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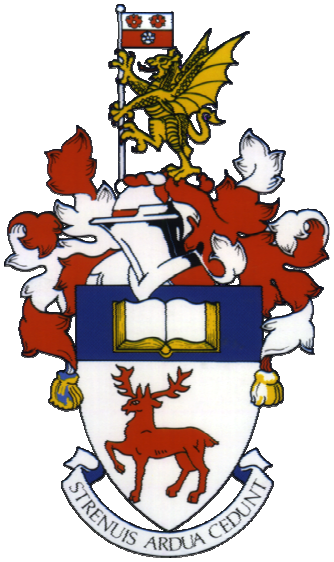
SCHOOL OF OCEAN AND EARTH SCIENCE



The influence of atmospheric organic carbon and organic nitrogen on biogeochemistry of the (sub-) tropical North Atlantic Ocean

by

Pornsri Mingkwan

Thesis for the degree of Doctor of Philosophy

September 2011

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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THE INFLUENCE OF ATMOSPHERIC ORGANIC CARBON AND ORGANIC NITROGEN ON BIOGEOCHEMISTY OF THE (SUB-) TROPICAL NORTH ATLANTIC OCEAN

By Pornsri Mingkwan

The (sub-) tropical North Atlantic Ocean is a region which is influenced by oceanic upwelling along the West African coast, an extensive oxygen minimum zone and also by atmospheric dust deposition from the Sahara and Sahel regions and the industrial continental regions of Europe and North America. In this study, samples from water column profiles were collected to investigate the distributions of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), including dissolved free amino acids (DFAA) and dissolved hydrolysable amino acids (DHAA). In addition, bulk aerosol samples were collected on the island of São Vicente (Cape Verde Islands) to quantify the deposition fluxes into the surface waters of the (sub-) tropical North Atlantic Ocean of atmospheric organic matter; namely leachable organic carbon (LOC), leachable organic nitrogen (LON), leachable free amino acids (LFAA) and leachable hydrolysable amino acids (LHAA). DOC, LOC, total dissolved nitrogen (TDN) and leachable total nitrogen (LTN) were determined using a high temperature combustion technique (HTC). DON values were defined as the difference between TDN and dissolved inorganic nitrogen (DIN). LON values were derived as the difference between LTN and leachable inorganic nitrogen (LIN). DFAA, LFAA, DHAA and LHAA were analysed using the method optimized in this study. The determination was performed using reversed-phase high performance liquid chromatography (RP-HPLC) combined with AQC pre-column derivatisation. Hydrolysis was conducted using vapour phase hydrolysis for 24 hours at a temperature of 110-115 ºC.

In general, DOC concentrations were high in surface waters of the (sub-) tropical North Atlantic Ocean and declined toward the thermocline, whereas DON exhibited non-typical vertical profiles, with the concentrations increasing with depth, in the tropical North Atlantic Ocean. Mean surface water DOC and DON concentrations ranged from 67.2 µM to 72.3 µM and 4.6 µM to 8.1 µM, respectively. Enhanced DON values in sub-surface waters were observed in the tropical North Atlantic Ocean along the 12ºN latitude section. The North Atlantic subtropical gyre and the tropical North Atlantic Ocean showed small contributions of DOC oxidation to apparent oxygen utilization (AOU) (14.1% - 16.5%) indicating that DOC is primarily re-mineralised in the surface waters and the bulk of respiration in sub-surface waters was supported by particulate organic carbon. In the Mauritanian shelf region, high particle sinking rates are primarily responsible for oxygen consumption. The stoichiometric ratios (C:N:P) indicated that the DOM pool in surface waters was enriched in carbon relative to nitrogen and phosphorus, and nitrogen relative to phosphorus, as a result of preferential mineralization of nitrogen and phosphorus relative to carbon in organic matter. The correlation between DOC and DON with density, chlorophyll a, bacterial abundance and DIN revealed that distributions of DOC and DON in the North Atlantic subtropical gyre and in the tropical North Atlantic Ocean were controlled by a combination of physical and biogeochemical processes. However, physical processes, particularly water mixing, appeared to be the main influence. DFAA and DHAA concentrations were below the limits of detection of the optimized method which are 0.07 and 0.66 µM, respectively.

The aerosols were classified into 6 types associated with air mass back trajectory analysis using the HYSPLIT model and according to the colour of the aerosols. The air masses were derived from different source regions; namely the Sahara region, the Sahel and Tropical Rainforest region, Europe and America. The aerosols in the air masses deposited organic carbon of 3.67 mmol/m^2/year, total nitrogen of 11.9 mmol/m^2/year and organic nitrogen 0.47 mmol/m^2/year into the (sub-) tropical North Atlantic Ocean. Air masses transported from the Saharan region supplied the highest LOC and LTN deposition fluxes, while LON fluxes were derived pre-dominantly from air masses of multiple origins including the Saharan and Sahel regions, Europe and North America. Aerosol leaching solutions contained low concentrations of LFAA (< 0.054 nmol/m^3) and LHAA (< 0.64 nmol/m^3). The aerosols from all origins were enriched in organic nitrogen in relation to phosphorus whereas the aerosols originating from the Saharan region were enriched in carbon relative to nitrogen. Relatively short residence times of LOC and LON were observed in the Mauritania Shelf region indicating the presence of rapid biotic removal processes coinciding with an enhanced abundance of heterotrophic bacteria and marine phytoplankton.

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DECLARATION OF AUTHORSHIP

I, Pornsri Mingkwan, declare that the thesis entitled:

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and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

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Acknowledgements

In the first place, I would like to record my sincere thanks to my supervisors Eric Achterberg and Martha Gledhill for the guidance and support from the early stage of the research enabled me to make my work possible. I am grateful to Richard Sanders, the chair of advisory panel, for the valuable advice on this work. I would like to thank Malcolm Nimmo for the review of this Thesis and for the helpful comments.

I would also like to thank all the people involved in the D326 and D338 cruises. Many thanks go in particular to the captain, officers and crews of *RRS Discovery*; Carol Robinson, PSO of the D338 cruise; Matthew Patey, Mark Stinchcombe, Brian Dickie, Claire Mahaffey and Malcolm Woodward for the nutrient and oxygen analysis; Mike Zubkov, Ross Holland and Glen Tarran for the microbial community measurements; Duncan Purdie and Claire Widdicombe for the chlorophyll *a* measurements and Mark Moore for the nitrogen fixation measurements; Vassilis Kitidis for the help with sample collection in the D338 cruise. I gratefully thank Jennifer Andrew, British Oceanographic Data Centre, for providing all the requested data.

I am also indebted to my colleagues at National Oceanography Centre Southampton; Matthew Patey for the pleasure working together, air mass back trajectory analysis and also help improve my writing; Wanwiwa Tumnoi for the image capture from the stereomicroscope; and many others for help, support and being my good friends.

I wish to thank Pornsook Chongprasith, Pollution Control Department, Thailand, for giving me the opportunity to further my knowledge at the PhD level. I gratefully acknowledge Ministry of Science and Technology, Thailand, for the financial support.

Finally, my profound thanks go to my mother Ngamtaa Prarakkamo, my brothers and my sister for their unconditional love, continual encouragement, and persistent confidence in me. And most of all for my loving and patient husband Sarawuth Mingkhwan whose love, dedication and understanding is deeply appreciated.

List of abbreviations

Aada	L- 2- aminoadipic acid
Aada	L- 2- aminoadipic acid
ACN	Acetonitrile
Al	Aluminium
Ala	Alanine
AMT	Atlantic Meridional Transect
AOU	Apparent oxygen utilisation
Apma	2-aminopimelic acid
AQC	6-Aminoquinolyl-*N*-Hydroxysuccunimidyl
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
C	Atmospheric aerosol mean concentration (μmol/m^3)
c	Coarse mode fraction
C18	Octadecyl-bonded silica gel
Ca_{BB}	Calcium related to biomass burning
Ca_{nss}	Non sea-salt aerosol calcium
Cd	Cadmium
CO_2	Carbon dioxide
CRM	Certified reference material
CTD	Conductivity Temperature Depth
CVFC	Cape Verde Frontal Zone
Cys	Cystine
DFAA	Dissolved free amino acids
DHAA	Dissolved hydrolysable amino acids
DIN	Dissolved inorganic nitrogen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EDTA	Ethylenediaminetetraacetic acid
f	Fine mode fraction
F	Dry deposition flux
F_{an}	Dry annual deposition fluxes

Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
HTC	High temperature combustion technique
HYSPLIT	HYbrid Single-Particle Lagrangian Integrated Trajectory
IBLC	*N*-isobutyryl-L-cysteine
IDL	Instrument detection limits
ILD 0.5	Isothermal mixed layer depth of 0.5ºC
Ile	Isoleucine
K	Potassium
K_{BB}	K related to biomass burning
KHP	Potassium Hydrogen Phthalate
L- aba	L- aminobutyric acid
Leu	Leucine
LFAA	Leachable free amino acids
LHAA	Leachable hydrolysable amino acids
LIN	Leachable inorganic nitrogen
LOC	Leachable organic carbon
LON	Leachable organic nitrogen
LPO	Leachable phosphate
LTN	Leachable total nitrogen
Lys	Lysine
M	Inventory
MDL	Method detection limits
Met	Methionine
MLD	Mixed layer depth
MLeu	Methyl Leucine
Mn	Manganese
NCD	Nitrogen chemiluminescence detector
NDIR	Non-dispersive infrared gas analyser
NEC	North Equatorial current
NECC	North Equatorial Countercurrent
NEUC	North Equatorial Undercurrent
NHS	N- hydroxysuccinimide
Ni	Nickel

nNECC	Northern North Equatorial Countercurrent
NO	Nitrogen monoxide
NO_2	Nitrogen dioxide
NO_x	Nitrogen oxides
OPA	Ortho-phthalaldehyde
Orn	Ornithine
Phe	Phenylalanine
PO	Persulfate oxidation
PO_4^{3-}	Phosphate
POC	Particulate organic carbon
Pro	Proline
PTFE	Polytetrafluoroethylene
PV	Potential vorticity
RP-HPLC	Reversed-phase high performance liquid chromatography
Ser	Serine
Si	Silicon
T	Residence time
TDN	Total dissolved nitrogen
TEA	Triethylamine
Thr	Threonine
TOC	Total organic carbon
Tyr	Tyrosine
UV	Ultraviolet oxidation
V	Vanadium
V	Deposition velocity
Val	Valine
VOC	Volatile organic compounds
L-aba	L- aminobutyric acid

Chapter 1

Introduction

1.1 Overview and plan of the thesis

Being one of the largest and least understood pools of organic matter on Earth, marine dissolved organic matter (DOM) has become one of the key topics in marine research. A wide variety of research on marine DOM has been carried out, focusing on various aspects of its composition, distribution and role in marine ecosystems. In this thesis, a study is made of the distribution of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), which are a subset of DOM, in the open ocean and leached aerosol solutions. In addition, method optimization for the determination of dissolved amino acids, a bioavailable form of DOM which naturally exists at nanomolar levels in the open ocean, is performed. There are five chapters in this thesis. *Chapter 1* introduces the roles of DOC and DON in the marine environment, and discusses their removal and production mechanisms. In addition, background information in relation to the study areas is provided. Aims and objectives of the overall PhD study are presented in this chapter. *Chapter 2* investigates DOC and DON distributions in the (sub-) tropical North Atlantic Ocean, including the Mauritanian shelf region. Sample collections were carried out on the *RRS Discovery* during cruise D326 in 2008 and during cruise D338 in 2009. Thus, the most recent data for DOC and DON distributions in the (sub-) tropical North Atlantic Ocean and factors controlling their distribution are reported. It has been reported widely that the (sub-) tropical North Atlantic Ocean receives high atmospheric aerosol inputs from the African continent (Prospero et al., 1996, Guerzoni et al., 1997). Several workers have reported that atmospheric aerosols supply essential nutrients to various marine environments (Krishnamurthy et al., 2010, Jickells et al., 2005, Mahowald et al., 2008). However, the work on atmospheric organic carbon and organic nitrogen is rather limited in the (sub-) tropical North Atlantic Ocean. The need to understand the aerosol-derived organic carbon and organic nitrogen and their influence on ocean biogeochemistry in this region leads to the study reported in *Chapter 3* where measurements of leachable organic carbon (LOC), leachable total nitrogen (LTN) and leachable organic nitrogen (LON) in

atmospheric aerosols are described. The aerosols were collected over the period of a year on the island of São Vicente, Cape Verde, and also during intense African dust events on cruise D326. *Chapter 3* reports not only the concentrations of LOC, LTN and LON in atmospheric aerosols but also describes air mass origins of those aerosol samples and possible impacts of the aerosols on ocean biogeochemistry. *Chapter 4* reports the results of the study on amino acid analysis, a fascinating yet challenging area of research. The methodology for amino acid measurement was optimized using pre-column derivatisation with AQC (6-aminoquinolyl-*N*-hydroxysuccunimidyl) and a binary reversed-phase high-performance liquid chromatography (HPLC) system. The results showed a significant progress on amino acid determination in oceanic samples containing very low amino acid concentrations. Finally, in Chapter 5 overall conclusions for this PhD research bring together the findings of the various studies. Proposals for future research are also presented in this chapter.

