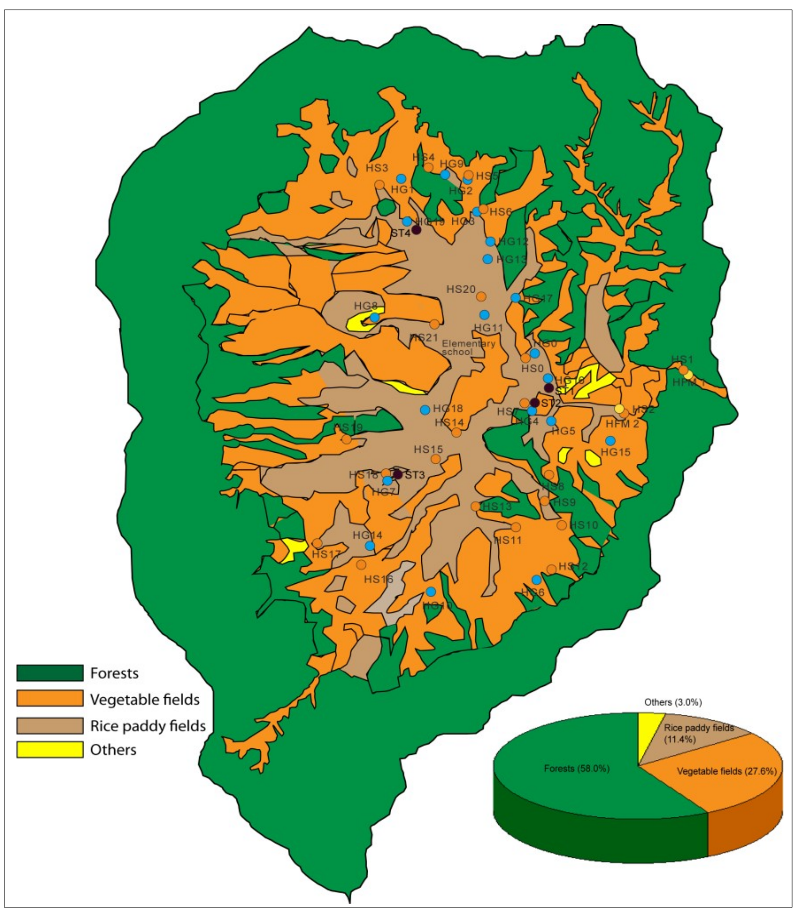

Figure 3. Land use and the monitoring points.

METHODS AND MATERIALS

Sampling and Chemical and Isotopic Analyses

The sample locations are shown in Figure 1 and the locations of 22 stream water sampling sites were classified according to land use patterns: 10 points (HS0, HS7, HS9, HS14, HS15, HS17, HS18, HS19, HS20, and HS21) were located in the rice paddy areas and 12 points (HS1, HS2, HS3, HS4, HS5, HS6, HS8, HS10, HS11, HS12, HS13 and HS16) were located in the vegetable field areas. The locations of 20 groundwater wells were also classified according to the same land use patterns: 11 points (HG0, HG5, HG7, HG9, HG11, HG12, HG13, HG14, HG16, HG18, and HG19) were located in the rice paddy areas and nine points (HG1, HG2, HG3, HG4, HG6, HG 8, HG 10, HG 15, and HG17) were in the vegetable field areas. The depths of the groundwater wells ranged from 6.7 to 200 m.

In total, 71 stream water and 72 groundwater samples were collected from the areas of vegetable fields and rice paddy fields in August and November 2011 and April, June, and September 2012. Water samples (each 100 mL) were transferred in acid-washed polypropylene bottles for chemical analysis of cations and anions after filtering through 0.45-μm membrane filters. Water temperature, pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), and electrical conductivity (EC) of groundwater and stream water were measured in the field using a portable meter (YSI556; YSI, USA).

Alkalinity, expressed as bicarbonate, was quantified with a digital auto-titrator with 0.05 N HCl and methyl orange as an indicator. Sodium, potassium, calcium, magnesium, and silica concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES/iCAP 6500 Duo; SPECTRO, USA), and sulphate, chloride, and nitrate concentrations were analysed by ion chromatography (761 Compact IC, Metrohm AG, Switzerland) at the Analytical Centre for Science Research of Sangji University in Wonju, Korea. The δD (Morrison et al., 2001) and $\delta^{18}O$ (Epstein and Mayeda, 1953) were analysed by stable isotope ratio mass spectrometer (Isoprime, GV Instruments, England) at the Korea Basic Science Institute (KBSI, Korea). The reliability of the chemical analyses was evaluated by the calculated charge imbalances between cations and anions, all of which were within ±10%.

The $\delta^{15}N$ and $\delta^{18}O$ values of dissolved nitrate were analyzed in the Isotope Science Laboratory at the University of Calgary (Alberta, Canada) using the denitrifier method (Casciotti et al., 2002). Furthermore, the N isotopic compositions of fertilizer sample were also determined. $\delta^{15}N$ values were analyzed using an elemental analyzer coupled to an isotopic ratios mass spectrometer (IRMS) in continuous flow mode achieving a precision of <±0.2‰. Sulfate in the groundwater was precipitated as $BaSO_4$ and $\delta^{34}S$ and $\delta^{18}O$ values were determined in the Isotope Science Laboratory at the University of Calgary as described in Shanley et al. (2005).

Data Treatment and Multivariate Analysis

Multivariate analyses using FA and CA techniques for the groundwater and stream water data sets were performed to determine the underlying processes of the water chemistry (Lee et al., 2001, 2009; Reghunath et al., 2002; Panda et al., 2006; Kim et al., 2009, 2014). A principal component provides information on the most meaningful parameters that describe the whole data set, allowing data reduction with minimum loss of original information. A factor analysis further reduces the contribution of less significant variables. CA, which is an unsupervised pattern recognition technique, reveals the intrinsic structure of a data set without making a prior assumption about the data to classify objects in the system into categories or clusters based on their nearness or similarity (Daughney and Reeves, 2006). The Euclidean distance usually indicates similarities between two samples, and a distance can be represented by the difference between analytical values from both samples.

For variables such as K, NO_3 and SO_4 of which analysis results were lower than the detection limits (0.01 μg/L for K; 0.01 μg/L for NO_3; 0.01 μg/L for SO_4), values equal to the half of the detection limits were assigned to the water chemistry data (Alley, 1993; Chae et al., 2004). Before FA and CA, the goodness of fit of the data to the normal distribution was checked by analyzing Kolmogorov-Smirnov statics (Shrestha and Kazama, 2007). According to the Kolmogorov-Smirnov statics test, all the parameters were normal distributed with 95% of higher confidence level (α = 0.05). After Kolmogorov-Smirnov statics test, the experimental data were standardized through a z-transformation to avoid misinterpretation due to wide differences in data dimensionality (Omo-Irabor et al., 2008). First, a Pearson correlation analysis was performed between the groundwater and stream water parameters to identify possible relationships. Then, a factor analysis of the physicochemical data was undertaken to quantify the contributions of anthropogenic inputs and natural weathering processes to the chemical composition of groundwater and stream water. The factor analysis technique extracted the eigenvalues from the co-variance matrix of original variables. The variables used for FA and CA were T, EC, ORP, DO, pH, Ca, K, Mg, Na, Si, Cl, NO_3, SO_4, and HCO_3. The calculation was performed using SPSS 18.

Seepage and Vertical Head Gradient (VHG) Measurement

The seepage meters (Lee, 1977) were used to measure the seepage rate at the HFM1 and HFM2 sites (see locations in Figure 1). The meters were driven into the streambed, open end down, to a depth of 10 cm. Size of the installed seepage meters was 0.3 m in diameter and 0.1 m in height. Also, vertical head gradients (VHG) were also measured on HFM1 and HFM2 sites. Installations of piezometers (Rosenberry et al., 2012) were adapted for measuring the vertical head gradients and direction of hyporheic water flow. Kim et al. (2014) suggested that the hyporheic zone depth of the basin is 9–15 cm using heat transfer analysis. A total of 35 piezometers were inserted at 0.1 m depth beneath the streambed adjacent to each seepage meter installation point at the regular interval. At each point, the seepage and VHGs were measured for 24 hours from August 13, 2011 (Figure 4).

We also analyzed soil grain size of the streambed sediments at the points where the seepage meter and piezometer were installed to obtain physical properties of the sediments. The streambed sediments were collected within the upper 0.1 m using an auger type sampler. The grain size distributions were determined by sieve analysis and a laser particle size analyzer at Kangwon National University. Table 1 shows the results of soil size distribution, soil texture, and porosity for HFM1 and HFM2. It shows the results of porosity 0.32, bulk density 1.4 g/cm^3, and composed of 90.44% sand, 0.18% silt, and 9.38% clay at HFM1 site and 0.32, 1.5 g/cm^3, and 88.45% sand, 0.22% silt, and 11.33% clay at HFM2 site, respectively. The soil texture is sand in HFM1 and loamy sand at HFM2.

Table 1. Results of soil size analyses and physical properties.

Sampling points	Particle size distribution			Soil texture	Bulk density (g/cm^3)	Porosity
	Sand (%)	Silt (%)	Clay (%)			
HFM1	90.44	0.18	9.38	sand	0.14	0.32
HFM2	88.45	0.22	11.33	loamy sand	0.15	0.32

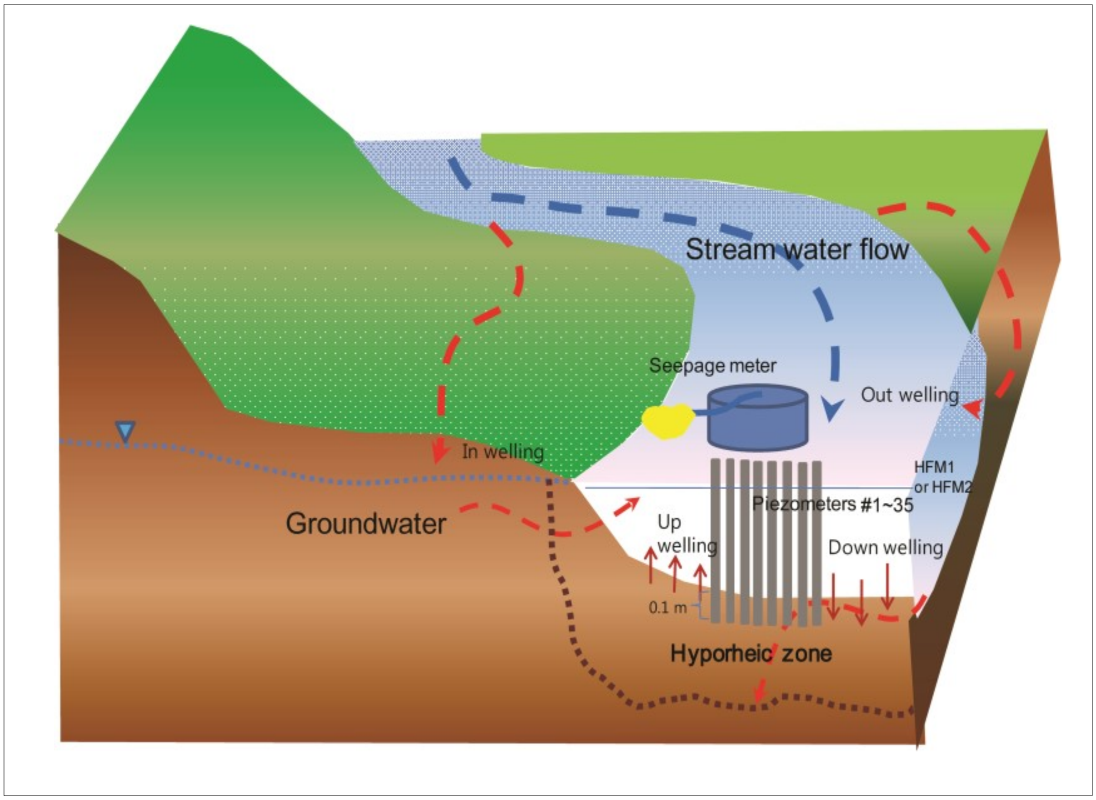

Figure 4. Seepage meter and piezometers for measuring water flux across the streambed and hydraulic head.

