Biosecurity of Upolu Fresh and Salt Environmental Water Resources

Final Report

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Research Partners: Ministry of Natural Resources of Environment, Samoa
National University of Samoa

Research was conducted within the framework of the partnership entered in by the United Nations Development Programme in Samoa and New York University with the participation of the Ministry of Natural Resources and Environment and the National University in Samoa

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I. EXECUTIVE SUMMARY

In 2019, the United Nations in Samoa secured a USD 1.0 mill financing from the UN-India Development Partnership Fund managed by the UN Office for South-South Cooperation (UNOSSC) to fund the joint programme titled Samoa-Knowledge Society Initiative (S-KSI) whose main objective is to facilitate access to knowledge as a public good and advance research-based sustainable development.

The Joint Programme is currently being implemented by UNDP and UNESCO under the close oversight of the UN Resident Coordinator Office and the UNOSSC. A National Steering Committee co-chaired by the Government of Samoa and the Resident Coordinator includes the Government of India as the main donor as well as relevant line ministries in Samoa and the National University of Samoa.

Several objectives have been set for the Joint Programme including (1) building the first Digital Library of Samoa (SADIL) to combine digitized local content with external knowledge through partnerships with major libraries, (2) developing the first lifelong learning platform for the people of Samoa to upgrade knowledge or acquire new skills, (3) create a digital environment for open research and (4) establish mechanisms to monitor use of knowledge in decision making and advocate for universal access to public information as a prerequisite for a viable democracy. To organize the digital resources that S-KSI aims to establish in Samoa, a digital platform is being developed to integrate components (1), (2), (3) within one system that will have a friendly interface, accessible from all devices and also through dedicated community centers.

The research titled *Biosecurity of Upolu Fresh and Salt Environmental Water Resources* is the first to have been produced through an open international partnership involving New York University (NYU New York) and New York University Abu Dhabi (NYUAD), the National University of Samoa and the Ministry of Natural Resources and Environment. The research aims to provide answers to questions regarding the underlying causes of declining biodiversity on land and in the waters of Samoa as well as of diseases that might be water borne.

The research has been conducted within the framework of a partnership that UNDP and NYU entered into for a 6-month period to include the field activity, lab tests, analysis of findings and the completion of the report. While the field mission took place in December 2019 as planned, the testing, analysis of findings and the development of the report have been affected by the COVID-19 pandemic and related movement restrictions that led to the closure of education institutions among others. The partnership has therefore been extended for another three months to allow for a thorough review of the results by partners and stakeholders.

While the results may need further interpretation for all correlations to be established, the research will inform the revision of environmental policies and the formulation of the adequate institutional and policy response to potential health threats identified.

The UN Resident Coordinator Office and the United Nations Development Programme in Samoa jointly express appreciation of the quality of the work conducted by NYU, the MNRE and the University of Samoa within this project.

I.1. Scope of work

Mission: The project mission is to collect and analyze fresh and salt-water resources of Upolu Island, Samoa, for absolute concentrations of elements across the breadth of the chemical periodic table, to assess water safety through analysis of microbial diversity and quality, and to assess the extent to which microplastics are present in the environment. We apply this novel "model system" of analysis to investigate environmental change in relation to human health and coral reef decline.

Model of Partnership: The Samoan Ministry of Natural Resources and Environment (MNRE), The National University of Samoa (NUS), and New York University College of Dentistry (NYUCD) and New York University Abu Dhabi (NYUAD) operated in joint teams within the project framework.

I.2. Project phasing

Phase 1: Fieldwork

The project team operated on Upolu Island for two weeks, December 1 to December 15, 2019 to collect samples. Two-hundred and nineteen water samples were collected from 35 rivers and 3 volcanic lakes, seawater samples from 39 inner reef, reef, and outer reef locations around Upolu Island, including each river estuary, and several rainwater samples, and Samoan bottled water samples. Water quality assessments performed on site included measurements of temperature, turbidity, dissolved O_2, specific conductivity, salinity, pH, oxidation-reduction potential, nitrate, and chloride, and detection of total coliform and *Escherichia coli* (*E. coli*) bacteria.

Phase 2: Laboratory measurements and data generation

At NYUCD, 71-element concentration data were acquired by next generation mass spectrometry technology. Microplastics analyses were undertaken by passing water samples through a filter paper, which accumulated microplastics that were then imaged by high-resolution scanning electron microscopy (EM). Confirmation of plastic (vs. other suspended materials) was accomplished using X-ray analytical chemistry technology attached to the SEM. Assessment of the bacterial relative abundance and composition in samples were accomplished by isolating DNA at NYUAD and using next-generation sequencing technology (Illumina MiSeq, USA) to sequence specific DNA markers of bacteria to estimate their abundance and diversity within samples. Elemental analyses, water quality assessments, microbial diversity results from testing at NUS, and microplastics data were sent to NYUAD for statistical processing.

Phase 3: Final Report and presentation of the findings

Researchers at NYU and NYUAD developed the report in consultation with the Ministry of Natural Resources and Environment and the National University of Samoa who provided valuable insights and data.

The report is subject to review with UNDP, the Government of Samoa and the National University of Samoa.

I.3. Results:

Key findings of the research are being summarized to inform recommendations that the Government of Samoa may consider in order to enhance the quality of the waters in Upolu, reverse biodiversity loss and improve quality of life. Further review of the findings will take place together with relevant line ministries including the Ministry of Natural Resources and Environment, Ministry of Health, Ministry of Women, Community and Social Development, Ministry of Agriculture and Fisheries as well as specific research institutions in Samoa.

Samples with elemental content above or below normal are listed for seawater and mangrove swamps in the Tables below.

Table 1 - Test results for seawater samples

Seawater				
Element	**Normal**	**Median**	**Range**	**% of Samples Above (+) or Below (-) Normal**
Ba	13.7	5.24	2.65-6.68	-100%
Br	67116	65600	25160-74080	36%
Ca	412824	372560	168880-532520	43%
Cu	5	6.16	1.2-12.44	64%
Mg	1293292	1091100	359400-2686320	27%
Ni	15	10.83	5.65-19.04	16%
Si	2809	868	44-2060	-100%
Sr	7885.8	4920	1886-7120	-100%
Zn	50	13.18	0.6-45.48	-100%

Table 2 - Test results for Mangrove swamps

Mangrove swamp				
Element	**Normal**	**Median**	**Range**	**% of Samples Above (+) or Below (-) Normal**
Ba	13.7	10.4	4-22.5	-71%
Br	67116	27493	2413-112023	27%
Ca	412824	87654	17316-339042	-100%
Cu	5	1.49	0.415-5.52	14%
Mg	1293292	386522	61746-1541911	14%
Ni	15	4.45	0.129-19	14%
Si	2809	10099.3	1522-14816	100
Sr	7885.8	1619	225-6736	-100%
Zn	50	4.53	0.383-14.5	-100%

Table 3 – Test results for microplastic particle density in Freshwater, Seawater and Mangrove Swamps

Microplastics				
Water Type	**Median**	**Minimum**	**Maximum**	
FW	3579	250	90925	
SW	3023	35	32709	
MS	7015	588	44130	

All values in μm^2 (square micrometers). Freshwater (FW), Seawater (SW), Mangrove Swamp (MS)

High concentrations of magnesium (Fig. 5), bromine (Fig. 8), and calcium (Fig. 9), are connected to widespread herbicide and pesticide chemical use on Upolu Island, which influence endocrine activity that may contribute to endemic obesity. The herbicides and pesticides are also highly toxic to aquatic life. Thus, there is an intimate connection between the role the environment on both human and aquatic health.

Magnesium (Fig. 5), copper (Fig. 6), calcium (Fig. 9), and nickel (Fig. 11) are ubiquitously or locally high around Upolu Island. These increases in concentrations of accumulated metals are associated with coral reef decline. In addition, nickel is associated with contact dermatitis in humans, which may increase the potential for additional vulnerabilities and exacerbation of other medically relevant insults through the skin. These associations between metal environmental contamination and human and aquatic health require study, analysis, and confirmation on Upolu Island.

Barium and strontium are essential for the growth and construction of stony corals, yet concentrations of these elements are very low around Upolu Island. Silicon is also essential for the construction of stony corals and for growth of important algal lineages such as diatoms; however the input from fresh water sources appears to be controlled at mangrove swamps, rendering an apparent insufficiency, potentially limiting the growth of important algae that support the marine food web and coral reefs (Fig. 7).

Pathogenic enteric bacteria, such as *E. coli*, *Klebsiella and Salmonella* are widely distributed around Upolu Island likely because of inadequate sanitation and are implicated in environmental degradation, human disease, and stress and mortality to corals. The prevalence of these bacteria in most samples poses severe health hazards to people if freshwater is ingested. In the marine environment, recreational and economic activities such as swimming and shellfish industry may suffer in the future if these bacteria increase further in abundance. This is particularly important during periods of hot weather, as increased temperatures tend to increase the abundance and activities of these pathogens.

The salinity of many seawater samples is very high, which if not evolutionarily adapted to by corals, can be lethal to corals.

Microplastics affect coral reef health and growth and have species-specific impacts that diminish coral biodiversity.

There is an indication along river systems that cumulative increases in concentrations of elements and microplastics from inland to the ocean is due to human activities.

I.4. Recommendations

To address factors deleterious to human, economic and recreational activities and coral reef health, the Government of Samoa may consider:

1. Mitigating environmental harm caused by use of herbicide and pesticide chemicals through alternative pest management, such as biological control alternatives, may be considered;
2. Identifying sources of metal contamination and mitigation of these sources using new or improved technologies and manufacturing processes for reducing their release to the environment;
3. Conducting specific investigations into barium, strontium, and silicone deficiencies. Historically this may be investigated by sampling coral skeletons retrieved at known dates in the past;

4. Introducing better sanitation practices to reduce incidence of pathogenic enteric bacteria, such as *E. coli,* and a monitoring system to keep track of the abundance of these microbes in both fresh and marine waters, particularly where fisheries and recreational activities take place. Most importantly, environmental-friendly waste management is critical in order to limit human harm to the quality of natural capitals.
5. Investigating causes of salinity variability of the inner reef and reef zone. Multivariate analyses of historical weather and ocean data with present day sampling through seasons is warranted;
6. Banning single use plastics as the largest contributor to microplastics needs to remain in force. Finding sustainable alternatives that take into account waste and pollution from pulp and paper manufacturing may be considered.
7. Future studies need to be performed drawing on the results of this research to identify potential medical cluster effects that correlate with water quality.

II. Biosecurity of Upolu Fresh and Salt Environmental Water Resources

II.1. MISSION

The project mission is to collect and analyze fresh and seawater resources of Upolu Island, Samoa, for absolute concentrations of elements across the breadth of the chemical periodic table, to assess water quality and microbial content, and to assess the extent to which microplastics are present in the environment. We advance this inclusive approach as a *model system* and major contribution to the biosecurity of fresh and seawater resources in search of explanations for human morbidity and to pursue drivers of terrestrial and aquatic decline in biodiversity, which can be used for monitoring the environment prior to, during, and following mitigation activities when indicated. Here, we apply this model system with particular emphasis on human health and the observed decline of coral reefs on Upolu Island.

II.2. MODEL OF PARTNERSHIP

The National University of Samoa (NUS), the Samoan Ministry of Natural Resources and Environment (MNRE), and New York University (NYU) operated in joint teams. Government of Samoa in-kind support included MNRE vehicles for teams sampling from rivers and lakes around the island, logistics, and geographical information systems (GIS) overlays. NUS provided logistics, team members, and laboratory space for processing all samples coming from the field. NYU Abu Dhabi (NYUAD) provided instrumentation onsite at NUS for sample processing and measurements (Masterflex L/S peristaltic pump, YSI ProDSS Multimeter for water analysis and IDEXX Colilert kits). In addition, DNA was collected on site and later extracted by NYUAD to be quantified and sequenced using the Illumina MiSeq platform. NYUAD also provided bioinformatic analyses and GIS processing (Esri, US). NYUCD provided specially prepared sampling tubes for water testing in New York. At NYUCD an ion meter was provided to obtain salinity values, a SPECTRO MS simultaneous-inductively coupled plasma-mass spectrometer (si-ICP-MS; SPECTRO, GER) was provided to perform the elemental analyses, and a Zeiss Gemini 300 field emission scanning electron microscope (FE-SEM; Zeiss, GER) was provided to quantify the microplastics analysis in conjunction with energy dispersive spectroscopy (EDS) to confirm plastic chemistry (Bruker, GER).

II.3. KEY PERSONNEL

Simona Marinescu, Ph.D., UN Resident Coordinator in Samoa, responsible for the Joint Samoa – Knowledge Society Initiative, Jorn Sorensen, UNDP Resident Representative and Verena Linneweber, UNDP Resident Representative who manage the UNDP-NYU partnership and project funding and liaising with all partners; Emarosa Romeo, Ph.D., MNRE Principal Hydrology Officer responsible for MNRE participation in the project; Patila Malua Amosa, Ph.D., Dean

Faculty of Science, National University of Samoa responsible for NUS participation in the project; Gary R. Goldstein, D.D.S., NYUCD responsible for project logistics in Samoa and liaison to the MNRE and NUS (pro-bono); Timothy G. Bromage, Ph.D., NYUCD, together with Khemet Calnek, B.A., Bin Hu, M.D., and Sasan Rabieh, Ph.D., were responsible for isotope and imaging facilities used to fingerprint the multi-element composition of water samples, and to assess the microplastics in the environment; Michael Ochsenkuehn, Ph.D., working with Shady Amin, Ph.D. of NYUAD responsible for water quality data, DNA sequencing bioinformatics and to evaluate water samples for their microbial content; Youssef Idaghdour, Ph.D. and Odmaa Bayaraa, student, of NYUAD responsible for statistical analysis and GIS processing.

II.4. PROJECT TIMELINE AND FINDINGS: The project was executed in three phases.

Phase 1: Fieldwork

The project team operated on Upolu Island for two weeks, December 1 to December 15, 2019 to collect samples.

Three sampling vessels were used to acquire all water samples from all locations, one for elemental testing, one for microplastics analysis, and one for the examination of microbial diversity and water quality assessments. Water samples were collected from rivers, lakes, and by boat at sea at designated locations identified around the study region. Surface water samples are taken from each of a river's headwater and its mouth and location(s) in between, from lakes, and from the sea that includes depths that capture water in contact with coral reefs. Water quality assessments were performed on site.

In all, 219 water samples were recovered from the land and sea surrounding Upolu island. These included: 96 water samples representing 35 rivers and 3 volcanic lakes, 109 salt water samples representing 39 locations around Upolu Island, including the lagoons of each river estuary, 2 rainwater samples acquired from specific rain events, 1 rainwater sample taken from a water reclamation system from a home, and 11 Samoan bottled water samples.

The facilities provided by NUS were used to acquire information on the presence of pathogenic bacterial contamination, physical and chemical water quality parameters, and to process the samples for microbial diversity analysis using genomic sequencing later. In laboratory tests at NUS, all water samples from the rivers, lakes, and sea were subject to initial study.

Directly after sampling, all samples were tested qualitatively for the presence of total coliform bacteria and fecal coliform (*E. coli)*, a commonly used method to assess suitability of fresh and seawater to human consumption and activities. The Colilert 18 (IDEXX, US) testing kit, which is US EPA approved, is able to detect a single pathogenic bacterial cell in 100 ml of water sample.

We found a consistent distribution of total coliform and fecal *E. coli* in rivers, lakes and inshore reefs. Tap and bottled waters were free of pathogenic bacteria, but non-pathogenic coliform were present in some bottled water samples.

The physical parameters for freshwater (pH, dissolved oxygen (DO), nitrate (NO_3^-), chloride (Cl), specific conductivity) and seawater (pH, DO, salinity, oxidation-reduction potential) samples were measured using a YSI ProDSS Multimeter (YSI Inc., US). These parameters are indicative of the health of the sampled water bodies.

To determine the microbial diversity of the samples and identify the distribution and relative abundance of harmful bacteria, DNA was isolated from all water samples and genomic sequencing was employed (NYUAD). The samples were concentrated at NUS onto Sterivex 0.2 µm (Merck, GER) filter cartridges and preserved (RNAlater, Invitrogen, US) for shipping and DNA isolation at NYUAD.

PHASE 2: LABORATORY TESTING AND DATA GENERATION

At the end of the fieldwork, samples were taken to NYU laboratories for analysis. Progress under Phase 2 began in the field, as per the Phase 1 findings above. At NYUCD, from December 15, 2019 to March 15, 2020, si-ICP-MS and FE-SEM laboratory testing and data generation were performed. Element concentrations were obtained by si-ICP-MS using analytical methods detailed in Bàuchle et al. [1]. For microplastics analyses, we passed 10 mL of each water sample through a 5 mm diameter gold-coated 0.4 µm filter paper for accumulating microplastics then imaged by FE-SEM using backscattered electron microscopy detection and EDS chemistry. These are high-resolution methods for imaging microplastic particles (e.g., to 1 µm) and confirming their chemistry. Microplastics were semi-automatically measured from tiled images of each filter using Adobe Photoshop (Adobe, US).