1.2 Cycling of DOM in the marine environments

In the open ocean, DOM accumulates mainly in the euphotic zone (Bronk, 2002). Marine plankton (phytoplankton and zooplankton) are the main DOM producers (Sundh, 1992a, 1992b), while heterotrophic bacteria are the main DOM consumers (Azam et al., 1983, Simon et al., 1998). DOM is often sub-divided into its constituent key elements namely DOC, DON and dissolved organic phosphorus (DOP). DOC is a major element of organic matter (Ogawa and Tanoue, 2003). There are several mechanisms through autochthonous and allochthonous processes responsible for DOM accumulation in marine environments (Figure 1.1).

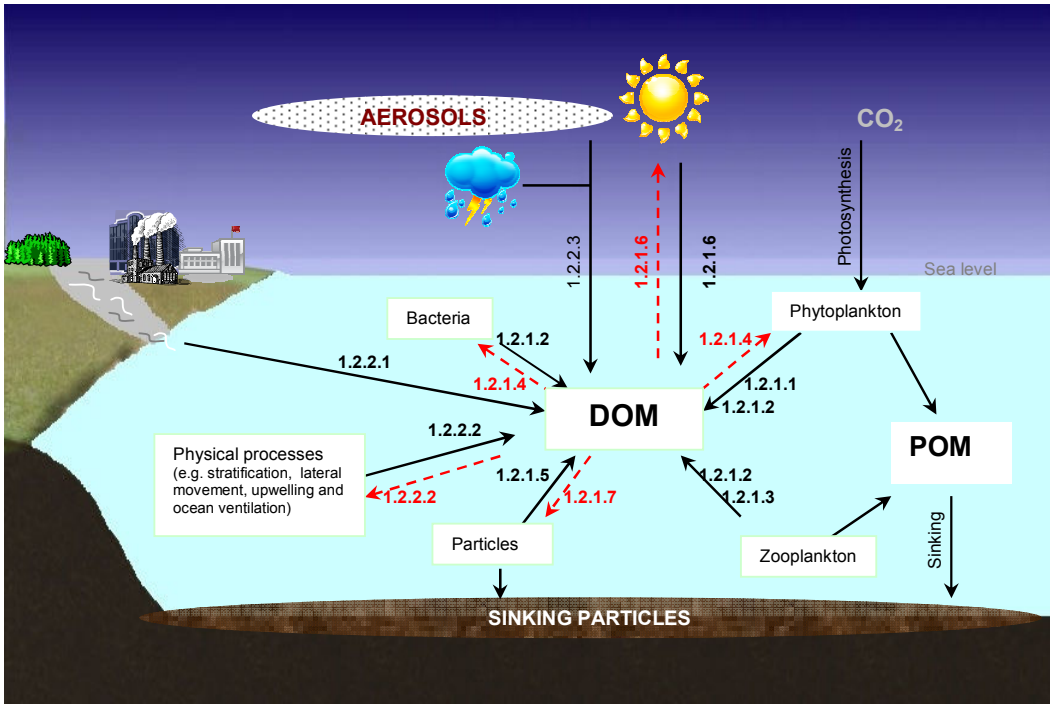


Figure 1.1 Production and removal pathways of DOM in marine system. The dark arrows denote production pathways. The broken arrows associate with removal pathways. The numerals represent the section in the text where the pathway is discussed.

1.2.1 Autochthonous activities

1.2.1.1 Extracellular release

Primary producers introduce DOM into the marine environment via extracellular releases (Zehr and Ward, 2002, Ogawa and Tanoue, 2003, Lapierre and Frenette, 2009) providing 23.1 x 10^{15} g C/year and representing 84.4 % of total organic matter (Millero, 2006). Marine organisms release DOM into the water column through mechanisms of metabolic waste removal and secretion (Bidigare, 1983, Nagata and Kirchman, 1991, Saba et al., 2009) associated with nutritional status. The highest release rates have been observed during the exponential growth phases (Nagata and Kirchman, 1991). These findings are in agreement with Gobler and Sanudo-Wilhelmy (2003) who revealed that DOM is mainly produced during phytoplankton blooms. After the blooming phase, bacterial density markedly increases, while DOM concentrations decrease as a result of bacterial consumption. In addition, elevated concentrations of DON are also observed during the blooming phase of *Trichodesmium*, a nitrogen fixer (Capone et al., 1994, Glibert and Bronk, 1994, Vidal et al., 1999). Approximately 80-90 % of fixed nitrogen is estimated to be released during nitrogen fixation (Mulholland and Bernhardt, 2005). Bronk et al. (1994) suggests 25 - 41% of dissolved inorganic nitrogen utilized by phytoplankton is released as DON in marine environments. In the Atlantic Ocean, Varela et al. (2006) reported that the percentage of bacterial extracellular DON release was within the ranged from 3 to 46% in relation to the gross uptake of ammonium, and within the range from 21% to 82% in relation to total nitrogen uptake.

1.2.1.2 Cell lysis and autolysis (apoptosis)

Release of DOM may occur from dead marine organisms following spontaneous autolysis, and cell lysis as the result of viral and bacterial attack (Fuhrman, 1999, Gobler et al., 1997). Viruses occur at a concentration of 10^6 - 10^8 cells/mL in seawater (Breitbart and Rohwer, 2005). Viruses can directly attack host cells following a lytic cycle in which the host cell is controlled by viral genome and subsequently lysed, releasing new viruses into the environment. Alternatively, viruses reproduce in the host cell by incorporation of its genetic material to the host genome and may remain dormant until the lytic cycle is induced (Middelboe, 2008). Viral lysis plays a significant role in supporting heterotrophic production by supplying bioavailable carbon, particularly in oligotrophic systems. Middelboe (2008) estimated that 5-30% of heterotrophic bacteria and cyanobacteria populations in marine environments are infected by viruses which is comparable to the values reported by Fuhrman (1999), who estimated that 10 - 50% of bacteria mortality is a result of viral infection. Infection by viruses affects approximately 3 - 7% of marine

phytoplankton population (Keller and Hood, 2011). However, phytoplankton cell lysis introduces DOM representing 30 - 70% of the net primary production in the eastern tropical North Atlantic according to Agusti et al. (2001).

1.2.1.3 Sloppy feeding, excretion and leakage from faecal pellet

Zooplankton play a vital role in contributing to the pool DOM in marine environments through various mechanisms (Moller, 2005) such as sloppy feeding, excretion and leakage of faecal pellets (Bronk, 2002, Moller et al., 2003, Moller, 2007). In the oligothophic ocean, Vargas et al. (2007) observed a high bacteria growth associated with copepod grazing. DOM production by sloppy feeding is dependent on size of prey (Moller, 2005) with large amount of DOM produced by zooplankton feeding on a very large prey (Hasegawa et al., 2001, Moller et al., 2003). For instance, copepods lose approximately 54 - 69 % of DOC when the prey is 25 - 30 times smaller (Moller and Nielsen, 2001), while DOC production is not detected when the prey is 39 times smaller than the copepods (Moller, 2007). Faecal pellets are another source of DOM, contributing in particular to the labile fraction (Urban-Rich, 1999). Quality and quantity of food consumed by zooplankton characterises the DOM production by excretion and leakage of faecal pellets. Enhanced dissolved free amino acids (DFAA) have been observed in waters surrounding faecal pellets (Roy and Poulet, 1990). According to incubation experiments with isotope technique, Urban-Rich (1999) observed that 86% of DOC was released from the faecal pellets within 6 hours. Excretion products of gelatinous zooplankton make a significant contribution to the DOM pool in Chesapeake Bay. DOM excreted by gelatinous zooplankton is rich in DOC, which contribute up to 18 - 28 % to labile DOC pool (Condon et al., 2010). Finally, faeces from seabirds seem to be a negligible source of DON for oceanic ecosystems but may be significant in shallow and small freshwater environments (Manny et al., 1994, Berman and Bronk, 2003).

1.2.1.4 Phytoplankton and microbial remineralization

DOM such as amino acids and urea can be consumed directly by bacteria and phytoplankton (Berman and Bronk, 2003). Gobler and Sanudo-Wilhelmy (2003) observed a rapid increase of bacterial densities and a decrease in DOM concentrations during the declining phase of an algal bloom suggesting that DOM may be remineralised by bacterial respiration. This observation agreed with the suggestion by Hansell et al. (2009) that microbial remineralisation is the main removal pathway of DOM. DFAA uptake contributed to 4 - 41% of bacterial nitrogen demand in open ocean environments, while the contribution reached 100% in coastal areas (Kirchman, 2000). Skoog et al. (1999) reported that dissolved glucose supported 5 - 10 % of bacterial production in the surface

water of the Gulf of Mexico, while less than 5% of bacterial carbon demand was supported by dissolved glucose in the Sargasso Sea (Keil and Kirchman, 1999). The uptake of DOM by cyanobateria has also been reported. For example, 25% and 33% of amino acids in surface water are taken up by *Prochlorococcus* in southern subtropical Atlantic (Zubkov et al., 2004) and the Arabian Sea (Zubkov et al., 2003), respectively. *Synechococcus* showed a lower demand (3% uptake) for amino acids in the Arabian sea (Zubkov et al., 2003).

1.2.1.5 Solubilisation of aggregates

Macroscopic organic aggregates (marine snow and lake snow) form colonization sites for autotrophic and heterotrophic bacteria (Grossart and Simon, 1998). DOM can be released from aggregates into the surrounding water as a result of the colonization and solubilisation of aggregates by attached bacteria (Smith et al., 1995, Anderson and Tang, 2010). Hydrolytic ectoenzymes produced by the aggregate-attached bacteria play a vital role in converting particulate organic carbon (POC) to DOC (Smith et al., 1992). Using a simple food web model of the mesopelagic zone, Anderson and Tang (2010) suggested that 70% of DOC production by bacteria in the water column is from the action of hydrolytic enzymes produced by aggregate-attached bacteria, while only 30% of DOC was produced by grazers. Smith et al. (1995) suggested that high ectohydrolase activities of bacteria attached to diatoms indicates the hydrolysis of diatom surface mucus could result in DOC production. However, the influence of hydrolytic ectoenzymes on pathways of organic matter needs further study.

1.2.1.6 Photochemical transformation

Photochemical transformation by UV irradiation in surface waters is a significant mechanism for transforming refractory DOM into more bioavailable forms (Mopper et al., 1991, Benner and Biddanda, 1998, Anderson and Williams, 1999, Vahatalo and Zepp, 2005, Ogawa and Tanoue, 2003). Degradation of DOM through photochemical reactions can increase biologically labile nitrogen by 20% (Bushaw et al., 1996). Photochemical transformation removes 2 - 3% dissolved organic carbon from the oceanic DOC pool per annum (Moran and Zepp, 1997). Vahatalo et al. (2011) suggested that the photochemical transformation not only stimulates auto- and heterotrophic bacteria biomass and production but also transfers photo-derived DOM through a higher trophic level.