Microbial Analysis

Microbial communities were analysed using streambed sediment (soil) samples collected at HFM1 and HFM2 in August 2011. The sediment samples from the upper 0.1–0.3 m of the streambeds were taken from the stream centre and the boundary between adjacent sites using a soil hand auger. The samples were brought to a laboratory and stored in a -70ºC refrigerator until soil DNA extraction and cloning analysis. The sampled soils were studied by using DNA-based analysis. The streambed soil was cloned from soil DNAs and cloning analysis. The DNA of sampled soil was extracted using a FastDNA Spin Kit (Qbiogene, USA) as specified by the manufacturer. The quality of extracted DNA was checked by standard agarose gel electrophoresis and stored at -20ºC. The DNA concentration was determined using a UV-VIS Spectrophotometer (Mechasys Co. Ltd., Korea).

The Polymerase Chain Reaction (PCR) was carried out using the primers 27F, 338F, 518R, 1522R, T7, and SP6. These primers were dissolved to a concentration of 10 pmol/µL. All primer sequences and references are given in Table 2. These primers were synthesized by Bioneer Co. Ltd., Korea. The PCR amplification conditions were as follows: 25 cycles with an initial denaturation of DNA at 94ºC for 8 min, followed by 25 cycles of 30 sec at 94ºC, 30 sec at 60ºC, and 30 sec at 72ºC. The PCR products were purified using a PCR Purification Kit (Bioneer Co. Ltd., Korea). The PCR products obtained from the soil DNA were cloned into the pGEM-T Easy Vector as recommended by the manufacturer (Promega, USA). The preparation of randomly selected clones, followed by PCR amplification of a cloned insert and purification of PCR product, was performed as described previously (Hengstmann et al., 1999). Sequencing was performed with an ABI prism BioDye Terminator Cycle Sequencing Ready Kit (Applied Biosystems, USA).

Table 2. 16S rDNA-targeted oligonucleotide primers used in this study.

Primer	Position	Primer sequence (5'-3')	References
27F	9-27	GAGTTTGATCCTGGCTCAG	Lane (1991)
338F	339-358	CTCCTACGGGAGGCAGCAGT	Muyzer et al. (1993)
518R	536-519	GTATTACCGCGGCTGCTG	Muyzer et al. (1993)
1522R	1522-1509	AAGGAGGTGATCCANCCRCA	Johnson (1994)
T7	pGEM T-Vector	TAATACGACTCACTATAGGG	Promega
SP6	pGEM T-Vector	ATTTAGGTGACACTATAGAA	Promega

The full sequences were analysed and compared with other known sequences that were available in the NCBI database. A search for sequence similarities with known genes was performed using a BLAST analysis. Identification of the conserved region and protein translations and analysis of amino acids were performed using BioEdit Sequence Alignment Editor (Ibis Biosciences, USA), and phylogenetic analysis was performed on amino acid sequences using Clustal and MEGA 4 (Larkin et al., 2007).

RESULTS AND DISCUSSION

Water Chemistry with its Spatial and Temporal Variations

Box and whisker plots of selected field parameters (water temperature, EC, ORP, DO, and pH) showing temporal trends are shown in Figure 5. The average stream water temperature was higher in August, June, and September than in November and April. The groundwater temperature also showed seasonal variations but its variation range is relatively narrow compared with stream water temperature. The EC levels of stream water varied less in August 2011 than in June 2012. However, the variation in EC levels followed an inverse pattern in groundwater. Precipitation had a large effect on the EC of stream water but a small effect on the EC of groundwater. The average ORP in stream water is higher in August 2011 and September 2012, than in June 2012. The average ORP in groundwater had the relatively narrow range compared with stream water. However, there was wide variation of groundwater ORP in April 2012 as compared to August and November 2011, and June and September 2012.

The DO of groundwater did not show a significant seasonal variation; however, the DO of stream water followed a distinct seasonal pattern. The inverse relationship between DO and temperature is a natural process (Knights et al., 1995). The DO of stream water was higher in November 2011 than in August 2011 and April, June, and September 2012. However, the temperature results in April 2012 were similar to those in November 2011. This similarity might have been due to a severe drought in 2012. The pH of groundwater and stream water showed the similar variation pattern over the study period. The major ions in water samples in different seasons are plotted on a piper diagram (Figure 6). The water samples mainly plot in the area of the Ca-HCO_3 water type and partly in the Ca-Cl type. The Ca-Cl type found in August and November 2011 was considered to indicate contamination by anthropogenic inputs, whereas the Ca-HCO_3 type represented relatively clean water (Prasanna et al., 2011).

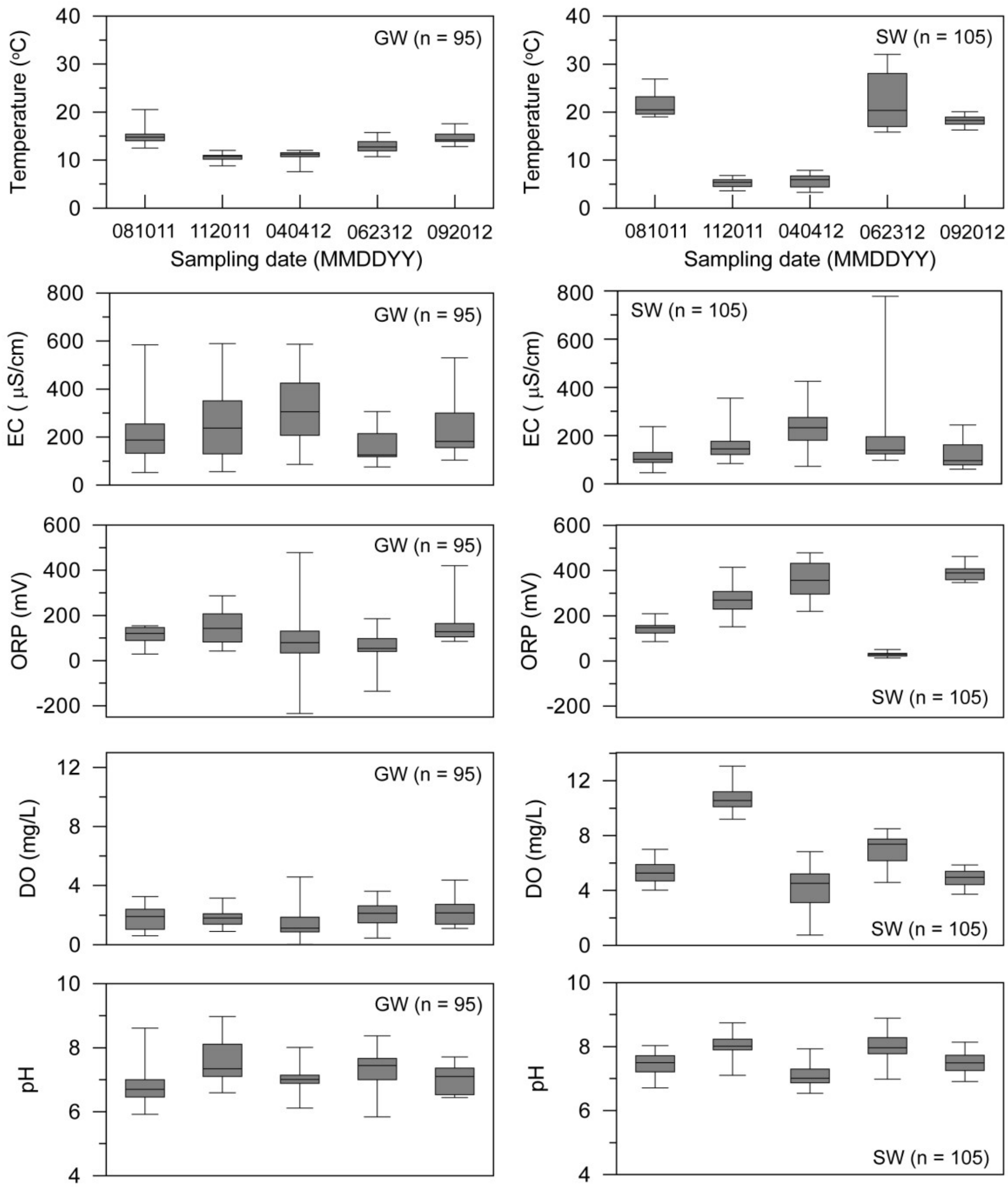

Figure 5. Spatial and temporal variations in temperature, EC, ORP, DO, and pH in stream water and groundwater in the Haean basin.

The basic statistics for all of the groundwater and stream water quality parameters measured during the 2-year sampling period at 20 groundwater sampling sites and 22 stream water sampling sites in an agricultural area of the Haean basin are summarized in Table 3. NO_3 and SO_4 as anions and Ca, K, and Mg as cations was dominant in groundwater, which can be typical of water contaminated with chemical fertilizers as [$(NH_4)_2SO_4$, K_3PO_4, and (Ca, Mg)CO_3]. The levels of the five ions in groundwater were more variable than those in stream water (see the coefficients of variation in Table 3). Levels of Ca and K ions in the groundwater were the most variable of the five ions. The mean nitrate concentrations were below than the maximum acceptable level of Korean water quality standards (44 mg/L NO_3). However, 18% of all groundwater samples (HG5, HG11, HG16, HG17, and HG19 in August 2011; HG5, HG8, and HG17 in April 2012; HG3, HG5, and HG11 in June 2012; HG18 and HG19 in September 2012) exhibited concentrations exceeding the maximum acceptable level.

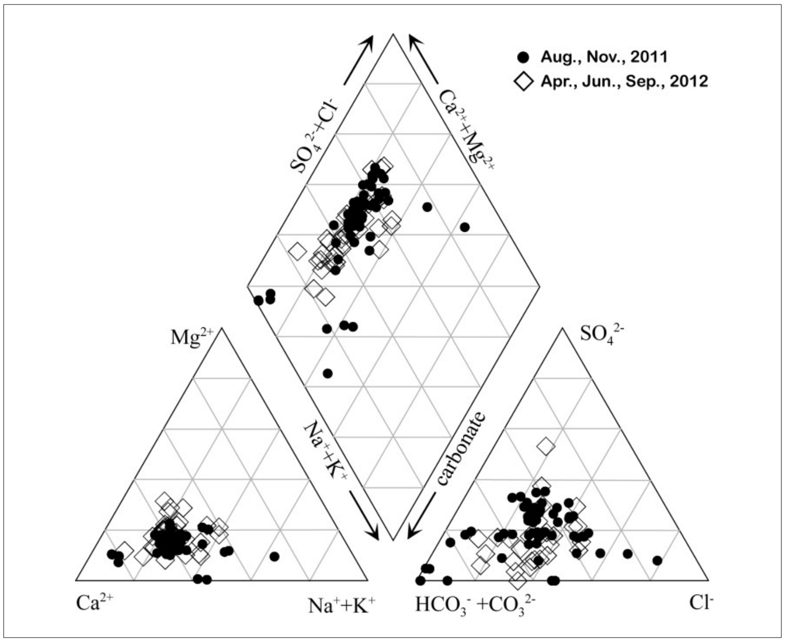

Figure 6. Piper plot showing the major chemical composition of water samples.

Furthermore, 38% of the groundwater samples contaminated with nitrate were collected during the heavy monsoon season in August 2011, 23% in the pre-monsoon season in April and June, respectively, and 15% after the monsoon season in September 2012. Soil conditions can be enhanced or retard nitrate leaching (Alhajjar et al., 1990). Leaching can also be enhanced by flood irrigation practices and by storm water (Domagalski and Dubrovsky, 1992). Among the stream water samples, 80% (HS8 in August 2011, HS3, HS5, HS6, and HS8 in April, and HS5 in September 2012) exceeded nitrate quality standards. Furthermore, 68% of stream water samples were contaminated in April 2012 when it is the pre-monsoon season.

