EFFECTS OF 2015 EARTHQUAKE ON BIOLOGICAL STABILITY OF WATER IN NEPAL

BY

SITAL UPRETY

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ABSTRACT

The April 2015 earthquake in Nepal resulted in the loss of 9000 lives, destroyed several infrastructures and displaced more than 2.3 million people. A study was conducted to analyze the perturbation in biological stability of the water sources compounded by poor sanitation practices in Kathmandu (severe earthquake damage) and Jhapa (no earthquake damage) using microbial source tracking and 16S rRNA sequencing. Samples from the same locations were taken before the earthquake in summer 2014, and then again in May to July, 2015 and in Dec. 2015, corresponding to one month and eight months after the 2015 earthquake. Microbial source tracking of human fecal contamination revealed the deteriorated sanitation practices in some specific sites in Kathmandu after the earthquake. The abundance of certain genera responsible for maintaining geobiochemical characteristics of water (Ammonia Oxidizing Archea and Bacteria, Nitrite Oxidizing Bacteria, Sulfate Reducing Bacteria), for example, *Methylobacter, Nitrospira, Methylomonas,* increased significantly right after the earthquake and decreased eight months later. This result indicated the disturbance in biostability of water right after the earthquake and also indicated the recovery of microbiomes with time. In addition, sudden spikes in some bacterial genera associated with opportunistic pathogens were observed after the earthquake, which associates with outbreaks observed after such event. This study highlighted the potential disruption of water microbiome after the earthquake and its restoration as a function of time and sanitation practices.

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CHAPTER 1

INTRODUCTION

Nepal is prone to earthquake because the country is located on the boundary of Indo-Australian and Asian tectonic plates [1]. The April 25, 2015 earthquake in Nepal measured 7.8 on the Richter scale and caused 8,959 fatalities and more than USD 5 billion in economic damage[2, 3]. According to the Department of Health Services (DoHS) of Nepal, there was a significant increase in waterborne infections in 2015 as compared to previous years. Specifically, there were 2.4 million cases of communicable waterborne infections from in the first six months of 2015 as compared to 3 million cases in the combined years of 2013 and 2014. This sudden rise in waterborne diseases suggests that microbial water quality changes may occur after an earthquake. However, no studies have examined water microbial communities longitudinally before and after earthquakes in Nepal.

Although multiple studies have examined microbial water quality in Nepal, most examined either total coliform or *Escherichia coli* as fecal indicators ([4] [5-7] One limitation with this approach is that a prolonged persistence and proliferation of coliforms in natural environment would not necessarily indicate the occurrence of a recent contamination event[8, 9]. Coliform tests can also result in anomalies which may not always relate to the presence of opportunistic or obligate pathogens [10, 11]. Finally, the presence of the coliforms alone do not provide a complete picture of the overall biological stability of water.

Biological stability of water is essential to maintain and minimize the risk of bacterial growth in the source or the distribution system. Water is considered biologically stable in a natural state when there is no change in the concentration or composition of the microbial community. Study of microbial ecology through the use of 16S rRNA gene-based amplicon sequencing helps better understand the biological stability in water as different bacterial genera responsible for maintaining the geobiochemical stability can be investigated. In this context, biological stability has mostly been studied on drinking water distribution systems (DWDS) to understand bio-corrosion or fouling resulting in the degradation of water quality at distribution points [12, 13]. This approach will also be very useful in investigating the degree in perturbation of the water before and after 2015 Nepal Earthquake.

To fill this gap in knowledge, source water samples were collected in Kathmandu and Jhapa, Nepal. Batch one was collected eleven months prior to the earthquake, batch two was collected one month after the earthquake and batch three was collected eight months after the earthquake. For each sample, 16S rRNA gene sequencing was performed and analyzed. For post-earthquake samples, water quality parameters, including coliform counts and nutrient levels, were also quantified. To the best of our knowledge, this is the first study investigating the impact of an earthquake on water microbiome dynamics.

CHAPTER 2

MATERIAL AND METHODS

2.1 Study Site:

Water samples were collected at seven schools in Kathmandu and six households in Jhapa throughout May 2014 (Table 1). Schools in Kathmandu were selected based on water quality data provided by our collaborator. Schools with water sources containing the highest concentrations of fecal and total coliform, among the selected government owned schools, were chosen for the study. The selected schools are located in central Kathmandu in a very urbanized area with high population density. Kathmandu samples (S1 to S7) were all groundwater samples. However, site S2 has unprotected bore holes and all other sites have unprotected dug wells. All selected schools are government owned and accommodate children mostly from lower middle class families. There were relatively poor sanitation facilities at the selected schools compared to privately owned educational institutions in Kathmandu. The toilets in the schools were located within 3m to 30 m from the sampled groundwater source. Although most schools had some sort of water purification system installed in the school by a private donor, most of them were either out of order or needed to be repaired which forced the students to drink groundwater directly from the source.