1.2.1.7 Sorption onto particles

Absorption to colloids, sub-micron particles, very small particles (e.g. transparent exopolymer particles and Coomassie Blue proteinaceous particles, protein-containing particles) and subsequent particle settling has been proposed as a removal pathway of DOM such as amino acids and proteins (Nagata and Kirchman, 1991, Berman and Bronk, 2003, Schuster et al., 1998, Alldredge et al., 1993). Hansell et al. (2009) estimate that 0.05 Pg C of refractory DOC per annum is removed by interaction with suspended particles. Recently, it was reported that marine gel surfaces, which were made of a three-dimensional polymer network imbedded in water, can possible serve as sites of organic matter absorption (Verdugo et al., 2004).

1.2.2 Allochthonous Activities

1.2.2.1 Fluvial inputs

Rivers deliver significant amounts of organic materials from terrestrial to marine environments (Carlson, 2002, Chen et al., 2004, Jennerjahn et al., 2004, Hawkins et al., 2006, Millero, 2006). The flux of DOM supplied by global fluvial inputs was estimated to be 0.25 Gt per year (Cauwet, 2002). The distribution and composition of fluvial DOM inputs by river vary depending on the nature of the catchment areas and sources (Berman and Bronk, 2003). In the running water of the pristine South American forest regions, DON account for 70 % of the TDN, while DON accounts only 2 % of the TDN observed for the polluted forest areas in North East of USA. Water run-off from urban or suburban areas supply higher bioavailable DON (ca. 59%) than that from agricultural areas (ca 30%) and forests (ca 23%) (Seitzinger et al., 2002). Fluvial riverine DOM is rich in glycine, alanine, aspartic acid and serine (Hubberten et al., 1994, Hubberten et al., 1995, Keil and Kirchman, 1999, Dittmar et al., 2001). Large quantities of DOC are transported to the Arctic Ocean through Arctic rivers (Dittmar and Kattner, 2003) which the majority of fluvial DOC flux occurred in spring freshet (Holmes et al., 2008). In Portugal, the annual DOC flux discharged from Douro River to coastal waters was estimated to be 420 kg C /m^2/ year (Magalhaes et al., 2008).

1.2.2.2 Water mass mixing processes

The variability of DOM accumulation is also controlled by physical forces such as water mass mixing, ocean stratification, Ekman wind-driven transport, meridional overturning circulation and ocean ventilation (Carlson et al., 1994, Suratman et al., 2009, Hansell et al., 2009, Ogawa and Tanoue, 2003, Hansell, 2002). Transfer of DOM into the subtropical gyre by Ekman wind-driven transport has been reported by Mahaffey (2004). Furthermore,

Ekman convergence of surface waters can export DOC accumulated in the subtropical gyres to the other oceanic basins (Hansell et al., 2009). Hansell and Carlson (2001) revealed that reduction in concentrations of total organic carbon (TOC) during winter periods in the Sargasso Sea is caused by convective overturning and mixing of the water column which agreed with the finding by Carlson et al. (1994). Stratification is one of the factors determining the vertical distribution of DOM in the oceans. Relatively high DOC concentrations are present in ocean regions with strong vertical stratification such as tropical and subtropical systems (Hansell et al., 2009), while low surface DOC concentrations are reported in the Sothern ocean where surface water is well mixed with deep water (Ogawa et al., 1999). Surface DOC and TOC concentrations can be affected by the different seawater temperatures. Hansell (2002) reported that relatively low DOC values are generally present in well ventilated, cold waters or in deep waters. For the western Pacific and Indian Oceans, TOC concentrations showed maxima in the warmest areas (Doval and Hansell, 2000).

1.2.2.3 Atmospheric deposition

Atmospheric deposition delivers DOM to open oceans and coastal areas in the form of precipitation, fog and cloud, aerosols, and gases (Cornell et al., 1995, Cornell et al., 2001, Mace et al., 2003a, Mace et al., 2003b, Mace et al., 2003c, Millero, 2006, Cape et al., 2011). It has been reported that atmospheric deposition supplies DON to the marine environment, of which 20 - 75% is in a bioavailable form (Timperley et al., 1985, Peierls and Paerl, 1997, Seitzinger and Sanders, 1999). Rain samples collected on the island of Tasmania contained organic nitrogen representing 25% of total nitrogen, while organic nitrogen in bulk aerosol samples represented 19% of total nitrogen (Mace et al., 2003b). Significant amounts of organic nitrogen were observed in rainwaters, 2.8 - 6.5 µmol/L, and aerosols, 3.3 - 28.5 nmol/m^3, collected in Hawaii (Cornell et al., 2001). Duarte et al. (2006) observed high dry deposition fluxes of organic carbon and total nitrogen in the subtropical northeast Atlantic, supplying on average 980 $\pm$ 220 µmol/m^2/d of organic carbon and 280 $\pm$ 70 µmol/m^2/d of total nitrogen. In the Mediterranean Sea, dry deposition fluxes of organic carbon ranged between 35.7 - 1200 µmol/m^2/d (Pulido-Villena et al., 2008). Temporal variations in dry deposition fluxes of organic nitrogen have been observed in the Eastern Atlantic Ocean, with a deposition of 34 µmol/m^2/d in June 1996, and 95 µmol/m^2/d in May 1997 (Spokes et al., 2000).

1.3 Classification of DOM

Dissolve organic matter in marine system can be divided into three categories, based on their biological residence times. There are labile, semi-labile and refractory fractions (Hansell et al., 2009, Amon and Benner, 1996). Figure 1.2 demonstrates typical vertical distribution of DOC in the ocean in relation to conceptual classification of biological resistance. The labile fraction such as DFAA and neutral monosaccharides (Carlson, 2002) represents the fraction which is rapidly oxidised within minutes to days by bacteria (Ogawa and Tanoue, 2003). This fraction can be utilized directly by primary producers (Bronk, 2002, Berman and Bronk, 2003, Bronk et al., 2007). The labile fraction accounts for 0-6% of the bulk DOM pool (Carlson and Ducklow, 1995, Cherrier et al., 1996) and is present at nanomolar concentrations in open oceans (Keil and Kirchman, 1999, Skoog et al., 1999, Keil and Kirchman, 1991, Kuznetsova and Lee, 2002, Yang et al., 2009). The most biologically resistant or so called “refractory territorial” fraction has undoubtedly the longest turnover time and represents the largest fraction of the DOC pool (Carlson, 2002), with an average residence time of hundreds to thousands of years (Ogawa and Tanoue, 2003). This fraction can be transformed to be partially bioavailable following photodegradation and bacterial degradation processes (Bronk et al., 2007). According to Druffel et al. (1992), the DOM refractory fraction exists uniformly throughout the water column so deep ocean DOC stocks are most representative of this biological refractory pool (Williams and Druffel, 1987, Bauer et al., 1992). The fraction with intermediate biological resistance is termed semi-labile. The semi-labile pool accumulates predominantly in the upper 500 m of the water column with removal time scales ranging from one year to several decades (Hansell et al., 2009).

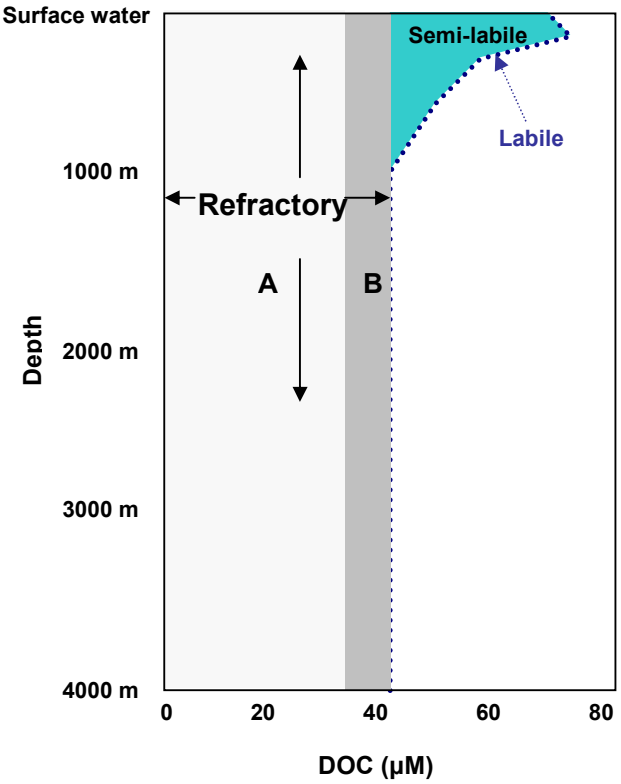


Figure 1.2 Conceptual classifications of refractory, semi-labile and labile DOC in open oceans reproduced from Carlson (2002) and Ogawa and Tanoue (2003). According to Hansell and Carlson (1998), the refractory pool A (light grey box) represents refractory DOC which turns over on time scales greater than that of ocean mixing (34 μM) and the refractory pool B (dark grey box) represents the fraction of the refractory pool that turns over on faster time scales than ocean mixing.

1.4 Dry atmospheric inputs of dissolved organic carbon and dissolved organic nitrogen

The review in this section focuses on dry atmospheric input rather than wet atmospheric input owing to the study region being characterised as having a dry tropical climate and subject to frequent droughts. Cape Verde, where aerosol sampling was performed, has a mean annual rainfall of 225 mm (Brooks, 2008).

1.4.1 Sources

Atmospheric aerosols originate from various natural and man-made sources. Size distributions of aerosol particles are generally arbitrary divided into 2 fractions: fine-mode aerosols (< 1 μm in diameter) and coarse-mode aerosols (> 1 μm in diameter) (Baker et al., 2010). Fine-mode aerosols provide slower dry deposition rates and can be transported further away from the origins than those in the coarser fraction (Cornell et al., 2001). Organic carbon and organic nitrogen-derived aerosols are generated by several sources including marine aerosols (Kuznetsova et al., 2005), deserts (Griffin et al., 2001, Mace et al., 2003c), biomass burning and other agricultural activities (Baker et al., 2006, Baker et al., 2010, Duan et al., 2004), combustion processes (Andreae et al., 1998, Liousse et al., 1996, Zheng et al., 2005), industrial and fossil fuel combustions (Cachier et al., 1995, Lewandowska et al., 2010) and forests (Graham et al., 2003).

Organic matter is present in both primary and secondary aerosols (Duan et al., 2004). The former is produced directly at its origin; for example fly ashes, sea-salt particles emitted by the ocean at the sea surface, plant pollens, leaf fragments and mineral dust (Haywood and Boucher, 2000, Duan et al., 2004, Fermo et al., 2006). Secondary organic aerosol are created through the oxidation of high molecular weight volatile organic compounds to form low-volatile organic products (Seinfeld and Pandis, 1997), particle phase heterogeneous reactions (Jang et al., 2003, Jang and Kamens, 2001), gas-particle partitioning of semi-volatile organic aerosols (Robinson et al., 2007) and also other atmospheric dynamic processes which change the physical and chemical characteristics of aerosols after their emission from combustion sources (Ning and Sioutas, 2010).