Groundwater and stream water nitrate concentrations varied spatially and temporally, including seasonally, in the study area. This indicates that there is large seasonal variability in the run-off of chemical fertilizers from agricultural fields (in this case, rice paddy and vegetable fields), resulting in seasonal effects on groundwater and stream water quality (Pitt et al., 1999). HG5 displayed constantly high nitrate concentrations (maximum concentration of 134.9 mg/L; see Table 3). HG5 was located at the confluence of two branches (from the west and south) of the stream, which flows eastward in the basin. HG5 represents the mixed hydrogeochemical properties of the western and southern parts of the study area and indicates that those areas were more contaminated by nitrate than the northern part of the study area. Chemical inputs from agricultural activities can substantially alter the quality of groundwater and stream water (Aravena and Robertson, 1998).

A cumulative frequency plot of NO_3 concentrations in the water samples can be used to evaluate the influence of agricultural activities. Cumulative probability technique was taken to investigate the NO_3-N background value (Sinclair, 1991; Panno et al., 2006). One of the most frequently cited studies of nitrate background values in groundwater is Madison and Brunett (1985). They suggested that the natural background concentration of NO_3-N was 0.2 mg/L and that concentrations over 3 mg/L could be attributed to anthropogenic effects. Also, the background concentration of NO_3-N in groundwater in Gangwon province of Korea was 2.7 mg/L (Kaown et al., 2007).

Table 3. Statistical summary of the measured parameters and major chemical constituents from 71 stream water and 72 groundwater samples.

Parameter	Source	Minimum	Maximum	Mean	Median	S.D	C.V
T	S	3.3	28.2	13.9	16.9	7.6	0.5
	G	7.4	20.5	13.2	13.6	2.3	0.2
EC	S	45.8	425.0	150.7	130.0	74.0	0.5
	G	56.3	2999.0	291.0	182.0	398.5	1.4
ORP	S	21.0	479.0	262.0	264.0	138.3	0.5
	G	-136.1	478.0	126.5	114.0	90.4	0.7
DO	S	0.8	13.1	6.5	5.5	2.8	0.4
	G	0.1	9.1	2.2	2.0	1.5	0.7
pH	S	6.6	8.7	7.7	7.7	0.5	0.1
	G	5.8	9.0	7.1	7.1	0.6	0.1
Ca	S	3.4	42.2	14.7	13.1	7.2	0.5
	G	4.1	273.5	32.0	20.6	46.6	1.5
K	S	0.0	13.3	1.8	1.2	2.1	1.1
	G	0.0	20.2	1.8	1.1	2.6	1.5
Mg	S	0.8	10.3	2.7	2.3	1.7	0.6
	G	0.1	28.3	4.2	3.1	4.5	1.1
Na	S	2.8	44.4	6.7	5.1	6.7	1.0
	G	4.8	52.0	10.3	8.2	6.4	0.6
Si	S	1.7	10.5	6.7	6.4	1.8	0.3
	G	4.3	40.5	11.9	10.4	7.2	0.6
Cl	S	1.3	106.0	10.4	6.9	14.5	1.4
	G	0.6	59.0	10.0	8.8	9.4	0.9
NO_3	S	0.1	76.1	21.9	16.8	15.8	0.7
	G	0.0	134.9	23.9	17.6	24.5	1.0
SO_4	S	2.0	37.3	7.6	6.1	5.5	0.7
	G	0.0	52.4	6.6	5.2	7.2	1.1
HCO_3	S	4.3	35.6	14.5	12.8	6.2	0.4
	G	9.6	411.1	44.1	26.0	73.1	1.7

Cumulative probability graphs of NO_3-N data for the groundwater samples are shown in Figure 7. The histogram of the log NO_3-N values showed a lognormal distribution. The inflection points on the probability graph indicate an interpretable breakdown of distribution of the logged values, as follows: (1) above a logged value of 1.08 (NO_3-N = 12.3 mg/L), the distribution was the skewed in the left with relatively short left-hand tail; (2) between the logged values of 0.46 and 1.08 (NO_3-N = 2.92 and 12.3 mg/L, respectively), the distribution was somewhat skewed to the right; and (3) between logged values of -0.16 and 0.46 (NO_3-N = 0.69 and 2.92 mg/L, respectively), the distribution was skewed in the opposite direction, to the right.

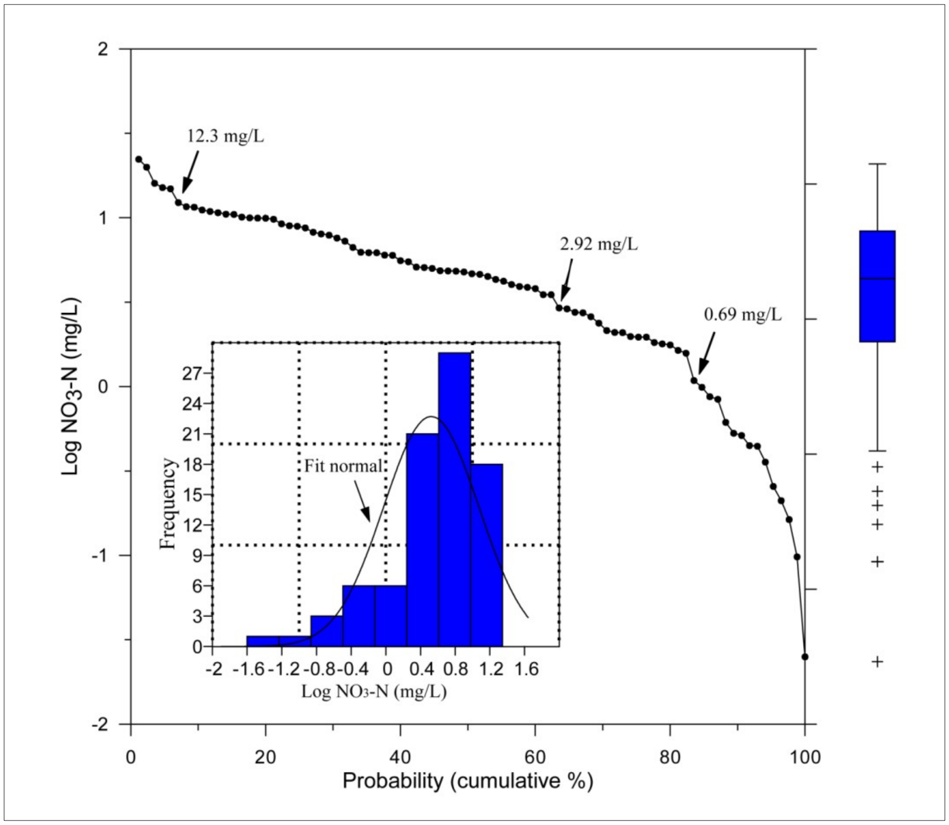

Figure 7. Cumulative probability graph for groundwater samples showing threshold values. A histogram and box and whisker plot of the data are also shown.

Three thresholds (inflection points or points where the slope change) were used to identify the NO_3-N background values for data sets in Haean basin. The threshold values for the groundwater samples were 0.69, 2.92, and 12.3 mg/L, respectively. The lowest NO_3-N threshold value (0.69 mg/L) was interpreted as the natural background concentration from precipitation. The threshold value of 2.92 mg/L was regarded as the upper limit of present-day background concentration, and concentrations above this value in the site were land-based anthropogenic sources. The water samples with concentrations exceeding 12.3 mg/L were probably dominated by septic and animal wastes. In the Haean basin, present-day background in the sampled groundwater was estimated to be 0.69 to 2.92 mg/L.

Correlation between Parameters

The results of the correlation analysis are presented in Table 4. In groundwater, Ca showed the strong positive correlations with Mg, Si, Na, and SO_4 and NO_3 had strong negative correlation with HCO_3. NO_3 and SO_4 were positively correlated with Na and Mg, which indicated that these components were derived from a similar source and moved together. Results for Na, Mg, NO_3, and SO_4 indicated their origin from chemical fertilizers. The strong positive correlation between Na, Ca, and Mg may be due to weathering, which is responsible for water mineralization. A negative correlation was found between HCO_3 and NO_3. The significant negative correlation could be explained by heterotrophic denitrification processes. In addition, the results of the correlation analysis for stream water are shown in Table 4. Ca had strong positive correlations with Mg, Na, Cl, SO_4, and HCO_3. However, there was no denitrification process occurring in the stream. All of the groundwater sampling

Table 4. Pearson correlation matrix for physicochemical data from groundwater samples (August and November 2011 and April, June, and September 2012) with correlation values given in the triangle. Values greater than 0.5 are shown in bold.

	T	EC	ORP	DO	pH	Ca	K	Mg	Na	Si	Cl	NO_3	SO_4	HCO_3
T														
EC	0.077													
ORP	-0.115	0.075												
DO	0.021	-0.121	0.259											
pH	-0.171	-0.211	0.066	0.133										
Ca	-0.130	0.419	-0.050	-0.088	-0.238									
K	-0.144	0.137	-0.029	0.047	-0.066	0.457								
Mg	-0.043	0.240	-0.144	-0.068	-0.189	**0.738**	0.409							
Na	0.029	0.201	-0.105	-0.050	-0.070	0.518	0.347	0.884						
Si	-0.057	0.330	-0.098	-0.100	-0.179	**0.890**	0.403	**0.710**	**0.565**					
Cl	-0.139	-0.054	0.164	0.135	-0.189	-0.038	0.231	0.448	**0.563**	-0.057				
NO_3	0.139	-0.146	-0.008	0.035	-0.095	-0.158	-0.064	**0.715**	**0.663**	-0.056	**0.618**			
SO_4	0.178	0.021	-0.189	-0.096	-0.041	-0.035	0.163	**0.690**	**0.645**	0.003	**0.600**	**0.506**		
HCO_3	-0.132	0.423	-0.063	-0.094	-0.199	**0.989**	0.474	**0.704**	0.475	0.883	-0.104	**-0.764**	-0.081	

Table 4. Continued for stream water samples.

	T	EC	ORP	DO	pH	Ca	K	Mg	Na	Si	Cl	NO_3	SO_4	HCO_3
T														
EC	-0.355													
ORP	-0.452	0.015												
DO	-0.217	0.024	-0.211											
pH	-0.123	-0.218	-0.285	**0.562**										
Ca	-0.169	0.421	-0.156	0.100	-0.134									
K	-0.208	0.296	-0.126	0.078	0.013	0.476								
Mg	-0.145	0.429	-0.096	0.021	-0.144	**0.913**	0.424							
Na	-0.172	0.398	-0.313	0.366	0.089	**0.693**	0.338	**0.597**						
Si	-0.010	-0.110	-0.133	0.472	0.326	**0.085**	-0.095	-0.057	0.225					
Cl	-0.294	0.469	-0.101	0.148	-0.091	**0.794**	**0.505**	**0.709**	**0.844**	-0.110				
NO_3	-0.295	0.248	0.383	-0.139	-0.203	0.391	0.209	0.324	-0.048	0.252	0.152			
SO_4	-0.096	0.381	-0.054	-0.057	-0.214	**0.778**	0.386	**0.900**	0.432	-0.172	**0.576**	0.274		
HCO_3	-0.105	0.254	-0.387	0.475	0.202	**0.719**	0.257	**0.623**	**0.664**	0.364	**0.517**	0.051	0.461	

wells and stream water sampling points were located around rice paddy or vegetable fields, and thus had a contaminant source at the surface. However, as mentioned earlier, 19% of groundwater samples and 8% stream water samples also exceeded the standard for drinking water in 2011–2012. This indicates that the nitrate contamination is related land use patterns and agricultural activities.