Households in Jhapa were purposely selected based on the use of biosand filter for water treatment. In the selected community, the river water flowing by the nearby village is collected in a reservoir and piped to individual houses for a monthly fee charged by the local government. Water samples (J1 to J6) were taken from the household pipes. Most of the

families in the selected households rely on subsistence farming and have little or no formal education. However, due to various Water, Sanitation and Hygiene (WASH) campaigns conducted in the area, community members seemed to be conscious about basic sanitation and water practices.

After several earthquakes in April and May of 2015 with epicenters marked in Figure 1, samples were collected from the same locations with some exceptions. The earthquake heavily affected Kathmandu and as a result two schools (S3 and S4) were excluded in the second Batch of sampling. There were very limited effects of the earthquake on Jhapa compared to Kathmandu. In December 2015, water samples were collected again from the same sampling sites in Kathmandu.

Table 1: Sampling location with GPS coordinates, water source type, level of earthquake damage and sampling Batches for each site. High earthquake damage indicates severe damage in infrastructure; low earthquake damage indicates minimum to no damage in infrastructure and damaged indicates the sampling site was inaccessible after the earthquake.

Site	Location	GPS Coordinates	Water Source Type	Location type	Earthquake Damage	Sampling Batches
S1	Kathmandu	N27°42'44" E 85°18'37"	Dug Shallow Well	School	High	Batch 1, 2 and 3
S2	Kathmandu	N27°42'53" E 85*18'27"	Borehole Deep well	School	High	Batch 1, 2 and 3
S3	Kathmandu	N 27°42'38" E 85°18'37"	Dug Shallow Well	School	Damaged	Batch 1
S4	Kathmandu	N 27°42'10" E 85°18'30"	Dug Shallow Well	School	Damaged	Batch 1
S5	Kathmandu	N 27°42'16" E 85°18'15"	Dug Shallow Well	School	High	Batch 1, 2 and 3
S6	Kathmandu	N 27°42'03" E 85°18'07"	Dug Shallow Well	School	High	Batch 1, 2 and 3
S7	Kathmandu	N 27°42'17" E 85°17'25"	Dug Shallow Well	School	High	Batch 1, 2 and 3
J1	Jhapa	N 26°45'05" E 88°11'22"	Surface water	Household	Low	Batch 1 and 2
J2	Jhapa	N 26°46'19" E 88°04'13"	Surface water	Household	Low	Batch 1 and 2
J3	Jhapa	N 26°46'19" E 88°04'19"	Surface water	Household	Low	Batch 1 and 2
J4	Jhapa	N 26°39'44" E 88°06'20"	Surface water	Household	Low	Batch 1 and 2
J5	Jhapa	N 26°42"44" E 88°05'21"	Surface water	Household	Low	Batch 1 and 2
J6	Jhapa	N 26°49'22" E 88°06'11"	Surface water	Household	Low	Batch 1 and 2

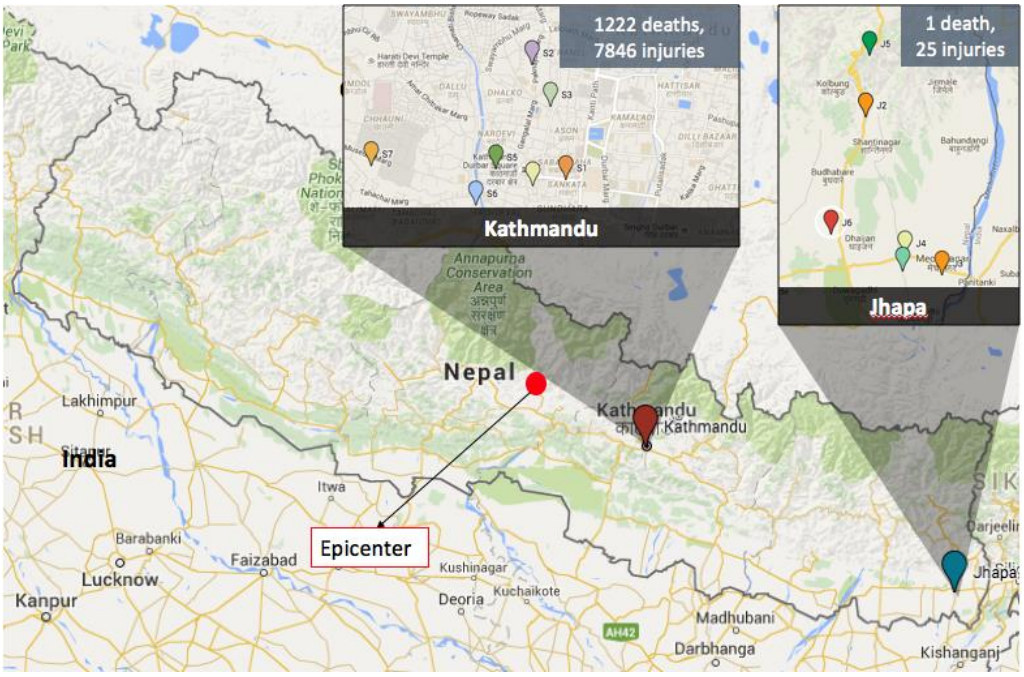

Figure 1: Sampling locations in Kathmandu and Jhapa, epicenter for 2015 Nepal earthquake and magnitude of damage in two sampling locations.

