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DISSOLVED ORGANIC MATTER (DOM)

AND HEAVY METALS IN WATERFALLS WATER IN

KELANTAN

Dissertation submitted in partial fulfillment for the degree of

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AKNOWLEDGMENTS

"It is He who sends down water from the sky. From it you drink and from it come the shrubs among which you graze your herds. And by it He makes crops grow for you and olives and dates and grapes and fruit of every kind. There is certainly a Sign in that for people who reflect". (An-Nahl: 10-11)

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ABBREVIATIONS

FAAS	-Flame Atomic Absorption Spectroscopy
AAS	-Atomic Absorption Spectroscopy
ppm	-Part per million
FT-IR	-Fourier Transfer-Infra Red Spectroscopy
DOM	-Dissolved Organic Matter
NOM	-Natural Organic Matter
DOC	-Dissolved Organic Carbon
HS	-Humic Substance
DON	-Dissolved Organic Nitrogen
FA	-Fulvic acid
HA	-Humic acid
WHYA	-Weak hydrophobic acids
HAA	-Hydrophilic acids
HYN	-Hydrophobic neutrals
HNs	-Hydrophilic neutrals
HYB	-Hydrophobic bases
HB	-Hydrophilic bases
SPE	-Solid Phase Extraction

ABSTRACT

The water samples were collected from Linag and Pasu waterfalls, and Top Bok hot spring, situated in Kelantan, Malaysia. The sampling and sample treatment was made according to EPA protocols. Physicochemical measurements of water samples were performed on site using YSI® 556 Multi-Probe System. DOM fractions were made with membrane filtration, and total DOM was trapped on C-18 and XAD-4 columns through the SPE technique. The metallic elements were determined in water samples using FAAS and GFAAS. Amberlite XAD-4 Resin was found to have more capacity to enrich total DOM than the C-18 column and membrane filter. The FT-IR spectra of the retentate showed bands for –OH, -CH, C=O and C-O-C stretching vibrations for phenolic, carboxylic and carbohydrate functional groups. The metals determined were Pb (II), Fe (II), Cu (II), Cd (II) and Zn (II); their concentration varied between samples, and overall concentrations were within the safe limits set by EPA and WHO for water used in bathing and tourism activities.

1.0

INTRODUCTION

Dissolve Organic Matter (DOM) is a common constituent of natural water, and is generated as organic matter decomposes (Durgin & Chaney, 1984; Hu *et al.* 1972). Organic matter in natural waters is of great importance in geochemical process, such as, solubilization, speciation and toxicity of metals in natural system. Organic matter in freshwaters exists as dissolved molecules, colloids, and particles (Ma *et al.*, 2001).

The term "dissolved organic matter" (DOM), as applied in environmental science, refers to the collection of organic compounds present in solution in surface waters, soil waters and ground waters. In freshwater and terrestrial systems the major constituents are humic substances (fulvic, humic and hydrophilic acids), while the minor components include carbohydrates, amino acids, carboxylic acids, hydrocarbons, sterols, alcohols, ketones, ethers, pigments and anthropogenic organic contaminants (Thurman, 1985).

It is increasingly recognized that DOM has a number of important ecological and geochemical functions, including light absorption, proton binding, binding of heavy metals, and radionuclides, binding of organic contaminants, adsorption at surfaces, aggregation and photochemical reactivity. Information about these functional properties had been obtained largely from laboratory experiments with isolated fractions, especially humic and fulvic acids. (Perdue and Gjessing, 1990; Kullberg et al., 1993; Hessen and Tranvik, 1998)

DOM is composed of a complex mixture of organic material, originating both within the aquatic environment (autochthonous) as well as through the transport of partially-degraded organic material from the surrounding terrestrial environment (allochthonous). DOM is often quantified in terms of dissolved organic carbon (DOC) concentrations, although

carbon only accounts for approximately 50% of DOM by weight (Thurman, 1985). Of the DOC, humic substances (HS) typically form 40-70% in rivers and streams and under certain circumstances, can account for up to 90% of the total DOC concentration (Thurman, 1985). HS are recalcitrant compounds and consist partly of aromatic structures which are joined by long-chained links (Francko, 1990; Lindell, 1996).