Factor Analysis

Table 5 summarizes the FA results after rotation, including the loadings, the eigenvalues, the amount of variance explained by each factor, and the cumulative variance. The results of factor analysis based on the four most significant factors indicated that these factors explain 58.97% of variance for groundwater chemistry and 76.80% of that for stream water chemistry. In this analysis, factor 1 of groundwater explained 21.80% of the variance and was strongly related with EC, Na, Cl, NO_3, and HCO_3. The variables of EC, Na and Cl have high positive loading on factor 1. The factor 1 group represent sea water intrusion into groundwater. However, sea water intrusion is less convincing because the study site is located in inland. Therefore, this factor clearly represents contamination with chemical deicer, as indicated by its strong correlation with EC, HCO_3, Na and Cl. Yanggu Country Office (2013) uses approximately 40,000 kg of sodium chloride for de-icing purposes on roads every winter. Factor 1 of groundwater was interpreted as groundwater contaminated by anthropogenic pollutants. Additionally, factor 1 described as affected by pollution sources related to agricultural activity. The constituents NO_3 could be derived from anthropogenic pollution. The NO_3 comes from anthropogenic pollution sources such as sanitation facilities, domestic effluents, atmospheric fallout, and agricultural fertilizer usage (Ritzi et al., 1993). The source has no known lithological source (Jeong, 2001). This factor 1 is attributed to anthropogenic influence of Na, Cl, and NO_3 in groundwater. The data shows that these ions have migrated from surface. When polluted surface water is infiltrated, the HCO_3 could be derived from natural processes such as the dissolution of carbonate minerals, soil CO_2, or bacteria degradation of organic materials (Jeong, 2001).

The factor 2 explained 16.55% of the total variance, with strong positive loading for Ca, K, Na, and NO_3. It can be inferred that the factor 2 are involved in determining the chemical fertilizer composition. Factors 3 and 4 of groundwater explained 10.54% and 10.08% of the variance, respectively. Factor 3 includes EC, pH, SO_4, and HCO_3 with a positive loading and the factor 4 includes EC, DO, and pH with a positive loading, respectively. These factors represent the typical characteristics of groundwater that has experienced a long water–rock interaction. Through the results of factor analysis of groundwater set are largely influenced by the contaminant source of agricultural area rather than natural processes.

For the data set representing the stream water, of four significant factors, factor 1 explained 34.80% of the total variance, with strong positive loading for EC, Ca, Mg, Cl, NO_3, and SO_4. As mentioned in the previous paragraph, EC, Ca, Mg, Cl, NO_3, and SO_4 could be supplied from chemical fertilizers. This factor represents the contribution of non-point source pollution from vegetable and rice paddy fields. Factor 2 explained 18.32% of the total variance and had a moderate positive loading for temperature, DO, ORP, pH, and HCO_3. Factor 3 explained 15.54% of the total variance and had negative loading for ORP and positive loading for temperature and HCO_3. The strong loading for ORP was attributed to the diurnal variation in atmosphere temperature and seasonal change in factors 2 and 3, respectively. Finally, factor 4 explained 8.14% of the total variance and had strong positive loadings for EC and SO_4 and moderate loadings for NO_3. These factors represent the contribution of chemical fertilizers from the agricultural area.

Table 5. Eigenvalues of factors extracted through principal component analysis, differences between the factors and the variance explained by the factors (August and November 2011 and April, June, and September 2012), and the rotated factor pattern of extracted factors after Varimax rotation (high loading values (>1.0) are shown in bold).

	Factors of GW				Factors of SW			
	1	2	3	4	1	2	3	4
Temp.	0.16	-0.90	0.70	0.54	-0.27	**1.90**	**1.77**	0.88
EC	**1.38**	0.59	**1.13**	**1.94**	**1.49**	0.33	0.56	**1.82**
ORP	-0.77	-0.46	0.26	0.93	0.32	**1.54**	**-2.57**	-0.42
DO	-0.37	0.21	-0.85	**2.12**	0.15	**1.84**	0.73	0.32
pH	0.90	0.54	**1.64**	**1.30**	-0.77	**1.88**	0.51	-0.22
Ca	0.83	**1.36**	0.39	-0.10	**1.02**	-0.28	0.36	-0.42
K	-0.44	**1.54**	-0.02	-0.61	0.81	0.16	0.24	0.52
Mg	-0.87	0.99	-0.15	0.26	**1.43**	0.06	0.25	-0.61
Na	**1.23**	**1.30**	0.16	0.09	0.85	-0.59	0.81	0.62
Si	0.31	-0.19	0.05	-0.72	-0.08	**-1.32**	0.76	-0.65
Cl	**1.58**	0.44	0.01	0.47	**1.75**	-0.28	0.25	0.80
NO_3	**2.22**	**1.04**	0.49	0.58	**1.99**	-0.25	-0.52	**1.08**
SO_4	-0.74	-0.04	1.35	-0.84	**1.29**	-0.49	-0.36	**1.87**
HCO_3	**1.10**	**-2.03**	**1.08**	-0.55	0.91	**1.01**	**1.10**	0.70
Eigenvalue	15.70	11.91	7.59	7.25	24.71	13.01	11.03	5.78
% variance	21.80	16.55	10.54	10.08	34.80	18.32	15.54	8.14
Cumulative	21.80	38.35	48.89	58.97	34.80	53.12	68.66	76.80

Factor scores estimated for groundwater and stream water are shown in Table 6. This table shows strongly negative scores of factor 1 at HG14 (-1.2) in August 2011. HG14 was located in the south-western part of the study area (see Figure 1). HG14 is vegetable fields (see Figure 2). All samples of groundwater in August and November 2011 and April 2012 also had negative factor scores for factor 1. Conversely, all samples in September 2012 had positive scores of factor 1. There were strongly positive factor scores of factor 1 at HG9 (2.0) in September. HG9 is located in the northern part of the study area. HG9 is vegetable fields. However, the samples had partially negative and a positive factor scores in June 2012. Table 6 also lists stream water factor scores. There were strongly positive and moderate negative factor scores of factor 1 at HS6 (2.2) in September 2012 and HS18 (-0.7) in June 2012, respectively. HS6 was located in the northern part (vegetable fields) of the study area and HS18 was in the south-western part (paddy fields). Groundwater and stream water factor scores have the strong relationship with the land use. This indicates that there was a little spatial variation due to land use. However, there were differences among the value signs (negative and positive) and the intensity of factor scores, as well as the temporal variations.

Table 6. Factor scores estimated for sampled groundwater. High values (>0.5) are shown in bold.

Factor score of sampled ground water														
Sample	1	2	3	4	Sample	1	2	3	4	Sample	1	2	3	4
HG00A	**-0.6**	0.3	**-0.7**	0.0	HG1N	0.0	-0.1	**0.9**	0.3	HG1Ap	0.0	-0.2	**0.5**	0.1
HG01A	0.0	**-0.5**	0.3	0.4	HG4N	-0.1	**0.7**	-0.1	-0.1	HG2Ap	0.3	-0.3	0.4	**-0.6**
HG04A	0.2	0.4	-0.3	-0.2	HG6N	**0.7**	-0.3	**0.5**	0.1	HG3Ap	0.4	-0.2	0.3	**-0.6**
HG05A	**-0.6**	**-0.6**	-0.3	-0.3	HG14N	**0.5**	-0.1	0.3	0.2	HG4Ap	-0.2	**0.9**	-0.1	-0.1
HG06A	**0.5**	**-0.7**	0.2	0.1	HG16N	-0.1	-0.2	0.4	**0.6**	HG5Ap	-0.4	-0.3	0.2	**-0.5**
HG07A	0.4	**-0.6**	0.1	0.1	HG18N	0.0	**0.8**	0.1	0.0	HG8Ap	**-0.6**	-0.4	0.4	0.2
HG08A	-0.1	-0.3	**-0.8**	**0.5**						HG14Ap	-0.4	-0.1	0.4	0.0
HG10A	0.4	**-0.6**	-0.3	**0.6**						HG16Ap	0.2	0.1	0.3	0.0
HG11A	**-0.7**	0.0	**-0.6**	-0.2						HG17Ap	**-0.5**	0.3	0.2	-0.4
HG12A	**0.5**	-0.3	**-0.5**	-0.2						HG18Ap	**-0.5**	**0.5**	0.3	-0.2
HG14A	**-1.2**	-0.1	0.1	-0.1						HG19Ap	0.2	0.3	-0.1	**-0.6**
HG15A	0.0	-0.1	**-0.9**	0.0										
HG16A	-0.4	**-0.7**	-0.1	-0.1										
HG17A	**-0.9**	-0.1	-0.2	0.3										
HG18A	-0.1	**0.6**	-0.2	-0.2										
HG19A	-0.3	**-0.8**	0.2	-0.1										
Sample	1	2	3	4	Sample	1	2	3	4					
HG0J	**-0.7**	0.4	-0.2	0.0	HG0S	**0.7**	0.1	-0.2	-0.1					
HG1J	**0.7**	-0.4	0.3	0.0	HG1S	**0.5**	**-0.6**	0.1	0.4					
HG2J	0.3	-0.1	0.0	**0.8**	HG2S	0.2	**-0.7**	-0.1	0.0					
HG3J	**-0.5**	**-0.5**	-0.1	**0.6**	HG3S	-0.3	**0.5**	-0.3	0.4					
HG4J	-0.3	-0.1	**-0.5**	**0.5**	HG4S	0.2	-0.3	0.1	0.3					
HG5J	**-0.6**	**0.5**	-0.2	0.1	HG5S	-0.1	**-0.8**	0.0	-0.1					
HG6J	0.1	-0.4	0.4	0.2	HG6S	-0.2	0.4	**-0.7**	0.1					
HG7J	0.0	0.0	0.2	**0.9**	HG7S	-0.1	0.0	-0.1	**0.5**					
HG8J	0.1	-0.1	**0.7**	-0.3	HG8S	-0.3	-0.1	0.0	**0.7**					
HG9J	**-0.6**	0.3	-0.4	-0.2	HG9S	**2.0**	0.1	-0.4	0.1					
HG10J	0.1	0.2	-0.2	**-0.6**	HG10S	-0.2	-0.2	-0.3	-0.3					
HG11J	**-0.8**	-0.2	0.1	0.1	HG11S	0.3	-0.4	-0.4	-0.4					
HG12J	**0.8**	0.1	0.2	-0.1	HG12S	**0.7**	0.0	-0.3	0.0					
HG13J	-0.1	**-0.6**	0.0	0.0	HG13S	0.2	-0.4	-0.3	**0.7**					
HG14J	0.3	0.3	0.2	0.3	HG14S	0.3	-0.1	0.3	0.4					
HG15J	**0.6**	0.3	-0.3	0.1	HG15S	0.2	0.2	0.1	0.1					
HG16J	**0.7**	-0.1	0.1	-0.1	HG16S	-0.1	**-0.7**	**-0.5**	0.0					
HG17J	-0.1	**0.6**	0.1	0.0	HG17S	0.2	**0.7**	-0.4	-0.2					
HG18J	**0.7**	-0.2	0.1	-0.1	HG18S	**-0.7**	-0.1	-0.4	0.2					
					HG19S	**-0.6**	-0.4	-0.1	-0.4					

Table 6. Factor scores estimated for sampled stream water. High values (>0.5) are shown in bold.

