CHAPTER 3
Materials and Methods

Chapter 3: Materials and Methods

3.1 Introduction

Present chapter describes the field sampling, chronology and various analytical protocols adopted to multiproxy studies of cave and lake deposits in this research work. The meaningful palaeoclimatic reconstruction of any area requires robust chronology and reliable proxies. The palaeoclimatology without chronology is similar to the history without dates (Blaauw and Heegaard, 2012). Hence a robust chronology helps in understanding links local, regional and global climatic events.

The chronology for the stalagmite samples, WSS-3, from the Wah Shikar cave, and MWS-1, collected from the Mawmluh cave NE Himalaya has been established using eight U-Th disequilibrium series dates. The carbonate subsamples from the speleothems were analysed for $\delta^{18}O$ and $\delta^{13}C$ ratios to understand precipitation variability in the NE Himalaya. The chronology of core TMC-1 from the Tso Moriri Lake is established using 16 AMS ^{14}C, and eighteen ^{210}Pb and ^{137}Cs ages. The sediment samples were analysed for multiple proxies including grain size measurements, elemental abundances, $\delta^{18}O$ and $\delta^{13}C$ ratios in bulk carbonate, $\delta^{13}C$ ratio in bulk organic carbon and total organic carbon (TOC) to understand climatic variability in the NW Himalaya.

3.2 Field sampling

3.2.1 Field sampling for speleothems

Two samples of cave deposits (speleothems), WSS-3 and MWS-1 were

collected from Meghalaya during our caving expedition in December, 2012. WSS-3 (23.5 cm long), was collected from the Wah Shikar cave. This sample was collected from a cave chamber around one km inside the cave entrance, having constant temperature of ~20.1°C and ~100% relative humidity, which does not appear to be affected by flooding during heavy rainfall months. The stalagmite was actively growing and fed by a soda straw stalactite.

Figure 3.1 Stalagmite sample WSS-3 in the Wah Shikar cave, NE Himalaya.

The stalagmite sample MWS-1 (18.5cm long) was collected from the Mawmluh cave, around 4km inside the cave entrance with no effect of flooding during summer monsoon season. The cave chamber encompassing MWS-1, had a constant temperature of ~20.5°C and ~100% relative humidity. This provides ideal conditions for the carbonate deposition in isotopic equilibrium with percolating cave water and avoid chances of kinetic fractionation due to changes in temperature

and humidity (Hendy, 1971; Wang et al., 2005; Sinha et al., 2011; Dutt et al., 2015). Both the samples were sliced in laboratory, into two halves and polished using sand papers of different sizes varying from 600 mesh to 2200 mesh successively and cleaned with high purity 18MΩ water.

Figure 3.2 Stalagmite sample MWS-1 in the Mawmluh cave, NE Himalaya.

3.2.2 Field sampling in the Tso Moriri Lake

A 5m long sediment core, TMC-1 was retrieved in September 2012 from the northern part of the Tso Moriri Lake using 'Livingstone piston corer'. The water depth at the coring site was 5 m. The core was split into two halves, one half was kept as archive and the other half was sampled at every 0.5 cm interval. The lithology was dominated by sand and silt size fractions. The samples were air dried and processed for detailed grain size, geochemical and isotopic analysis.

Figure 3.3 Sediment coring in the Tso Moriri Lake, NW Himalaya during Lake coring expedition in September-October 2012.