Natural organic matter (NOM) contributes to the most part of dissolved organic carbon in aquatic environment. The partitioning behavior of NOM at the solid–water interface plays an important role in many physiochemical phenomena. For example, the surface-adsorbed NOM will increase the stability of colloidal particles (Chandrakanth & Amy, 1998) and impose negative effect on their aggregation and precipitation process (Hundt & O'Melia, 1988). The transport of organic or inorganic pollutants in the aqueous environment can also be extended by the adsorbed NOM (Murphy et al., 1990).

DOM in the sea is one of the largest reservoirs of organic matter on the earth's surface (others include soil organic matter and plant biomass on land), holding approximately as much carbon as is available in atmospheric carbon dioxide (Hedges, 1992). The fact that DOM is a huge organic reservoir on the earth's surface has continued to influence ocean scientists to investigate what DOM is, in terms of its source, chemical nature and function in marine environments, from early in the 20th century.

Recognition of the importance of the microbial loop also has given us new insight into the role of DOM in marine ecosystems (Pomeroy, 1974; Azam et al., 1983). Primary production is the ultimate source of organic matter in the sea, but living biomass forms less than 1% of total organic carbon in seawater, while more than 90% of organic carbon occurs as non-living DOC (Cauwet, 1979). In contrast to the organic reservoirs on land, the processes

by which DOM has been formed are unclear, and actual sources and the chemical nature of DOM are not well known. Recent advancements in DOM study have been covered by reviews of the results of various approaches, trying to understand the dynamics and chemical nature of DOM (Trumbore & Druffel, 1995; Guo and Santschi, 1997; Nagata, 2000; Williams, 2000; Kepkay, 2000; Myklestad, 2000; Ogawa, 2000; Benner, 2002; Hansell & Carlson, 2002; Hedges, 2002).

DOM has various functions and plays important roles in chemical, biological and even physical oceanography. For example, DOM interacts with trace metals or radionuclides and controls their dynamics, it fuels the microbial loop, generates gases (CO, CO2) and nutrients with biological and photochemical reactions, absorbs and extinguishes light, and affects satellite images, etc. The terrestrial input of DOM is also an important topic in the global carbon budget as well as carbon dynamics in coastal environments.

Research on agricultural soils showed that addition of organic materials increased the concentration of water-extractable organic carbon (Rochette & Gregorich, 1998; Gregorich et al., 1998). DOM is considered as an environmental amphiphile and is similar both structurally and functionally to surfactants. DOM can, therefore, enhance the solubility of poorly water-soluble compounds (Kile & Chiou, 1989; Cho et al., 2002). As such, a potential untold consequence of using organic fertilizers is to enhance chemical transport by DOM.

Dunnivant et al. (1992) reported that DOC could readily move through soil columns without preferential flow pathways. Therefore, DOM can serve as a carrier in soil solutions that could result in the rapid flow of organic chemicals through soil profiles. Nelson et al. (1998) reported that leaching rates of napropamide from sewage sludge amended soil was

twice that of soils without sewage sludge due to enhanced transport by sewage sludge-derived DOM in column experiments.

The aim of the present work is to fractionate different kinds of DOM through the use of Amberlite XAD-4 Resin, C-18 column and membrane filtrate. Besides, this study is to determine the heavy metals in waterfalls and hot springs water. These waterfalls and hot springs sites are located in Kelantan.