2.2 Sampling Protocol:

Two-liter water samples were collected at each sampling site in a sterile Whirl-pak® sampling bag (Nasco, WI) and were processed within 24 h of collection. The collected water samples were vacuum filtered through 0.45 um sterile cellulose acetate filter membrane after coagulation using 25 mM magnesium chloride ($MgCl_2.6H_2O$) for Batch 1 samples. However, this process clogged the 0.45 um cellulose acetate filters very rapidly and was not feasible for practice after the earthquake. Hence, Batch 2 and Batch 3 samples were vacuum filtered through a 1.6 um glass fiber membrane followed by a 0.45 um cellulose acetate membrane after coagulation using 25 mM Magnesium Chloride. Each membrane, in all Batches, was treated with RNAlater for storage at -20^{o}C prior to extraction. Water samples

in Batch two were analyzed for typical water quality parameters including nitrate, nitrite, ammonia, phosphate, conductivity, total dissolved solids (TDS), salinity and fecal and total coliform.

2.3 Field testing of water quality parameters:

Eleven water quality parameters were measured onsite using dye-based kits and probes. APIfishcare kits (Mars, NJ) were used to measure nutrient levels of nitrite, nitrate, ammonia and phosphate. The accuracy of the kits was verified by testing standard concentrations using the Hach DR/890 Portable Colorimeter. The standard curves from the experiment are provided in the supplementary information. Temperature, conductivity, salinity and TDS of the samples were measured using EC100-ExStik® Conductivity/TDS/Salinity Meter manufactured by EXTECH® (Massachusetts, USA). Fecal coliform and total coliform was measured using 3M™ Petrifilm™ Coliform Count Plates (Minnesota, USA).

2.4 DNA extraction:

Total DNA for the biomass retained on 0.45 um membrane was extracted using the MoBio PowerWater RNA Isolation Kit[14] , removing the DNase step to ensure the collection of both DNA and RNA. RNA is then removed by treating the extracted nucleic acids with RNAse, followed by standard sodium acetate – ethanol precipitation to concentrate the DNA. Total DNA for the biomass retained on 1.6 um membrane was extracted using the

MPI FastDNA Kit for Soil Extraction [15] with minor modifications. For Batch one samples, DNA from 0.45 um filter membrane was used for analysis of microbial community. For Batch 2 and Batch 3, combined DNA in equal volumes from both 1.6 um and 0.45 um filter was used for microbial community analysis.

2.5 PCR-based fecal source tracking*:*

Microbial source tracking was performed using three primer pairs that target human-associated *Bacteroides uniformas, Bacteroides fragilis* and *Bacteroides vulgatas* and a primer pair that targets cow-specific uncultivated *Bacteroidales.* Gene inserts were obtained from *B. vulgatus* BCRC12903, *B. uniformis* JCM5828, *B. fragilis* BCRC10619 and from a cow-specific uncultivated *Bacteroidales* clone obtained from an earlier study [16] . qPCR standards were prepared by first cloning the gene inserts into pCR4 TOPO vector (Invitrogen, Carlsbad, CA, USA). Plasmid DNA was extracted using PureYield^{TM} Plasmid Miniprep System (Promega, Madison, WI, USA). The extracted plasmids were sequenced to verify the oligonucleotide sequences of gene inserts, and quantified for their copy numbers per μL. Amplifications to obtain standard curves were performed in triplicate, while test amplifications and negative blanks were run in duplicates. Each reaction volume of 20 μL contained 10 mL of FAST SYBR Green master mix, 0.4 μL of each primer (10 μM), 1 mL of DNA template and 8.2 μL molecular biology grade water. The Applied Biosystems 7900 HT Fast protocol was used for thermal cycling. The protocol includes 40 cycles of 1 s denaturation at 95°C and 60 s of annealing and extension. Dissociation curve analysis was included to detect non-specific amplification.

2.6 16S rRNA gene-based amplicon sequencing

Illumina MiSeq amplicon sequencing was performed for all the samples to provide information on the total microbial community. To prepare the 16S rRNA gene amplicon libraries, 515F (5'- Illumina overhang- GTGYCAGCMGCCGCGGTAA- 3') and 907R (5'- Illumina overhang- CCCCGYCAATTCMTTTRAGT- 3') primers were modified to encode the overhang adaptor sequences, and used to amplify the 16S rRNA genes. The thermal cycling program included an initial denaturation stage at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension period at 72 °C for 5 min. PCR amplicons were purified by AMPure XP beads (Beckman Coulter, CA, USA) prior to the index PCR assay. Nextera XT Index (Illumina, CA, USA) was incorporated into each of the individual samples during PCR. The thermal cycling program included denaturation stage at 95 °C for 3 min, followed by 8 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension period at 72 °C for 5 min. The final indexed PCR amplicons were again purified by AMPure XP beads and nucleic acid concentrations were quantified using Invitrogen Qubit® 2.0 fluorometer. The controls for all PCR reactions were negative for amplification. Purified amplicons were submitted to KAUST Genomics Core lab for unidirectional sequencing read on an Illumina MiSeq platform.