Factor score of sampled stream water														
Sample	1	2	3	4	Sample	1	2	3	4	Sample	1	2	3	4
HS00A	-0.4	**0.6**	0.2	**-0.5**	HS0N	0.3	**-0.8**	0.2	0.0	HS0Ap	0.1	0.0	-0.2	**0.8**
HS01A	-0.3	**0.6**	**0.5**	**-0.5**	HS1N	**0.5**	**-0.6**	0.3	-0.2	HS1Ap	0.3	**-0.5**	**-0.7**	0.0
HS02A	-0.3	**0.7**	0.4	**-0.5**	HS2N	**0.5**	**-0.6**	0.2	0.3	HS3Ap	0.4	**0.5**	**-0.5**	0.3
HS04A	**-0.9**	0.1	-0.2	-0.1	HS4N	-0.2	-0.3	0.1	**0.6**	HS4Ap	-0.4	0.4	**-0.6**	-0.1
HS05A	**-0.6**	**0.5**	0.0	**-0.5**	HS5N	-0.2	**-0.6**	0.2	0.1	HS5Ap	**0.9**	0.2	-0.2	0.0
HS06A	0.5	0.2	**0.6**	-0.2	HS6N	0.1	**-0.7**	0.4	-0.1	HS6Ap	**0.8**	-0.1	-0.3	0.2
HS07A	**-0.8**	0.4	0.2	-0.4	HS7N	**0.5**	-0.4	0.3	0.4	HS8Ap	**0.8**	-0.1	-0.2	0.2
HS08A	**0.7**	0.2	0.2	0.1	HS11N	0.1	**-0.8**	0.4	0.0	HS9Ap	**0.9**	-0.1	0.1	-0.1
HS11A	-0.3	**0.7**	0.1	-0.3	HS12N	**-0.9**	-0.2	-0.2	0.2	HS14Ap	**0.9**	0.1	0.0	0.1
HS12A	**-0.9**	0.2	-0.2	-0.2	HS13N	-0.1	**-0.9**	0.0	0.0	HS16Ap	0.2	0.3	-0.2	**0.6**
HS13A	**-0.5**	**0.5**	0.2	-0.4	HS14N	0.1	**-0.9**	0.0	-0.1	HS17Ap	**0.5**	0.4	-0.1	0.2
HS15A	**-0.8**	0.2	0.0	-0.4	HS15N	-0.1	**-1.0**	0.0	-0.1	HS19Ap	0.3	0.4	-0.4	**0.6**
HS16A	**-0.8**	0.2	-0.1	0.0	HS16N	-0.4	**-0.6**	-0.2	0.3	HS20Ap	0.4	**0.6**	-0.4	0.3
HS17A	**-0.9**	0.1	-0.1	0.1	HS18N	**-0.6**	**-0.7**	-0.1	-0.1	HS21Ap	-0.2	-0.4	**-0.8**	0.0
HS18A	**-0.8**	0.3	-0.2	-0.2	HS19N	-0.4	**-0.8**	-0.1	-0.1					
HS20A	-0.4	**0.6**	0.4	-0.4	HS20N	**0.5**	**-0.6**	0.2	-0.2					
HS21A	**-0.5**	0.4	0.1	0.3	HS21N	0.3	**-0.9**	-0.2	0.0					
Sample	1	2	3	4	Sample	1	2	3	4	Sample	1	2	3	4
HS0J	0.2	**-0.5**	**0.7**	0.0	HS0S	**-0.6**	0.3	-0.4	0.3	HS8S	**0.9**	0.0	0.1	-0.4
HS1J	**0.5**	-0.4	**0.7**	0.0	HS1S	0.2	0.0	-0.2	0.0	HS9S	**-0.6**	0.3	-0.4	0.4
HS2J	0.1	-0.1	**0.9**	0.1	HS2S	**-0.8**	0.1	-0.3	0.0	HS10S	**0.5**	-0.3	-0.3	**-0.6**
HS3J	0.1	-0.4	**0.7**	0.0	HS3S	**-0.6**	0.4	**-0.5**	0.1	HS14S	**-0.7**	0.2	**-0.6**	-0.3
HS10J	0.1	-0.1	**0.9**	-0.2	HS4S	-0.5	0.2	**-0.6**	**-0.5**	HS16S	**0.5**	0.2	-0.3	**-0.6**
HS15J	-0.1	0.1	**0.9**	-0.1	HS5S	**0.9**	0.2	-0.1	0.0	HS17S	-0.4	-0.1	**-0.5**	**-0.6**
HS18J	**-0.7**	0.0	**0.5**	0.2	HS6S	2.2	0.1	-0.4	-0.2	HS18S	**-0.5**	0.1	**-0.5**	0.0
HS19J	0.1	-0.1	**0.9**	0.0	HS7S	-0.3	**0.6**	**-0.5**	-0.3	HS18S	**-0.5**	0.1	**-0.5**	0.0

Clustering into Similar Compositions

As shown in Figure 8, all water samples were classified into four statistically significant clusters. Cluster I included 47 stream water samples and 42 groundwater samples in August and November 2011 and April 2012, respectively. All samples from 2011 were included in cluster I. In contrast, samples from 2012 were divided into either cluster III or IV. These results have a close relationship with the precipitation records. Cluster II included eight stream water samples and 27 groundwater samples in 2012. Cluster I included most of the stream water sampled in 2011. However, cluster II included small parts of the stream water. This is because the amount of precipitation in 2011 was greater than in 2012.

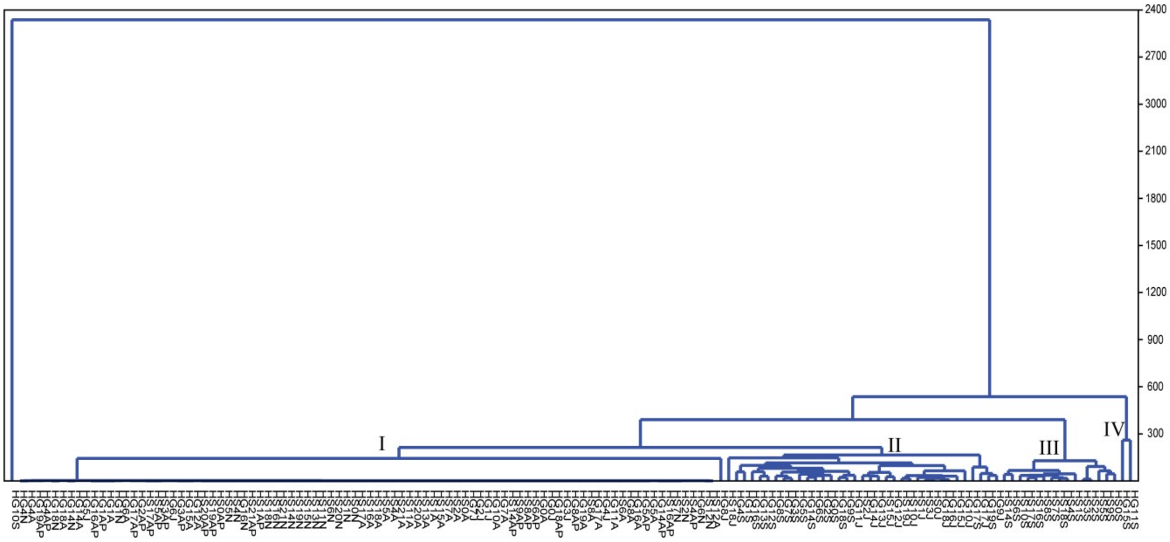

Figure 8. Dendrogram showing the clustering of sampling sites according to water quality characteristics of the Haean basin.

Groundwater and stream water mixing was significantly affected by precipitation. Clusters I and II indicated that groundwater and stream water mixing occurred in the study site. These results also varied depending on the intensity of the precipitation.

Isotopic Compositions and Water Flux for Determining Groundwater–Stream Water Mixing

Figure 9 shows δD versus $\delta^{18}O$ values for the groundwater and stream water. The δD and $\delta^{18}O$ values of the groundwaters and stream waters are plotted between or near the two local meteoric water lines (LMWLs) for dry and wet season precipitation indicating that aquifer and stream are hydraulically connected (Jorgensen and Banoeng-Yakubo, 2001). The groundwater in the Haean basin is more depleted in δD and $\delta^{18}O$ values than stream water. This can be explained that the stream water was experienced a little evaporation or was influenced by irrigation return flows. The water in the Haean basin is mostly supported from precipitation because the study area is surrounded mountain (up to 1304 m). $\delta^{18}O$ and δD in hydrologic cycle had been discussed by Gat (1996).

Figure 10 shows a schematic view of groundwater flow in the basin. It contains information of groundwater chemistry data, groundwater well information such as well depth and elevation, and groundwater flow direction. The spatial and temporal distributions of isotopic compositions (e.g. δD, $\delta^{18}O$, $\delta^{15}N$, and $\delta^{34}S$), and NO_3-N in the aquifers are shown. There are relatively little temporal variations of NO_3-N concentrations in the HG9 which is located high elevation part. The groundwater in the HG12 (located in the middle elevation part) and HG0 (located lower elevation part) were characterized by elevated NO_3-N concentrations. The Haean basin is largely responsible for the distinct isotopic signature of stream water and precipitation as compare with groundwater. Nitrate concentration of the well HG0 was high which is influenced by the geomorphology (e.g. elevation and slope), land use pattern, and agricultural activities of the basin. Furthermore, it can be inferred that the NO_3 contaminant is derived from surface and loaded in groundwater through the hyporheic exchange.

Figure 11 shows the strong loss that occurred in the hyporheic exchange at HFM1 and HFM2 in the wet season. This represents that stream water intruded into the groundwater through the hyporheic zone. This is important information for the maintenance and management of water quality in an intensive agricultural area. Chemical contaminants could be loaded from the stream water to the groundwater through the hyporheic zone more in the wet season than in the dry season (Kim et al., 2013).

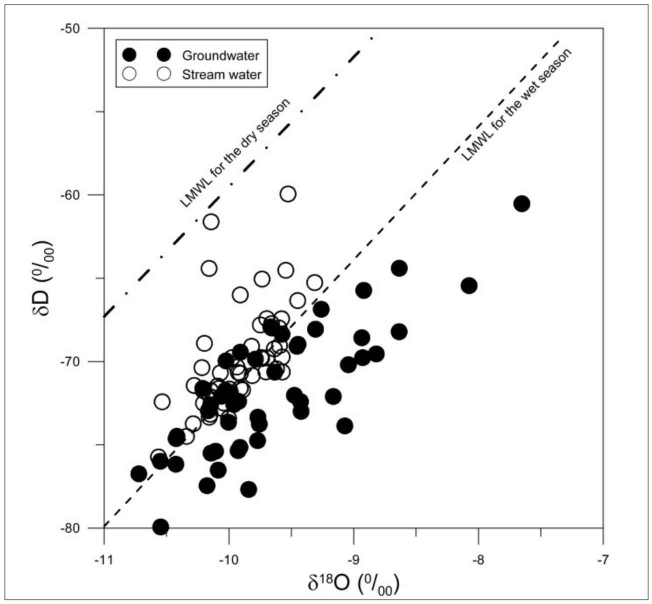

Figure 9. Plot of δD versus $\delta^{18}O$ values of the sampled groundwater and stream water.

Microbial Community

The mixing zone between stream waters and the adjacent groundwater system plays an important role in biogeochemical processes and the biodiversity of both ecosystems (Gibert et al., 1990; Griebler and Lueders, 2009). A phylogenetic tree was used to illustrate the relationship between organisms in the GenBank database and those in the soil samples in the groundwater-stream water mixing zone (Figure 12). A neighbour-joining (NJ) un-rooted phylogenetic tree based on 16S rRNA gene sequences was created to show the relationship among 25 bacteria. The numbers at branch nodes are bootstrap percentages based on 1000 replications with only values > 50% shown. The closed circles indicate that the corresponding branches were also recovered in the NJ and Kimura two-parameter/maximum likelihood trees. A bar value of 0.02 represents substitutions in the nucleotide position. The phylogenetic tree described for the groundwater and stream water mixing zone with molecular methods was essentially dominated by the lineages detected in the cultivation of a number of proteobacteria and firmicutes. It revealed bacterial sequences belonging to β-proteobacteria, γ-proteobacteria, and firmicutes.