1.4.1.1 Natural sources

1.4.1.1.1 Marine

Marine aerosols consist of two aerosol types which are primary sea-salt aerosols and secondary aerosols. Marine aerosols are present in bimodal size distributions (Miyazaki et al., 2010). Primary sea-salt aerosols are generated through the mechanical disruption of

the ocean surface through processes such as the bursting of air bubbles at the sea-surface microlayer. Primary sea-salt aerosols are typically present in the coarse-mode fraction lying in the range of 1-100 μm (Odowd et al., 1997, Kuznetsova et al., 2005, Gorzelska and Galloway, 1990). Secondary marine aerosols are formed by gas-to-particle conversion processes such as binary homogeneous nucleation, heterogeneous nucleation and condensation, which are generally present in environments in the nanometre size range, although particle sizes can increase through condensation and heterogeneous conversion processes (Twomey, 1977). Relatively high organic carbon and nitrogen concentrations have been observed in marine aerosols collected in the ocean regions with high biological productivity (Miyazaki et al., 2010). Furthermore, it has been reported that marine aerosols are enriched in DFAA and dissolved combined amino acids (DCAA) (Kuznetsova et al., 2005). These authors reported that marine aerosols collected at Stony Brook Harbour contained a low D/L ratio reflecting the amino acids were generated by phytoplankton rather than by bacteria which is in agreement with Wedyan and Preston (2008) who suggested that approximately only 8 % of leachable free amino acids derive from bacteria sources.

1.4.1.1.2 Desert

Mineral dust emitted by desert regions represents about 45% of the global aerosol emission (Andreae, 1995). Global dust models show that the Sahara desert is the greatest contributor to the global dust budget accounting for 62% of the total global dust load to the atmosphere, while dust generated from deserts in East Asia account for 6% of the total global dust load (Tanaka and Chiba, 2006). Therefore, Saharan dust is critically important for the deposition of potential essential nutrients to surface seawater (Duce et al., 1991). Most Saharan mineral particles are transported directly to the northern tropical and subtropical Atlantic (Romero, 1999) influencing oceanic ecosystems as they contain bioavailable nutrients such as iron, nitrate, phosphate (Baker et al., 2003, Moore et al., 2009, Mahowald et al., 2009). Contributions of Saharan dust to dissolved organic matter depositions have been reported (e.g. Violaki et al., 2010; Baker et al., 2010). Griffin et al. (2001) has observed a connection between primitive biological organisms, bacteria and fungi, and African dust which is a likely source of amino acids (Mace et al., 2003c).

1.4.1.1.3 Forest (Biogenic aerosols)

Biogenic sources such as plant spores, pollens or soil organic matter have been observed to be important in tropical regions. Graham et al. (2003) identified sources of Amazonian aerosols using the composition of organic compounds and reported that Trehalose,

arabitol, mannitol and fatty acids were detected at night-time possibly contributed by yeasts and small fungal spores, while glucose, fructose and sucrose during the daytime are connected to the release of pollen, fern spores, pollen grains and other bio-aerosols.

1.4.1.2 Man-made sources

1.4.1.2.1 Biomass burning and agricultural activities

Biomass burning introduces significant amount of aerosols and gases containing organic matter into the atmosphere (Mace et al., 2003a). Biomass burning is a significant activity at low latitudes and introduces primary carbonaceous aerosols to the atmosphere and marine environment (Liousse et al., 1996, Zheng et al., 2005, Sapkota et al., 2004). For instance, the North Atlantic Ocean receives significant aerosol deposition originating from agricultural biomass burning in Africa. The estimation of the biomass burning emissions of organic carbon and black carbon in Sahel/Savanna region was 0.35 $\pm$ 0.09 Tg, contributing 2.2% to the global emission (Vermote et al., 2009). Furthermore, Ansmann et al. (2009) observed, using a Lidar observation system, long–range transport of aerosol material, potentially of biomass burning origin, over the Atlantic Ocean towards the Amazon basin. Biomass burning is a probable source of urea to the Amazon basin (Mace et al., 2003a), while in Beijing biomass burning introduces significant atmospheric organic carbon (Zheng et al., 2005). The organic carbon emitted during biomass burning is swiftly converted into a hydrophilic form (Ducret and Cachier, 1992, Hurst et al., 1994). Biomass burning originating from tropical forests releases large quantities of particles and gases to the atmosphere (Andreae, 1991, Houghton and Skole, 1990). As carbon accounts for approximately 40 % of total tropical forest biomass (Houghton and Skole, 1990), the particles potentially contain organic carbon. Besides biomass burning, agricultural activities such as cattle and sheep farming are possible sources of the organic nitrogen contained in the aerosols collected at Cape Grim, Tasmania (Mace et al., 2003b).

1.4.1.2.2 Industrial and fossil fuel combustion

Combustion sources such as vehicle emissions, wood smoke, oil refineries and power plants represent some of the most significant sources of primary aerosols (Duan et al., 2004, Ning and Sioutas, 2010). Disselkamp et al. (2000) suggested that approximately two thirds of carbonaceous aerosol emitted from combustion processes are organic carbon, in agreement with Cornell et al. (2003) who suggest that approximately 70% of carbonaceous aerosols emitted from combustion sources were reactive organic carbon compounds and only up to 4 % of aerosols generated by combustion sources emitted as organic nitrogen (Cachier et al., 1995). However, an even higher proportion of reactive

organic carbon is observed in the emissions from biomass burning than those from industrial combustion sources (Cachier et al., 1995).

1.4.2 Influence of atmospheric aerosol

Atmospheric aerosols contain various nutrients and trace metal constituents depending on their origins and sources (Mahowald et al., 2005, Cornell et al., 2001, Herut et al., 2002). Organic compounds derived from aerosols have been found to be bioavailable (urea, amines and amino acids) and/or bio-toxic (N-substituted polycyclic aromatic hydrocarbons) (Cornell et al., 2003, Reche et al., 2009). Effects of atmospheric aerosol deposition on marine and freshwater biogeochemistry have been documented. Duarte et al. (2006) observed an increase in oceanic phytoplankton biomass and production associated with aerosol inputs in subtropical northeast Atlantic. In addition, the deposition of Saharan dust is reported to stimulate bacterial growth and abundance in Mediterranean freshwater systems (lakes and reservoirs) (Reche et al., 2009). Atmospheric organic carbon contributed up to 18% of the total carbon flux in the coastal zone of the southern Baltic Sea, which receives air masses transported over agricultural and industrial centres (Lewandowska et al., 2010). Atmospheric deposition constitutes a significant source of phosphorus and consequently has been found to affect primary producers (Seitzinger and Sanders, 1999) and heterotrophic communities in the western Mediterranean (Pulido-Villena et al., 2008). Furthermore, Eurasian atmospheric aerosols transport significant amounts of nitrogen and phosphorus containing aerosols to Sea of Japan, Seto Inland Sea, and the East China Sea (Hartmann et al., 2008). Herut et al. (2002) reported deposition of nitrogen to surface waters in the southeast Mediterranean. Besides adding bio-available nutrients and trace metals into the ocean environments, atmospheric aerosols also physically interfere with solar radiation and change the structure of clouds leading to hydrological system alteration (Graham et al., 2003). It has been reported that carbonaceous aerosols (black carbon and organic carbon) absorb and scatter solar radiation (Haywood and Boucher, 2000). In addition, atmospheric aerosols are of concern as an air quality and human health problem (Schlesinger et al., 2006, Prospero, 1999, Griffin et al., 2007, Laitinen et al., 2010).

1.5 The (sub-) tropical North Atlantic Ocean

The North Atlantic subtropical gyre is characterised as an oligotrophic region exhibiting low surface water nutrient concentrations and low primary productivity (chlorophyll *a* <0.25 mg/m^3) (Poulton et al., 2006, Rees et al., 2006, Dixon, 2008, Kahler et al., 2010). The tropical North Atlantic Ocean is considered as a productive region (chlorophyll a > 0.25 mg/m^3) (Varela et al., 2006). The Mauritanian coastal area is classified as one of the most biologically productive upwelling regions in the world ocean (Schafstall et al., 2010). The Mauritanian upwelling system is driven by north-easterly trade winds blowing along the coast. The upwelling off African coast between 20ºN and 25ºN is present throughout the year, while upwelling system off Mauritanian displays the strongest activity in winter and spring (Mittelstaedt, 1991, Schafstall et al., 2010). In addition, these regions receive massive dust inputs from the Sahara desert and Sahel region (Prospero, 1996 and Guerzoni et al., 1997) (Figure 1.3) which are the sources of the world's highest concentrations of aeolian soil dust (Goudie and Middleton, 2001). Furthermore, these regions are located downwind of highly industrialised continents (Zamora et al., 2010) and also influenced by biomass burning emissions in Africa (Vermote et al., 2009).

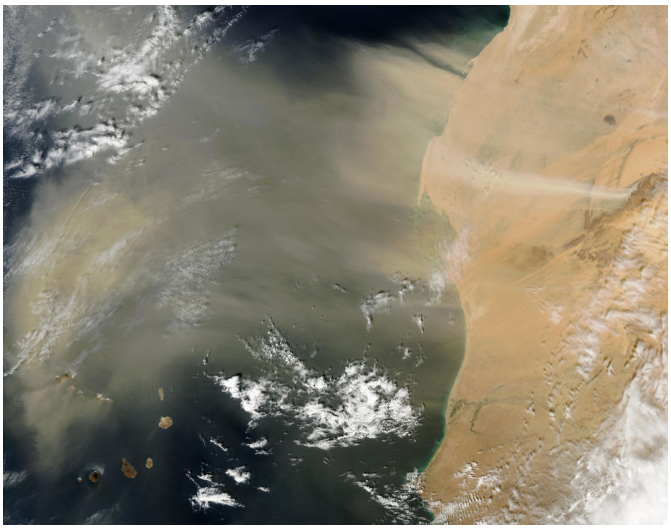
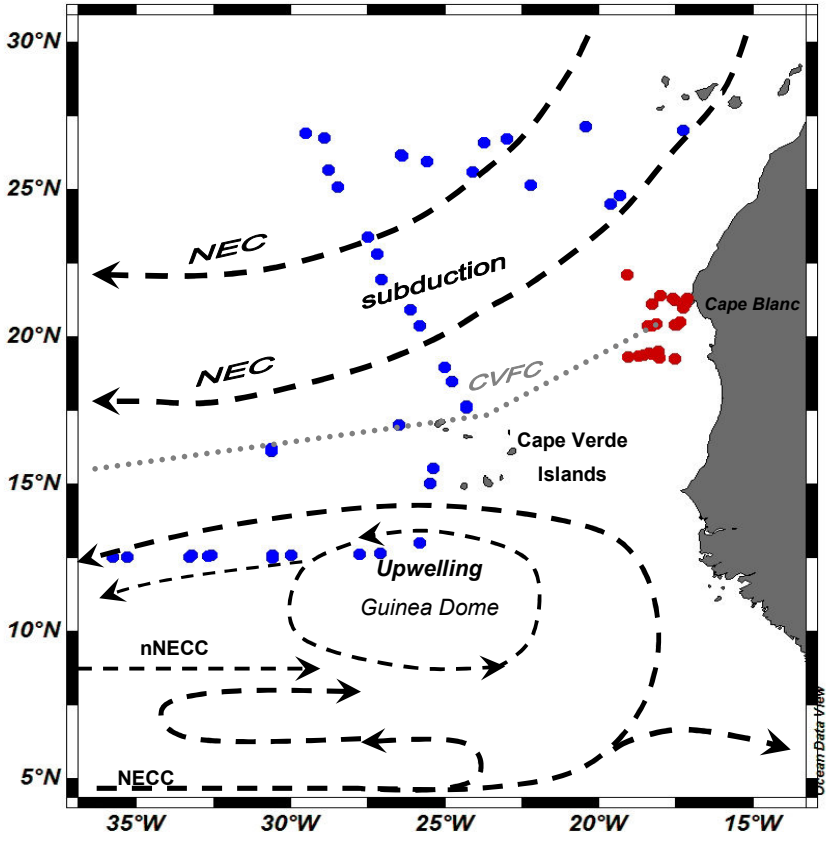


Figure 1.3 Dust transported from the African continent over the (sub-) tropical North Atlantic Ocean. The image obtained from http://lance-modis.eosdis.nasa.gov.

Water current measurements and hydrographic observations in the tropical eastern North Atlantic have been recently reported by Stramma et al. (2005, 2008). Figure 1.4 demonstrates water currents in the (sub-) tropical northeast Atlantic. The North Equatorial current (NEC) forms in the north of Cape Verde Islands where subduction takes place. The tropical eastern Atlantic is physically governed by several oceanographic systems.