2.7 Data analysis:

Raw sequences were first trimmed off for the primers, barcodes and adaptor sequences. Trimmed sequences that were < 300 nt in length and with Phred score < 20 were removed. Chimeras were checked for on UCHIME [17] by referencing to a core reference set that was downloaded from Greengenes (i.e. gold strains gg16—aligned.fasta, last modified on 19 March 2011). The relative abundances of the bacterial and archaeal genera were then calculated, collated and square-root transformed. The transformed data set were computed for their Bray–Curtis similarities and represented graphically for spatial distribution in a multidimensional scaling (MDS) plot using Primer-E version 7.

CHAPTER 3

RESULTS

3.1 Differences in the microbial community among Kathmandu and Jhapa water samples

The relative abundance of specific bacterial genera and unclassified bacterial groups in different samples from Kathmandu and Jhapa were analyzed based on their Bray-Curtis similarities (Figure 2). Samples collected before the earthquake were clustered into four distinct subclusters (Figure 2). All J sources were clustered in a single region with similarity of 55% indicating the relatedness in the microbial communities in all the sources. However, the Kathmandu sources were clustered in two different groups; S1, S5 and S6, were clustered in one region with a similarity of 43% and S3, S4 and S7 in the other region with similarity of 51% and S2 was unique from the other groups (Figure 2). Samples collected in S2 were shared a low similarity of 38% from all other samples and were uniquely subclustered by itself with 85% similarity.

When further analyzed on a multidimensional scaling plot coupled with vector-based analysis, main bacterial genera that were predominant among Jhapa samples were unclassified *Burkholderiales* which accounted for 26.7% of the total microbial community. In addition, unclassified *Comamonadaceae* accounted for 12.7% of the total microbial community.

In Kathmandu samples, S1, S5 and S6, on average, unclassified *Gammaproteobacteria* and unclassified *Betaproteobacteria* accounted for 22.8% and 19.9% of the total microbial community, respectively. In the other group comprising, S3, S4 and S7, *unclassified_Rhodobacteraceae* accounted for 1.65% and *unclassified_Microbacteriaceae for* accounted for 0.65%. As seen in the Figure 3, *Zooglea* and *Massilia* were dominant in S2 with 4.01% and 2.43% respectively. This indicates the large contrast in the dominant microbes within sites of Kathmandu and also between Kathmandu and Jhapa (Figure 3).

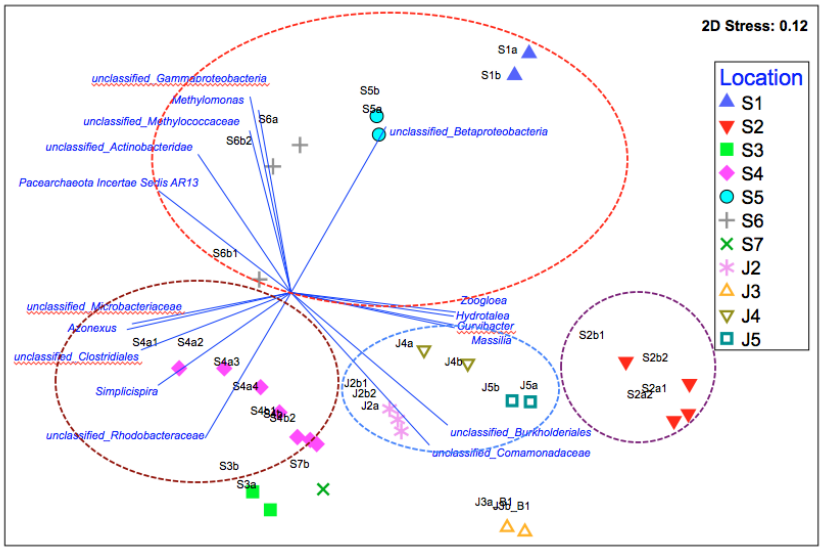

Figure 2: Multidimensional scaling (MDS) plot for the microbial community in Kathmandu (S) and Jhapa (J) samples before the earthquake. Relative abundance of genera and unclassified bacterial groups revealed distinct microbial community between Kathmandu and Jhapa and also within Kathmandu samples. Vector-based analysis overlay the genera that showed significant correlation with the clustering patterns.