We observed a bacterium, *Pseudacidovorax* sp., in HFM2. This bacterium was 97% related to *Pseudacidovorax intermedius*, which is Gram negative, has a fine rod shape, occurs singularly or in pairs, is 0.5 µm × 1.5-2.0 µm in size, and motile by means of a flagellum. This bacterium has the ability to fix nitrogen (Zhang and Chen, 2012). *Massilia* sp. was also observed in the soil of HFM2. The bacterium in HFM2 had 97% similarity to *Massilia suwonensis*. It was Gram negative, occurred singularly or in pairs, had a straight rod shape, and was 1.0 µm ×1.6-3.0 µm in size. This bacterium can be isolated from human, soil, and environmental samples (Gallego et al., 2006). At HFM2 the soil contains thiosulfate oxidation bacteria. The bacterium in HFM2 had 94% similarity to *Comamonas thiooxidans* (Narayan et al., 2010). A denitrifying bacterium, *Achromobacter xylosoxidans,* was found in the hyporheic soil of HFM1 and HFM2. The bacterium in HFM2 had 94% similarity to

Achromobacter xylosoxidans. This bacterium can be observed under denitrifying and non-denitrifying conditions (Yoshimura et al., 1993).

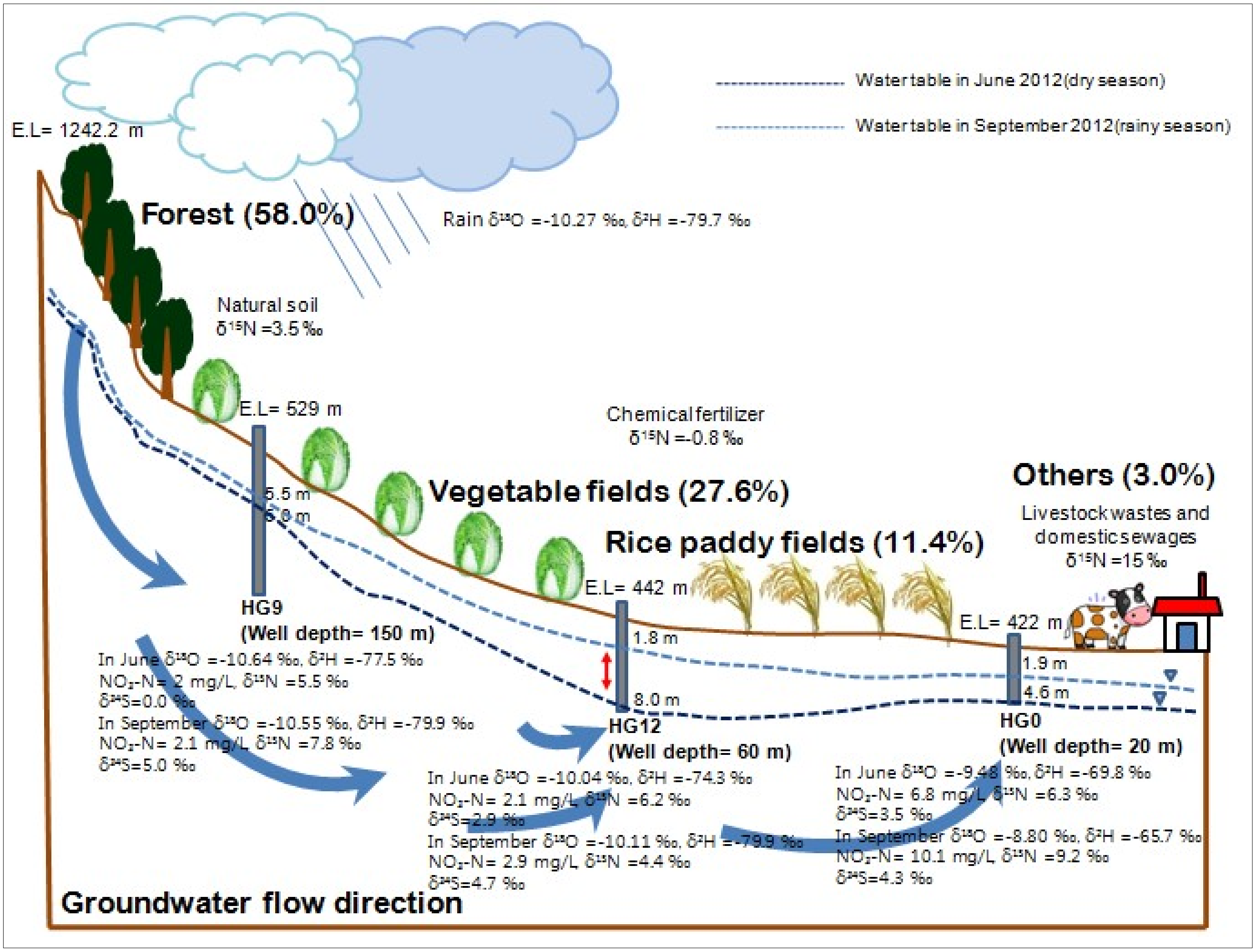

Figure 10. Schematic view of groundwater flow.

Furthermore, HFM2 also contains bacteria from ginseng cultivation and BTEX-contaminated groundwater. The *Pseudoxanthomonas* sp. was a Gram negative, strictly aerobic, non-spore-forming bacterium, which was motile by means of a single polar flagellum and was rod shaped (Yoo et al., 2007). *Pseudoxanthomonas suwonensis* and the *Lysobacter ginsengisoli* strain Gsoil were related to the HFM2 bacterium. This bacterium was a Gram negative, aerobic, non-motile, rod-shaped bacteria (Yoon and Im, 2007). A bacterium capable of surviving in groundwater contaminated with oil was also identified at HFM2. *Pseudoxanthomonas spadix* was reported to be able to tolerate degraded BTEX compounds in groundwater (Lee et al., 2012). This genus is characterized as Gram negative and aerobic with non-spore-forming rods. HFM1 and HFM2 contained the pathogenic bacteria *Acinetobacter johnsonii* (Spear et al., 1988) and the wetland bacteria *Clostridium saccarobutylicum* and *Clostridium puniceum* (Drake et al., 2009). Bacteria in hyporheic soils have 94% similarity to *Acinetobacter* *johnsonii*, 99% similarity to *Clostridium saccarobutylicum*, and 94% similarity to *Clostridium puniceum*.

Most of the bacteria clones were related to living microorganisms in groundwater and stream water. In addition, the microorganism communities reflected the agricultural conditions of the study site. The microbial diversity of the groundwater and stream water mixing zone reflected the groundwater and stream water ecological environment as well as agricultural and human activities.

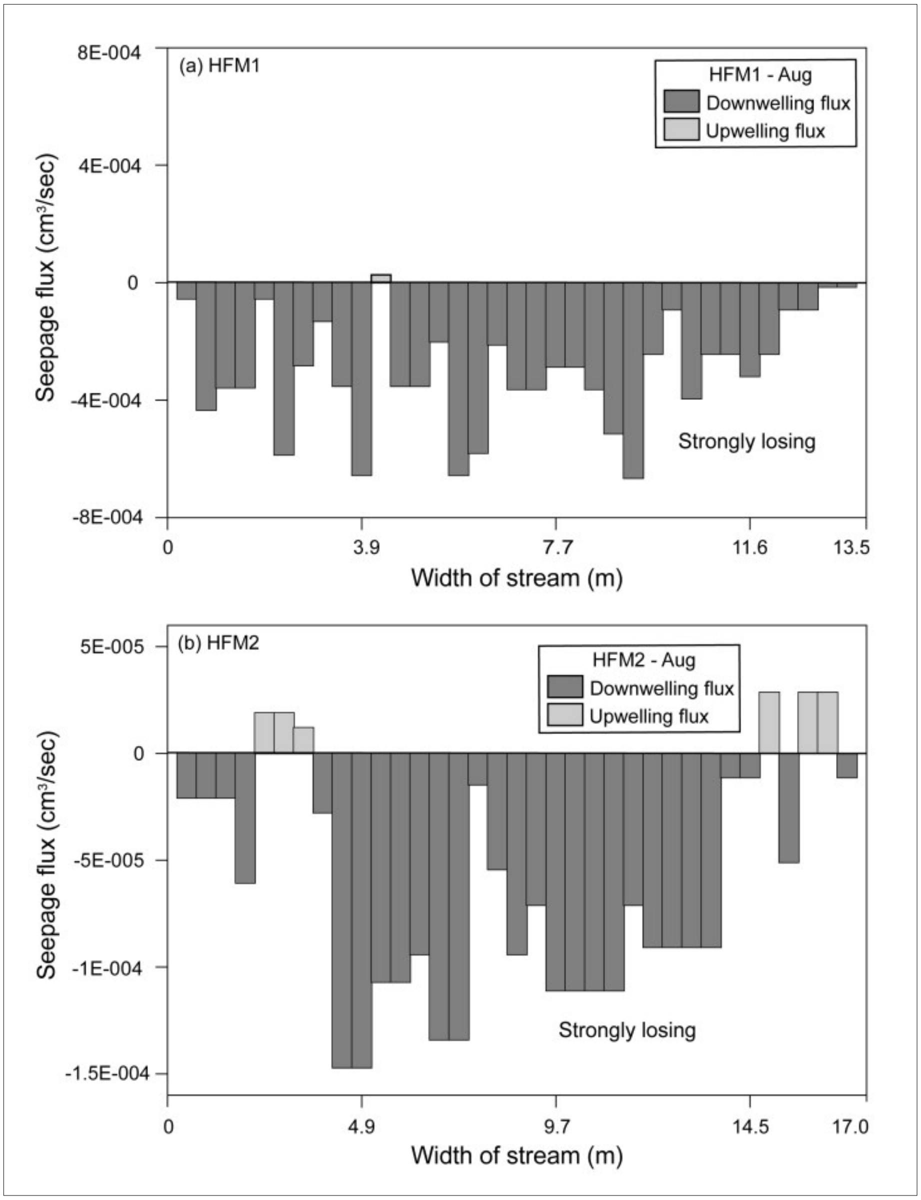

Figure 11. Calculated seepage flux in August 2011.

CONCLUSIONS

In this study, multivariate statistical techniques and pyrosequncing analysis were used to evaluate the groundwater and stream water quality of an intensive agricultural area in the Haean basin, Korea. The statistical analysis revealed the spatial and temporal properties of the hydrochemistry. The