3.3 Chronology

3.3.1 Chronology of stalagmite WSS-3, Wah Shikar cave

Chronology of stalagmite WSS-3 from the Wah Shikar cave has been established using eight dates acquired by Uranium-Thorium (U-Th) disequilibrium series dating technique (Figure 3.4, Table 3.1). For dating, sample was polished and washed with clean $m\Omega$ water. The subsamples of ~150 mg weight were drilled parallel to the growth of layers. The subsamples were treated with 0.1 N HCl. The subsamples were then spiked with the known amount of ^{236}U, ^{233}U and ^{229}Th spikes for the accurate measurements of U and Th concentrations (Cheng et al., 2013). U and Th fractions were separated and purified with column

chromatography (Chen and Wasserburg, 1981). The isotopic compositions of U and Th were analysed using Thermo Finnigan 'Neptune' multi-collector inductively coupled plasma mass spectrometry (MC-ICPMS) at Department of Earth Sciences, University of Minnesota, U.S.A. The dating results indicate that WSS-3 has grown continuously from AD 1026 to the time of collection i.e. AD 2012. WSS-3 age depth model has been established by linear interpolation between adjacent age measurements. The strong correlation for age-depth bivariate plot for WSS-3 (R^2= 0.86) (Figure 3.3), suggesting a continuous deposition of WSS-3 sample, without any hiatus. The growth rate of WSS-3 ranges from 0.015 to 0.21 mm/yr.
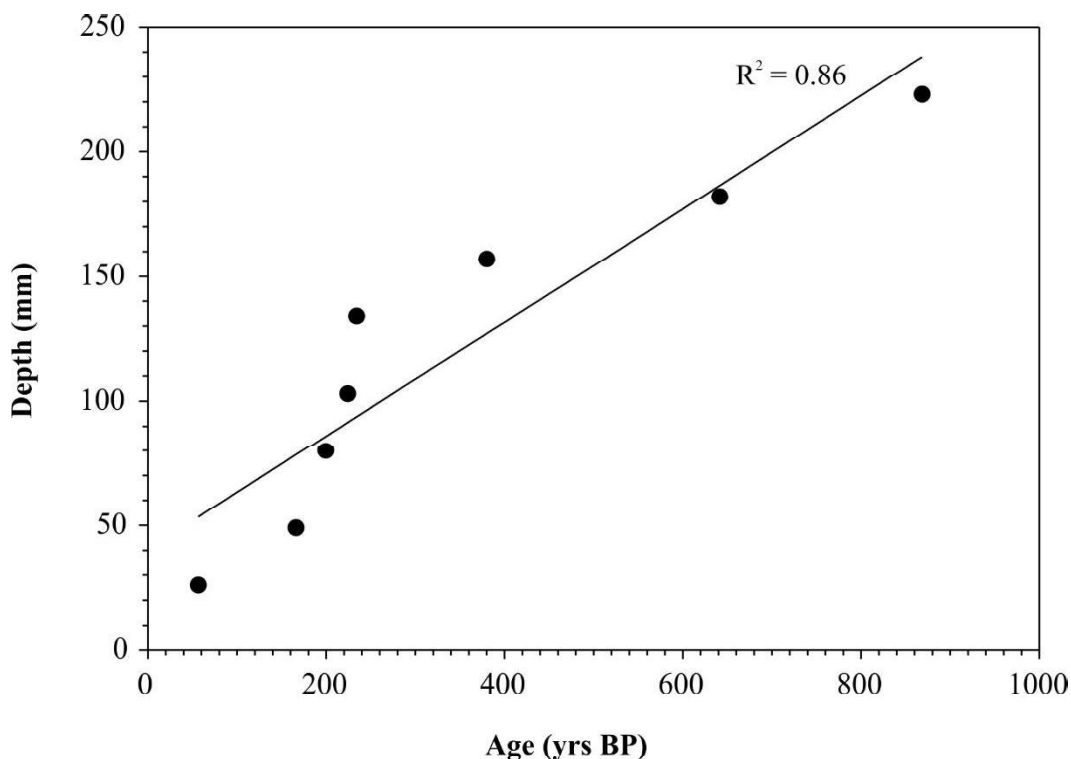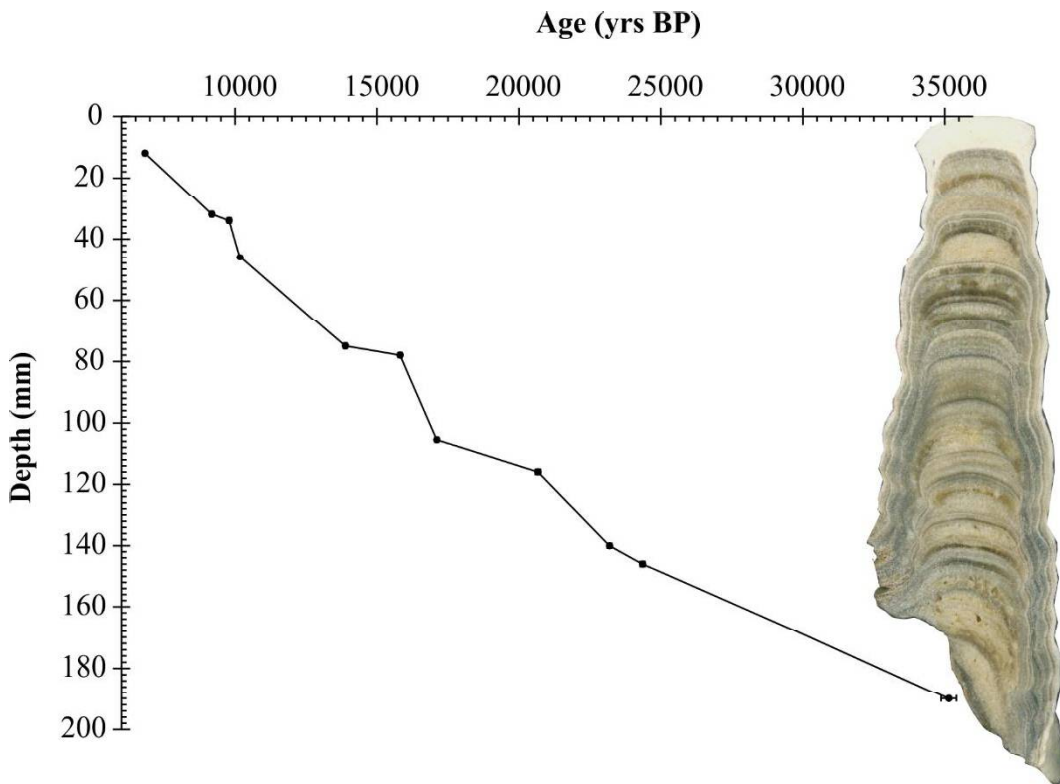