3.2 Baseline abundance of geochemically relevant microbial populations

The above sequencing data showed specific patterns in the bacterial genera associated with geochemical characteristics (Table 2). In Kathmandu samples, there was 1.33 fold ammonia oxidizing archaea (AOA), *Nitrososphaera*, compared to Jhapa samples. In both

Kathmandu and Jhapa, *Nitrospira* was the only nitrite-oxidizing bacteria (NOB) detected in relative abundance greater than 0.08% of the total microbial community. Methane-oxidizing bacteria (MOB) and sulphate-reducing bacteria (SRB) were also found in Kathmandu samples and were undetectable in Jhapa samples (Table 2). Except *Nitrospira* and *Nitrososphaera*, other geochemically relevant microbial populations were present in higher relative abundance in Kathmandu samples when compared to Jhapa samples (Table 2).

Table 2: The average relative abundance of genera associated with geochemical characteristics of water in Kathmandu and Jhapa and fold difference between two locations. ND denotes not detected.

Bacterial Genera	Type of Bacteria/Archaea	Average Kathmandu	Average Jhapa
Nitrospira	NOB	0.182%	0.086%
Nitrososphaera	AOA	0.007%	0.003%
Nitrosopumilus	AOA	0.045%	ND
Methylobacter	MOB	0.005%	ND
Methylomonas	MOB	0.025%	ND
Desulfovibrio	SRB	0.004%	ND

3.3 Comparison of microbial communities pre- and post-earthquake

Additional water samples were collected in June 2014, one month after the earthquake, from selected sites in Kathmandu and Jhapa (Table 1). Multivariable analysis was performed to compare the microbial community between samples from each site (Figure 3). For batch 1, ten dominant bacterial genera made up of 60% of the batch microbial community belong to *Proteobacteria* family. These same ten genera also made of 72% of batch 2. Given that the epicenter of the earthquake was 145 km away from Jhapa, the differences in microbial communities of water samples before and after earthquake were not anticipated.

However, there were differences in microbial communities in S2 and S5 samples where the earthquake impact is more significant (Figure 3). For example, in the S2 samples, there was a substantial increase in the relative abundance of geochemically relevant microbial populations (Table 3). This suggested that the earthquake disturbed the biological stability of the water (Table 3). *Methylobacter* and *Methylomonas* were undetectable pre- earthquake. In contrast, 16S rRNA genes of both MOBs were detected after the earthquake. In addition, there was a 220 fold increase in *Desulfovibrio. Nitrospira* was also not detected before the earthquake but accounted for 0.182% of the total microbial community after the earthquake (Table 3).

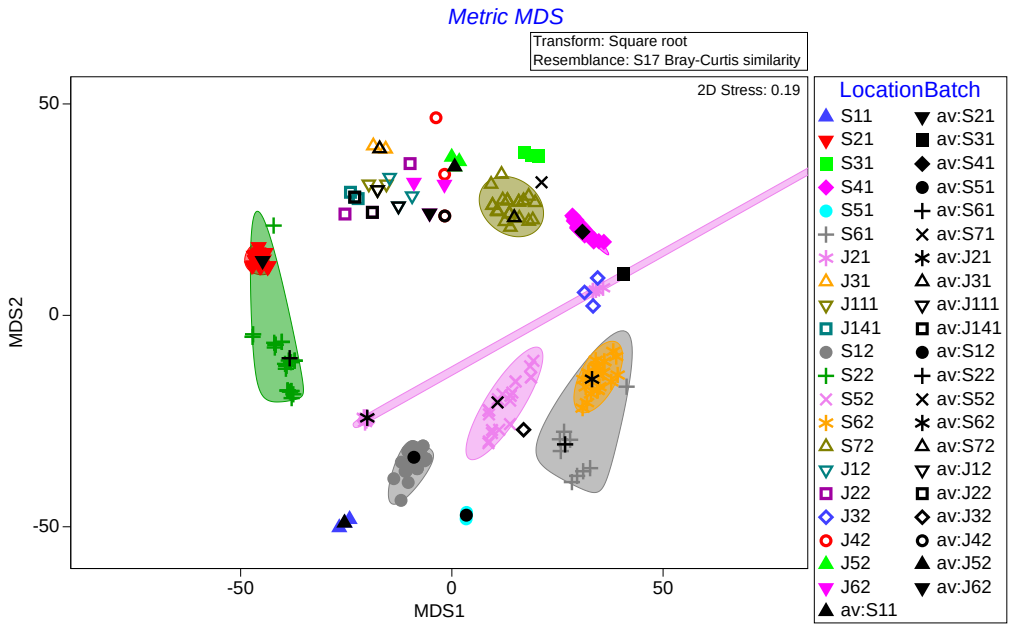

Figure 3: Multidimensional Scaling (MDS) plot comparing the microbial community between two batches, before and after the earthquake. S and J represent sampling sites Kathmandu and Jhapa respectively followed by sampling location and sampling batch consecutively.