2.0

LITERATURE REVIEW

In water systems, many hazardous contaminants, such as trace metals and most organic micropollutants, are predominantly transported in association with suspended particulate matter. The suspended particles accumulate in regions of low turbulence and thus leads to the formation of highly polluted sediments. The accumulation of pollutants in sediments depends on many factors such as the size of pollutant sources, abundance and composition of particulate matter, and production and cycling of organic matter. Humic substances are natural organic compounds arising from the decomposition of plant and animal tissues. They can influence the chemical and physical characteristics of the marine ecosystem as they play an important role in light attenuation, pH buffering and in the transport of both water soluble and insoluble species. (Petronio et al, 2006)

The dissolved organic nitrogen (DON) is an important source of N for microorganisms in many aquatic ecosystems. As such, understanding the source, bioavailability, and environmental dynamics of DON is crucial for better understanding of biogeochemical processes in aquatic environments. (Bushaw et al., 1996)

Heavy metals present in the form of free cations or in labile complexes (which can easily dissociate) will generally have a more harmful effect on aquatic organisms than metal in non-labile complexes, since the free metal form is both very mobile and can easily absorb and pass into, the tissue of marine organisms (Peterson, 1982; Campbell, 1995; Stumm & Morgan, 1996; Foulkes, 2000; Batley et al., 2004, 2005).

Durgin & Chaney (1984) fractionated DOM according to molecular size and chemical characteristics into acids, neutrals, and bases of the hydrophilic and hydrophobic groups. The dominant fraction causing dispersion included the hydrophobic acids and organics with molecular sizes greater than 104 nominal molecular weight. Partial oxidation of the DOM increases its carboxylic acid content and dispersion potential. Molecular composition of DOM is poorly characterized, largely due to a lack of analytical methods with adequate versatility to separate and identify the hydrophobic portions of DOM and the sensitivity necessary in bioassays to detect biological uptake without extended incubations.

Horowitz et al., (1996) had reported that 0.45 μm is generally considered to be the cut-off point between dissolved and particulate matter in aquatic systems.

Øygarda et. al., (2006) had reported that the major cations Ca, Mg, K, and Mn were present mainly as free cations/labile complexes < 0.45 μm, while As and Mo were present to a large degree (70–90%) as free anions/nonlabile complexes < 0.45 μm. Aluminium was present mainly as particulate and colloidal matter > 0.45 μm. The particulate and colloidal matter > 0.45 μm was mainly inorganic; indicating that the metals present in this fraction were bound as inorganic compounds. The fractionation gives important information on the mobility and potential bioavailability of the metals investigated, in contrast to the total metal concentrations reported.

Leenheer & Noyes (1984) had outlined a comprehensive method for NOM fractionation using the isolation-fractionation approach. By this procedure, NOM can be fractionated into eight classes: fulvic acid (FA), humic acid (HA), weak hydrophobic acids (WHYA), hydrophilic acids (HAA), hydrophobic neutrals (HYN), hydrophilic neutrals (HNs), hydrophobic bases (HYB), and hydrophilic bases (HB). Other NOM isolation-

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fractionation studies include those by Wong et al., (2002), Martin & Mousset et al., (1997), Marhaba & Van (1999), Marhaba et al., (2003), and Egeberg & Alberts (2003).

Lindell (1996) reported that this material covers a broad range in terms of molecular size (which can vary from a few hundred to hundreds of thousands of Daltons) and due to the presence of carboxylic and phenolic groups, is slightly acidic.

McKnight et al., (2001) reported that HS can be further separated into humic acids (HA, insoluble below pH 2) and fulvic acids (FA, soluble at all pH) as well as humins (insoluble at all pH). Aquatic FA is itself a major fraction of the DOC in natural waters and variations in the chemical characteristics of FA have been related to differences in precursor organic matter. Generalizations concerning DOM are difficult due to its complexity, however, several analytical techniques have provided useful information. For example, XAD resins have provided an operational method for isolating aquatic HS based on hydrophobicity.

Mysore & Amy (1998) investigated the ozonation of NOM sources in the presence of $Ca(NO_3)_2$. They indicated that ozone can change the functional groups of NOM to carboxylic groups, and calcium binding can enhance the removal of NOM.