Figure 3.4 Chronology of the stalagmite WSS-3 from the Wah Shikar cave, Meghalaya, NE Himalaya.

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Table 3.1 ^{230}Th dating results for stalagmite WSS-3, from the Wah Shikar cave, NE Himalaya. The ages are reported with two sigma

(2σ) uncertainty. BP stands for the 'Before Present' where Present corresponds to year 1950.

Sample Number	Depth (mm)	^{238}U (ppb)	^{232}Th (ppt)	^{230}Th / ^{232}Th (atomic x10^{-6})	δ^{234}U (measured)	^{230}Th / ^{238}U (activity)	^{230}Th Age (yr) uncorrected	^{230}Th Age (yr) (corrected)	δ^{234}U$_{Initial}$ (corrected)	^{230}Th Age (yr BP) (corrected)
WSS-3-3	26±1	350±1	186±5	38±6	-3.3±2.3	0.0012±0.0002	135±19	120±22	-3±2	57±22
WSS-3-8	49±1	426±1	2022±41	12±1	5.0±2.1	0.0034±0.0002	367±18	229±99	5±2	166±99
WSS-3-4	80±1	300±1	759±15	20±1	-7.8±2.9	0.0031±0.0002	337±17	262±55	-8±3	199±55
WSS-3-9	103±1	338±1	561±11	30±2	-1.7±2.5	0.0031±0.0002	336±23	287±41	-2±3	224±41
WSS-3-5	134±1	220±1	433±9	27±2	-7.5±3.2	0.0032±0.0002	355±22	297±46	-7±3	234±46
WSS-3-10	157±1	303±1	795±16	30±1	-0.1±2.7	0.0048±0.0002	520±20	443±58	0±3	380±58
WSS-3-6	182±1	163±1	559±11	35±1	-12.1±3.4	0.0073±0.0002	806±26	704±76	-12±3	641±76
WSS-3-2	223±1	223±1	2030±41	20±2	-4.1±2.1	0.0109±0.0009	1198±101	931±214	-4±2	868±214