There are two current systems which form the southern part of a tropical gyre including the North Equatorial Countercurrent (NECC) and the North Equatorial Undercurrent (NEUC). The water masses subducted in the eastern subtropical region and inserted into the thermocline by Ekman transport are characterised by a potential vorticity (PV) minimum (Schott et al., 2004). The water masses in this region are characterized by tropical oxygen minimum zone which appear to have expanded and intensified. Stramma et al. (2008) observed that integrated oxygen in the tropical North Atlantic between 10º to 14ºN and 20º to 30ºW has been declining at a rate of 0.34 ± 0.13 µmol/kg/year since 1996. A strong exchange between the Northern North Equatorial Countercurrent (nNECC), which carries oxygen rich water from the southern hemisphere, and NECC is present in the region between 22ºW and 32ºW (Stramma et al., 2005). Upwelling exists in the region near the coast of Mauritania and Senegal and in the Guinea Dome south of the Cape Verde Islands (Schott et al., 2004, Schafstall et al., 2010).



NEC : North Equatorial current **nNECC : Northern North Equatorial Countercurrent**

NECC : North Equatorial Countercurrent **CVFC : Cape Verde Frontal Zone**

Figure 1.4 Water currents in the upper tropical northeast Atlantic off northwest Africa. Figure is modified from Stramma et al. (2005). The blue dots denote the sampling stations during cruise D326 and the red dots denote the sampling stations during cruise D338.

1.6 Aims and objectives

DOC and DON are considered to be important sources of bioavailable carbon and nitrogen for marine phytoplankton and bacteria (Berman and Bronk, 2003) and the role of DOC and DON in the ocean biogeochemistry has been a challenging topic for marine scientists for more than two decades. In the Atlantic Ocean, the distributions of DOC and DON have been examined from a number of different points of view (e.g. Vidal et al, 1999, Kahler and Koeve, 2001, Robinson et al. 2006, Torres-Valdes et al., 2009). However, there are no adequate, up-to-date data on the distribution of DOC and DON in the (sub-) tropical North Atlantic Ocean, an area which is strongly impacted by atmospheric dry deposition of aerosols from Africa and industrial continents. This project therefore aims to investigate the distribution of DOC and DON including amino acids in the (sub-) tropical North Atlantic Ocean, and also to determine the role of atmospheric dry deposition in supplying organic carbon and nitrogen to the region.

The principal research objectives of the project are:

- To determine the distribution of dissolved organic carbon and dissolved organic nitrogen and the factors controlling their distributions in the (sub-) tropical North Atlantic Ocean and ,
- To determine the magnitude of organic carbon and nitrogen supplied by atmospheric aerosols in the air masses transported over the (sub-) tropical North Atlantic Ocean,
- To optimise a method to determine dissolved amino acids at low concentrations using reversed-phase high-performance liquid chromatography with a binary gradient system.

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Chapter 2

Distributions of Dissolved Organic Carbon and Dissolved Organic Nitrogen in the (sub-) tropical North Atlantic Ocean

Abstract

Samples from water column profiles collected during two cruises in the (sub-) tropical North Atlantic Ocean and the Mauritanian shelf region were analyzed to investigate the distributions of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in relation to hydrological and biogeochemical measurements. DOC and total dissolved nitrogen (TDN) were determined using a high temperature combustion technique (HTC). DON values were derived as the difference between TDN and dissolved inorganic nitrogen (DIN). In general, elevated DOC and DON values were present in surface waters and the values decreased with increasing depth. Mean surface water DOC and DON concentrations ranged from 67.2 μM to 72.3 μM, and 4.6 μM to 8.1 μM respectively. Enhanced DON values in sub-surface waters were observed in the tropical North Atlantic Ocean along the 12ºN latitude section. Contributions of DOC oxidation to apparent oxygen utilisation (AOU) in sub-surface water were low, ranging from 14.1% in the North Atlantic subtropical gyre and 16.5% in the tropical Atlantic Ocean, implying that DOC is primarily re-mineralised in the surface waters and the bulk of respiration in sub-surface waters was supported by particulate organic carbon (POC). There was no statistical correlation between DOC and AOU in sub-surface waters at the Mauritanian shelf region leading to the assumption that in this region high particle sinking rates are primarily responsible for the observed AOU. The surface water C:N molar ratios of the bulk organic pool ranged from 9.0 to 14.8 in our study region, which deviated from the canonical Redfield ratio of 6.6. The C:N molar ratios increased with increasing depths indicating that the more refractory DOM is carbon rich. The surface water C:P molar ratios of bulk organic pool varied between 241 and 620, while N:P molar ratios ranged from 26.9 to 42.4. The stoichiometric patterns indicated that dissolved organic matter (DOM) pool in surface waters was enriched in carbon relative to nitrogen and phosphorus, and nitrogen

relative to phosphorus, as a result of preferential mineralization of N and P relative to C in organic matter. Distributions of DOC and DON in the North Atlantic subtropical gyre and in the tropical North Atlantic Ocean were controlled by a combination of physical and biogeochemical processes. However, physical processes, particularly water mixing, appeared to be more important according to the covariance of DOC and DON with density. There was no covariance observed of DOC and DON with other variables in the data set collected in the Mauritanian shelf region.

2.1 Introduction

DOM plays a vital role in chemical and biological oceanography (Ogawa and Tanoue, 2003). Dissolved organic matter is sub-divided into labile, semi-labile and refractory fractions in relation to their turnover times (Hansell et al., 2009). The labile fraction has rapid turnover time, within minutes to days, while the refractory fraction has a longest turnover time, with an average of hundreds to thousands of years (Ogawa and Tanoue, 2003). The semi-labile fraction has intermediate removal time scale ranging from a year to decades (Hansell et al., 2009). In seawater, the refractory fraction dominates (Hansell and Carlson, 2002), accounting for approximately 70% and 60% of the DOC and DON pools, respectively, in the Mid-Atlantic Bight (Hopkinson et al., 2002). Although labile DOC is part of an important biological carbon flux in marine waters, the pool of labile DOC represents less than 1% of the total DOC pool in the ocean as a result of its rapid turnover rate (Hansell et al., 2009). The semi-labile DOC fraction makes up nearly 40% of the total DOC pool and is primarily responsible for the variations in DOC concentration in oceanic waters.

DOM provides bioavailable carbon (Moran and Hodson, 1990) and nitrogen to phytoplankton and bacteria (Berman and Bronk, 2003). Dissolved organic nutrients are introduced into water column through a variety of mechanisms. Marine organisms release DOM into water column through metabolic waste removal and secretion (Nagata and Kirchman, 1991, Saba et al., 2009). The release of DOM by microplankton is associated with nutritional status, with highest release found during exponential growth (Nagata and Kirchman, 1991). Enhanced DOC concentrations can furthermore be linked to excess carbon released from phytoplankton as a result of inorganic nitrogen deficiency (Williams, 1995). Another mechanism involves the production of DOM from dead marine organisms by spontaneous autolysis and cell lysis resulting from viral and bacterial attack (Fuhrman, 1999, Gobler et al., 1997). Furthermore, DOM can also be introduced into marine waters by “sloppy feeding” by zooplankton (Bronk, 2002). DOM such as porin P, exopolymers, peptidoglycan and hydrolytic enzymes can be released directly by bacteria (Tanoue et al., 1995, McCarthy et al., 1998). Allochthonous DOM inputs including atmospheric deposition deliver DOM to open ocean and coastal areas (Cornell et al., 1995, Cornell et al., 2001, Mace et al., 2003a, Mace et al., 2003c, Mace et al., 2003b, Millero, 2006). In addition, fluvial inputs form another significant source of DOM, transporting organic compounds from terrestrial environments to coastal waters (Carlson, 2002, Chen et al., 2004, Jennerjahn et al., 2004, Hawkins et al., 2006, Millero, 2006).

There are two main removal mechanisms of DOM from marine waters: biotic and abiotic removal. The first mechanism is recognised as the dominant sink of DOM and involves DOM utilisation by prokaryotes (Carlson, 2002). Heterotrophic bacteria are major consumers of DOM in the ocean (Berman and Bronk, 2003, Garcia et al., 2005, Hansell and Carlson, 2001). POC and DOC fluxes to waters below the surface mixed layer support respiration of heterotrophic bacteria (Aristegui et al., 2002). The correlation between DOC and AOU has been used as a measure of the contribution of DOC to respiration. Aristegui (2002) observed a small contribution of DOC (8.4%) towards respiration processes in the global mesopelagic ocean. However, the contribution varies from one ocean region to another. For example, Thomas et al. (1995) in the equatorial Atlantic Ocean suggested that approximately 20% of DOC contributed to microbial respiration, while in the North Pacific Ocean up to 82% of DOC supported microbial degradation (Druffel et al., 1992). Pan et al. (in Press) reported that the contribution of DOC to AOU in the Atlantic Ocean decreased towards the equatorial regions. Furthermore, by applying an isotope dilution approach, Rosenstock and Simon (2001) observed that amino acids and proteins in Lake Constance were utilised mainly through bacterial consumption. These amino acids and proteins supported 58% and 80% of carbon and nitrogen demand of bacteria, respectively. In some regions, such as the Baltic Sea (Berg et al., 2001), Georges Bank (Berman and Viner-Mozzini, 2001) and Lake Kinneret (Townsend and Thomas, 2002), DON supplies nitrogen nutrition that can lead to blooms of phytoplankton.

The second abiotic removal mechanisms involve photochemical transformation and sorption onto particles. Photo-degradation of DOM generates labile nitrogen compound such as ammonium and amino acids (Moran and Zepp, 2000). Photochemical transformation removes 2-3% dissolved organic carbon from the oceanic DOC pool per annum (Moran and Zepp, 1997). According to Nagata and Kirchman (1991) and Schuster et al. (1998), absorption to colloids and particles and subsequent particle settling is considered a removal pathway for significant amounts of organic compounds such as amino acids and proteins (Berman and Bronk, 2003).

Distributions of DOM in the ocean are regulated by several biogeochemical processes as mentioned above in addition to physical factors such as ocean stratification, Ekman wind-driven transport, meridional overturning circulation and ocean ventilation (Carlson et al., 1994, Suratman et al., 2009, Hansell et al., 2009, Ogawa and Tanoue, 2003). Generally, relatively low DOC values are present in well ventilated, cold waters or in deep waters (Hansell, 2002). According to Mahaffey et al. (2004), the lateral transfer of DOM plays a

role in balancing the loss of nitrate and phosphate over the North Atlantic subtropical gyre implied from the inverse studies by Rintoul and Wunsch (1991) and Ganachaud and Wunsch (2002), and maintaining the level of export production. DOM is transported northwards from the equator to the North Atlantic subtropical gyre through the Ekman wind-driven circulation and overturning circulation (Roussenov et al., 2006). In the northern South China Sea, Hung et al. (2007) investigated how DOM values were linked to physical processes by considering the statistical correlation with density, temperature and salinity, and investigated biological effects by considering the significant correlation between DOC and chlorophyll *a*.

The North Atlantic subtropical gyre is characterised as an oligotrophic region (Poulton et al., 2006, Rees et al., 2006, Dixon, 2008, Kahler et al., 2010), while the tropical North Atlantic Ocean is considered a productive region (Varela et al., 2005). The Mauritanian shelf region is described as an upwelling region (Schafstall et al., 2010) where the upwelling is driven by north-easterly trade winds blowing along the coast. The upwelling system south of 20ºN displays the strongest activity in winter and spring, while the upwelling system north of 20ºN is pronounced all year round (Mittelstaedt, 1991). These areas receive significant atmospheric dust inputs from the Saharan desert and Sahel region (Prospero et al., 1996, Guerzoni et al., 1997), the world's largest source of aeolian soil dust (Goudie and Middleton, 2001). Atmospheric deposition has been recognised as a source of essential nutrients (Jickells et al., 2005, Krishnamurthy et al., 2010) such as nitrogen, iron, phosphorus and silicon, controlling marine phytoplankton production which is one of the key sources of DOM in marine environments. The main aim of this study is to investigate the distributions of DOC and DON in the (sub-) tropical North Atlantic Ocean including the Mauritanian shelf region and to determine the processes controlling their distributions.