Humic substances have a substantial capacity to complex dissolved species such as metal ions and cationic organic molecules and to interact with mineral surfaces (Burba, 1994; Hering, 1995; Allen and Hansen, 1996; Haitzer et al., 1998; Takahashi et al., 2002). Consequently, a range of metal ions can efficiently be removed from water and the bioavailability of metals acting as toxicants can be reduced (Meinelt et al., 2001; Shinozuka & Lee, 1991; Lores & Pennock, 1998).

Wershar et. al. (2005) fractioned NOM into the following nine different fractions by the isolation procedure:

(1)   Coarse particulate

(2)   Fine-particulate organics

(3)   Solvent-extractable organics

(4)   Hydrophobic neutrals (HPON fraction)

(5)   Dissolved colloids

(6)   Bases

(7)   Hydrophobic acids (HPOA)

(8)   Transphilic acids + neutral (TPI-A+N)

(9)   Hydrophilic acids + neutrals (HPI-A+N).

Each of these fractions, with the exception of the first and the third which were too small for the complete series of analyses, was characterized by elemental, carbohydrate and amino acid analyses, and by nuclear magnetic resonance and infrared spectrometry.

The data obtained from these analyses indicate that :-

(1)   Fine particulate organics and colloids are mainly composed of peptidoglycans,   and lipopolysaccharides derived from algal, bacterial, and fungal cell walls.

(2)   HPO-N fraction most likely consists of a mixture of alicyclic, terpenes   and carbohydrates.

(3)   HPOA   fraction   consists   mainly   of   lignin   components   conjugated   to carbohydrates.

(4)   TPI-A+N and the HPI-A+N fractions most likely   represent   complex   mixtures of relatively low molecular weight carbohydrates, and peptides, and

(5)   Base fraction is composed of free amino acids,   browning   reaction   products, and   peptide fragments.

Leenheer et. al., (2000) also has developed a comprehensive procedure for the fractionation and characterization of NOM. In this procedure particulate organic matter is separated from DOM, and the DOM is fractionated according to polarity. This procedure, which has been applied so far to only a few natural waters, allows one to isolate and characterize hydrophilic and colloidal fractions that were lost by the procedures used previously.

Wershaw et. al., (2005) reported that the basic principles behind the isolation and fractionation procedure are relatively simple. The particulate and colloidal fractions are isolated by filtration and dialysis. The truly dissolve NOM (DOM) is then fractionated according to polarity by sequential sorption chromatography on XAD-8 and XAD-4 resin (Aiken,1985).XAD-8 resin is an acrylic ester resin that is more hydrophilic than XAD-4

(styrene divinyl-benzene resin).The least polar DOM fraction, the hydrophobic neutral fraction (HPON fraction),is sorbed on an XAD-8 resin column without pH adjustment; by reducing the pH of the water passes through the column a second more polar fraction, the hydrophobic acid fraction (HPOA),is isolated. A third event even more polar fraction, the transphilic acids + neutrals fraction (TPIA+N), is isolated by sorption on an XAD-4 column. The most polar fraction, the hydrophilic acid plus neutral (HPIA+N),is isolated after sorption of the transphilic fraction by a multi-step precipitation procedure, volatilization of some in organic ions, and ion exchange. The base fraction is isolated on a cation exchange resin.

Mounier et. al., (2000) reported that solid phase extraction is based on the hydrophobic behaviour of dissolved organic compounds. This reverse-phase liquid chromatographic technique has been employed recently for the isolation of DOM, and metal-organic complexes, from natural waters using pre-concentration columns. The weak interactive forces involved in partition chromatography minimize the possibility of denaturing the isolated material. Therefore, the likelihood of more complete study on DOM appears to be greater with this technique.

When C18 columns were introduced, they were intended for reverse phase extraction of non-polar to moderately polar compounds such as antibiotics, barbiturates, caffeine, drugs, essential oils, fat soluble vitamins, fungicides, herbicides, pesticides, hydrocarbons, phenols, phthalate, esters, steroids, surfactants, and water soluble vitamins.