3.3.2 Chronology of stalagmite MWS-1 from the Mawmluh cave

The chronology of Mawmluh Stalagmite-1 (MWS-1) was constrained by 11 absolute U-Th series dates determined by MC-ICPMS at the University of Minnesota, USA. The analytical procedure is similar to that described in section 3.3.1. All the ages are in correct stratigraphical sequence and lies close to regression line (R^2=0.95), indicating the continuous growth of the stalagmite MWS-1 between 33,800 and 5,500 years (yrs) BP. Sample ages were interpolated linearly between adjacent age measurements.

Figure 3.5 Chronology of the stalagmite MWS-1 from the Mawmluh cave, Meghalaya, NE Himalaya.

Table 3.2 ^{230}Th dating results for stalagmite MWS-1, from the Mawmluh cave, NE Himalaya. The ages are reported with two sigma (2σ) uncertainty.

Sample Number	Depth (mm)	^{238}U (ppb)	^{232}Th (ppt)	^{230}Th / ^{232}Th (atomic x10^{-6})	δ^{234}U (measured)	^{230}Th / ^{238}U (activity)	^{230}Th Age (yr) (uncorrected)	^{230}Th Age (yr) (corrected)	δ^{234}U$_{Initial}$ (corrected)	^{230}Th Age (yr BP) (corrected)
MWS-1-T	12±1	2122±7	2758±56	625±13	-198.8±1.8	0.0493±0.0002	6937±38	6889±51	-203±2	6826±51
MWS-1-3	32±1	1967±2	2167±44	989±21	-187.8±1.0	0.0661±0.0003	9280±47	9241±54	-193±1	9178±54
MWS-1-4	34±1	2003±2	3621±73	647±13	-181.7±1.1	0.0709±0.0003	9918±44	9853±63	-187±1	9790±63
MWS-1-5	46±1	2146±2	248±7	10501±281	-175.5±1.1	0.0737±0.0002	10241±32	10237±33	-181±1	10174±33
MWS-1-7	75±1	1574±2	635±14	4109±94	-159.9±1.2	0.1006±0.0004	13960±56	13946±57	-166±1	13883±57
MWS-1-8	78±1	2945±4	3477±70	1577±32	-164.9±1.3	0.1129±0.0003	15915±52	15874±60	-172±1	15811±60
MWS-1-9	105±1	3738±7	367±8	20161±459	-171.4±1.5	0.1201±0.0003	17169±54	17165±54	-180±2	17102±54
MWS-1-10	116±1	1748±2	916±21	4448±101	-179.5±1.3	0.1413±0.0005	20749±85	20730±86	-190±1	20667±86
MWS-1-11	140±1	4796±9	237±9	51005±1870	-197.8±1.4	0.1528±0.0004	23254±77	23252±77	-211±1	23189±77
MWS-1-12	146±1	3311±5	601±15	14414±361	-202.8±1.4	0.1586±0.0004	24422±84	24416±84	-217±1	24353±84