For microbial diversity, from December 20, 2019 to February 10, 2020 freezer-preserved filter cartridges were used to isolate total DNA. DNA isolation was performed using a protocol optimized by the Amin Lab at NYUAD to isolate microbial DNA from water samples using an SDS-based method and AMPure XP beads (Beckmann Coulter, US). DNA samples were then shipped for sequencing at the Roy J. Carver Genomics Center (Illinois, USA) using the Illumina MiSeq platform and utilizing a method to sequence all bacterial groups in a given sample. From June 10 to June 13, sequencing data were acquired and analyzed using a modified dada2 pipeline (https://benjjneb.github.io/dada2/index.html) to assess microbial diversity and abundance on the Amin Lab computing cluster.

From March 15 to June 13, 2020, all statistical analyses of elements, water quality assessments, microbial diversity results from testing at NUS, and microplastics data were done at NYUAD.

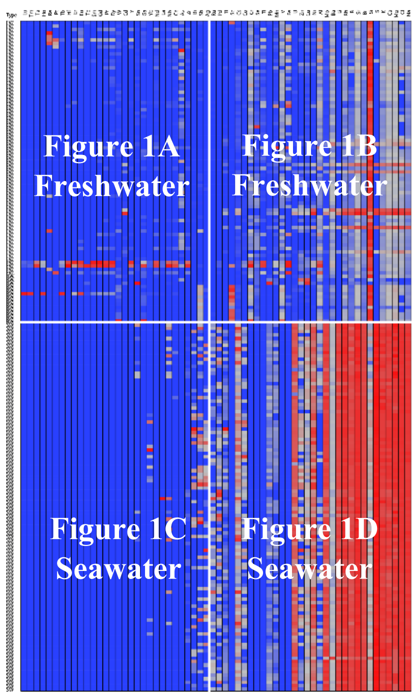
PHASE 3: FINAL REPORT AND PRESENTATION OF THE FINDINGS

What follows is a description of the statistical approaches taken to the "big data" set of element concentrations, the distributions of elements over geography, and water quality and microbial diversity analyses followed by implications for the health of humans and aquatic life, and a summary.

II.5. MAIN FINDINGS

II.5.1. Analyses of Water Chemistry

In Figure 1 on this and the following page are plotted the element concentration data for 66 of 71 elements (5 elements lacked sufficient data) so that we may obtain a sense of variability in the study sample. The data have been transformed (log transformation), which is a statistical technique to improve the visualization of the full spectrum of elements. Light shades and shades of blue are elements at low concentrations, while elements in higher concentrations are shown in shades of red.



Inspection of a thumbnail of Figure 1 at left indicates that seawater contains more elements at higher concentrations than fresh water sources.

To permit further scrutiny, Figure 1A-D have been enlarged on the following pages. On each figure the element name using its formal one or two letter chemical abbreviation is above its own colored column. Each row in the plot is an individually collected water sample. At the left margin is given the sample type for all rows: FW for freshwater, RW for rainwater, BW for bottled water, and SW for seawater.

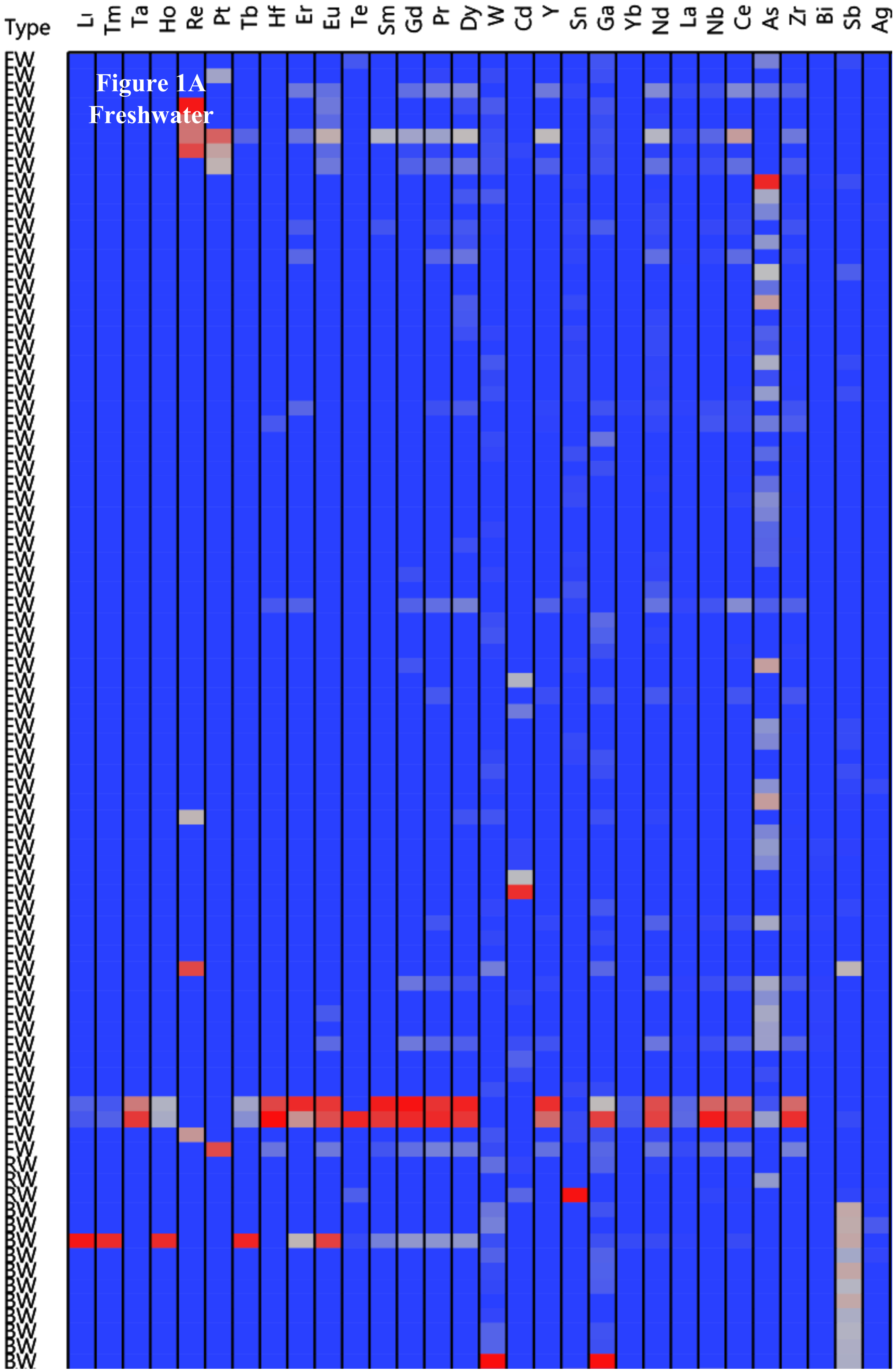


Figure 1A
Freshwater
Type
Li Tm Ta Ho Re Pt Tb Hf Er Eu Te Sm Gd Pr Dy W Cd Y Sn Ga Yb Nd La Nb Ce As Zr Bi Sb Ag
FW
RW
BW

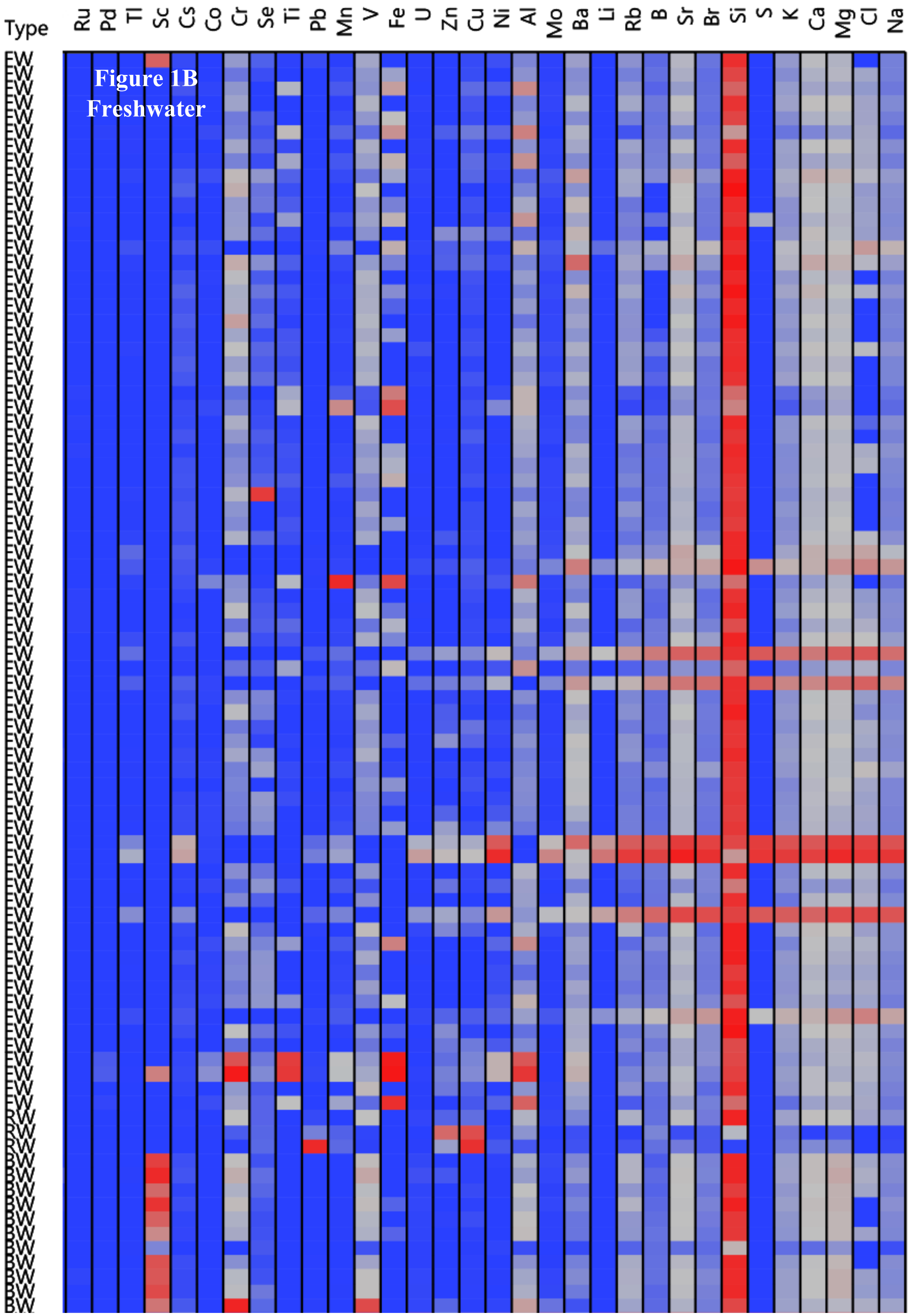


Figure 1B
Freshwater
Type
Ru Pd Tl Sc Cs Co Cr Se Ti Pb Mn V Fe U Zn Cu Ni Al Mo Ba Li Rb B Sr Br Si S K Ca Mg Cl Na
EW
RW
BW

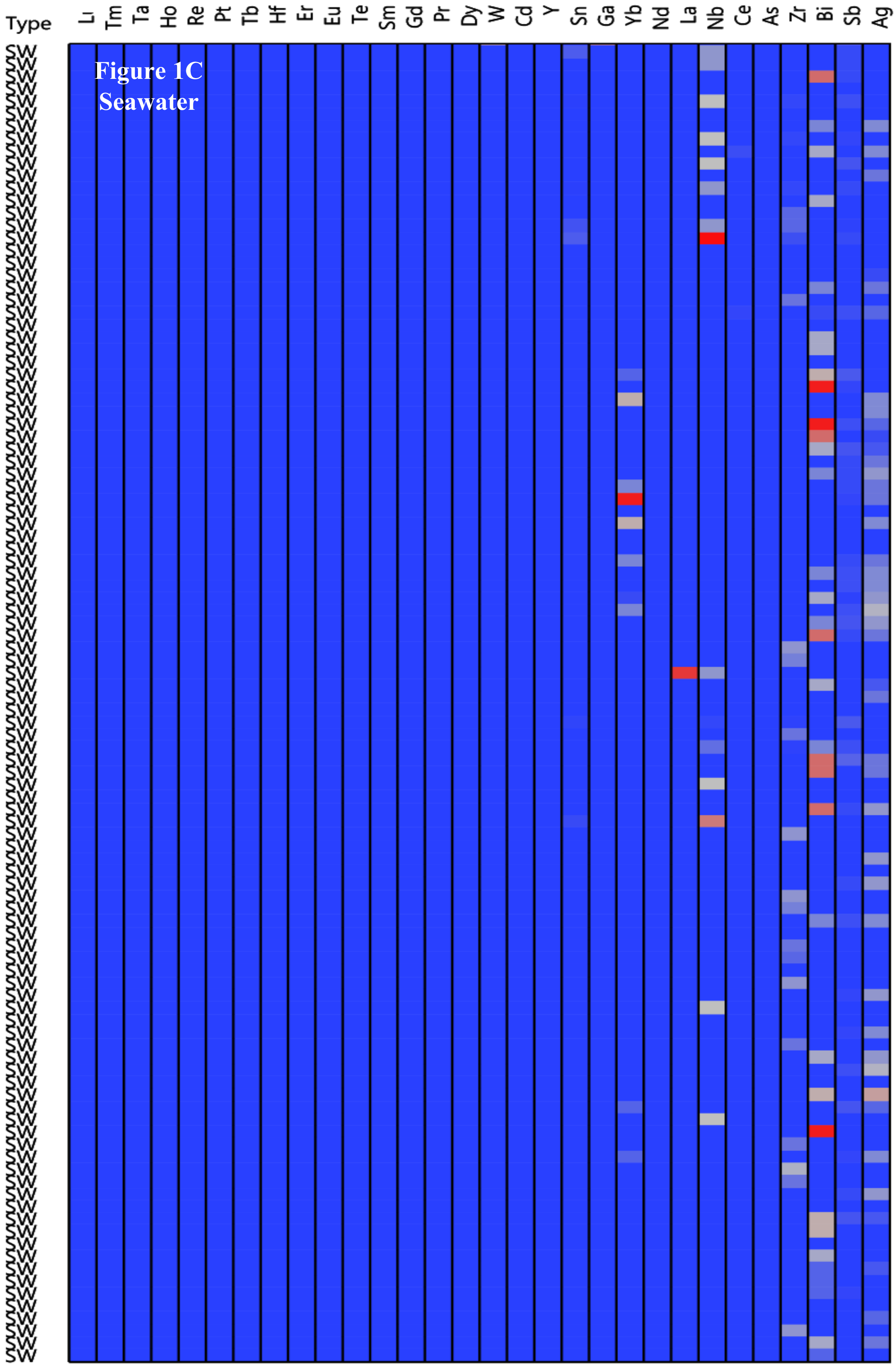


Figure 1C
Seawater
Type
Li
Tm
Ta
Ho
Re
Pt
Tb
Hf
Er
Eu
Te
Sm
Gd
Pr
Dy
W
Cd
Y
Sn
Ga
Yb
Nd
La
Nb
Ce
As
Zr
Bi
Sb
Ag
SW