The use of solid phase extraction (SPE) for the extraction and isolation of DOM has not been investigated much although the extraction of fuel oil and synthetic organic material has been studied (Mills & Quinn,1981).The protocol of DOM extraction is more problematic on account of the small amount of organic matter available. In addition, selectivity,

contamination during extraction and modifications of the retained compounds are other challenges (Thurman et al., 1988).

3.0

MATERIALS AND METHODS

3.10 Materials

Chemical and Reagent

AnalaR or UHP (ultra-high purity) grade chemicals were purchased from Merck (Germany) or Sigma/ Aldrich (USA). The chemicals were used as such or stated otherwise.

Glassware

All the glassware was from Brand (Germany) or Pyrex (England). The glassware was soaked overnight in 10% HNO_3, rinsed with double distilled water and dried in oven at 110°C.

Instrumentation

Fourier Transform Infrared (FT-IR), Atomic Absoprtion Spectrometry (AAS), membrane filtration set (using 0.45μm and 0.25μm membrane filter, YSI® 556 Multi-Probe System

3.20 Methods

Sample collection and preparation

1.5 L polyethylene bottles were cleaned by using 10% HNO3. The bottles then rinsed with distilled water and E-pure water before drying for overnight.

Water samples from three location in Kelantan; Jeram Pasu, Jeram Linang, and Hot Spring in Tok Bok, Machang were collected. Six samples were collected from each location. Whatman GF/F filters (Grade 1, nominal pore size 11µm) were used for filtration onsite. The sample were treated with 10% HNO_3 and stored in the refrigerator at 4°C.

Table 1: Sample collected in Jeram Pasu (labeled as JP)

JP 1(top level)		JP 2(Middle level)		JP 3(Low level)	
1 Sample filtered on site	1 Sample unfiltered	1 Sample filtered on site	1 Sample unfiltered	1 Sample filtered on site	1 Sample unfiltered

Table 2: Sample collected in Jeram Linang (labeled as JL)

JL 1(top level)		JL 2(Middle level)		JL 3(Low level)	
1 Sample filtered on site	1 Sample unfiltered	1 Sample filtered on site	1 Sample unfiltered	1 Sample filtered on site	1 Sample unfiltered

Table3: Sample collected in Hot Spring, Tokbok Machang (labeled as HS)

HS 1			HS 2		
1 sample filtered on site	1 sample unfiltered	1 sample not treated with HNO3	1 sample filtered on site	1 sample unfiltered	1 sample not treated with HNO3

All the samples were treated with HNO_3 except two samples from the Hot spring water. The distance from one level in the waterfall to another level was about 30-50 meter . The sample which were filtered were treated with 5-6 drops of 10% HNO_3 while to the unfiltered

sample, 8-10 drops of 10% HNO₃ were used for treatment. Different filter papers were used for samples filtration on site.

Physicochemical properties of the natural waters in the different location were recorded by using YSI® 556 Multi-Probe System .

Chart 1: Flow chart of SPE process using XAD-4 & C-18 column & metal detection in water