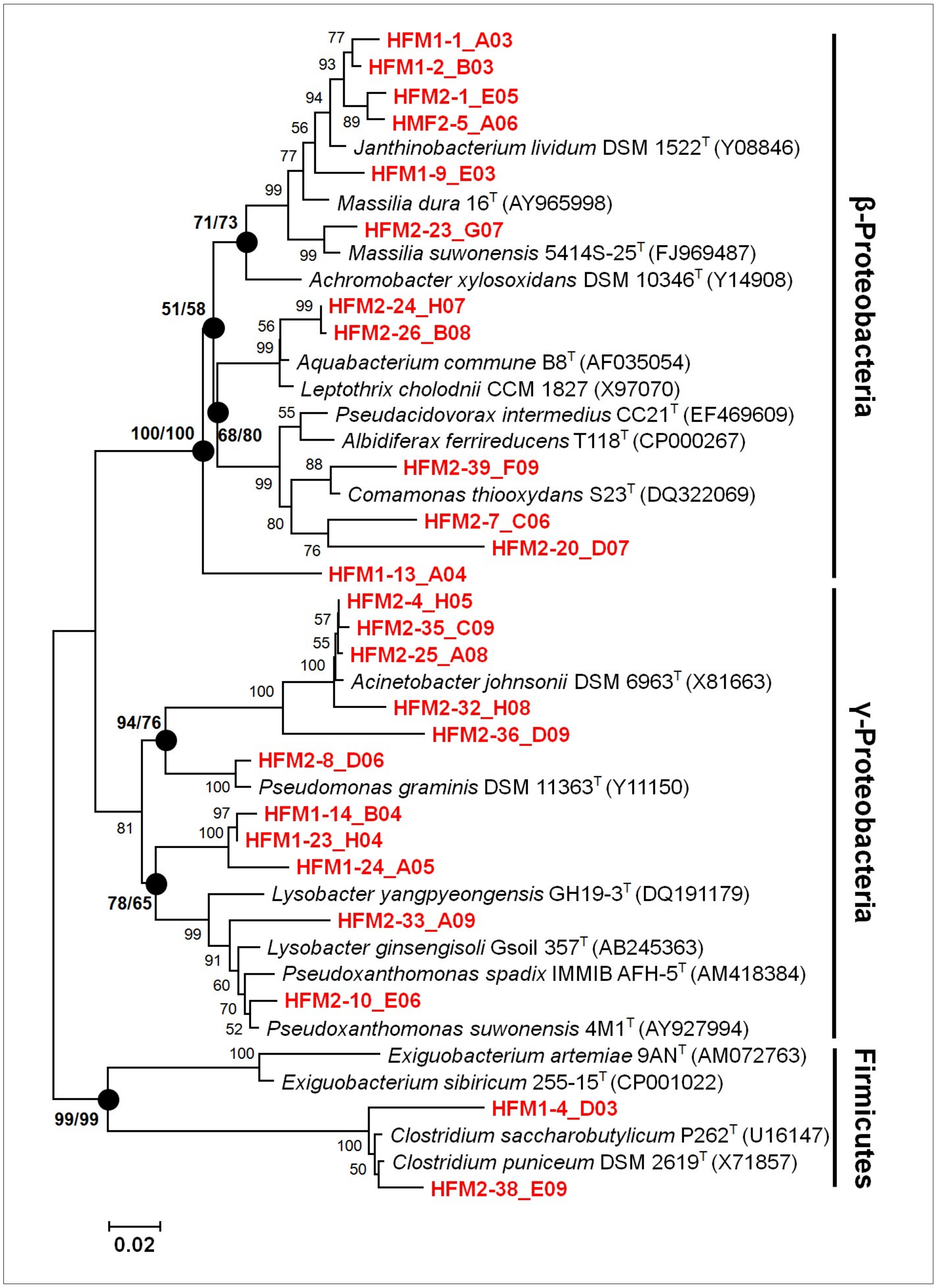

Figure 12. Phylogenetic tree illustrating the relationships between organisms in the GenBank database and those in soil samples from the groundwater-stream water mixing zone.

processes responsible for hydrochemical variations in water qualities and bacteria communities are hyporheic exchanges, domestic and agricultural pollution, and geologic deposits.

First, the FA results indicated four latent factors controlling groundwater and stream water chemistry. In groundwater, 58.97% of total variance was explained by four factors. These latent factors were identified as being responsible for the physicochemical weathering and anthropogenic factors. In stream water, 76.80% of total variance was explained by four factors, related to the rainfall and anthropogenic activities. Therefore, the FA could be useful for evaluation of potential environmental contaminants in the region.

The results of CA showed four different groups according to similarity of geochemical properties. All samples collected from 2011 were included in cluster I. In contrast, samples collected from 2012 were divided into either cluster III or IV. These results have a close relationship with the precipitation records. Cluster II included eight stream water samples and 27 groundwater samples in 2012. Cluster I included most of the stream water sampled in 2011. However, cluster II included small parts of the stream water. This is because the amount of precipitation in 2011 was greater than in 2012. Groundwater and stream water mixing was significantly affected by precipitation. Clusters I and II indicated that groundwater and stream water mixing occurred in the study site. These results also varied depending on the intensity of the precipitation.

We also used microbiological analysis to investigate the hydroecological characteristics of groundwater stream water interaction. The contaminated stream water intruded into the groundwater at interaction zone of the groundwater and stream water. Furthermore, most of the bacteria clones were related to living microorganisms in the groundwater and stream water. The microorganism communities of the hyporheic zone reflected the agricultural conditions of the study site. The zone can act as a sink of contaminants in contaminated stream water depending on the flux and microbial activity.

This study showed that various methods based on hydrogeology, hydrochemistry, and hydroecology is necessary to provide useful information regarding the quality and quantity of stream and groundwater and the design of effective pollution control and management techniques in an intensive agricultural area.

ACKNOWLEDGEMENTS

This paper was reviewed by Dr. Youngyun Park at Kangwon National University in Chuncheon, Korea and Dr. Myeong-Jae Yi at GeoGreen21 Co., Ltd. in Seoul, Korea. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIP) (No. NRF-2015R1A4A1041105).

REFERENCES

Alhajjar, B.J., G.V. Simsiman, and G. Chesters. 1990. Fate and transport of alachlor, metalachlor and atrazine in large columns. Water Science and Technology, Vol. 22, pp. 87-94.

Ally, W.M. 1993. Regional groundwater quality. New York; Van Nostrand Reinhold.

Aravena, R., and W.D. Robertson. 1998. Use of multiple isotope tracers to evaluate denitrification in ground water: study of nitrate from a large-flux septic system plume. Ground Water, Vol. 36(6), pp. 975-982.

Boulton, A.J., S. Findlay, P. Marmonier, E.H. Stanley, and H.M. Valett. 1998. The functional significance of the hyporheic zone in streams and rivers. Annual Review of Ecology and Systematics, Vol. 29, pp. 59-81.

Casciotti, K.L., D.M. Sigman, M.G. Hastings, J.K. Böhlke, and A. Hilkert. 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Analytical Chemistry, Vol. 74, pp. 4905-4912.

Chae, G.T., K. Kim, S.T. Yun, K.H. Kim, S.O. Kim, B.Y. Choi, H.S. Kim, and C.W. Rhee. 2004. Hydrogeochemistry of alluvial groundwaters in an agricultural area: An implication for groundwater contamination susceptibility. Chemosphere, Vol. 55(3), pp. 369-378.

Chae, G.T., S.T. Yun, B. Mayer, B.Y. Choi, K.H. Kim, J.S. Kwon, and S.Y. Yu. 2009. Hydrochemical and isotopic assessment of nitrate contamination in an alluvial aquifer underneath a riverside agricultural field. Agricultural Water Management, Vol. 96(12), pp. 1819-1827.

Chapman, D.V. 1996. Water quality assessments - a guide to the use of biota, sediments and water in environmental monitoring - second edition. London; E&FN Spon.

Choi, H.M., and J.Y. Lee. 2010. Groundwater level distribution and rainfall response characteristics in Haean basin of Yanggu. Journal of Soil and Groundwater Environment, Vol. 15(6), pp. 1-8.

Daughney, C.J., and R.R. Reeves. 2006. Analysis of temporal trends in New Zeeland's groundwater quality based on data from the National Groundwater Monitoring Programme. Journal of Hydrology (NZ), Vol. 45(1), pp. 41-62.

Domagalski, J.L., and N.M. Dubrovsky. 1992. Pesticide residues in groundwater of the San Joaquin Valley, California. Journal of Hydrology, Vol. 130(1-4), pp. 299-338.

Drake, H.L., M.A. Horn, and P.K. Wüst. 2009. Intermediary ecosystem metabolism as a main driver of methanogenesis in acidic wetland soil. Environmental Microbiology Reports, Vol. 1(5), pp. 307-318.

Epstein, S., and T.K. Mayeda. 1953. Variation of ^{18}O content of waters from natural sources. Geochimica et Cosmochimica Acta, Vol. 4, pp. 213-224.

Esmaeili, A., and F. Moore. 2012. Hydrogeochemical assessment of groundwater in Isfahan province, Iran. Environmental Earth Sciences, Vol. 67(1), pp. 107-120.

Gallego, V., C. Sánchez-Porro, T. García, and A. Ventosa. 2006. *Massilia aurea* sp. nov., isolated from drinking water. International Journal of Systematic and Evolutionary Microbiology, Vol. 56(10), pp. 2449-2453.

Gat, J.R. 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. Annual Review of Earth and Planetary Sciences, Vol. 24(1), pp. 225-262.

Gibert, J., M.J. Dole-Olivier, P. Marmornier, and P. Vervier. 1990. Surface - groundwater ecotones. In: The Ecology and Management of Aquatic-Terrestrial Ecotones, Naiman, R.J., and H. Decamps (eds). Carnforth, UK; UNESCO Paris and Parthenon Publishing Group, pp. 199-225.

Grieber, C., and T. Lueders. 2009. Microbial diversity in groundwater ecosystems. Freshwater Biology, Vol. 54(4), pp. 649-677.

Hayashi, M., and D.O. Rosenberry. 2002. Effects of ground water exchange on the hydrology and ecology of surface water. Ground Water, Vol. 40(3), pp. 309-316.

Hengstmann, U., K.J. Chin, P.H. Janssen, W. Liesack. 1999. Comparative phylogenetic assignment of environmental sequences of genes encoding 16S rRNA and numerically abundant culturable bacteria from an anoxic rice paddy soil. Applied and Environmental Microbiology, Vol. 65(11), pp. 5050-5058.

Hooda, P.S., A.C. Edwards, H.A. Anderson, and A. Miller. 2000. A review of water quality concerns in livestock farming areas. Science of the Total Environment, Vol. 250(1), pp. 143-167.

Huang, F., X. Wang, L. Lou, Z. Zhou, and J. Wu. 2010. Spatial variation and source apportionment of water pollution in Qiantang River (China) using statistical techniques. Water Research, Vol. 44(5), pp. 1562-1572.

Jeong, C.H. 2001. Effect of land use and urbanization on hydrochemistry and contamination of groundwater from Taejon area, Korea. Journal of Hydrology, Vol. 253(1), pp. 194-210.

Jin, Z., Z. Pan, M. Jin, F. Li, Y. Wan, and B. Gu. 2012. Determination of nitrate contamination sources using isotopic and chemical indicators in an agricultural region in China. Agriculture, Ecosystems and Environment, Vol. 155, pp. 78-86.

Johnson, J.L. 1994. Similarity analyses of rRNAs. In: Methods for General and Molecular Bacteriology, Gerhardt, P., R.G.E. Murray, W.A. Wood, and N.R. Kreig (eds). Washington DC; American Society for Microbiology, pp. 683-700.

Jorgensen, N.O., and B.K. Banoeng-Yakubo. 2001. Environmental isotopes (^{18}O, ^{2}H and $^{87}Sr/^{86}Sr$) as a tool in groundwater investigation in the Keta Basin, Ghana. Hydrogeology Journal, Vol. 9(2), pp. 190-201.

Kaown, D., Y. Hyun, G.O. Bae, and K.K. Lee. 2007. Factors affecting the spatial pattern of nitrate contamination in shallow groundwater. Journal of Environmental Quality, Vol. 36, pp. 1479-1487.

Kaown, D., D.C. Koh, B. Mayer, and K.K. Lee. 2009. Identification of nitrate and sulphate sources in groundwater using dual stable isotope approaches for an agricultural area with different land use (Chuncheon, mid-eastern Korea). Agriculture, Ecosystems and Environment, Vol. 132(3-4), pp. 223-231.

Kazi, T.G., M.B. Arain, M.K. Jamali, N. Jalbani, H.I. Afridi, R.A. Sarfraz, J.A. Baig, and Q. Shah. 2009. Assessment of water quality of polluted lake using multivariate statistical techniques: A case study. Ecotoxicology and Environmental Safety, Vol. 72(2), pp. 301-309.

Kettering, J., J.H. Park, S. Lindner, B. Lee, J. Tenhunen, and Y. Kuzyakov. 2012. N fluxes in an agricultural catchment under monsoon climate: A budget approach at different scales. Agriculture, Ecosystems and Environment, Vol. 161, pp. 101-111.

Kim, H., J.Y. Lee, and K.K. Lee. 2013. Spatial and temporal variations of groundwater-stream water interaction in an agricultural area, case study: Haean basin, Korea. Research Journal of Earth and Planetary Sciences, Vol. 3(1), pp. 1-12.

Kim, H., K.K. Lee, and J.Y. Lee. 2014. Numerical verification of hyporheic zone depth estimation using streambed temperature. Journal of Hydrology, Vol. 511, pp. 861-869.

Kim, K.H., S.T. Yun, B.Y. Choi, G.T. Chae, Y. Joo, K. Kim, and H.S. Kim. 2009. Hydrochemical and multivariate statistical interpretations of spatial controls of nitrate concentrations in a shallow alluvial aquifer around oxbow lakes (Osong area, central Korea). Journal of Contaminant Hydrology, Vol. 107(3-4), pp. 114-127.

Korea Metrological Administration. 2013. KMA (http://www.kma.go.kr). Accessed on February 5, 2013.