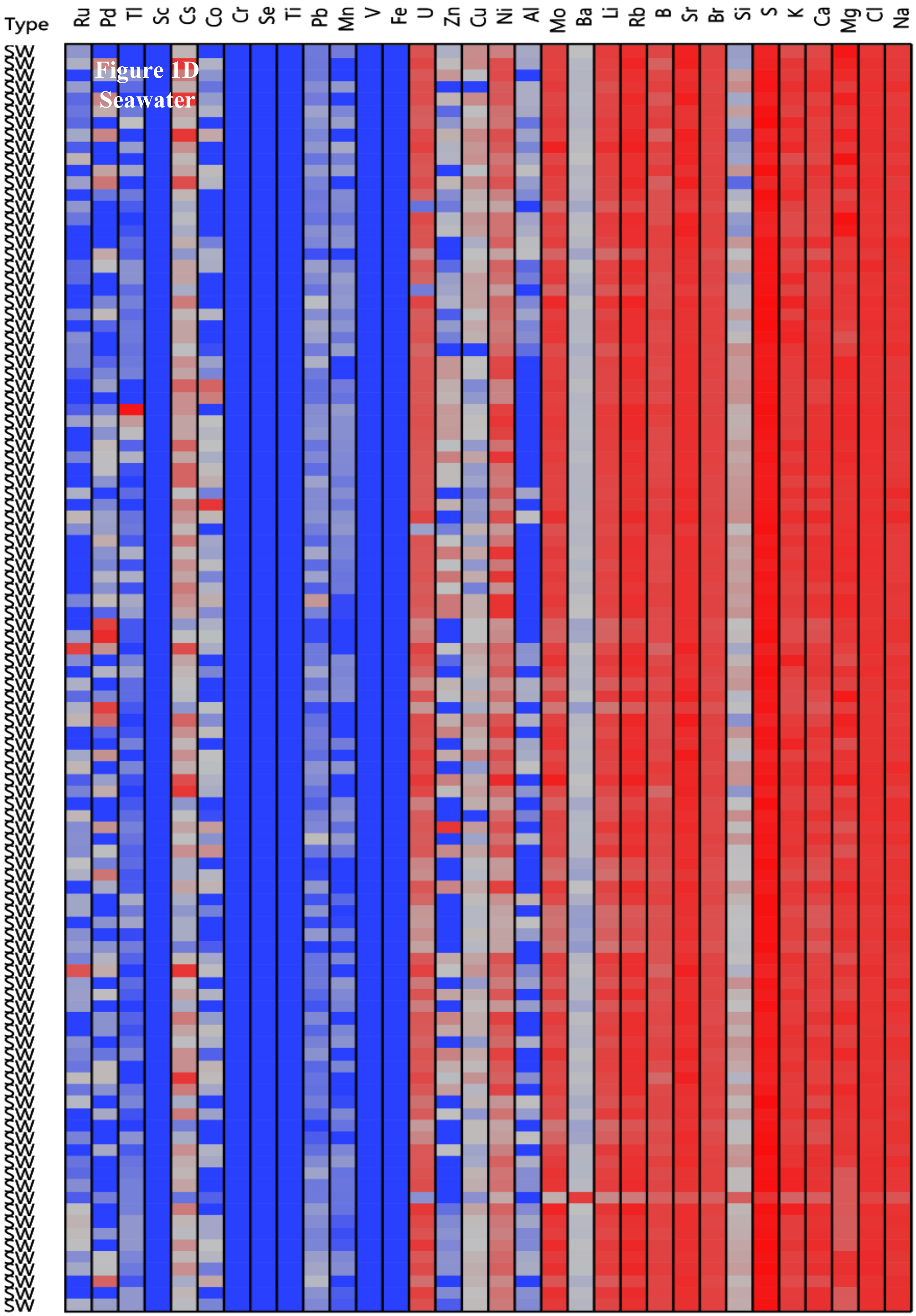
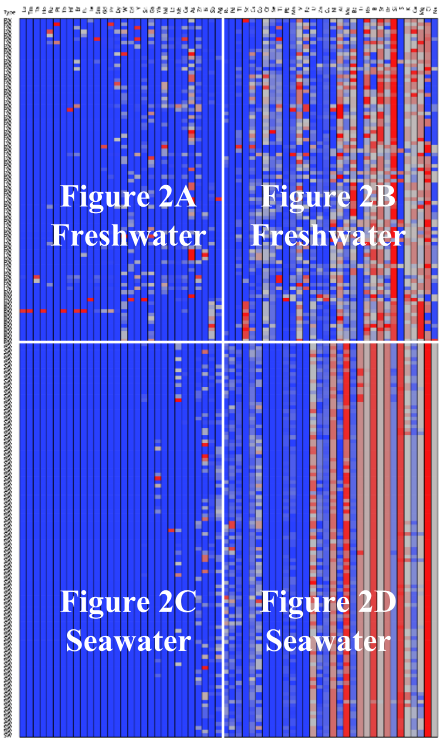


Figure 1D
Seawater
Type
Ru
Pd
Tl
Sc
Cs
Co
Cr
Se
Ti
Pb
Mn
V
Fe
U
Zn
Cu
Ni
Al
Mo
Ba
Li
Rb
B
Sr
Br
Si
S
K
Ca
Mg
Cl
Na
SW

Because the range of concentrations in Figure 1 is so large, the log transformation we applied is still not enough to reveal by eye all element concentrations, particularly among the freshwater sources, which have very low concentrations for some elements. For this reason, we applied another statistical procedure to the data represented in Figure 1 called "quantile normalization". This statistical procedure generates a common unit for all element concentrations and spreads them out between the values 0 and 1; i.e., from no concentration detected to the maximum concentration respectively. This reveals structure in the thumbnail of Figure 2 below that can now be more easily visualized. There is still separation of seawater from other water types, but concentrations in the freshwater sources are now more visible.



As for Figure 1, to permit further scrutiny, Figure 2A-D have been enlarged on the following pages. On each figure the element name using its formal one or two letter chemical abbreviation is above its own colored column. Each row in the plot is an individually collected water sample. At the left margin is given the sample type for all rows: FW for freshwater, RW for rainwater, BW for bottled water, and SW for seawater

Very noticeable in Figure 2A-D is silicon (7th column from right), which is especially high in freshwater sources (bright red cells) but low in seawater. Elements further revealed in this analysis include arsenic, chromium, vanadium, uranium, aluminum, barium, and strontium. We will return and scrutinize some of these elements by their geographic distribution later in this report.

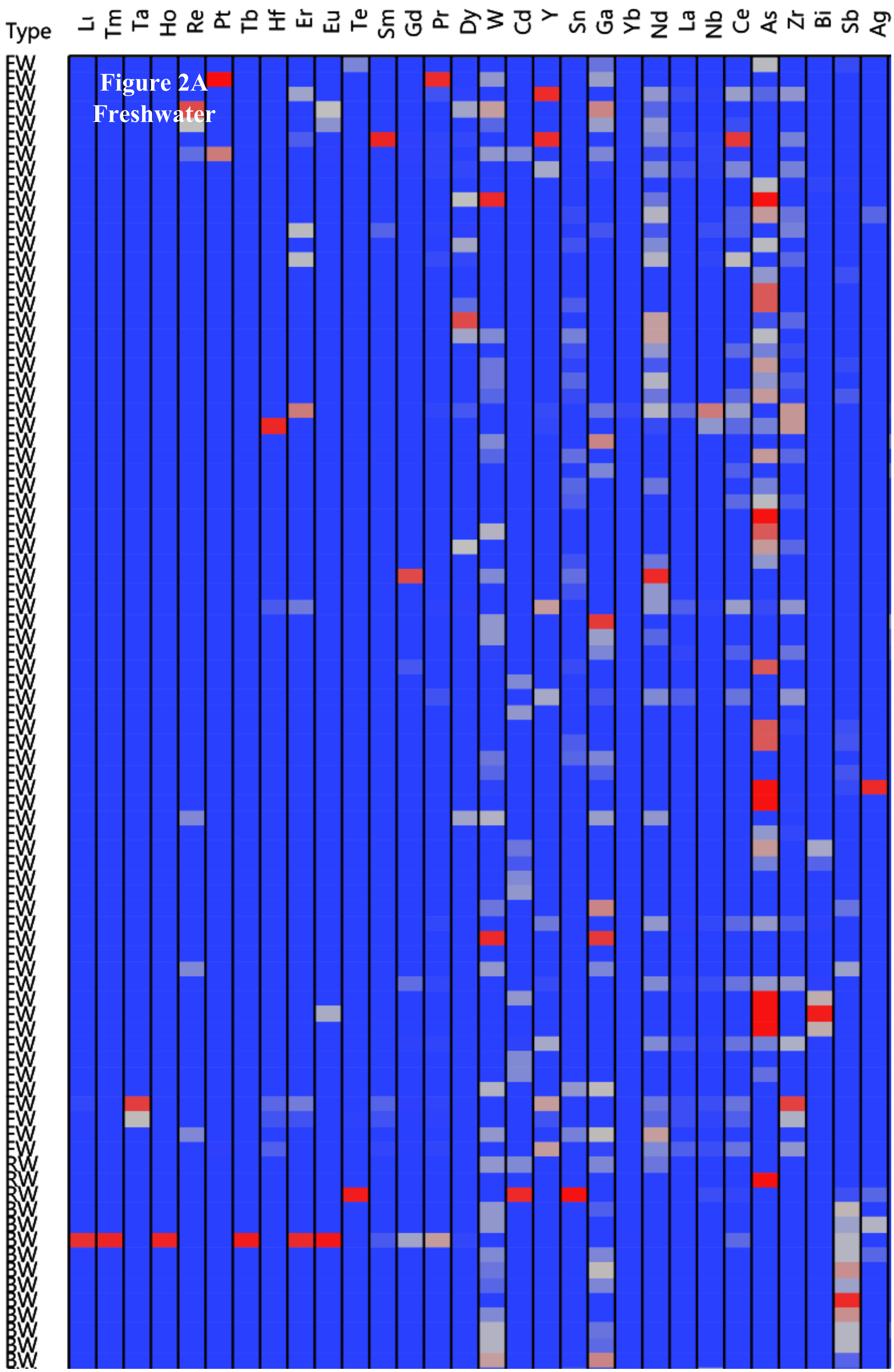


Figure 2A
Freshwater
Type
Lu
Tm
Ta
Ho
Re
Pt
Tb
Hf
Er
Eu
Te
Sm
Gd
Pr
Dy
W
Cd
Y
Sn
Ga
Yb
Nd
La
Nb
Ce
As
Zr
Bi
Sb
Ag
FW
RW
BW

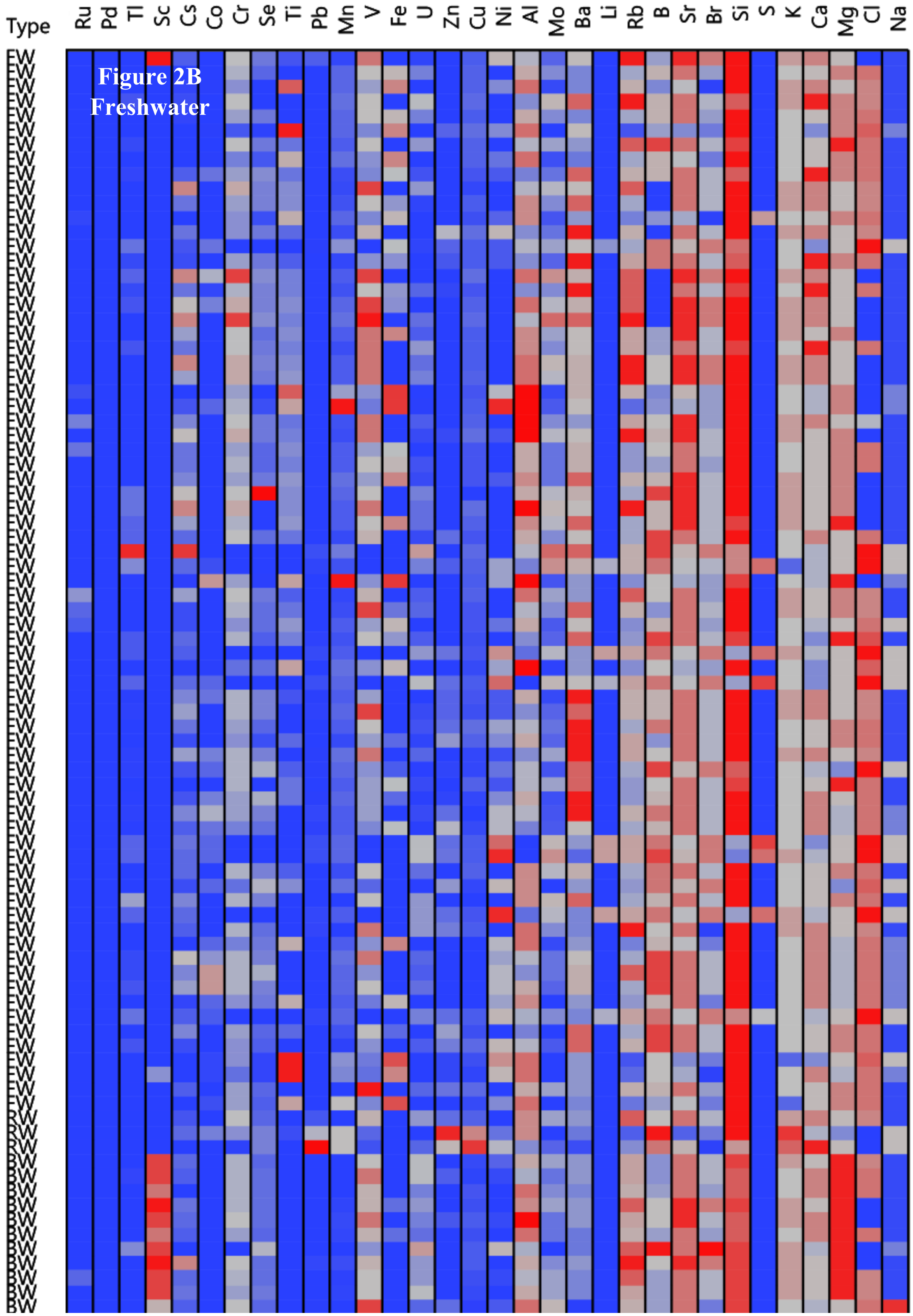


Figure 2B
Freshwater
Type
Ru Pd Tl Sc Cs Co Cr Se Ti Pb Mn V Fe U Zn Cu Ni Al Mo Ba Li Rb B Sr Br Si S K Ca Mg Cl Na
FW
BW

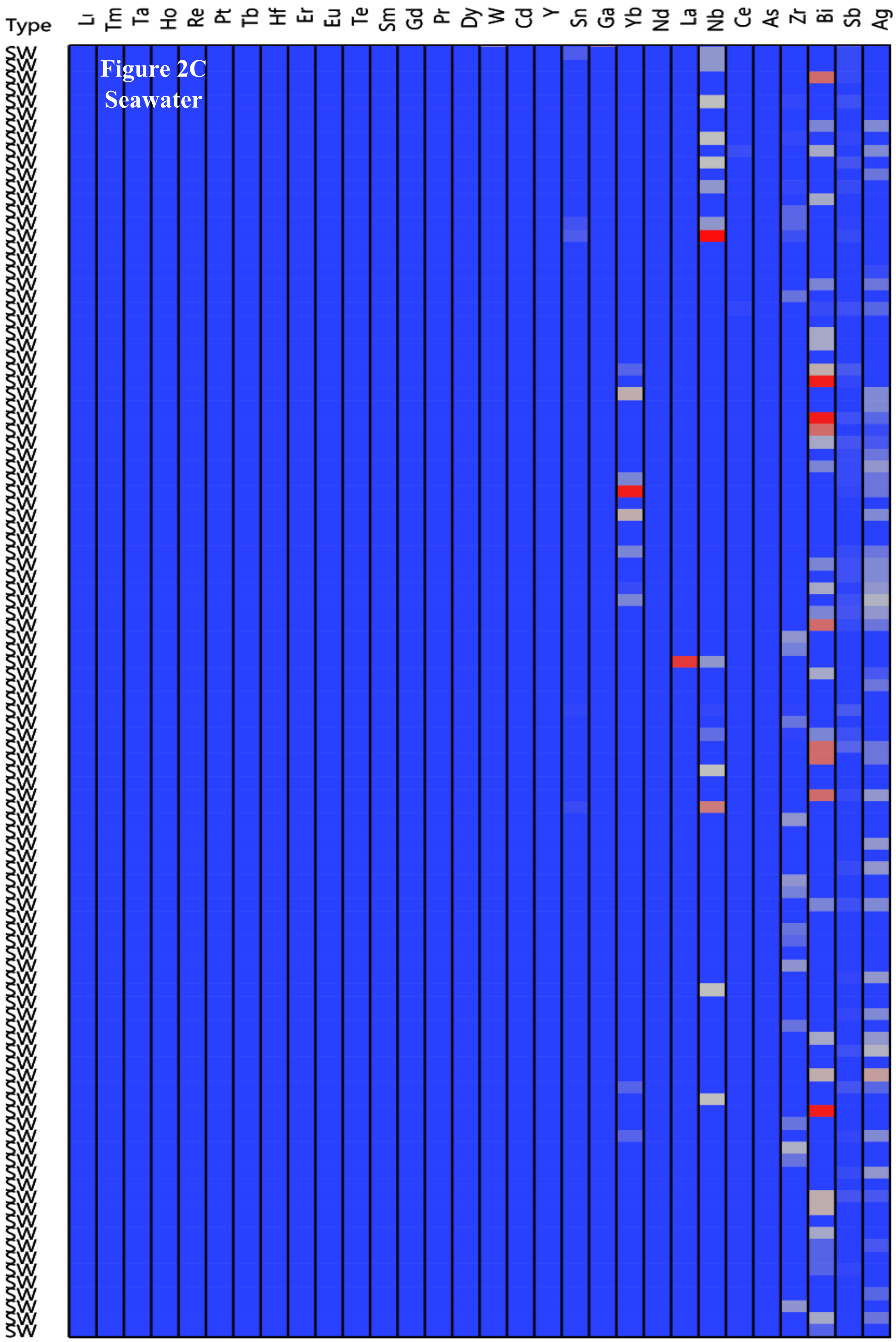


Figure 2C
Seawater
Type
Li
Tm
Ta
Ho
Re
Pt
Tb
Hf
Er
Eu
Te
Sm
Gd
Pr
Dy
W
Cd
Y
Sn
Ga
Yb
Nd
La
Nb
Ce
As
Zr
Bi
Sb
Ag
SW