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│     │  Sample collection   │                                             │
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│                 ▼                                                         │
│     ┌──────────────────────┐          ┌──────────────────────────┐       │
│     │  Samples unfiltered  │────────▶ │  AAS determination of Cd, │       │
│     │  ( HNO₃ treatment)   │          │  Cu, Zn, Ni, Pb, Fe       │       │
│     └──────────────────────┘          └──────────────────────────┘       │
│           │              │                                                │
│           ▼              ▼                                                │
│     ┌───────────┐   ┌──────────────┐                                     │
│     │ SPE on XAD-4│  │ SPE on C-18  │──────▶ ┌──────────────────┐        │
│     │ column      │  │ cartridge    │        │ ΔW after SPE     │        │
│     └───────────┘   └──────────────┘        │ on C-18 cartridge │        │
│           │                                  └──────────────────┘        │
│           ▼                                                               │
│     ┌──────────────┐                                                     │
│     │ ΔW after SPE on│                                                   │
│     │ XAD-4          │                                                   │
│     └──────────────┘                                                     │
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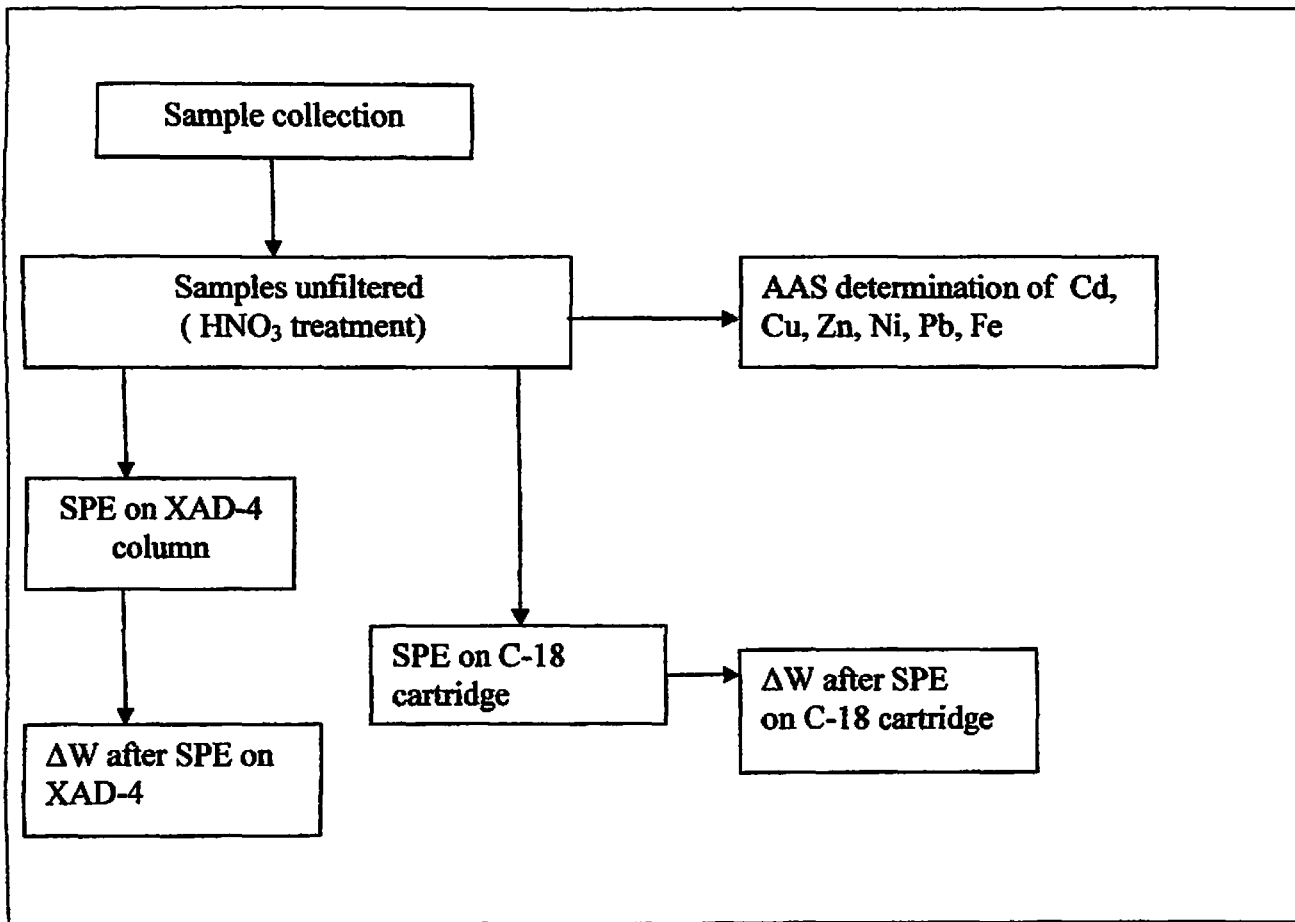
Chart 2 : Flow chart for the filtration of H_2O through membrane filtration and detection of elements