3.3.3 Chronology of core TMC-1 from the Tso Moriri Lake

The chronology of the sediment core TMC-1 from the Tso Moriri Lake is based on eighteen ^{210}Pb and ^{137}Cs dates (Table 3.2) and sixteen AMS [14]C (Figure 3.6, Table 3.3) on bulk sediment samples. Eight AMS ^{14}C age measurements were carried out at NOSAMS facility, Woods Hole Oceanographic Institution, U.S.A. and other eight in the Beta Radiocarbon Inc., U.S.A. (Table 3.3). The lakes of Ladakh and Tibet demonstrate very high "reservoir age effect" (Leipe et al., 2014; Hou et al., 2016); hence the AMS ^{14}C age of the top sample i.e.1,820 yrs BP has been considered as reservoir age and subtracted from all the AMS ^{14}C age measurements assuming constant reservoir age effect. Since the concentration of ^{14}C has not been constant throughout and changed due to variation in ^{14}C production in atmosphere and admixture of CO_2 due to fossil fuel burning, the ages were calibrated for their formation/deposition timing. The age calibration was performed using CALIB 7.0.4 programme in yrs BP using the INTCAL 13 calibration curve (Stuiver and Reimer, 1993) (http://calib.org/calib/download/). The Tso Moriri time series is established using Bacon age model prepared using weighted mean values of calibrated ages (Blaauw and Christen, 2011) http://chrono.qub.ac.uk/blaauw/bacon.html).

Table 3.3 ^{137}Cs and ^{210}Pb measurements of core TMC-1 from the Tso Moriri

Lake, Ladakh, NW Himalaya.

Sl. No.	Depth (mm)	^{137}Cs (Bq/Kg)	^{210}Pb$_{ex}$(Bq/Kg)	^{226}Ra(Bq/Kg)	^{210}Pb$_{t}$(Bq/Kg)
1.	10	0	251.27	308.57	559.83
2.	15	0	253.45	510.26	763.71
3.	20	0	153.58	299.64	453.22
4.	25	0	161.44	219.53	380.97
5.	30	0	148.31	330.58	478.89
6.	35	0	158.56	457.34	615.90
7.	40	0	76.10	132.25	208.35
8.	45	0	67.21	160.92	228.13
9.	50	0	62.49	183.50	245.99
10.	55	0	289.38	197.18	486.56
11.	60	0	84.55	130.88	215.43
12.	65	0	9.38	226.62	236.0
13.	70	0	9.83	260.82	270.65
14.	75	0	148.39	311.77	460.16
15.	80	0	24.10	262.76	286.85
16.	85	0	17.23	312.92	330.15
17.	90	0	3.05	228.36	231.41
18.	95	0	10.74	223.46	234.20

Table 3.4 AMS ^{14}C ages, corrected and calibrated weighted mean age measurements of core TMC-1 from the Tso Moriri Lake, Ladakh, NW Himalaya using Bulk organic matter (BOM) and Plant material. The calibrated ages are reported in years BP with respect to AD 1950 with 2σ uncertainty in ages using CALIB 7.0.4 software. '*' indicates samples analyzed at Beta Radiocarbon Inc.