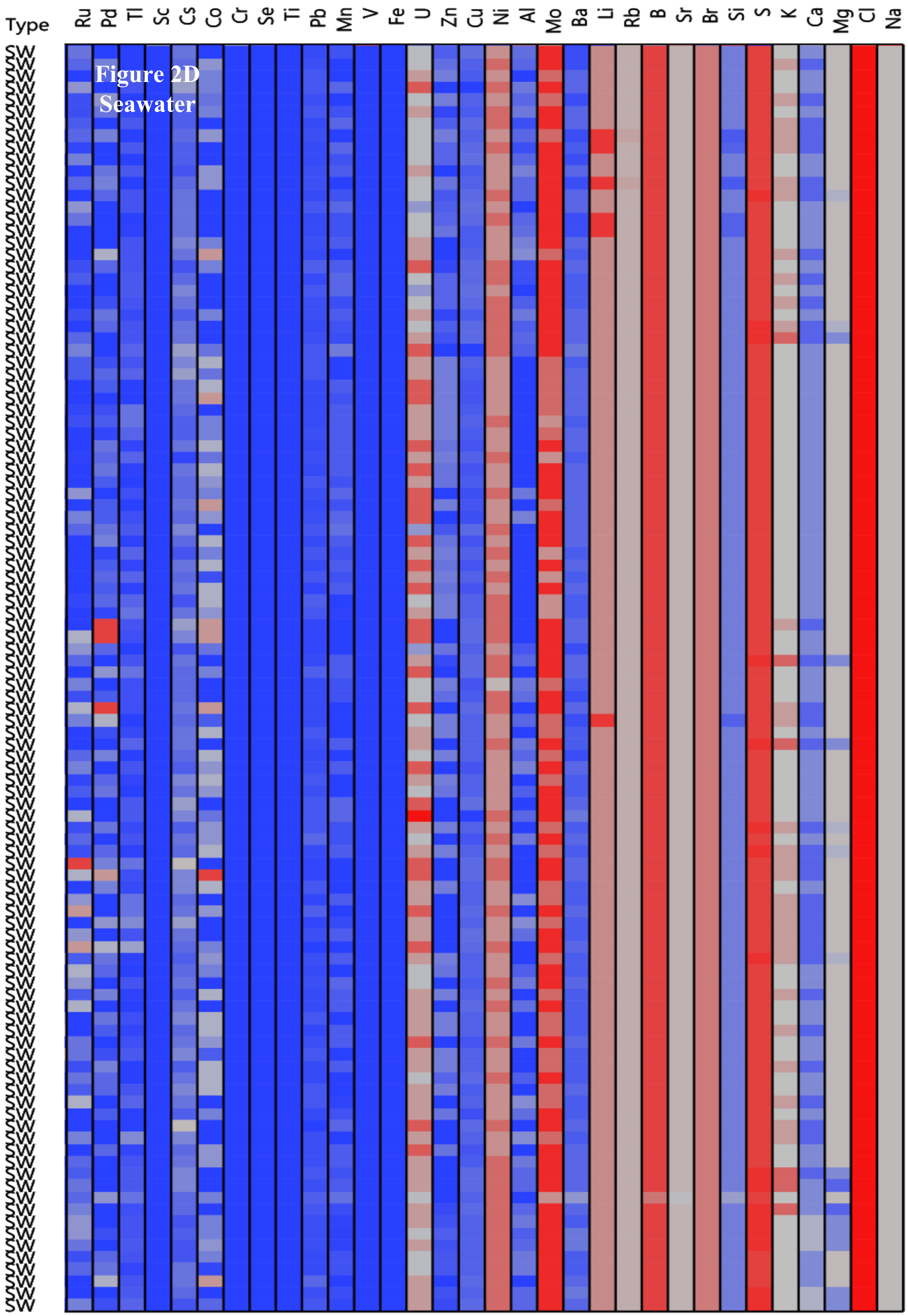


Figure 2D
Seawater
Type
Ru Pd Tl Sc Cs Co Cr Se Ti Pb Mn V Fe U Zn Cu Ni Al Mo Ba Li Rb B Sr Br Si S K Ca Mg Cl Na
SW

Another statistical approach called principal components analysis (PCA) was employed in Figure 3. This method, like its name implies, extracts the largest explainable variation in element concentrations into the first "component" (component 1) on the X axis, and then the next most amount of explainable variation in component 2 on the Y axis. This two-dimensional graphical method helps to visualize primary phenomena residing in the data that we want to understand. The top two figures, Figure 3A-B, use the data from Figure 1 (log transformed), and the bottom two figures, Figure 3C-D, use the data from Figure 2 (log transformed, quantile normalized) in order to compare the two statistical techniques used to obtain finer-grained visualizations of the data. Figure 3A reveals a separation on the X axis of the water types, and in Figure 3B, in which all freshwater and seawater samples are combined, that same data expressed in terms of individual elements. Upon quantile normalization of this data that we employed in Figure 2, water types are better separated by both the first and second principal components and we can see and evaluate more structure in element concentrations in Figure 3C. Noticeable are several FW samples that cluster near to the seawater cluster in Figure 3C (arrow). These are mangrove swamp samples that share characteristics of seawater; we did not know a priori beforehand how mangrove swamp water would cluster, so when we subjected the data to statistical analyses we placed them into the FW category. The complementary element plot to Figure 3C is Figure 3D, where we see that most elements clearly noticed by eye that have relatively high concentrations in Figure 2 can be seen to plot between the 3:00 and 5:00 o'clock position. Element clustering links the combined freshwater and seawater water sample types by their presence of potassium, calcium, magnesium, sodium, and chlorine between the 3:00 and 5:00 o'clock, together with a connected cluster containing rubidium, boron, strontium, bromine, and sulphur between the 8:00 and 10:00 o'clock positions. Elements clustering at the 1:00 position are in low concentration.

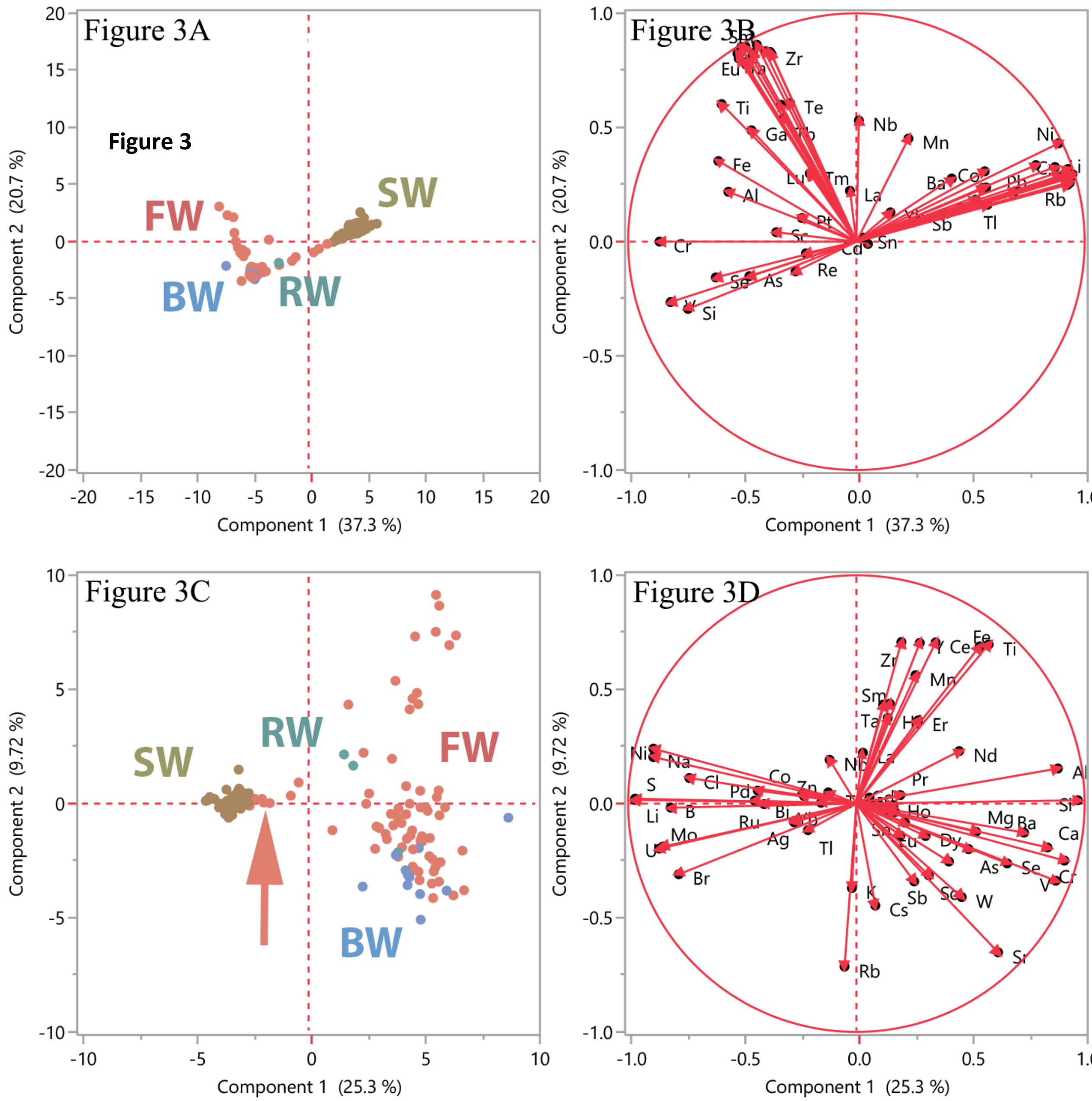


Figure 3A
Figure 3
FW
SW
BW
RW
Component 1 (37.3 %)
Component 2 (20.7 %)
Figure 3B
Zr
Eu
Ti
Te
Nb
Mn
Ni
Fe
Al
Ba
Co
Rb
Tm
La
Sb
Tl
Cr
Sn
Cd
Re
As
Si
Figure 3C
Component 1 (25.3 %)
Component 2 (9.72 %)
Figure 3D
Ce
Fe
Ti
Zr
Mn
Sm
Ta
Er
Nd
Na
Al
S
Cl
Pr
Pd
Co
Li
B
Ho
Mg
Ba
Ca
Mo
Ru
Ag
Tl
U
Br
Dy
As
Se
Cr
V
K
Sb
Cs
W
Rb
Sr

II.5.1.1. Elements in the environment. The statistical analyses informed and helped to target the element analyses below. The narrative observes the comparison between normal freshwater and seawater values for selected elements (Table 1) and from the mangrove swamps (Table 2) of the Upolu Island study sample

Table 4 - Element concentrations compared between normal and Upolu Island water sources

Atomic Sym.	FW Normal	Median Conc.	Range	SW Normal	Mean Conc.	Range	% Above (+) or Below (-) Normal
As		0.11	0.001-0.46	1.73 [2]	ND	ND	
Ba		3.46	1.1-22.2	13.7 [2]	5.24	2.65-6.68	-100%
Br		10.45.08	5.17-853	67116 [2]	65600	25160-74080	36%
Ca		6732	915-24663	412824 [2]	372560	168880-532520	43%
Cd	0.2-2.0 [3]	0.01	0.008-0.019	2 [3]	ND	ND	
Cu	2.-5 [3]	0.18	0.013-1.58	5 [3]	6.16	1.2-12.44	64%
Hg	0.1 [3]	ND	ND	0.1 [3]	ND	ND	
Mg		6082	893.24-19571	1293292 [2]	1091100	359400-2686320	27%
Ni	15–150 [3]	0.17	0.006-5.15	15 [3]	10.83	359400-2686320	16%
Pb	1-5 [3]	0.01	0.006-0.11	5 [3]	0.22	0.019-0.771	
Si		10001	1564-15887.3	2809 [2]	868	44-2060	-100%
Sr		55.35	7.68-252	7885.8 [2]	4920	1886-7120	-100%
Zn	5–50 [3]	0.74	0.024-4.72	50 [3]	17.34	0.6-45.48	-100%

All values in µg/l (equivalent to parts per billion). Freshwater (FW), Seawater (SW), no data (ND). Element codes given in the text.

Table 5- Element concentrations of Upolu Island Mangrove Swamps.

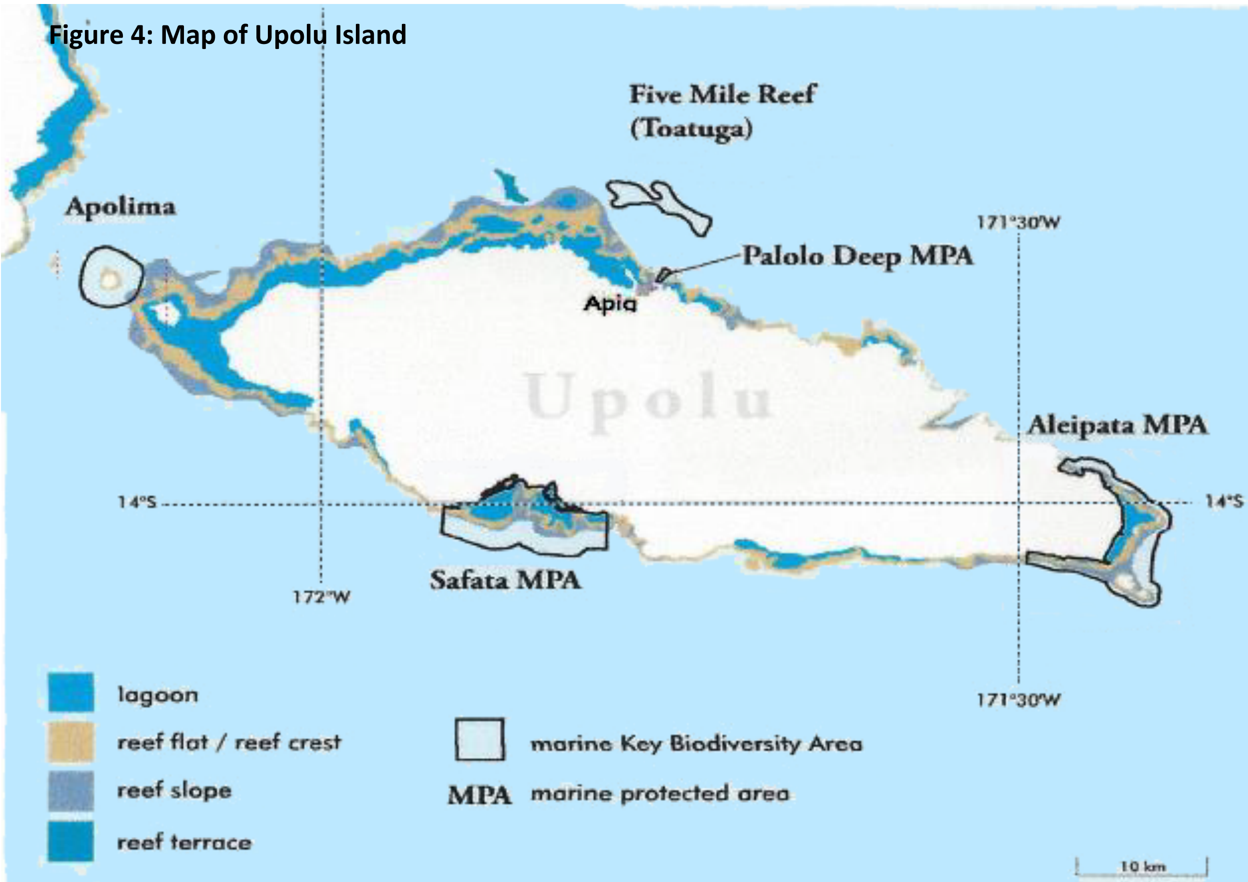
Element	Normal	Median	Range	% of Samples Above (+) or Below (-) Normal
Ba	13.7	10.4	4-22.5	-71%
Br	67116	27493	2413-112023	27%
Ca	412824	87654	17316-339042	-100%
Cu	5	1.49	0.415-5.52	14%
Mg	1293292	386522	61746-1541911	14%
Ni	15	4.45	0.129-19	14%
Si	2809	10099.3	1522-14816	100
Sr	7885.8	1619	225-6736	-100%
Zn	50	4.53	0.383-14.5	-100%

All values in µg/l (equivalent to parts per billion). Element codes given in the text. Literature values for normal element concentrations may be found in Table 1.