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                    ┌─────────────────────┐
                    │  Sample collection  │
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              ┌──────────────────────────────────┐
              │ Filtered on site (11 μm filter paper) │
              └──────────────────┬───────────────┘
                                 │
                                 ▼
                    ┌──────────────────────┐
                    │  Acid treatment      │
                    │  (HNO₃ 5-6 drops)    │
                    └──────────┬───────────┘
                               │
                               ▼
          ┌───────────────────────────────────┐
          │ AAS determination of Cd, Cu, Zn, Ni, │
          │ Pb, Fe                            │
          └──────────────────┬────────────────┘
                             │
                             ▼
          ┌───────────────────────────────┐      ┌──────────────────┐
          │ Filtration (0.45μm size)      │─────▶│ AAS              │
          └──────┬────────────────┬───────┘      │ determination Cd, │
                 │                │              │ Cu, Zn, Ni, Pb, Fe │
                 ▼                │              └──────────────────┘
      ┌────────────────────┐     │
      │ ΔW of retentate    │     │
      └────────────────────┘     │
                                 ▼
                    ┌──────────────────────────┐
                    │ Filtration (0.25μm size) │
                    └──────────┬───────────────┘
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                    ┌──────────────────────┐
                    │ ΔW of retentate      │
                    └──────────────────────┘
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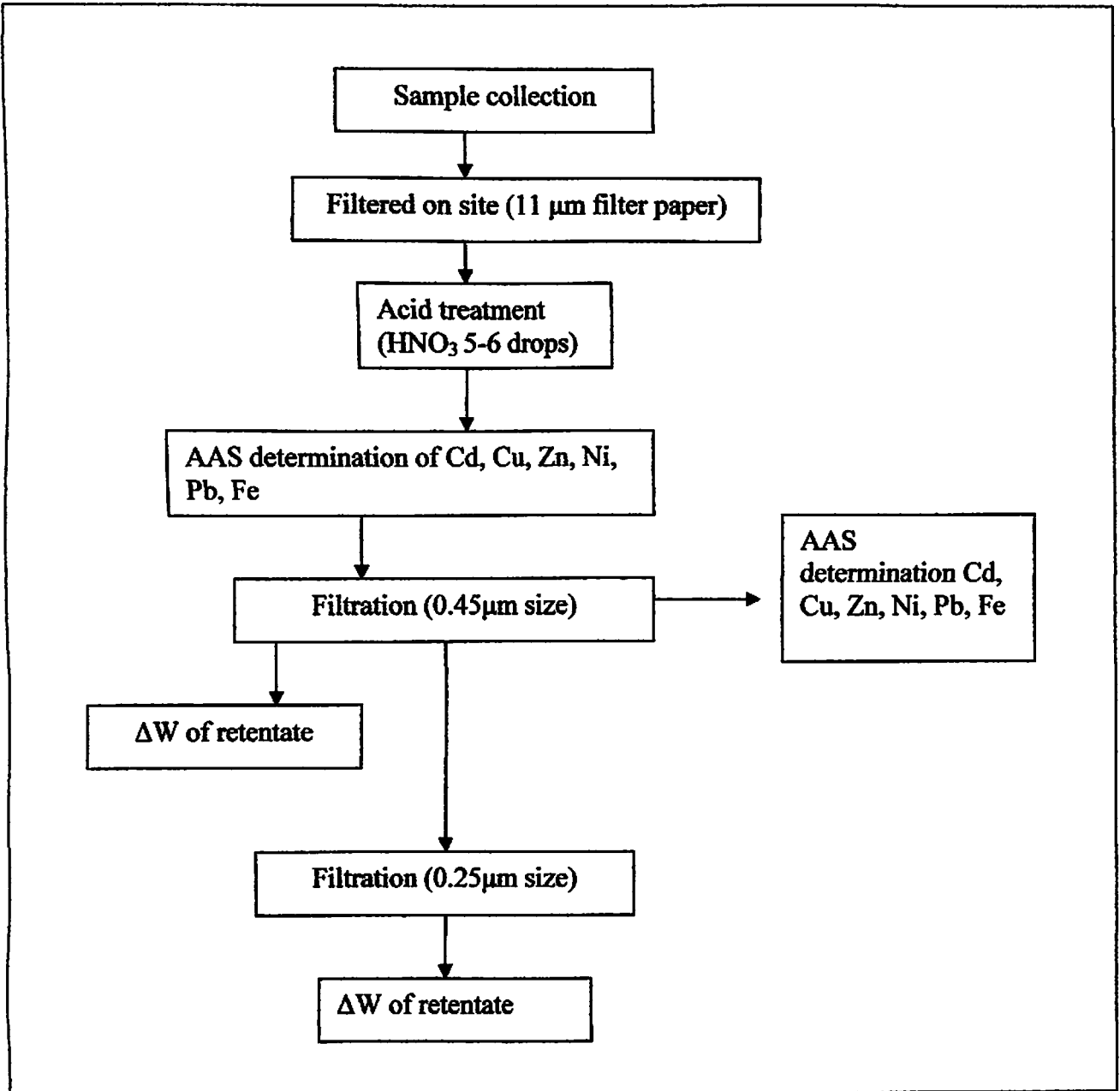
4.0