| Lab. Number	Depth (mm)	Sample Type	AMS ^{14}C Age (years B.P.)	Corrected age (years B.P.)	Calibrated weighted mean age (years B.P.) 2σ
112150	0	BOM	1820±25	0	-62
441300*	155	BOM	1860±30	40±55	122±55
113460	310	BOM	2600±35	780±60	726±73
441301*	410	BOM	2270±30	450±55	470±60
441302*	500	BOM	1510±30	-310±55	Cannot be calibrated
112321	640	BOM	2500±40	680±65	628±82
441303*	780	Plant Material	440±30		440±30
441304*	1020	BOM	5250±30	3430±55	3701±135
112152	1170	BOM	2620±35	800±60	742±68
441305*	1545	BOM	4440±30	2620±55	2758±78
112322	1725	BOM	2190±35	370±60	408±102
112323	2115	BOM	3160±40	1340±65	1262±94
441306*	2200	BOM	3440±30	1620±55	1507±117
113461	2360	BOM	1870±30	50±55	123±56
441307*	2375	BOM	2470±30	650±55	610±67
112155	2535	BOM	5840±45	4020±70	4523±195
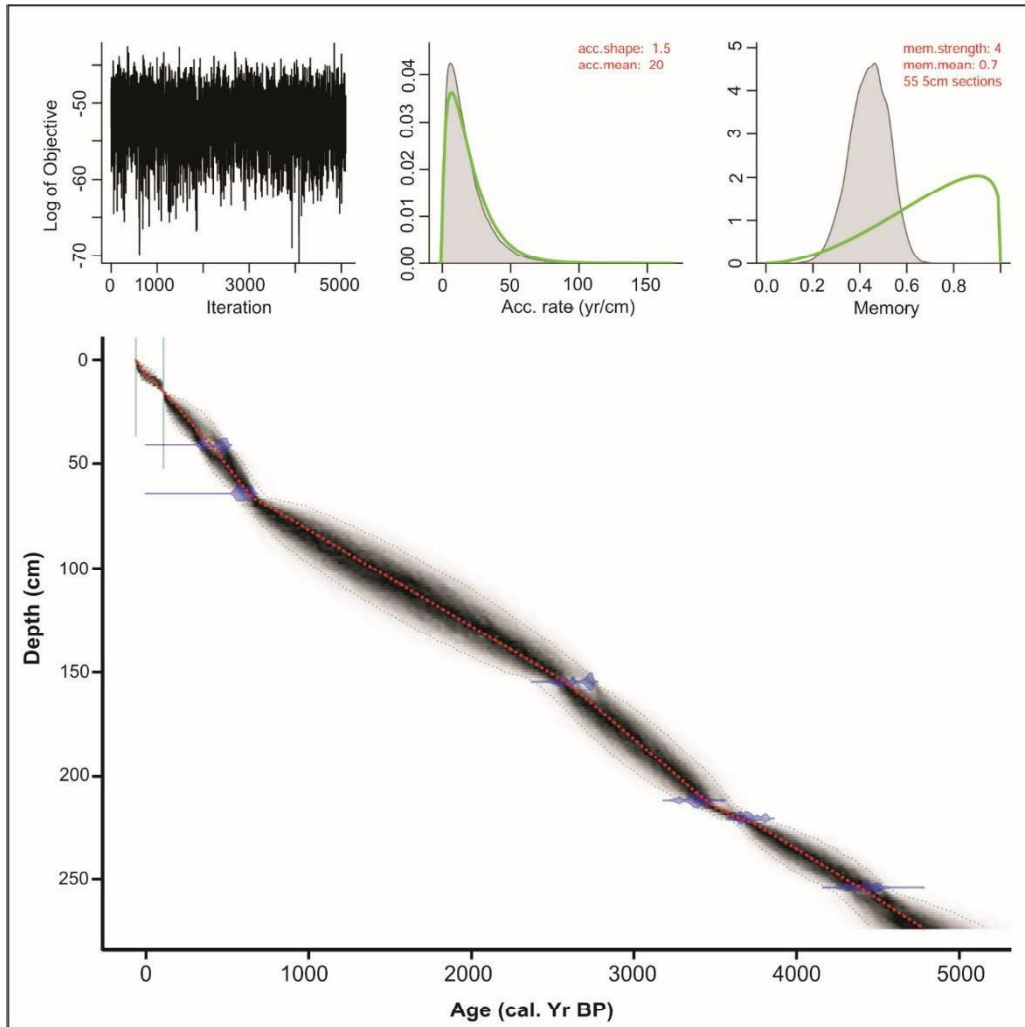
Figure 3.6 Age Depth plot of core TMC-1 from the Tso Moriri Lake, Ladakh, NW Himalaya, based on Bacon age model using calibrated mean ages. The main graph shows the 2 sigma (95%) confidence level of the calibrated weighted mean ages (blue dots) before the present (BP) with respect to AD 1950 plotted against depths. The shadowed area between the dotted lines shows the probability range of the ages (minimum and maximum ranges). The red line in the middle of the shadowed area is the best fit used for age depth calculation.