A similar difference was noticed between the two Batches in S5 as well. There was 43.5 fold increase in *Methylobacter*. Undetected *Desulfovibrio* before the earthquake was present immediately after the earthquake, and there was 9 fold and 15.3 fold increase in *Nitrospira* and *Methylomonas*, respectively. Besides an increase in the bacterial genera related to geochemical characteristics of the water post-earthquake, there was also an increase in the ratio of different opportunistic pathogens in overall microbial communities after the earthquake. In S2, there was an increase of 9.73 fold of *Acinetobacter*, 0.19 fold of *Aeromonas* and undetected *Legionella* before earthquake represented 0.02% of the total microbial community after the earthquake. *Acinetobacter*, *Aeromonas* and *Legionella* were not detected in S5 before the earthquake but were present in relative abundance of 0.73%, 0.21% and 0.01% in samples collected immediately after earthquake (Table 4).

Table 3: Fold difference in samples S2 and S5 in Kathmandu before and immediately after the earthquake.

Bacterial genus	Type	Fold Change in S2	Fold Change in S5
Methylobacter	MOB	ND to 0.28%*	43.5
Desulfovibrio	SRB	220.6	ND to 0.02%
Nitrospira	NOB	ND to 0.05%	9
Methylomonas	MOB	ND to 0.02%	15.3
Acinetobacter	Opportunistic pathogenic genera	0.73	ND to 0.73%
Aeromonas	Opportunistic pathogenic genera	0.19	ND to 0.21%
Legionella	Opportunistic pathogenic genera	ND to 0.02%	ND to 0.01%

* Percentage of the total microbial community

3.4 Microbial community eight months after the earthquake:

Samples S2 and S5 samples collected from the same locations in Kathmandu in December 2015, eight months after the major quake (Batch 3) to investigate if the microbial communities would approximate towards pre-earthquake relative abundance. For both sets of samples, there was a observable decrease in the relative abundance of geochemically relevant microbial populations and those associated with opportunistic pathogens (Figure 4). In S2, *Methylobacter,* which was not detected in Batch 1 but was abundant in Batch 2 contributing to 0.28% of total microbial community, decreased by 57.8% in Batch 3. Similarly, for *Desulfovibrio* in S2, there was 99.1% increase in Batch 2 compared to Batch 1 but was decreased by 96.3% in Batch 3 again returning closer to the baseline level. There was 59.9% increase in *Aeromonas* in Batch 2 from Batch 1 but was not detected in Batch 3 at all. *Legionella* in S2 was not detected in Batch 1, accounted for 0.021% of total microbial community in Batch 2 and was not detected in Batch 3 again. Similarly, in S5, *Desulfovbrio* and *Acinetobacter,* were not detected in Batch 1, accounted for 0.016% and 0.738% in total microbial community in Batch 2 respectively, were not detected in Batch 3 again. *Aeromonas*, which was not detected in Batch 1 and contributed to 0.21% of total microbial community in Batch 2, decreased by 91.95% in Batch 3 returning closer to the baseline level. This indicates the pattern of perturbation in biostability of water after extreme

natural events like an earthquake and a possible recovery to the original state after some amount of time.

3.5 PCR-based Fecal Source Tracking:

Host-associated *Bacteroidales* had been previously used as bacterial indicators to examine the origins of fecal contamination. Human fecal contamination was determined positive when two or more human-associated *Bacteroides* spp. were present in the sample [16]. Samples from site S2 were tested negative for all three human associated *Bacteroides* marker before, immediately and eight months after the earthquake. However, samples in S2 tested positive for cow specific *Bacteroidales* marker immediately after the earthquake. Samples form site S5 tested positive for two human specific markers before the earthquake and eight months after the earthquake but tested positive for all three markers right after the earthquake. No cow markers were detected on S5 at any sampling time.

Table 4: Presence or absence of human-associated *Bacteroides* spp. and cow-specific *Bacteroidales.*

	Sample	Positive for all 3 markers	Positive for 2 markers	Positive for 1 marker	No markers detected	Positive for marker
Batch 1 (before earthquake)	S2	-	-	-	+	-
	S5	-	+	-	-	-
Batch 2 (immediately after earthquake)	S2	-	-	-	+	+
	S5	+	-	-	-	-
Batch 3 (eight months after earthquake)	S2	-	-	-	+	-
	S5	-	+	-	-	-