Coral reefs are distributed around Upolu Island, to which a small proportion were assigned to Key Biodiversity Areas and Marine Protected areas (MPA) in a recent hydrographic risk assessment (Fig. 4; modified from [4]). We used these areas as geographic references and highlighted them in yellow in the Geographic Information System (GIS) maps that follow (Figures 5-11).

It is widely known that many heavy metals have no biological function and are harmful to life in even relatively moderate concentrations. Arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are particularly egregious to life, and their distributions and concentrations are typically a result of industrial and human activity. However, these elements are in low concentrations on Upolu Island (Table 1) and are unlikely the cause of harm to the Samoan population or the environment. This is good news.

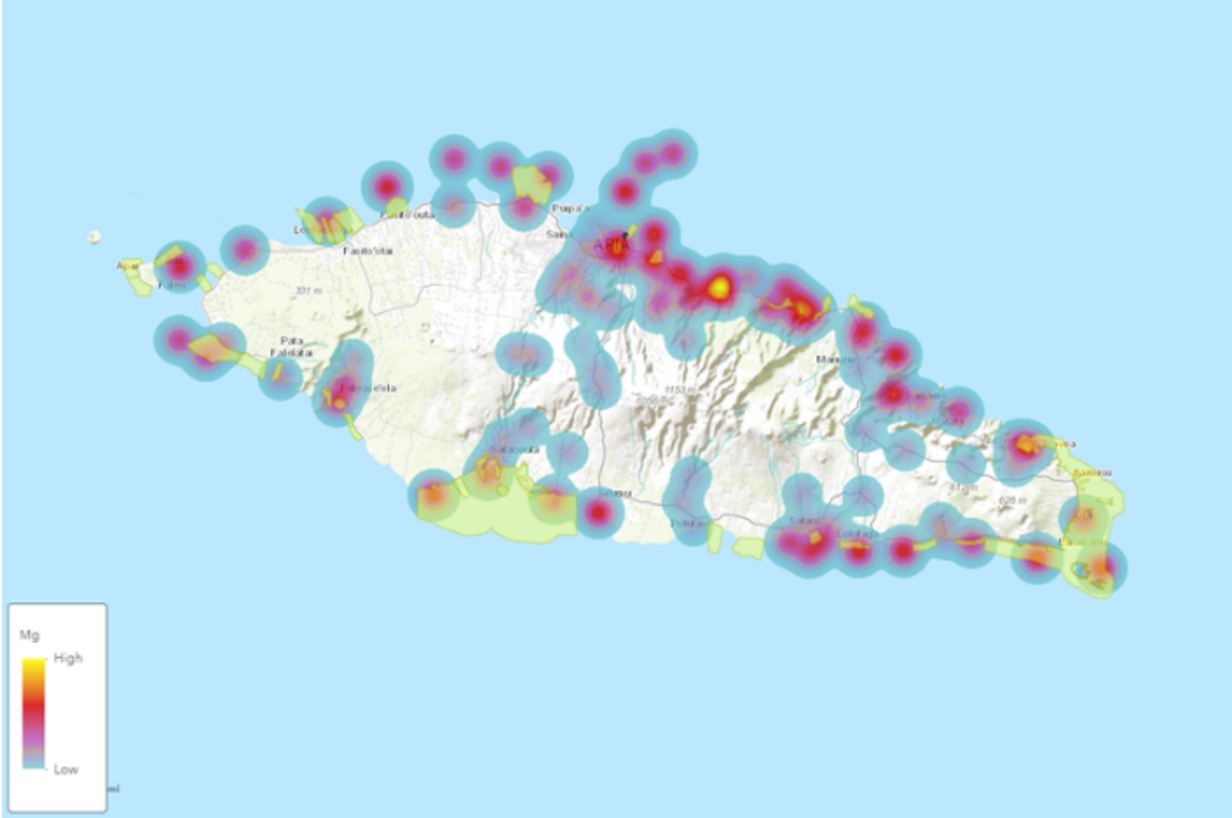
Figure 4: Map of Upolu Island



Several alkaline earth metals are essential to life, such as strontium (Sr) and barium (Ba), yet all of the sample concentrations are below the normal environmental limits (Table 1). Having said this, though they are very low compared to average SW values, they are needed at extremely low amounts for organisms and are unlikely to be limiting.

The median value for another alkaline earth metal, magnesium (Mg; Fig. 5), is near the seawater normal, but 27% of seawater samples are elevated above normal, some concentrations being double the level of that which is typical in seawater (Table 1). These high values (yellow intensities inside red circular spots) are unevenly distributed around the island, which indicates local toxicity due to industrial and other human activities.

Figure 5: Magnesium



Copper (Cu; Fig.6) distributed around the coastline in moderate-to-high concentrations, 67% of the sample values increased above normal, with some concentrations double that which is typical in seawater (yellow intensities inside red circular spots) (Table 1). The median value of zinc is well below normal seawater concentration, all of the samples below typical concentration values in seawater (Table 1). In the marine environment the major anthropogenic sources of copper, combined with transient decreases in zinc, are antifouling paints used to coat ship hulls, buoys, other underwater surfaces, and from decking, pilings and marine structures in which treated timbers are present [5, 6].

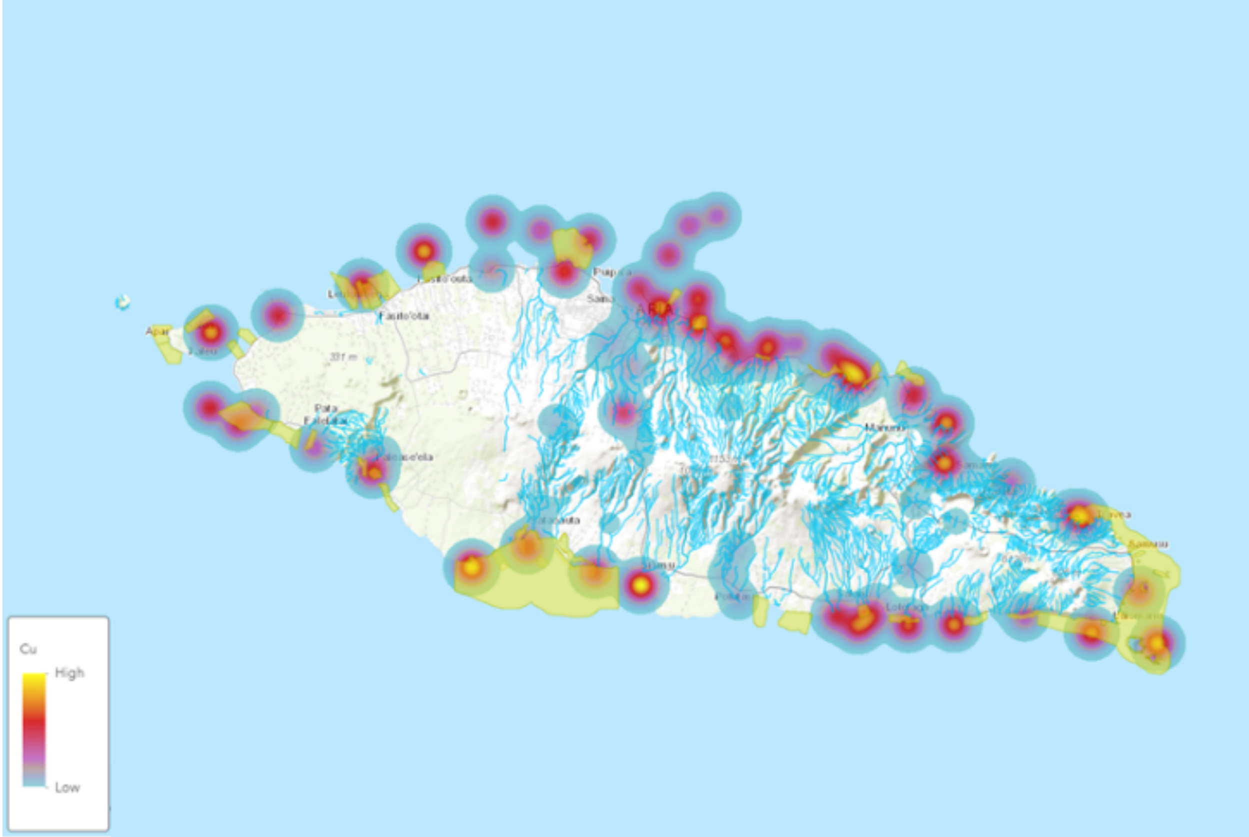


Figure 6: Copper

Siliceous igneous rocks are the primary source of silicon on Upolu. All seawater concentrations of silicon (Si; Fig. 7) are below normal concentrations values in seawater (Table 1). However, the Si concentrations in mangrove swamps is very high (Table 2), thus this dynamic needs further investigation.

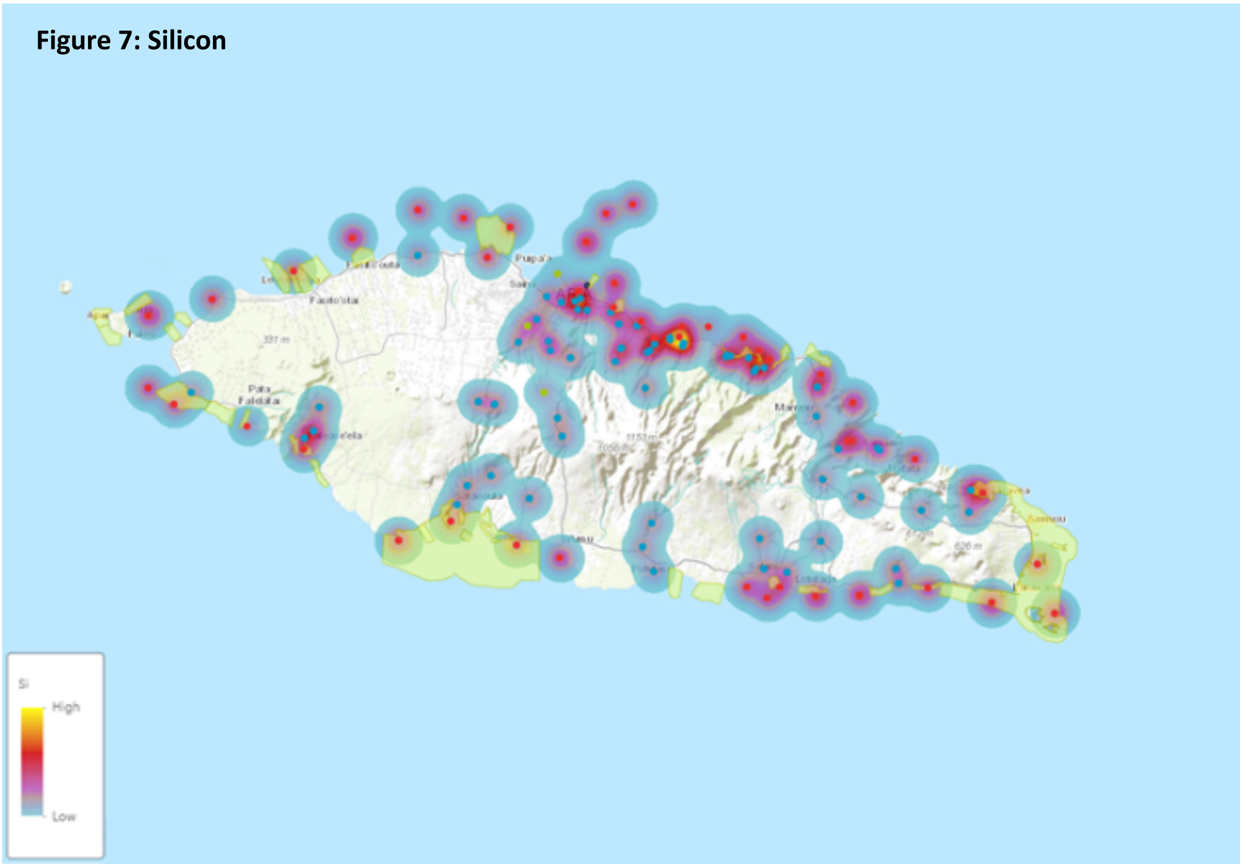
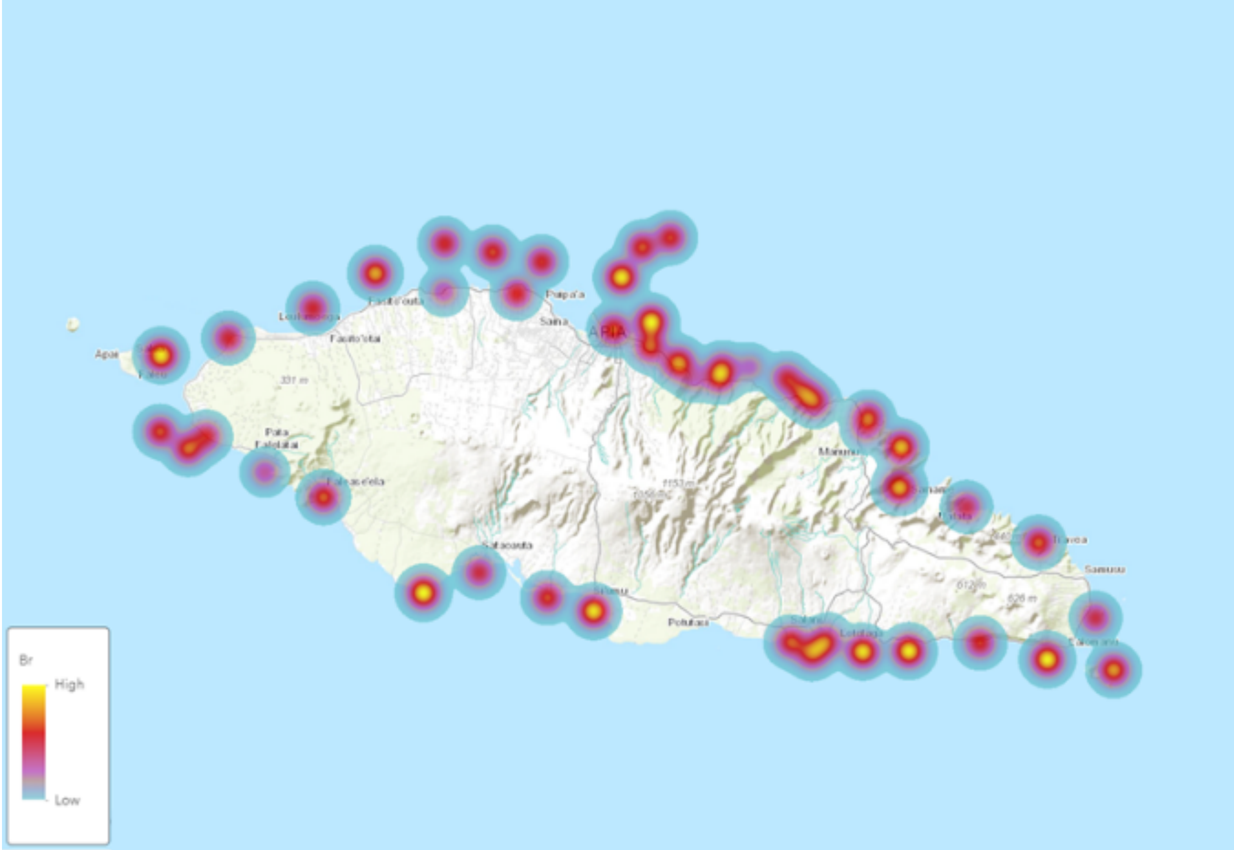


Figure 7: Silicon

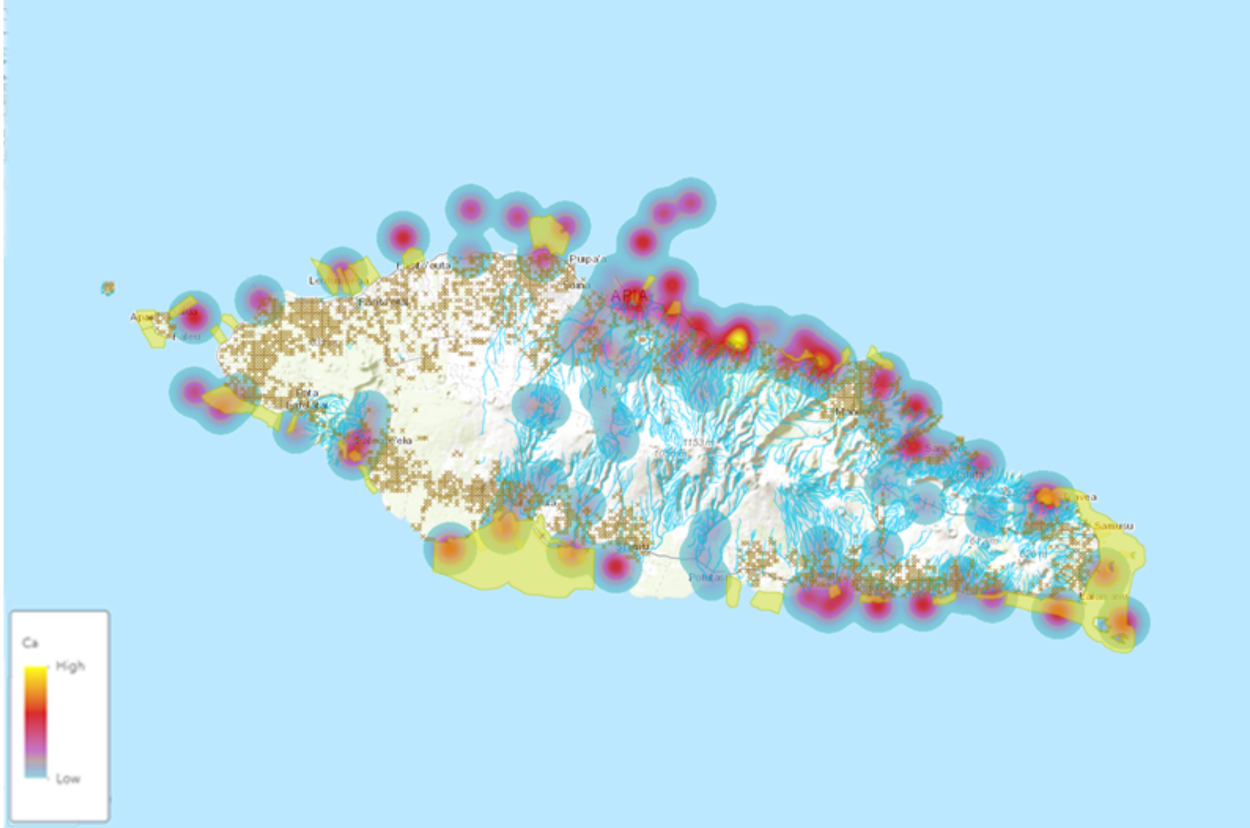
The median bromine (Br; Fig. 8) concentration is a little below normal for seawater (yellow intensities inside red circular spots), but 36% of the samples values are above normal, reaching concentration values as much as ca. 12% above typical in seawater (Table 1).