2.2 Methods

2.2.1 Sampling areas

The study region covers an area between 12ºN - 27ºN and 17ºW - 36ºW, which is subject to African continent dust inputs, and between 19ºN - 22ºN and 17ºW - 19ºW, which is influenced by the Northwest African upwelling system. Seawater sampling was performed on two cruises onboard the *RRS Discovery*. The sampling was conducted in January and February 2008 on cruise D326 and in April and May 2009 on cruise D338. The D326 and D338 were undertaken as part of the international SOLAS project (Surface Ocean – Lower Atmosphere Study, www.solas-int.org) which aims to achieve understanding of the biogeochemical-physical interactions between the ocean and atmosphere, and how this coupled system affects and is affected by climate and environmental change. The main objective of D326 cruise is to improve understanding of the atmospheric transport, cycling and deposition of dust and nutrients into the North Atlantic including the consequence of the dust inputs on the surface microbial community (Rijkenberg and Achterberg, 2008), while the main objective of D338 is to determine the impact of coastal upwelling on the microbiological activity in the ocean and chemical interactions between the ocean and the atmosphere (Robinson, 2009).

The sampling stations and cruise track on D326 were decided on the basis of emerging dust plumes during the cruise period. In this study, the results are reported based on topographic and hydrographic conditions and have been split into three areas, namely the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region (Figure 2.1).

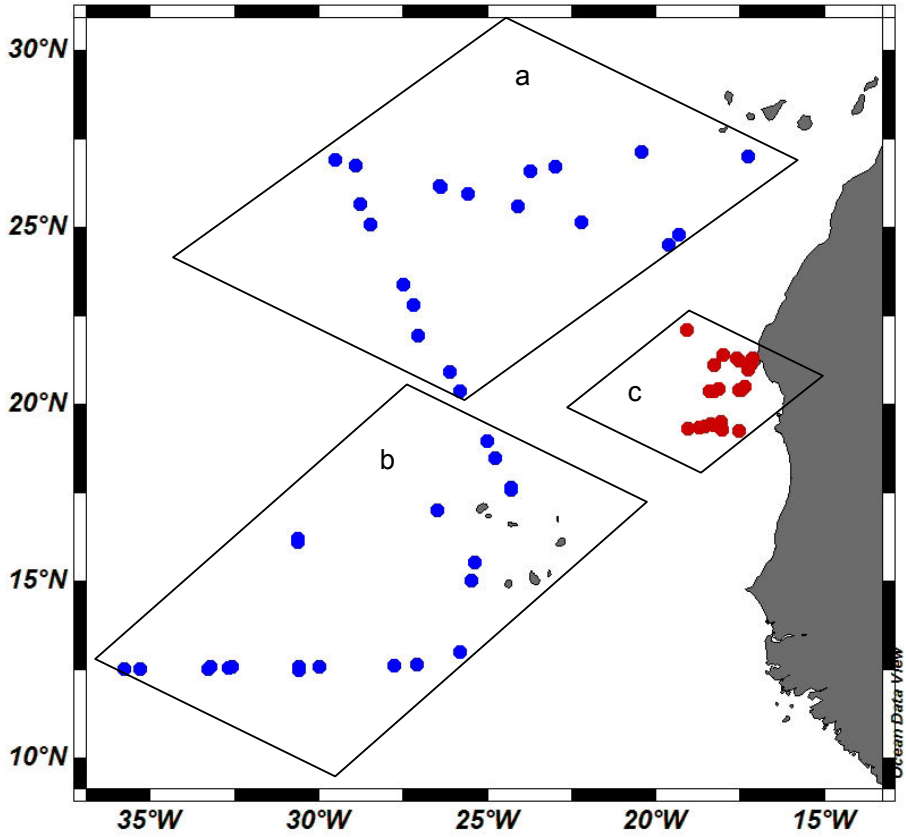

Figure 2.1 Sampling stations in a) the North Atlantic subtropical gyre between 20.4ºN - 26.9ºN and 17ºW – 29.5ºW, b) the tropical North Atlantic Ocean between 12ºN - 18.9ºN and 24.2ºW – 35.8ºW and c) the Mauritanian shelf region between 19.2ºN - 22.1ºN and 17.1ºW – 19.1ºW. The blue dots denote the sampling stations during cruise D326 and the red dots denote the sampling stations during cruise D338.

2.2.2 Seawater sample collection

Seawater collection for DOC and TDN was conducted using a stainless steel CTD rosette frame with 24 Niskin (General Oceanics, 20 L) bottles. Seawater profiles were collected from the surface to a depth of 300 metres in the (sub-) tropical regions. For the upwelling area, seawater profiles were collected from surface up to 50 - 200 metres. The samples were filtered through pre-combusted (450ºC, 4 - 6 h) glass fibre filters (MF300 Fisher; nominal pore size 0.7 µm) and transferred into clean glass ampoules (combusted in a furnace by combustion at 450ºC for 4 - 6 h). After acidifying to pH 2 with 50% v/v hydrochloric acid (final concentration was 0.1% v/v, analytical reagent grade), the ampoules were flame-sealed using a butane/propane burner and stored in a refrigerator at 4ºC until analysis. The reason for acidification is to prevent further biological processes from occurring in the samples and to convert inorganic carbon to CO_2.

2.2.3 Chemical Analysis

2.2.3.1 DOC and TDN measurements

DOC and TDN in seawater samples were analysed using HTC technique (Badr et al., 2003). The analyses were undertaken using a coupled system comprising of a Shimadzu TOC 5000A and an Antek model NCD 705D total nitrogen analyser (Figure 2.2). The method is used to analysed organic carbon concentration ranging from 0.0 to 250 µM, with the detection limit of 0.33 µM. Principally, the acidified seawater is purged by using high purity oxygen in order to drive off the inorganic carbon contained in the acidified seawater sample. The acidified and purged sample is then injected into a total carbon combustion tube filled with an oxidation catalyst (Pt) and heated to 680ºC where CO_2 is consequently produced. High purity oxygen is supplied into the combustion tube as combustion and carrier gas at a flow rate of 150 mL/min. The carrier gas together with combusted products from the combustion tube are dehumidified and purified prior to moving to a non-dispersive infrared gas analyser (NDIR) where the CO_2 is detected. For TDN determination, nitrogen monoxide (NO) contained in the combustion gas flows from the NDIR into a nitrogen chemiluminescence detector (NCD) facilitated by a vacuum pump. The excited nitrogen dioxide (NO_2) species produced by the reaction between the NO and ozone in the instrument are detected in the NCD by emission of photons during their return to the ground-state. This signal provides us with the measurement of TDN. A summary of instrumental conditions is presented in the Table 2.1. Dissolved organic nitrogen (DON) was derived as the difference between TDN and DIN (ammonium and nitrite + nitrate) according to the following equation.

$$DON = TDN - (NH_4 + NO_2 + NO_3)$$

Standard calibration curves of DOC and TDN were generated using mixtures of Potassium Hydrogen Phthalate (KHP) and glycine. Fresh deionised water was used as a system blank to evaluate the system contamination. For this, fresh deionised water was acidified and analysed as a sample. The performance of the HTC technique and the accuracy of the measurement were verified with a certified reference material (deep Sargasso seawater CRM) obtained from the Hansell laboratory, University of Miami (Badr et al., 2003). The CRM was analysed with every sample run. The run started with standard solutions, a system blank and CRM analysis. Then 10-12 samples were analysed, followed by another system blank. This sequence was repeated until all samples for that run were analysed. The run ended with a system blank and CRM analysis. The analyses of the deep Sargasso seawater for average carbon and nitrogen were 41.8 $\pm$ 3.8 µM and 33.1 $\pm$ 2.0 µM respectively which were in good agreement with

the certified values (41 - 43 μM for C and 32.25 - 33.75 μM for N Batch 8). Negative DON derived from the difference between TDN and DIN at a number of the North Atlantic Ocean stations indicates the error of measurement of the analytical system (approximately 0.58 μM) is greater than the concentration of DON. The negative DON concentrations in the data set were not included in any calculation.

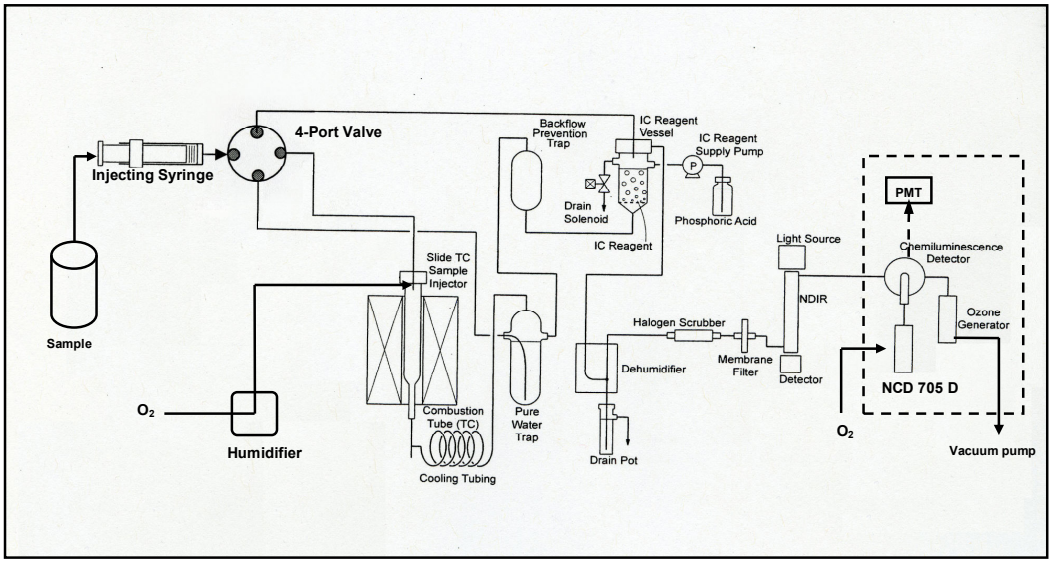

Figure 2.2 Schematic diagram of the Shimadzu TOC 5000A total organic carbon analyser coupled with an Antek NCD 705D nitrogen chemiluminescence detector using a high temperature combustion technique (HTC) (adapted from Badr et al. (2003)).

Table 2.1 Instrumental conditions of the Shimadzu TOC 5000A and an Antek model NCD 705D instrument.

Parameters	Conditions
Combustion temperature	680 ºC
Carrier gas	Oxygen (ultra pure, 99.999%)
Carrier gas flow rate	150 mL/min
Sample sparge time	8.0 mins
Minimum number of injections	3
Maximum number of injections	5
Number of washes	2
Standard deviation maximum	0.100
CV maximum	2.00%
Injection volume	100 μL

2.2.3.2 Relevant data

Additional relevant data using in this study obtained from various sources are shown in Table 2.2, or otherwise stated in context.

Table 2.2 List of relevant parameters and analytical approaches.