Knights, B.C., B.L. Johnson, and M.B. Sandheinrich. 1995. Responses of Bluegills and Black Crappies to dissolved oxygen, temperature, and current in Backwater Lakes of the upper Mississippi River during winter. North American Journal of Fisheries Management, Vol. 15(2), pp. 390-399.

Krishna, A.K., M. Satyanarayanan, and P.K. Govil. 2009. Assessment of heavy metal pollution in water using multivariate statistical techniques in an industrial area: A case study from Patancheru, Medak District, Andhra Pradesh, India. Journal of Hazardous Materials, Vol. 167(1-3), pp. 366-373.

Kwon, Y.S., H.H. Lee, U. Han, and W.H. Kim. 1990. Terrain analysis of Haean basin in terms of earth science. Journal of Korea Earth Science Society, Vol. 11(3), pp. 236-241.

Lane, D.J. 1991. 16S/23SrRNA sequencing. In: Nucleic Acid Techniques in Bacterial Systematics, Stackebrandt, E., and M. Goodfellow (eds). Chichester, England; John Wiley and Sons, pp. 115-148.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettingan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, and D.G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, Vol. 23(21), pp. 2974-2978.

Lee, D.R. 1977. A device for measuring seepage flux in lake and estuaries. Limnology and Oceanography, Vol. 22(1), pp. 140-147.

Lee, J.Y., J.Y. Cheon, K.K. Lee, S.Y. Lee, and M.H. Lee. 2001. Factors affecting the distribution of hydrocarbon contaminants and hydrogeochemical parameters in a shallow sand aquifer. Journal of Contaminant Hydrology, Vol. 50(1-2), pp. 139-158.

Lee, J.Y. 2009. Importance of hydrogeological and hydrologic studies for Haean basin in Yanggu. Journal of Geological Society of Korea, Vol. 45, pp. 405-414 (in Korean with English abstract).

Lee, J.Y., H.S. Kim, S.T. Yun, and J.S. Kwon. 2009. Factor and cluster analyses of water chemistry in and around a large dam: implications for water leakage. Journal of Geotechnical and Geoenvironmental Engineering, Vol. 135(9), pp. 1254-1263.

Lee, J.Y., K.S. Lee, Y. Park, H.M. Choi, and Y.J. Jo. 2013. Chemical and isotopic compositions of groundwater and stream water in a heavy agricultural basin of Korea. Journal of the Geological Society of India, Vol. 82(2), pp. 169-180.

Lee, S.H., H.M. Jin, H.J. Lee, J.M. Kim, and C.O. Jeon. 2012. Complete genome sequence of the BTEX-degrading bacterium *Pseudoxanthomonas spadix* BD-a59. Journal of Bacteriology, Vol. 194(2), pp. 544.

Li, S., J. Li, and Q. Zhang. 2011. Water quality assessment in the rivers along the water conveyance system of the Middle Route of South to North Water Transfer Project (China) using multivariate statistical techniques and receptor modelling. Journal of Hazardous Materials, Vol. 195, pp. 306-317.

Madison, R.J., and J.O. Brunett. 1985. Overview of the occurrences of nitrate in groundwater of United States. In: National Water Summary 1984: Hydrologic Events, Selected Water-quality Trends, and Ground-water Resources, USGS Water Supply Paper 2275, Washington DC; U.S. Government Printing Office, pp. 93-135.

Morrison, J., T. Brockwell, T. Merren, F. Fourel, and A.M. Phillips. 2001. On-line high precision stable hydrogen isotopic analyses on nanoliter water samples. Analytical Chemistry, Vol. 73(15), pp. 3570-3575.

Muyzer, G., A. Teske, C.O. Wirsen, and H.W. Tannasch. 1993. Profiling of complex microbial ecology populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, Vol. 59(3), pp. 695-700.

National Academy of Agricultural Science. 2013. Korean Soil Atlas (http://soil.rda.go.kr). Accessed on February 5, 2013.

Narayan, K.D., S.K. Pandey, and S.K. Das. 2010. Characterization of *Comamonas thiooxidans* sp. nov., and comparison of Thiosulfate oxidation with *Comamonas testosteroni* and *Comamonas composti*. Current Microbiology, Vol. 61(4), pp. 248-253.

Olsen, R.L., R.W. Chappell, and J.C. Loftis. 2012. Water quality sample collection, data treatment and results presentation for principal components analysis-literature review and Illinois River watershed case study. Water Research, Vol. 46(1), pp. 3110-3122.

Omo-Irabor, O.O., S.B. Olobaniyi, K. Oduyemi, and J. Akunna. 2008. Surface and groundwater water quality assessment using multivariate analytical methods: A case study of the Western Niger Delta, Nigeria. Physics and Chemistry of the Earth, Vol. 33(8-13), pp. 666-673.

Otero, N., C. Torrento, A. Soler, A. Mencio, and J. Mas-Pla. 2009. Monitoring groundwater nitrate attenuation in a regional system coupling hydrogeology with multi-isotopic methods: The case of Plana de Vic (Osona, Spain). Agriculture, Ecosystems and Environment, Vol. 133(1-2), pp. 103-113.

Panda, U.C., S.K. Sundaray, P. Rath, B.B. Nayak, and D. Bhatta. 2006. Application of factor and cluster analysis for characterization of river and estuarine water systems – A case study: Mahanadi River (India). Journal of Hydrology, Vol. 331(3-4), pp. 434-446.

Panno, S.V., W.R. Kelly, A.T. Martinsek, and K.C. Hackley. 2006. Estimation background and threshold nitrate concentrations using probability graphs. Ground Water, Vol. 44(5), pp. 697-709.

Peters, N.E., and M. Meybeck. 2000. Water quality degradation effects on freshwater availability: Impacts of human activities. Water International, Vol. 25(2), pp. 185–193.

Pitt, R., S. Clark, and R. Field. 1999. Groundwater contamination potential from stormwater infiltration practices. Urban Water, Vol. 1(3), pp. 217-236.

Prasanna, M.V., S. Chidambaram, G. Senthil Kumar, A.L. Ramanathan, and H.C. Nainwal. 2011. Hydrogeochemical assessment of groundwater in Neyveli Basin, Cuddalore District, South India. Arabian Journal of Geosciences, Vol. 4(1-2), pp. 319-330.

Pretty, J.L., A.G. Hildrew, and M. Trimmer. 2006. Nutrient dynamics in relation to surface-subsurface hydrological exchange in a groundwater fed chalk stream. Journal of Hydrology, Vol. 330(1-2), pp. 84-100.

Reghunath, R., T.R.S. Murthy, and B.R. Raghavan. 2002. The utility of multivariate statistical techniques in hydrogeochemical studies: an example from Karnataka, India. Water Research, Vol. 36(10), pp. 2437-2442.

Ritzi, R.W., S.L. Wright, B. Mann, and M. Chen. 1993. Analysis of temporal variability in hydrogeochemical data used for multivariate analyses. Ground Water, Vol. 31(2), pp. 221-229.

Rosenberry, D.O., P.Z. Klos, and A. Neal. 2012. In situ quantification of spatial and temporal variability of hyporheic exchange in static and mobile gravel-bed rivers. Hydrological Processes, Vol. 26(4), pp. 604-612.

Scanlon, B.R., I. Jolly, M. Sophocleous, and L. Zhang. 2007. Global impacts of conversions from natural to agricultural ecosystems on water resources: Quantity versus quality. Water Resources Research, Vol. 43(3), W03437.

Shanley, J.B., B. Mayer, M.J. Mitchell, R.L. Michel, S.W. Bailey, and C. Kendall. 2005. Tracing sources of streamwater sulfate during snowmelt using S and O isotope ratios of sulfate and 35S activity. Biogeochemistry, Vol. 76(1), pp. 161-185.

Shrestha, S., and F. Kazama. 2007. Assessment of surface water quality using multivariate statistical technique: A case study of the Fuji river basin, Japan. Environmental Modelling & Software, Vol. 22(4), pp. 464-475.

Simeonov, V., J.A. Stratis, C. Samara, G. Zachariadis, D. Voutsa, A. Anthemidis, M. Sofoniou, and Th. Kouimtzis. 2003. Assessment of the surface water quality in Northern Greece. Water Research, Vol. 37(17), pp. 4119-4124.

Sinclair, A.J. 1991. A fundamental approach to threshold estimation in exploration geochemistry: Probability plots revisited. Journal of Geochemical Exploration, Vol. 41(1), pp. 1-22.

Smith, J.W.N., B.W.J. Surridge, T.H. Haxton, and D.N. Lermer. 2009. Pollutant attenuation at the groundwater-surface water interface: A classification scheme and statistical analysis using national-scale nitrate data. Journal of Hydrology, Vol. 369(3-4), pp. 392-402.

Singh, K.P., A. Malik, D. Mohan, and S. Sinha. 2004. Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of Gomti River (India)–a case study. Water Research, Vol. 38(18), pp. 3980-3992.

Spear, J.B., J. Fuhrer, and B. Kirby. 1988. *Achromobacter xylosoxidans* (*Alcaligenes xylosoxidans* subsp. *xylosoxidans*) bacteria associated with a well-water source: Case report and review of the literature. Journal of Clinical Microbiology, Vol. 26(3), pp. 598-599.

Varol, M., B. Gökot, A. Bekleyen, and B. Şen. 2012. Spatial and temporal variations in surface water quality of the dam reservoirs in the Tigris River basin, Turkey. Catena, Vol. 92, pp. 11-21.

Wang, Y., P. Wang, Y. Bai, Z. Tian, J. Li, X. Shao, L.F. Mustavich, and B.L. Li. 2013. Assessment of surface water quality via multivariate statistical techniques: A case study of the Songhua River Harbin region, China. Journal of Hydro-environment Research, Vol. 7(1), pp. 30-40.

Yanggu County Office. 2013. One-Stop Service (http://yanggu.go.kr). Accessed on March 10, 2013.

Yoo, S.H., H.Y. Weon, B.Y. Kim, J.H. Kim, Y.K. Beak, S.W. Kwon, S.J. Go, and E. Stackebrandt. 2007. *Pseudoxanthomonas yeongjuensis* sp. nov., isolated from soil cultivated with Korean ginseng. International Journal of Systematic Evolutionary Microbiology, Vol. 57(3), pp. 646-649.

Yoon, M.H., and W.T. Im. 2007. *Flavisolibacter ginsengiterrae* gen. nov., sp. nov. and *Flavisolibacter ginsengisoli* sp. nov., isolated from ginseng cultivating soil. International Journal of Systematic Evolutionary Microbiology, Vol. 57(8), pp. 1834-1839.

Yoshimura, T., S. Shidara, T. Ozaki, and H. Kamada. 1993. Five coordinated nitrosylhemoprotein in the whole cells of denitrifying bacterium, Achromobacter xylosoxidans NCIB 11015. Archives of Microbiology, Vol. 160(6), pp. 498-500.

Yun, S.Y., Y.J. Jo, and J.Y. Lee. 2009. Comparison of groundwater recharges estimated by water level fluctuation and hydrograph separation in Haean basin of Yanggu. Journal of the Geological Society of Korea, Vol. 45(4), pp. 391–404 (in Korean with English abstract).

Zhang, B., X. Song, Y. Zhang, D. Han, C. Tang, Y. Yu, and Y. Ma. 2012. Hydrochemical characteristics and water quality assessment of surface water and groundwater in Songnen plain, Northeast China. Water Research, Vol. 46(8), pp. 2737-2748.

Zhang, L.H., and S.F. Chen. 2012. *Pseudacidovorax intermedius* NH-1, a novel marine nitrogen-fixing bacterium isolated from the South China Sea. World Journal of Microbiology and Biotechnology, Vol. 28(9), pp. 2839-2847.

ADDRESS FOR CORRESPONDENCE
Jin-Yong Lee
Department of Geology
Kangwon National University
Chuncheon 200-701, Korea

E-mail: hydrolee@kangwon.ac.kr