Figure 8: Bromine



The median value of calcium (Ca; Fig. 9) is a little below normal to those of seawater, but 43% of the sample values are above normal (yellow intensities inside red circular spots), reaching concentration values more than 20% above typical concentration values in seawater (Table 1). Crop farming is a potential anthropogenic source, because calcium is associated with herbicides and pesticides, as is Mg (note the similarity in its concentration map with Mg of Fig. 5).

Figure 9: Calcium



Finally, the median concentration of nickel (Ni; Fig. 10) is below normal to those of seawater, but 16% of the samples are above normal, reaching concentration values of more than 20% above typical concentration values in seawater (Table 1).

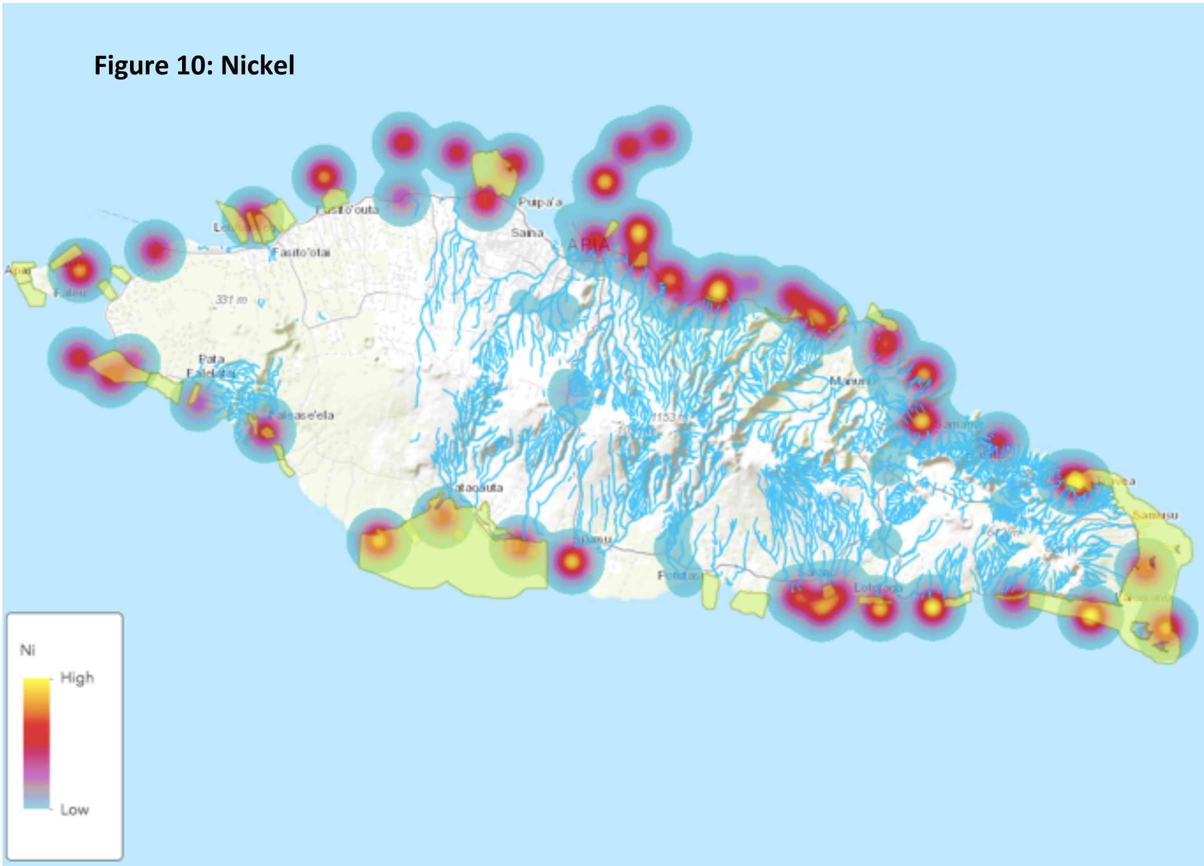


Figure 10: Nickel

II.5.1.2. Salinity. Salinity in ocean water derives mainly from sodium chloride and is measured in parts per thousand. Salinity of South Pacific water is in equilibrium at around 35-36 parts per thousand [8]. During a recent survey of salinity around American Samoa at an average depth of 11.45 m, the average salinity was 34.63 with a range of only 1.39 [9]. Around Upolu Island, ca. 30% of all seawater samples are well above this, in the range of 52-54 parts per thousand (Fig. 11).

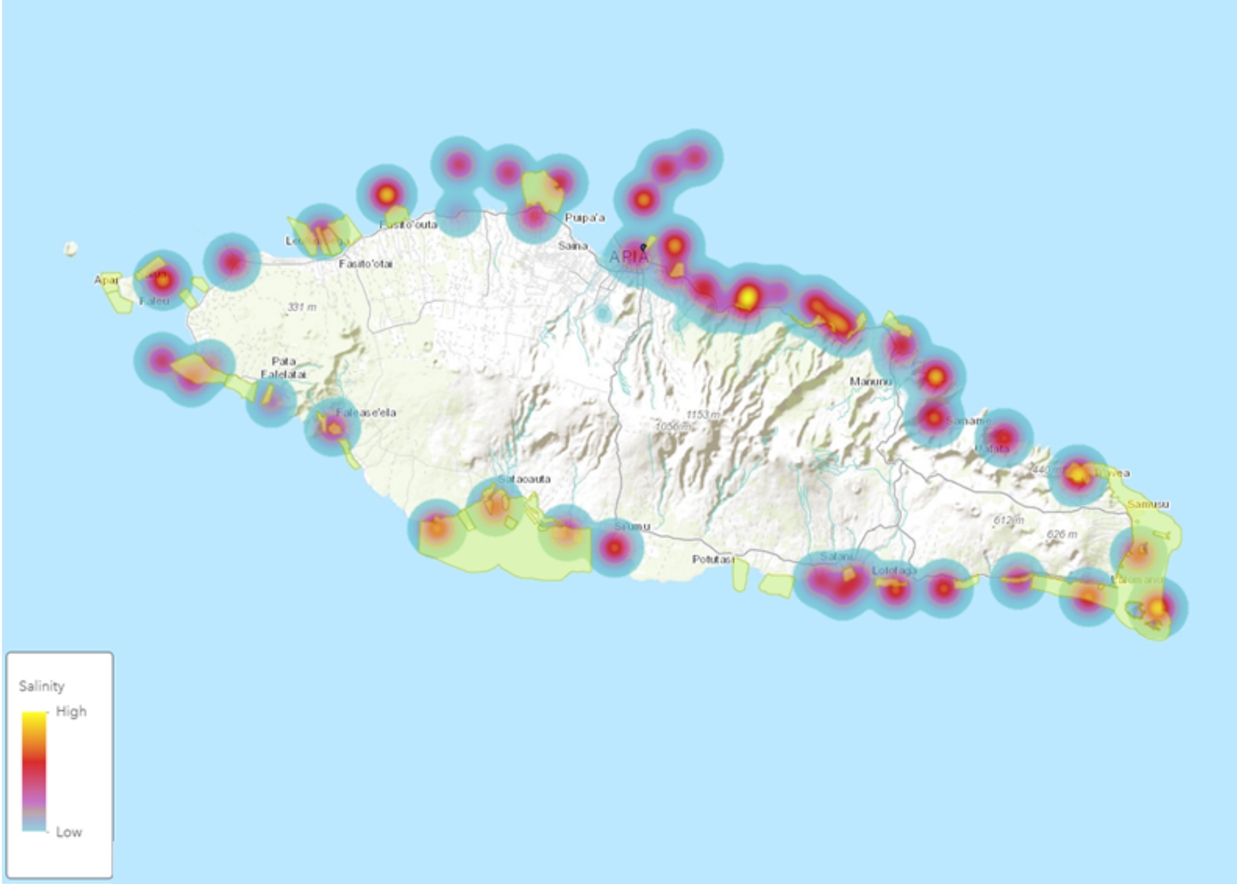


Figure 11: Salinity

II.5.2. Analyses of Water Quality

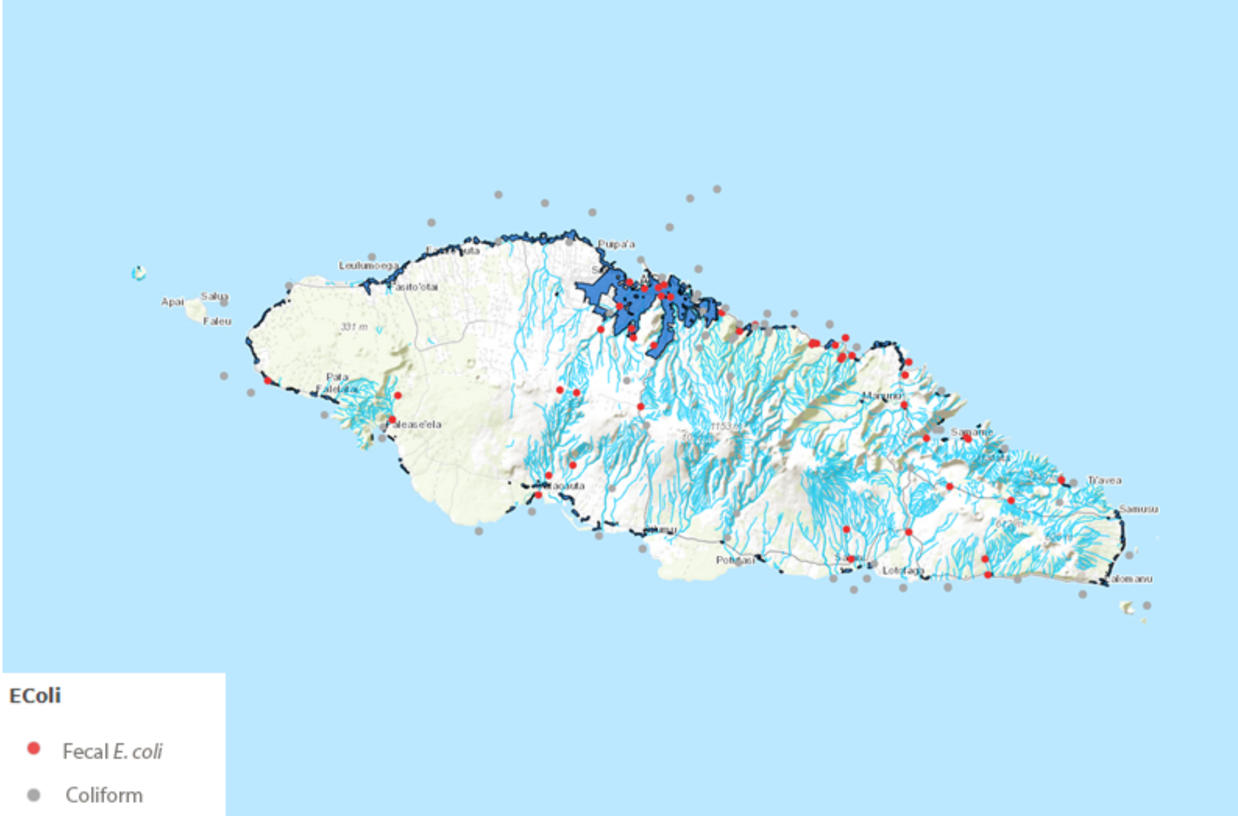
II.5.2.1. Water's capacity to maintain balance with organics- Some water quality assessments are within normal limits. The oxidation-reduction potential (ORP) on which dissolved oxygen levels depend are good, typically ranging between 100-150 mV [7]. This, together with low nitrate levels indicates that Upolu is not causing eutrophication of the surrounding sea (i.e., excessive nutrients that drive the growth of phytoplankton and other algae and reduction of dissolved oxygen), which would otherwise pose a threat to aquatic life.

II.5.2.2. Bacterial contamination -*Escherichia coli* (*E. coli*) and other pathogenic bacteria can persist in aquatic environments [10] and often lead to development of gastrointestinal diseases in humans if water is ingested or fish and shellfish are consumed. All freshwater samples contained coliforms and pathogenic fecal-derived *E. coli*, which are main culprits of disease (Fig. 12). The Colilert-18 kit used to test for coliform cannot distinguish between pathogenic vs non-pathogenic coliforms and thus this data should be viewed with caution. The findings on *E. coli*, on the other hand, suggest consistent contaminations that may cause health problems if enough *E. coli* cells are ingested by a human. Each seawater sample tested also tested positive for either coliform or *E. coli*, which are often a result of water run-off, wastewater discharge or due to near-shore human activities.

Population concentrations appear as dark blue shading in the vicinity of Apia and around the periphery of the island and the pattern suggests where there are concentrations of people there is pathogenic *E. coli* contamination (red dots in Fig. 12).