Cruise	Parameters	Analytical approaches	Personnel
D 326	Micromolar Nitrate + Nitrite	Nutrient autoanalyser (segmented flow analysis + colourimetric chemistry)	M. Stichcombe[a]
	Nanomolar Nitrate + Nitrite	Nutrient autoanalyser (segmented flow analysis + miniaturise spectrophotometers)	M. Patey[a]
	Ammonium	OPA fluorescence	B. Dickie[a]
	Dissolved organic phosphorus	UV Oxidation	C. Mahaffey[b]
	Heterotrophic bacteria	Flow cytometry	M. Zubkov[a] and R. Holland[a]
	Chlorophyll *a*	Acetone extraction and fluorometry	D. A. Purdie[a]
	Oxygen	Winkler titration	M. Stinchcombe[a]
D 338	Nitrate+ Nitrite and Ammonia	Colorimetric autoanalysis	M. Woodward[c]
	Ammonium	Colorimetric autoanalysis	M. Woodward[c]
	Heterotrophic bacteria	Flow cytometry	G. Tarran[c]
	Chlorophyll *a*	Acetone extraction and fluorometry	C. Widdicombe[c]

[a] University of Southampton
[b] University of Liverpool
[c] Plymouth Marine Laboratory

2.3 Result and discussion

2.3.1 Hydrographic and Chlorophyll a features

Pycnoclines were relatively shallow in the Mauritanian shelf region and relatively deep in the North Atlantic subtropical gyre (Figure 2.3). The nitracline, defined as the water depth at above which the nitrate concentrations were 1 mmol/m^3 (Robinson et al., 2006), showed similar distributions to the pycnocline. The depth of the nitracline was exceptionally shallow in the Mauritanian shelf region and deepest in the North Atlantic subtropical gyre (Figure 2.3). Contour plots of temperature and salinity are depicted in Figure 2.4 and Figure 2.5. The strong upward movement of water masses in Figure 2.4c and Figure 2.5c are characteristic of strong upwelling regions. The relatively low temperature of surface water in Mauritania shelf region is a consequence of the advection of cold, nutrient-rich deep water rising to the surface (Teira et al., 2001). The tropical North Atlantic Ocean was strongly stratified during the study period (surface minimum density was below 25 kg/m^3). Mixed layer depths were estimated based on an isothermal mixed layer depth of 0.5ºC (ILD 0.5) (Hooker et al., 2000), defined as the shallowest water depth at which the temperature differs from the surface layer by more than 0.5ºC. The result showed the maximum mixed layer depth was in the North Atlantic subtropical gyre (down to 100 -150 metres), while the tropical North Atlantic Ocean and the Mauritanian shelf region had mixed layer depths of approximately 30 - 90 m and 20 - 110 m, respectively. Chlorophyll *a* concentrations showed maximum values (up to 6.18 µg/L) in the Mauritanian shelf region (Figure 2.6a), where there is intense upwelling (Torres-Valdes et al., 2009), with the minimum value ($\leq$ 0.5 µg/L) observed in the North Atlantic subtropical gyre (Figure 2.6c), an oligotrophic region. The chlorophyll *a* profiles in the (sub-) tropical regions are consistent with observations from the Atlantic Meridional Transect (AMT) Programme (Robinson et al., 2006). Vertical profiles of oxygen concentrations demonstrated typical decreases in concentration with increasing water depth in all areas (Figure 2.7).

The distributions of chlorophyll *a* in Figure 2.6 and the nitracline in Figure 2.3 suggest that nutrients were one of the factors controlling biomass and phytoplankton communities in the study areas. The upwelling off the Northwest African coast yields relatively shallow nitraclines, corresponding with enhanced surface concentrations of chlorophyll *a* (Figure 2.6c). Remarkable low oxygen concentrations below the mixed layer depths of the tropical North Atlantic Ocean and the Mauritanian shelf region (Figure 2.7) can be linked to the presence of an extended oxygen minimum zone. Low oxygen concentrations are typically found underneath upwelling regions owing to their productive nature, generating large amounts of organic matter which is subsequently oxidised during its export to deeper water (Schafstall et al., 2010).

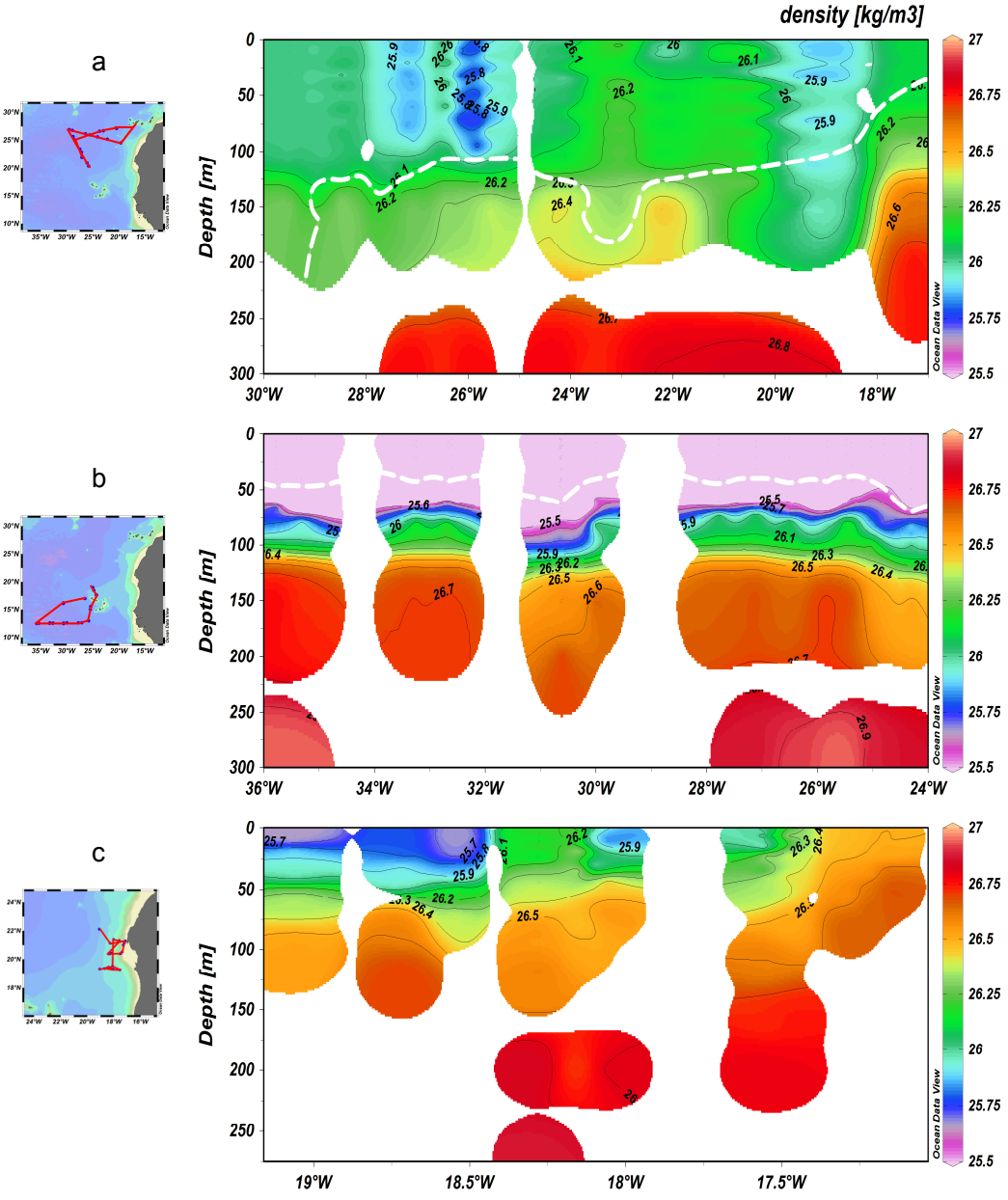

Figure 2.3 Vertical density contours of a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region. The white dashed-lines denote the nitracline.

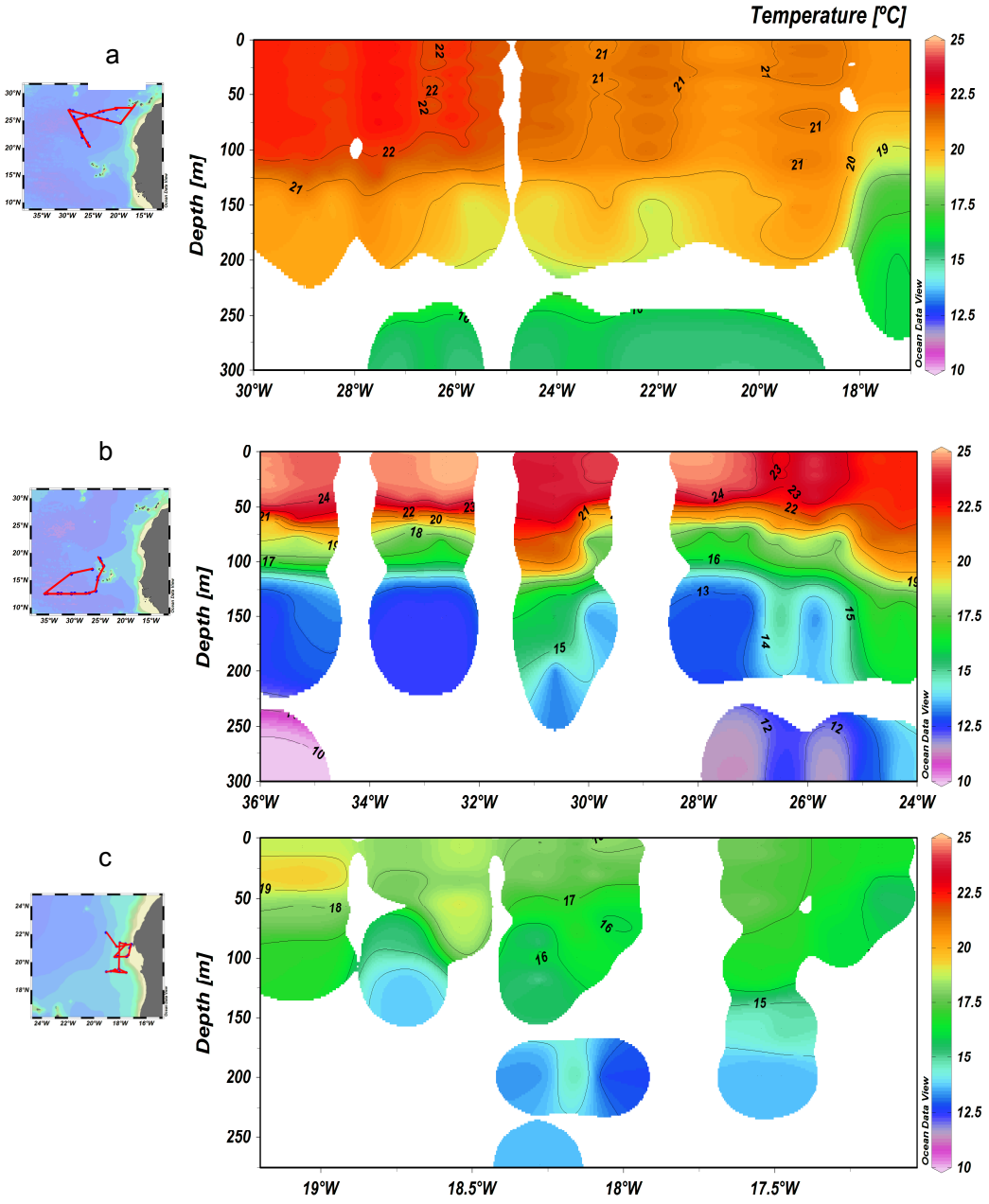

Figure 2.4 Depth contours of temperature for a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