RESULT AND DISCUSSION

4.10 Pysicochemical Parameters

The physicochemical parameters of the water samples collected from Linang, Pasu waterfalls and Tok Bok hot-spring water were measured on site. The data obtained by using a YSI® 556 Multi-Probe System is listed in Table 4.

Table 4: Physical properties of natural water

Location 1 (Jeram Pasu)	Temp. (°C)	Salinity	pH	TDS (%)	Conductivity (mS/cm)	DO (%)	DO (mg/L)
High level (JP 1)	23.97	0.02	6.94	0.028	0.043	582.9	50.44
Medium level (JP 2)	23.92	0.02	7.84	0.028	0.042	378.5	31.92
Low level (JP 3)	24.28	0.02	7.16	0.029	0.044	403.9	33.85
Location 2 (Jeram Linang)	Temp. °C	Salinity	pH	TDS (%)	Conductivity mS/cm	DO (%)	DO (mg/L)
High level (JL 1)	24.78	0.01	6.79	0.020	0.031	512.5	42.50
Medium level (JL 2)	24.48	0.01	6.83	0.020	0.030	535.6	43.88
Low level (JL 3)	24.40	0.01	7.08	0.020	0.030	470.4	38.34

Table 4(contd.): Physical properties of natural water

Location 3 (Hot Spring) Machang	Temp. °C	Salinity	pH	TDS (%)	Conductivity mS/cm	DO (%)	DO (mg/L)
Location A (HS1)	44.23	0.15	8.67	0.207	0.435	373.8	22.46
Location B (HS2)	43.51	0.15	8.68	0.207	0.432	170.5	9.95

The physicochemical parameters measured on site do not significantly differ in the water from waterfalls, while they differ in the case of hot spring water. The notable character among these parameters is dissolved oxygen. It varies from sample location to location, as well as within the areas (levels) of the water falls and hot spring water. Since the dissolve oxygen is temperature dependent, and it changes with the turbulence or aeration in water bodies, therefore, the values of DO are different at different places.

As the hot spring water is concerned, the variation in temperature, dissolved oxygen, salinity and pH were observed. This may me attributed to the location of the hot spring water, and subsequent phenomena taking place.

4.20 Membrane Filtration

The samples collected from waterfalls and hot spring water were filtered onsite using Whatman Grade 1 filter paper (11 μm pore size). Another batch of sub-samples were added an appropriate amount of conc. HNO_3 and then brought to laboratory for DOM fractionation