3.4 Analytical procedures

3.4.1 $\delta^{18}O$ and $\delta^{13}C$ ratios in speleothem calcite from NE Himalaya

A total of 465 subsamples for oxygen isotopic measurement were drilled along the central growth axis using the hand held dental drill (Marathon-3). Around 500 μg powder was extracted at every 0.5 mm interval and analysed at Stable Isotope Laboratory, Wadia Institute of Himalayan Geology (WIHG), using Thermo Finnigan Delta IV Stable Isotope Ratio Mass Spectrometer (SIRMS) attached with the Gas Bench facility. Before analysis, the instrument was also tuned for the linearity correction using different amount of Merck-1 (an in house standard for carbonates). Around 200 μg of each sample was taken into a glass vial and flushed with the 'He' gas to avoid atmospheric contamination. About 200 μl of phosphoric acid was then added which reacted with the carbonate sample to produce CO_2. This CO_2 was further introduced into using SIRMS and analysed. Signals corresponding to molecular masses 44, 45 and 46 were measured for carbonate subsamples as well as reference gas. Thereafter, the isotopic composition was calculated. Five pulses of reference gas were allowed to enter SIRMS through reference open split, followed by single pulse of sample CO_2. The raw delta value of sample was calculated with reference to the 'Reference CO_2' to get the final results. To maintain the precision and accuracy of the results, several blanks and standards NBS-18 and Merck-1 were run repeatedly in between the samples. Reproducibility of results for the oxygen isotopes is 0.1‰ and for carbon isotope is 0.05‰. The results are reported against Vienna Pee Dee *belemnites* (VPDB) in standard delta (δ) notation

$$\delta^{18}O \text{ or } \delta^{13}C \text{ (‰)} = [(R_{sample}/R_{standard})-1] * 1000$$

Where R_{sample} and $R_{standard}$ are the ratios of heavy isotope to lighter isotope in sample and standard, respectively.

$\delta^{18}O$ and $\delta^{13}C$ of 365 samples from the Mawmluh cave was determined at Brown University, U.S.A. The measurements were made using MAT 252 Isotope Ratio Mass Spectrometer (IRMS) system attached with Kiel III carbonate device. In house standards Brown Yule Marble (BYM) and Carrara Marble (CM) were used as reference material. Repeated measurements of the standards were carried out to maintain the accuracy of the results. The reproducibility of BYM is 0.01‰ and 0.07‰; and CM 0.02‰ and 0.06‰ for stable carbon isotope and oxygen isotope, respectively.

3.4.2 Sediments from the Tso Moriri Lake, NW Himalaya

3.4.2.1 Grain size measurements

The sediment samples were treated with 10% HCl solution for two hours and 30% H_2O_2 for 24 hours to remove carbonates and organic contents, respectively. After treatment, samples were centrifuged with distilled water at r.p.m. 5000 for five minutes, to remove excess acid and H_2O_2 from the samples. A total of 94 samples were analysed for particle size distribution in the Sedimentology Laboratory, WIHG using the Laser Particle Size Analyser (Malvern Mastersizer 2000). Prior to measurements, the samples were disaggregated in distilled water, with 2300 rpm stirrer and 40% ultrasonic vibrator for 45 seconds. The grain size distribution parameters include the sediment size (μm) and its volume percentage.

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Five measurements were taken on each sample to obtain the best results. The grain size data was then used for extracting meaningful end members to understand various hydrological processes (Weltje and Prins, 2007).