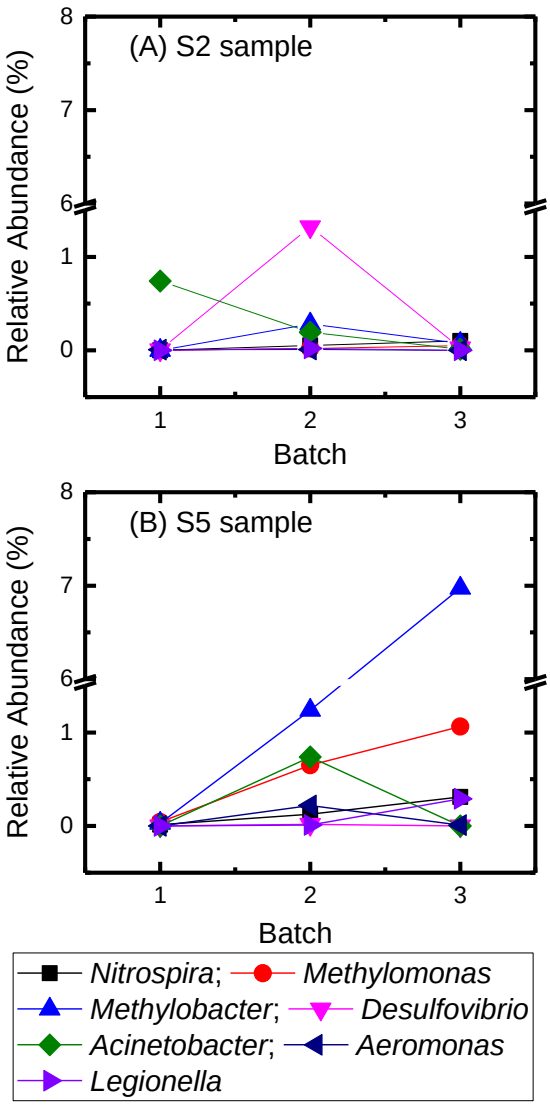

Figure 4: Change of relative abundances of different bacterial genera associated with geochemical characteristics of water and genera associated with opportunistic pathogens in samples S2 and S5 for Batch 1, Batch and Batch3.

3.6 Chemical and microbial water quality in samples post-earthquake:

Batch 2 and 3 samples were tested for chemical and physical parameters (pH, nitrite, nitrate, ammonia, phosphate, temperature, salinity, total dissolved solids (TDS) and conductivity) and microbial parameters (total coliforms and fecal coliforms). Kathmandu (S samples) generally had higher nitrate, ammonia and phosphate content than the Jhapa (J samples), with nitrate concentration up to 70 mg/L in S7 and ammonia concentration up to 8 mg/L in S1 and S2. Phosphate concentrations in S samples were also up to 79-fold higher than that in J samples (Table 5). In most of the Batch 2 samples, fecal coliforms counts were too numerous for counting (TNTC), but both fecal and total coliform count decreased in the samples taken eight months after the earthquake (Batch 3). The number of fecal coliforms detected in Batch 3 samples was generally less than 2 CFU/mL (Table 5).

Table 5: Chemical and microbial quality of water samples from Kathmandu and Jhapa immediately after the earthquake and eight months after the earthquake. These measurements were not taken during the sampling before the earthquake. The measurements without average and standard deviation indicate single measurements. TNTC indicates too numerous colonies to count.

		Nitrite	Nitrate	Ammonia	Phosphate	Total Coliform	Fecal Coliform	pH	Temperature	Salinity	TDS	Conductivity
	Sample Name	Average (mg/L) ± Standard Deviation				CFU/1mL observed highest		Average ± Standard Deviation	Average (°C) ± Standard Deviation	Average (ppm)) ± Standard Deviation		Average (µS) ± Standard Deviation
Batch 2 (immediately after earthquake)	S1	ND	ND	7.75±0.4	9.5±0.8	TNTC	TNTC	7±0	27.3±1.04	286.6±18.6	305±86	600±34.9
	S2	0.12±0.21	ND	7.5±0.9	1.6±0.6	10	ND	6.1±1.1	26.7±2.	197.3±3.7	507.6±317	438±37.2
	S5	ND	24±32.4	1.5±0.5	10±0	TNTC	TNTC	7.1±1	25.4±1.2	470.6±4.9	671±6.23	954±7.3
	S6	ND	25±32.7	3.5±0.9	1.75±0.8	26	10	7.1±0.7	25±1.1	574±74.5	597±216.5	1166±139.5
	S7	ND	70±54.4	0.3±0.1	0.7±0.32	TNTC	TNTC	7.25±.4	23.9±0.6	212.7±116.1	404.6±138.2	444±237.6
	J1	ND	ND	0.1±0.1	0.4±0.1	52	4	7±0	32.4	55.6	82.5	118.2
	J2	ND	ND	0±0	0.4±0.1	65	2	6.5±0	29.8	48	71	101.2
	J3	ND	ND	0.1±0.1	0.4±0.1	TNTC	TNTC	5.7±0.3	30.8	44.4	65.8	94
	J4	ND	ND	ND	0.12±0.1	15	ND	6.2±0.8	32.3	63	93.8	134
	J5	ND	ND	ND	ND	25	ND	6±0.5	31.5	61.5	91.1	130.8
	J6	ND	ND	0.1±0.1	ND	31	2	6.5±0.5	30.6	46	68	98
Batch 3 (eight months after earthquake)	S1	ND	ND	8	10	1	0	7.5	22.1	307	451	676
	S2	ND	ND	8	0.5	17	2	7	18.3	200	295	421