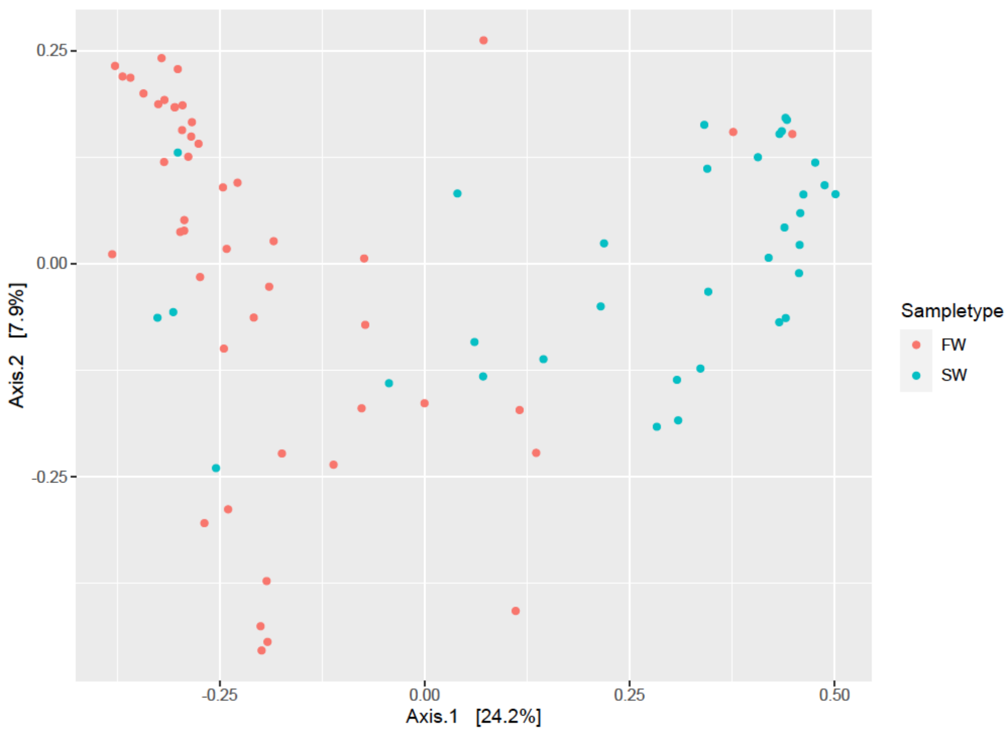
II.5.2.3. Pathogenic Bacterial Diversity and Abundance. To examine the similarities between all samples collected for DNA sequencing, we performed an analysis similar to the principal component analysis (PCA) shown in Fig. 3 and called Bray-Curtis dissimilarity analysis. This analysis can be seen in Figure 13. Each point represents a sample (FW or SW) and the distances between points represent how similar or dissimilar these samples are. Generally, the bacterial composition of freshwater (FW) cluster together but away from seawater bacteria (SW) with the

Figure 12: ***E. coli***



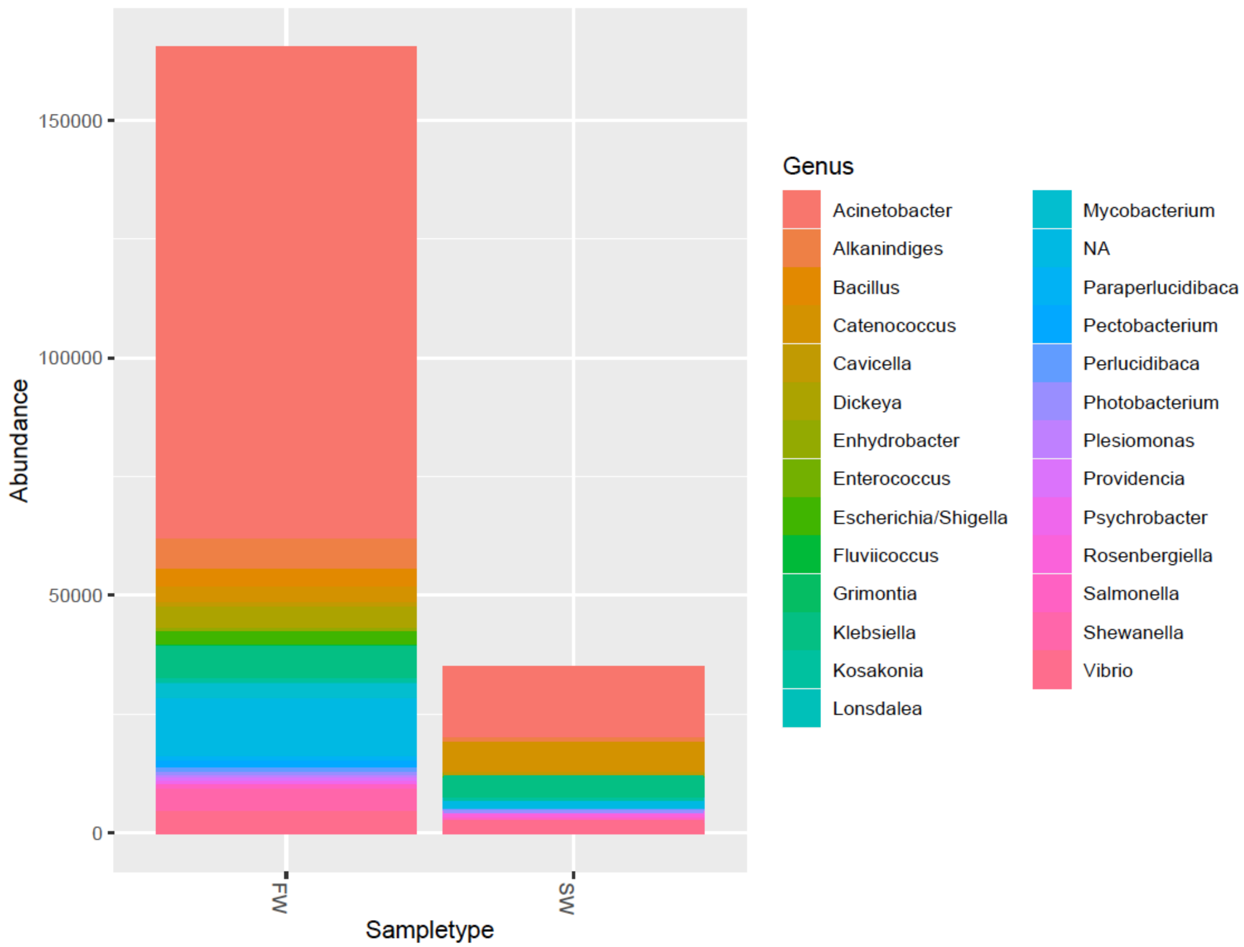
exception of a few samples that cross the divide between fresh and seawater. Because one of the major selection factors for bacterial adaptation is salinity, seawater bacteria always tend to be starkly different from freshwater bacteria, and this is evident from Figure 13. The few samples that appear to cross the divide between both environments are brackish water, typically seawater that receives large freshwater input from shores or freshwater that is brackish.

Figure 13: Bacterial Diversity



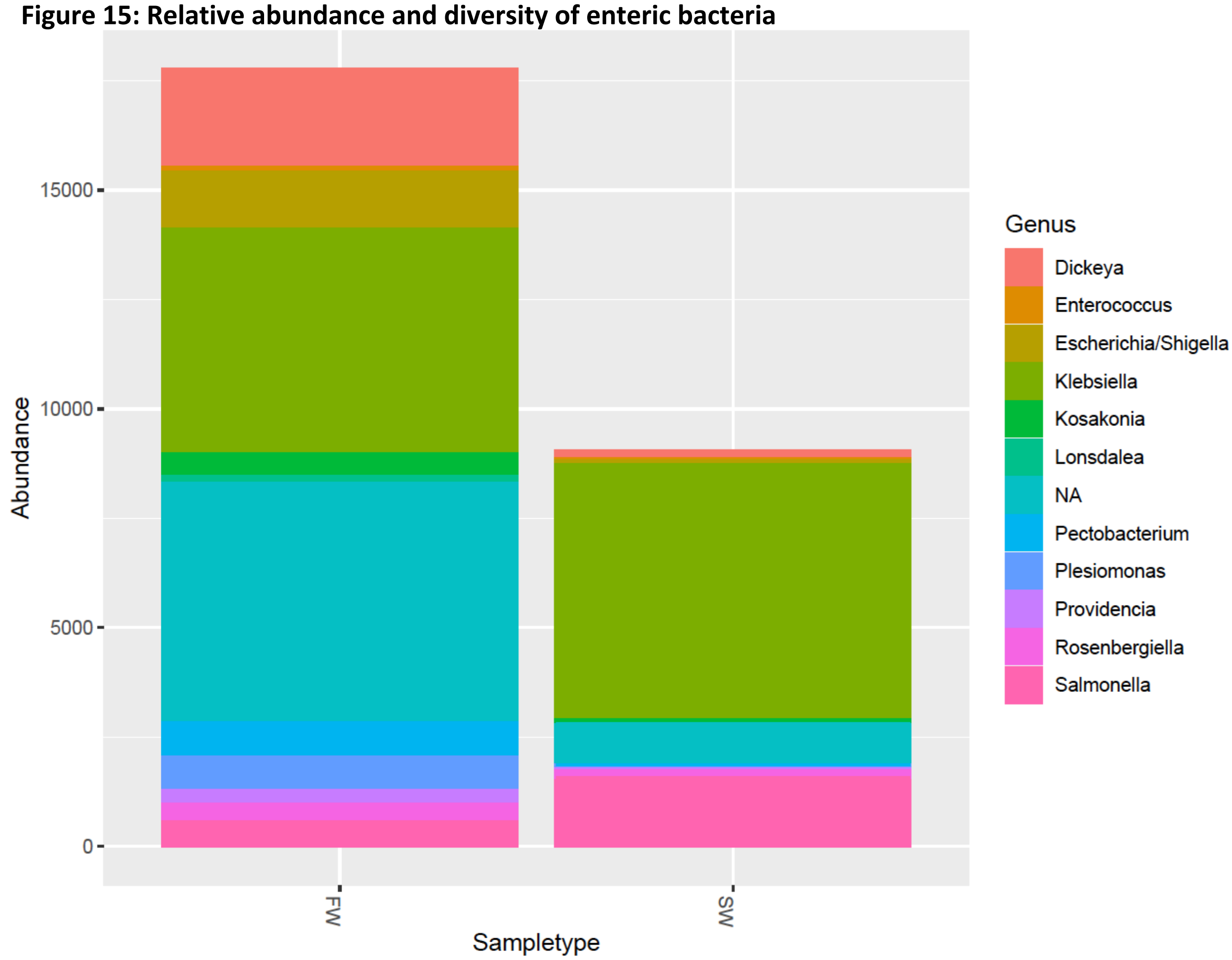
More importantly, analysis of the bacterial taxa in all water samples shows a significantly higher abundance of bacteria in freshwater (FW) relative to seawater (SW) (Fig. 14), an observation that is consistent with the fact that freshwater tends to have a higher input of organics from soil that fuel bacterial growth. The different colors represent different bacterial groups (genus, pl. genera). One clear pattern in both groups of samples is that enteric bacteria that are typically the cause of disease when present in aquatic environments are present in both groups of samples. For example, *Escherichia* where *E. coli* belongs is present in both samples and this is consistent with analysis done on-site using the Colilert kit (Fig. 12). Other groups of potential pathogenic bacteria are also evident, such as *Bacillus*, *Mycobacterium*, *Enterococcus*, *Salmonella* and *Vibrio* although some of these groups typically are dominated by non-pathogens and may not be a health concern.

Figure 14: Bacterial taxonomy and relative abundance



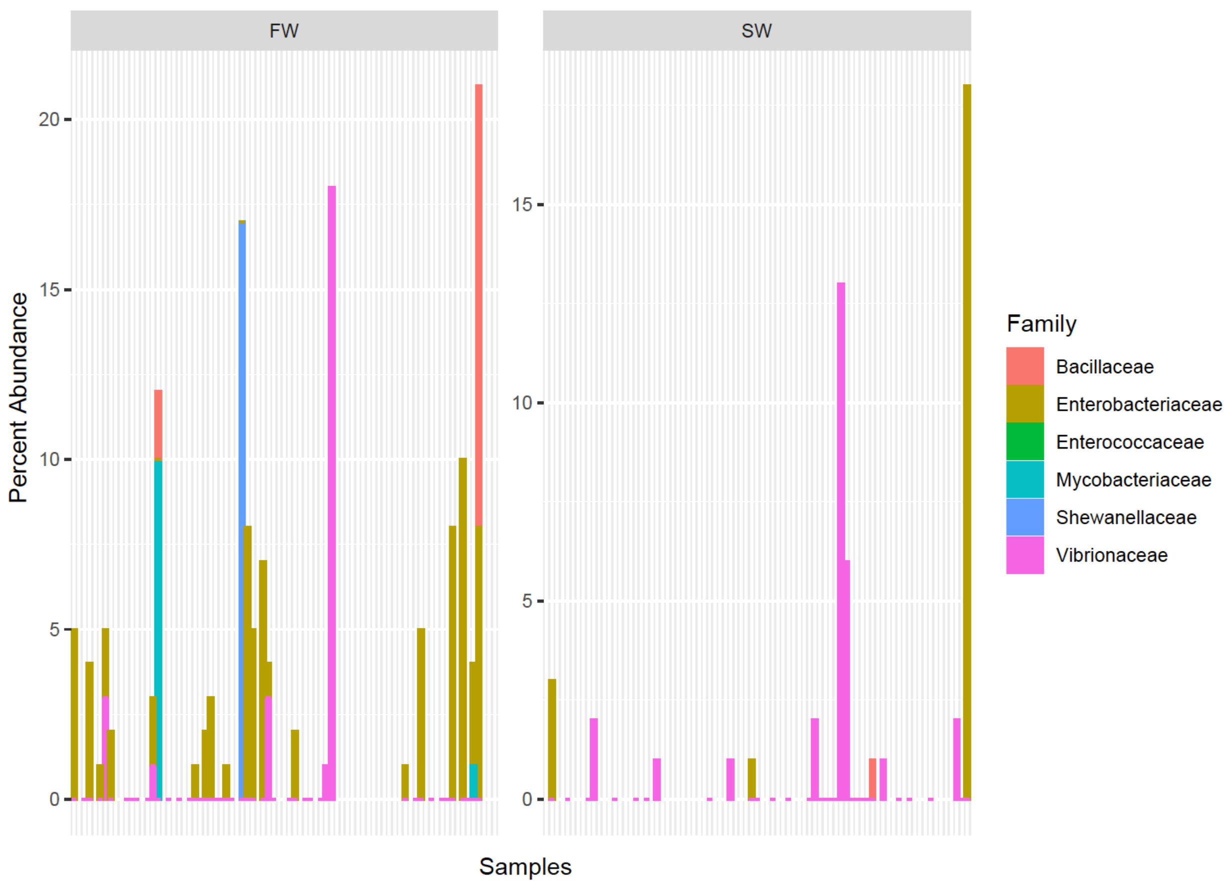
To get a closer look at pathogens in collected samples, we limited further analysis to the enteric bacteria that are typically associated with gastrointestinal diseases found in our samples (Fig. 15). The most abundant genus of enteric bacteria in both fresh and seawater was *Klebsiella*. Some members of this genus are pathogenic towards humans and/or mammals and can cause diseases such as pneumonia and urinary tract infections. While the current data cannot tell us whether these *Klebsiella* are pathogenic or not, their relative high abundance in samples suggest some may be. Of particular importance is the *Escherichia* genus, where *E. coli* belongs. The abundance of *Escherichia* was highest in freshwater samples, consistent with their ability to survive in terrestrial environments more so than marine. This finding is also consistent with Colilert *E. coli* detection performed on-site in Upolu. Another important group of bacteria that tends to harbor pathogens is *Salmonella*. This group cannot not be detected by the Colilert kit and its detection here highlights the power of DNA sequencing.

Figure 15: Relative abundance and diversity of enteric bacteria



To examine the distribution of these enteric bacteria across samples and how abundant they are, we plotted the number of sequences belonging to enteric bacteria recovered from each sample in Figure 16. While some sites lacked or had negligible number of sequences belonging to enteric bacteria, mostly seawater samples, some sites were significantly enriched in enteric bacteria. These sites are likely to be influenced by near-shore human activities and may be a good target for the Samoan government to conduct regular monitoring of pathogens. Several freshwater samples contained enteric bacterial sequences that surpassed 10% of total reads recovered, with Enterobacteriaceae (where *E. coli* belongs) being the most consistently distributed across samples. Improved sanitation practices may help curb these trends.