3.4.2.2 Elemental abundances

A total of 210 samples were analysed for elemental abundances using the Inductively coupled plasma mass spectrometer (ICPMS) (Perkin Elmer, ELAN-DRC-e) at WIHG. Samples were prepared by the open acid digestion method as described in Khanna et al. (2009). 100 mg of each powdered sediment sample (~200 mesh) was kept in silica crucibles and 25 ml solution of HF (48%), HNO_3 (concentrated) and $HClO_4$ solution in the ratio of 8:4:3, respectively, added to it. The solution was digested at 180 °C in the open crucibles and contents were allowed to evaporate till the incipient dryness. The process was repeated thrice to ensure the complete digestion and finally evaporated till the incipient dryness. The dried mass was dissolved in 10 ml of 10% Nitric acid and warm gently till clear solution is obtained. Final solution is made up to 100 ml volume by adding high quality $18m\Omega$ water. The international standard for Marine sediment (MAG-1) and Green River Shale (SGR-1) were used to calibrate the machine and validation of results, respectively. Elemental abundances are given in weight percentage of total dry mass of sediments. The results produced have an analytical error of ±0.1-0.4 %.

3.4.2.3 The $\delta^{18}O$ and $\delta^{13}C$ ratios in lake carbonates

A total of 428 samples were analysed for the $\delta^{18}O$ and $\delta^{13}C$ ratios in bulk lake carbonates. From top to the depth of 213 cm, samples were analysed at Brown

University, U.S.A. and from depth 213 to 270 cm at WIHG. Prior to analysis, samples were air dried and powdered finely after discarding plant fragments.

At Brown University, the measurements were made using MAT 252 IRMS system attached with Kiel III carbonate device. In house standards BYM and CM were used as reference material. The reproducibility of BYM is 0.01‰ and 0.07‰; and CM 0.02‰ and 0.06‰ for stable carbon isotope and oxygen isotope, respectively.

At WIHG, the analyses were carried out using "Thermo Finnigan Delta IV" SIRMS attached with the gas bench facility. About 300 µg of finely powdered sample was used for each sample. The analytical procedure is describes in detail in subsection 3.4.1 Repeated analysis of Merck-1 indicate the reproducibility of results for the oxygen isotopes is 0.2‰ and for carbon isotope is 0.1‰.

3.4.2.4 The $\delta^{13}C_{org}$ ratio in organic carbon and total organic carbon (TOC) analysis

174 finely powdered samples were treated twice with 1N HCl for two hours for removing carbonates, subsequently centrifuged with high quality MΩ water and dried. 20-30 µg of carbonate free powdered sample was combusted at 1200°C in an oxygenated environment in the "Flash Elemental Analyser". The CO_2 produced was then analysed using "Thermo Finnigan Delta IV" SIRMS at WIHG. The results are reported with respect to VPDB standard using conventional δ (‰) notations. Replicate analysis of several samples suggest an analytical error of 0.1 ‰ in the measurements. The TOC was calculated from the peak areas obtained from the sum

of m/z 44, 45 and 46 signals measured in the SIRMS (Rawat et al., 2015). The reproducibility of the measurements were checked by the replicate analysis of several samples. The TOC results are expressed as percentage of dry weight (Wt. %).

3.4.3 Spectral analysis:

Spectral analysis of proxy records in this research work has been determined using "Oversample size 1'' and "Number of Segments 1'' in the PAST software (Hammer et al., 2001), available on http://folk.uio.no/ohammer/past/.

To determine the periodicity in the paleoclimatic time series from the Wah Shikar cave, spectral analysis was performed on entire $\delta^{18}O$ time series from speleothem WSS-3 for the period between AD 1026 and 2007 and also in three different phases spanning AD 1026 to 1320; AD 1320 to 1710 and AD 1710 to 2007. These intervals correspond to the MWP, LIA and CWP, respectively. The periodicity was determined with 95% confidence level using PAST Red Fit and Monte Carlo methods.

The periodicities of $\delta^{18}O$ proxy record of the ISM from the Mawmluh cave during 33,800 to 5,500 yrs BP, was determined with 95% confidence level using PAST Red Fit method.

Spectral analysis of Al/Ca time series from core TMC-1 from the Tso Moriri Lake was carried out for the period between 4,500 and -62 yrs BP, with 90% confidence level using PAST Red Fit and Monte Carlo methods.