CHAPTER 4

DISCUSSIONS

Distinct difference in microbial communities in samples from Jhapa and Kathmandu shown in Figure 2 can be explained by water sources, environmental conditions and human activities. Source water in Kathmandu are from a single aquifer [18] , accessed by a deep or shallow well, whereas households in Jhapa rely on river water, stored and distributed through a reservoir. Geochemical characteristics, including anthropogenic contaminants of both surface water and groundwater has been shown to influence the bacterial and archaeal communities [19-21]. Surface water and groundwater environments have distinct indigenous microbial communities [22]. In addition to the water sources of these samples, these two locations have vastly different human activities. Per capita use of the source water in Kathmandu was much higher compared to that of Jhapa as two hundred people use water in sampled schools of Kathmandu and only five family members use sampled water in Jhapa, everyday. Human activities and settlements could affect the overall microbial communities and specially nitrifying bacteria and archea as reported in previous studies looking specifically at *Nitrosomonas* and *Nitrospira* [23, 24].

Within Kathmandu, before the earthquake, the observation of three different microbial communities can be explained by groundwater contamination related to human activities. All sampling sites in Kathmandu were from a shallow/dug wells except S2, which is a

deep borehole (80 ft) well. The microbial communities from the S2 samples are distinct from other samples.

Deep wells, such as S2, are less likely to be affected by contamination associated with human activities compared to shallow wells. Studies in the past have shown that consumption of water from the deep wells are less likely to cause diarrheal diseases [25, 26]. Among the samples from the shallow wells, the two clusters formed by S1, S5 and S6, and by S3, S4 and S7 can possibly be explained by difference in sanitation practices at these locations. The shallow well in S4 was uncovered. S3 and S7 samples were from the wells that are only ~10 m and ~4 m, respectively from the toilet. The fecal source tracking also confirmed this observation as S3 was found positive for one human associated *Bacteriodes* spp and S4 and S7 were found positive for all three markers affirming human fecal contamination. All, S1, S5, S6 wells were protected wells and toilets were located at more than ~30 m away. This baseline study provided information regarding the biological stability of the water before the earthquake in the two selected locations and assisted in the comparative analysis to understand the stability perturbation following the earthquake.

Although the cases of waterborne diseases increases tremendously after major natural disaster [27, 28], there has been very limited research on effect of natural disaster (including earthquake) on water microbiota. Transfer of several microorganisms including pathogenic bacteria from flood water to tap water was observed after 2011 flood in Thailand [29]. In addition, metagenomic analysis of microbes in soil after 2011 Japan earthquake revealed loss of siderophore-synthesis

genes from *Arthrobacter* strains along with over-representation of denitrification related genus and the presence of pathogenic genera.

[30]. Similarly, more pathogenic bacteria were found in water samples collected from earthquake-affected area in Pakistan compared to the areas that were not affected by earthquake [31]. Even though, these studies provide some insight about disturbance in microbiota after an extreme natural event, there still exists a knowledge gap on if these microbiota adapt with time and go back to its neutral state and if so what is the timeline. The study was thus designed to fill that knowledge gap.

Consistent with previous findings of in biological stability of water [28, 30, 31], there was significant increase in relative abundance of bacterial genera responsible for maintaining geobiochemical characteristics of water and also pathogenic genera right after the earthquake for S2 and S5 in Kathmandu (Figure 4). Site S2 and S5 were being used as temporary camps for the victims of earthquake, increasing the human activities in the area and unhealthy sanitation practices like open defecation, introducing contamination to the wells. Human settlement activities have been shown to influence the presence of nitrifying bacteria and archea [25], which in turn could affect the overall biological stability of the water [32]. The sharp drop in overall abundance of bacterial genera was observed eight months after the earthquake in S2 and S5. This observation indicates that perturbation observed in biostability of water that occurred immediately after the earthquake was restoring

back to its original state. Moreover, the temporary settlements in those sites were removed and the contamination due to overload sanitation was stopped.

This study presented new knowledge on the dynamics of water microbiota after extreme natural events like earthquake and its restoration with time. However, there were certain limitations to this study, including (i) 16S rRNA sequencing can mostly characterize bacterial genera but leaves out all eukaryotes including fungal and parasitic genera and viruses, which could help understand the big picture with perturbation in water microbiota [33, 34]. Although we are trying to study the perturbation as function of time, (ii) sampling right after the earthquake and eight months after the earthquake might not be enough to characterize all important genera as some genera might require longer to go back to original state compared to others. To overcome these limitations, shotgun sequencing can be performed on the samples over period of time to understand the overall microbial diversity, including viruses, rather than be limited with 16S, 18S and 23S [35-37]. We have also scheduled number of sampling trips in next three years to better understand the timeline microbial community's restoration after major natural disaster like earthquake.

The results of this study on understanding the biological stability of water after extreme natural events like earthquake can provide information to scientists and engineers on how the perturbation occurs and how it restores as a function of time. This study can be a foundation on further studies on the perturbation studies, especially regarding opportunistic pathogens that causes several causalities after

extreme natural event and may eventually help with Water Sanitation and Hygiene (WASH).

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