Figure 16: Abundance of enteric bacteria in individual samples



Below are maps showing the distribution and relative abundance of three of the most important groups of enteric bacteria found on and around Upolu. Figure 17, distribution of *Escherichia/Shigella*; Figure 18, distribution of *Klebsiella*; Figure 19, distribution of *Salmonella.*

Figure 17: Distribution and abundance of *Escherichia/Shigella*

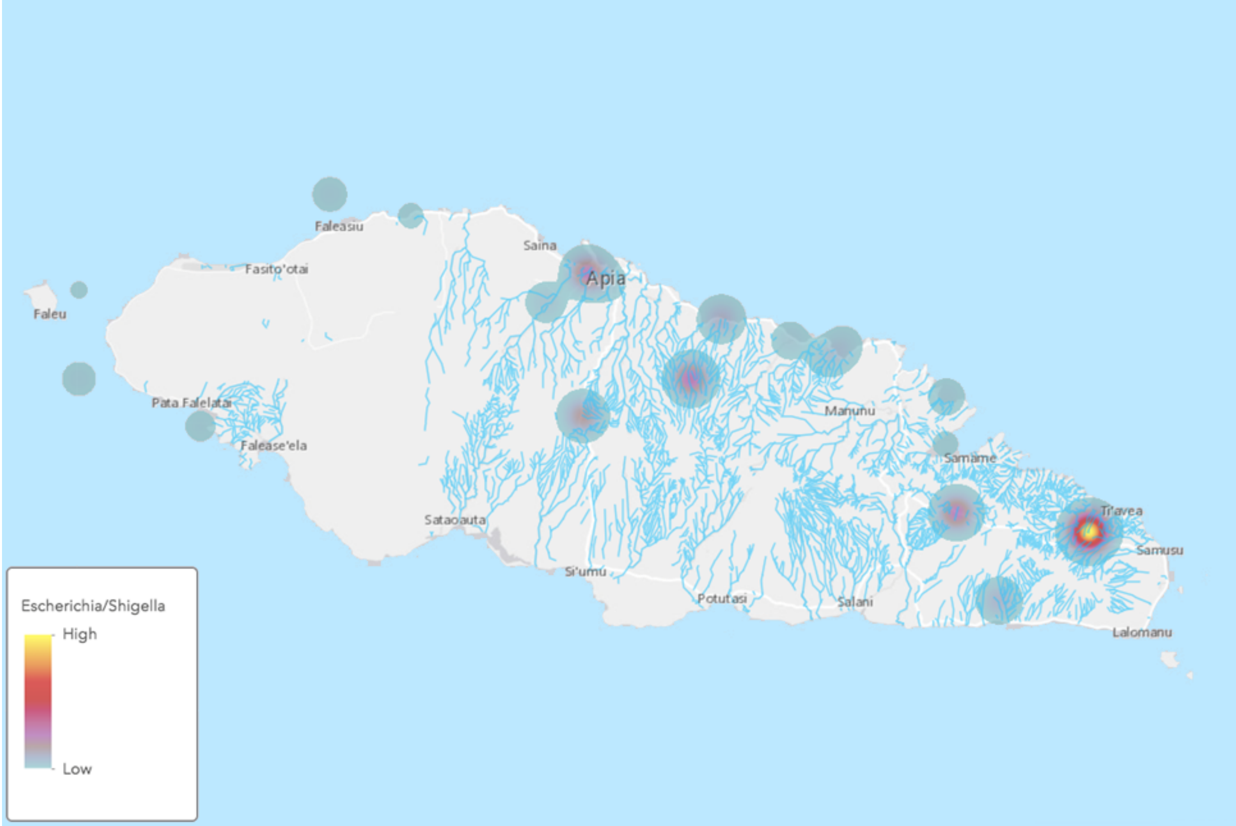


Figure 18: Distribution and abundance of *Klebsiella*

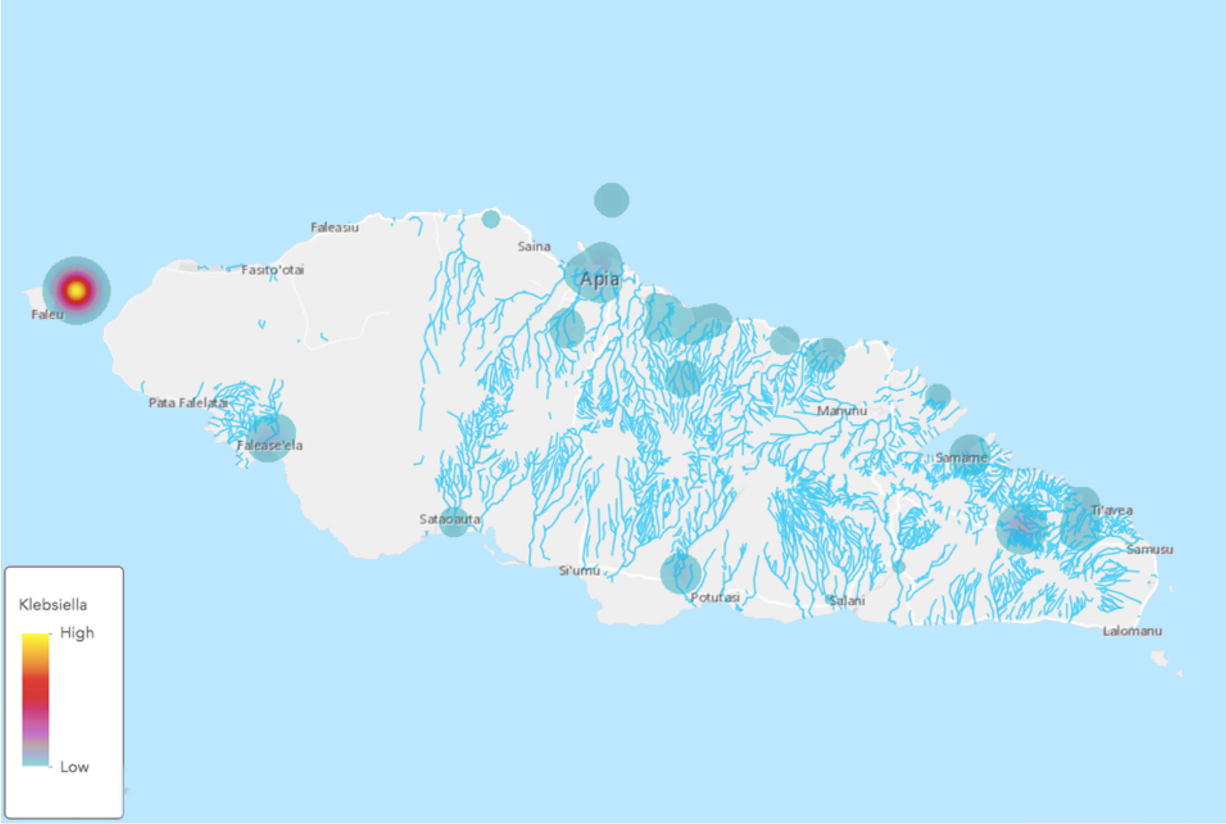


Figure 19: Distribution and abundance of *Salmonella.*

II.5.3. Microplastics

Nearly all freshwater and seawater samples contain microplastics, illustrated here on a major road map of Upolu Island (Fig. 20, Table 3). Distributions of microplastics follow population concentrations, with highest concentrations in the northern settled coast and in the vicinity of Saleilua town in the south of Upolu Island.

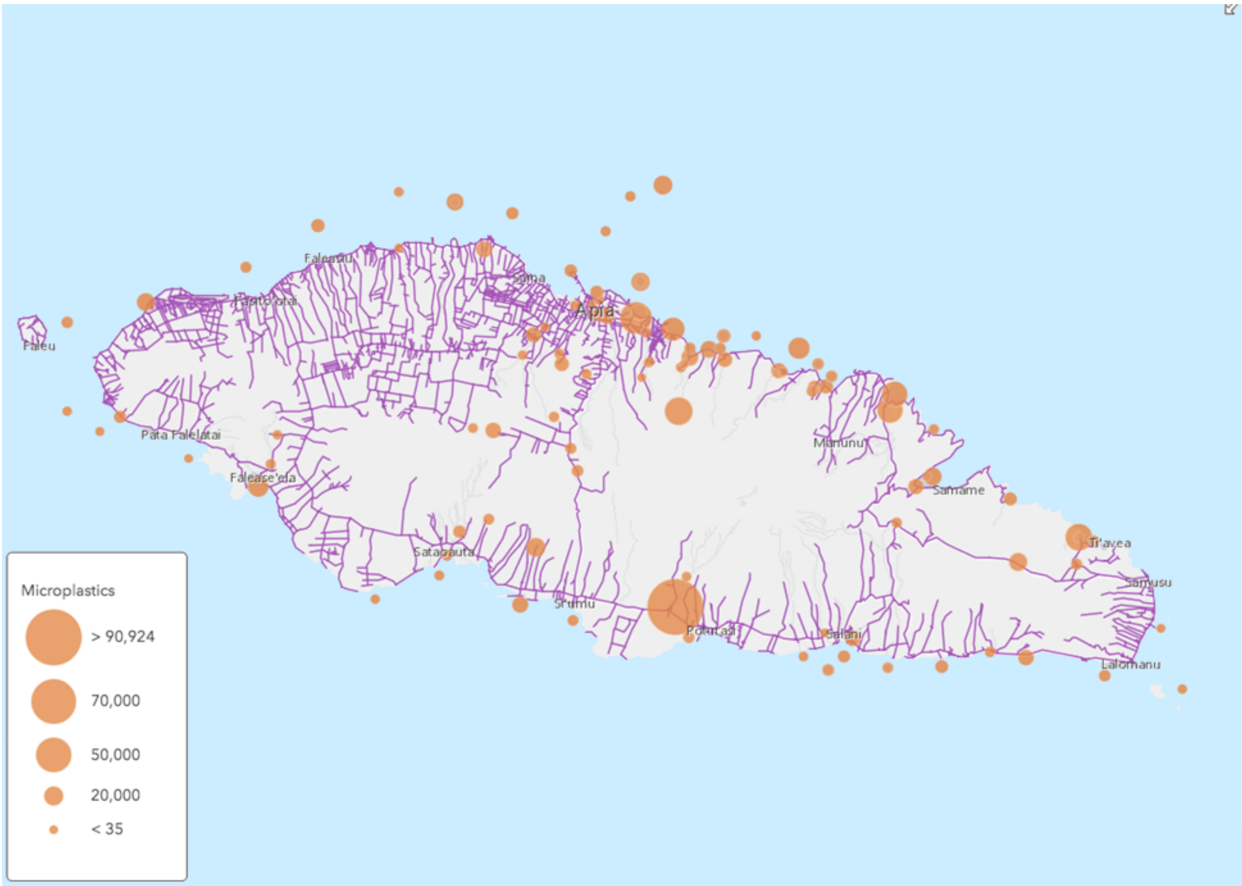


Figure 20: Microplastics

Table 6 -Microplastic particle density in Freshwater, Seawater and Mangrove Swamps

Water Type	Median	Minimum	Maximum
FW	3579	250	90925
SW	3023	35	32709
MS	7015	588	44130

All values in μm^2 (square micrometers). Freshwater (FW), Seawater (SW), Mangrove Swamp (MS).

III. FINDINGS IN RELATION TO HUMAN HEALTH AND THE ENVIRONMENT

A 1994 survey of South Western Pacific waters cited overfishing, pollution, eutrophication, and erosion leading to sedimentation as a major threat to lagoons and coral reefs [11]. A more recent report on the status of the coral reefs around Upolu Island speculate that a series of tropical cyclones and the 2009 tsunami have exacerbated these conditions leading to substantial coral reef decline [12]. Missing from these studies is a model system for identifying the fine-grained detail laying at the foundation of threats to the coral reefs, which, in an integrated ecosystem should be visible in other areas of the system such as may be expressed in human health problems.

The incidence of obesity in the island state of Samoa is among the highest in the world [13], which includes all of the well-recognized concomitant complications of the condition. Locally high levels of magnesium (Fig. 5), bromine (Fig. 8), and calcium (Fig. 9) are connected to widespread herbicide and pesticide chemical use on Upolu Island, which have endocrine effects that may be contributing to endemic obesity [14]. For instance, the nonuniformity of Bromine suggests that it may be supplemented by water and waste treatment, as a fungicide, and as an agricultural pesticide against snails [15]. The primary means of exposure to herbicides and pesticides is by dermal exposure [16], and airborne incidences and exposure while swimming and bathing in contaminated waters is likely responsible.

The herbicides and pesticides, that include magnesium (Fig. 5) and calcium (Fig. 9) are also highly toxic to aquatic life. Thus, there is an intimate connection between the role the environment on both human and aquatic health. This is a major justification for the employ of the model system employed in this study on Upolu Island.

Magnesium (Fig. 5), copper (Fig. 6), calcium (Fig. 9), and nickel (Fig. 10) are ubiquitously or locally high around Upolu Island. These increases in concentrations of accumulated heavy metals - even those essential to life - are associated with coral reef decline [17]. In a further example of the relationship between assessments of the environment and human health, we note that nickel is associated with contact dermatitis in humans [18], which may increase the potential for additional vulnerabilities and exacerbation of insults through the skin. These associations between metal environmental contamination and human and aquatic health require study, analysis, and confirmation on Upolu Island.

Barium and strontium are essential for the growth and construction of stony corals [19, 20], yet the concentrations of these elements are very low around Upolu Island. There is no explanation for how these elements can be sequestered to such levels. Silicon is also essential for growth of unicellular algae (diatoms) that support marine food webs in coastal areas and islands and for the construction of stony corals, however the input from fresh water sources appears to be controlled at mangrove swamps, rendering an insufficiency potentially limiting the growth of coral reefs. The fate of river-born silicon upon entering Upolu Island lagoons, requires further study.

The salinity (Fig. 11) of about one third of all seawater samples is high, and while this has no direct relationship to human health, these levels may be lethal to coral organisms. Values should be checked against historical records to determine if this is a recent phenomenon or whether values have always been high, in which case the corals have adapted.

Pathogenic enteric bacteria, including *E. coli* (Fig. 12), *Salmonella* and *Klebsiella* species (Figs. 15, 16) are widely distributed around Upolu Island in both fresh and seawater likely because of inadequate sanitation. Human infection with these pathogens causes abdominal pains and cramps, diarrhea, pneumonia, urinary tract infections and bloody stools, fatigue, loss of appetite, and vomiting. Pathogenic *E. coli* are also implicated in environmental degradation, known to cause stress and mortality to coral organisms [21, 22].

Microplastics are predominant on Upolu Island (Fig. 13). While there is uncertainty about the consequences of microplastics to human health, microplastics do absorb toxic chemicals, and eating contaminated seafood is a risk requiring further research [23]. Microplastics also affect coral reef health and growth, and have species-specific impacts that diminish coral biodiversity [24].

Finally, a feature of most maps is the increase in river elemental concentrations from inland to the ocean, the main exceptions being relatively high copper (Fig. 6) and calcium (Fig. 9) in the vicinity of Malololelei town in the center of Upolu Island (red intensities inside blue circular spots). Blue circular spots represent low concentrations, which can often be seen in the interior of the island (if not present, then the concentration is too low to visualize). These blue spots frequently become tinged with red intensities at the coastline, denoting higher concentrations. This is an indication of cumulative increases due to human activities.

IV. SUMMARY

Complex ecosystems are characterized by having many diverse parts, which are interdependent and connected, and that adapt to circumstances as they evolve. System failures occur when perturbations - potentially over extended periods of time - diminish the capacity of the ecosystem to adapt. Tipping points to cascade failure of environmental health follow when delays in system responses and lack of mitigation conspire. The coral reefs of Upolu represent such a complex ecosystem, and the human health burden, diminishing biodiversity, and failing coral reef health are indications that the island is nearing a tipping point.

Herbicide and pesticide chemical use on Upolu Island is widespread and magnesium, bromine, and calcium are the likely signatures. These chemicals have endocrine effects that may contribute to endemic obesity and they are also highly toxic to aquatic life. Mitigation efforts may include alternative pest management, such as biological controls

In our study of the biosecurity of Upolu Island freshwater and seawater environmental resources we have identified several environmental challenges that individually or, more likely, cumulatively, may explain problems that require mitigation. Metal contributions to the coastline of Upolu Island require attention. Copper from antifouling paints - an apparently unavoidable feature of marine infrastructure - requires discussion by the Samoan government and the international community. Identification of specific sources of metal contamination and mitigation using new or improved technologies and manufacturing processes for reducing their release to the environment are needed. Significant research and development on this issue is anticipated.

Among the elements essential to life, silicon, bromine, barium, and strontium are of particular interest. Regarding silicon, the contrast between high freshwater sources and low seawater concentrations suggests that excess inputs due to farming activities and/or climate effects may be dumping silica from the rivers into the sea, which falls from surface waters to the sea floor and potentially obscuring the coral reef surface, thus decreasing the coral reefs' ability to thrive. Bromine is essential for some coral organisms, but in the general environment it is linked to ozone depletion [25] and is expected to be reduced by international agreements. Ironically, Samoa became free of chlorofluorocarbons, a leading cause of diminished ozone in 2003, yet the country's increased demand for products containing bromine for health concerns [26] requires a fresh discussion by Government. Barium and strontium, also essential to the lives of corals, but are in surprisingly low concentrations around Upolu Island. These insufficiencies require investigation. Historically this may be investigated by sampling coral skeletons retrieved at known dates in the past

The oxygenation of Samoa waters is favorable for aquatic life, but salinity of coastal waters should be investigated. If increased salinity is found to be recent, it may be due to climate induced decreased freshwater flow into the sea and/or evaporation from relatively sequestered landward sides of coral reefs. Causes of salinity variability of the inner reef and reef zone requires investigation, and multivariate analyses of historical weather and ocean data with present day sampling through seasons is warranted.

The near universal distribution of enteric bacteria throughout sampling sites from both fresh and seawater and using two independent methods suggests a general sanitation problem around the island. In freshwater samples, the family of bacteria where *E. coli* belongs constituted >10% of all bacteria recovered from these samples. In terrestrial environments, these bacteria can cause severe human disease if water is consumed. In marine environments, they can also cause health hazards if water is ingested or if filter-feeders, such as shellfish are consumed. Regular monitoring by the local government of beaches, fishing sites and drinking water sources are important to prevent disease outbreak, particularly in hot months when these bacteria tend to increase in abundance.

Finally, the presence of microplastics in all Samoa water types is extensive. Exposure of microplastics to coral reef organisms is linked to compromised health. Reductions of disposable plastic on Upolu Island are indicated. Single use plastic is the largest contributor to microplastics. Mitigation is only possible by an enforceable ban and finding alternatives that take into account waste and pollution from pulp and paper manufacturing.

In sum, our model system has revealed several potential causes of coral reef decline relatable to element toxicity, water quality anomalies, and the ubiquitous presence of microplastics, which may individually or, more likely, collectively, relate to a deterioration of the health of Upolu's coral reefs, akin to what has been observed from other locations [27].

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