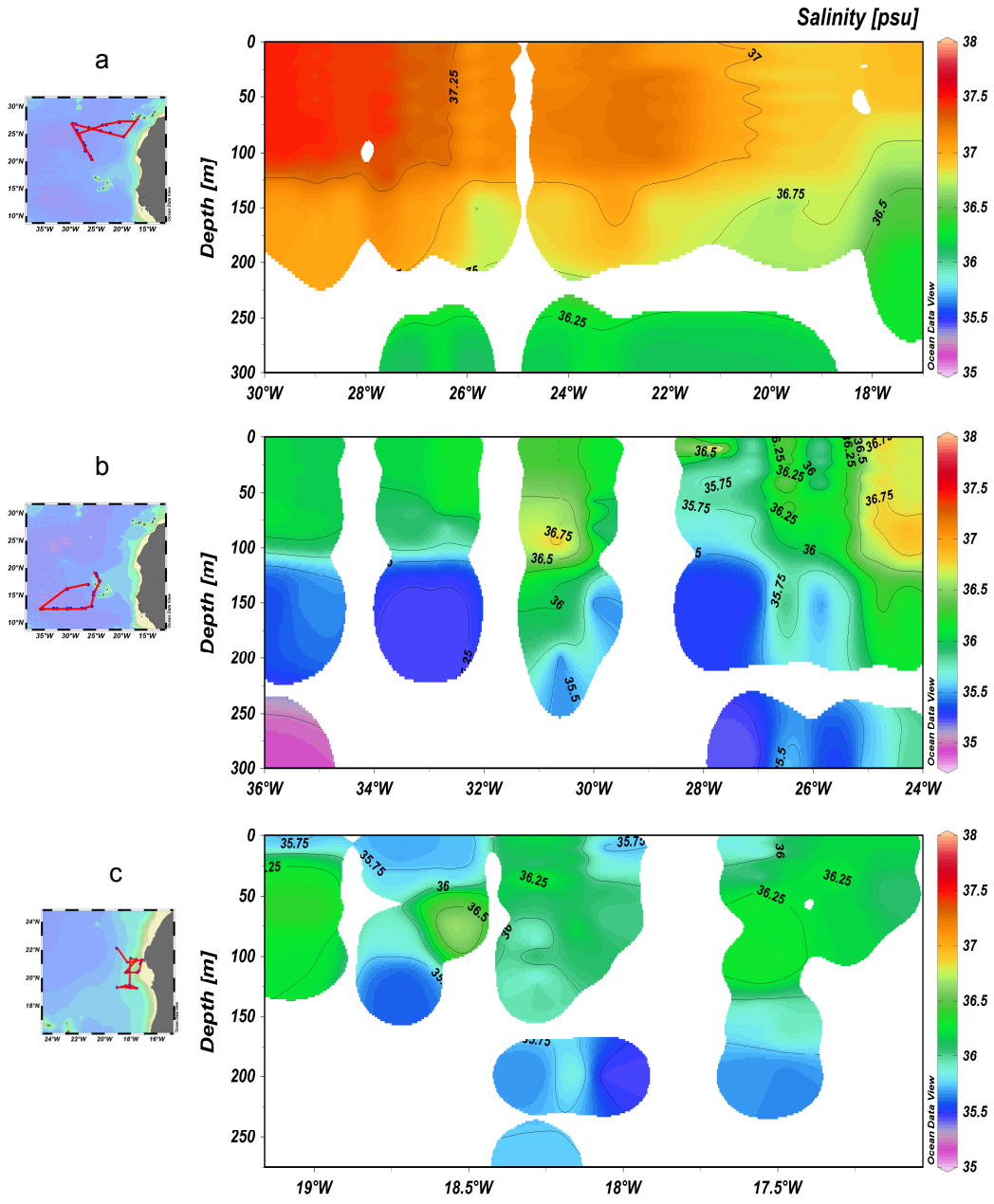

Figure 2.5 Depth contours of salinity for a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

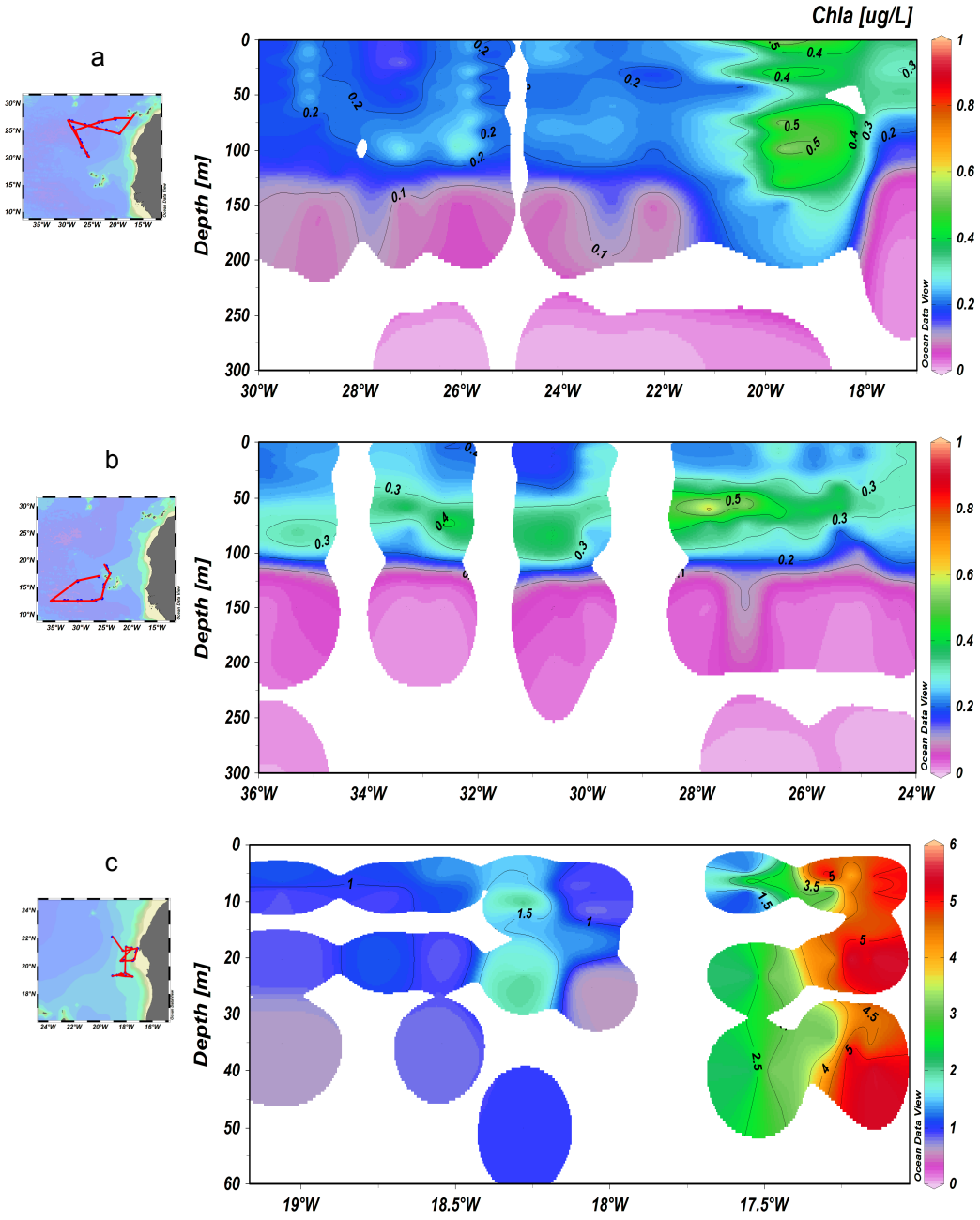

Figure 2.6 Depth contours of chlorophyll *a* concentrations for a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

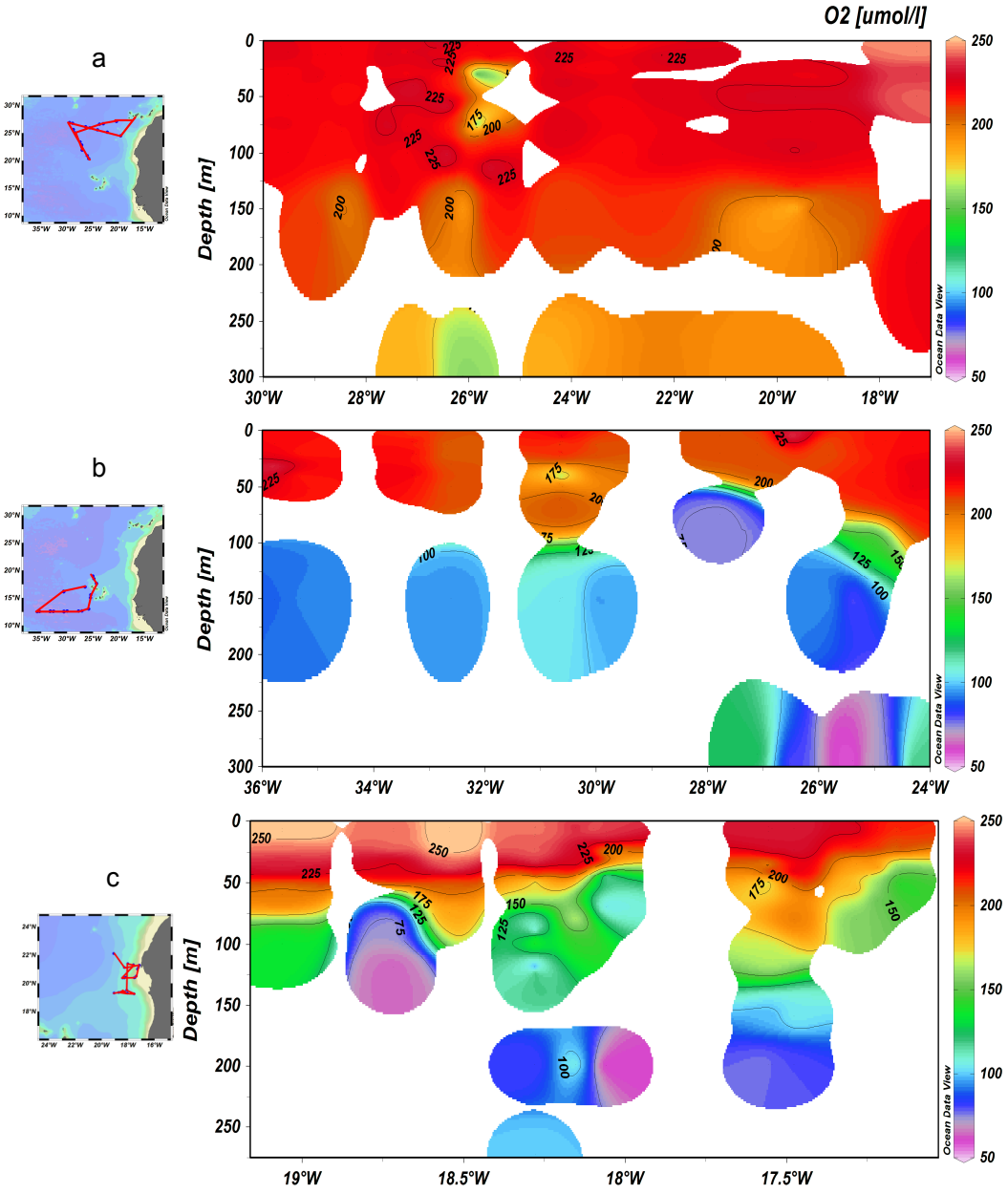

Figure 2.7 Depth contours of oxygen concentrations for a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

2.3.2 Distributions of DOC and DON

2.3.2.1 DOC concentrations

Distributions of DOC in the (sub-) tropical Atlantic Ocean are shown in Figure 2.8. Generally, DOC concentrations showed enhanced concentrations in the upper water column (< 40 m) and decreasing concentrations with depth. The typical depth profiles are depicted in Figure 2.9. Our observations are consistence with the findings reported for previous studies (Table 2.3). In the North Atlantic subtropical gyre, DOC concentrations in the surface layer ranged from 52.8 to 76.3 μM with a mean of 67.2 ± 5.6 μM. Concentrations decreased in sub-surface water (200 - 300 m), ranging between 43.3 to 56.7 μM with a mean of 48.6 ± 3.8 μM. In the tropical North Atlantic Ocean, DOC concentrations in surface water varied between 68.6 and 100.6 μM, with a mean of 77.0 ± 6.7 μM. The statistical analysis revealed that the DOC concentrations in surface water of the North Atlantic subtropical gyre was significantly lower than those in the other areas (ANOVA, $p<0.05$). However, there was no statistically difference (t-test, $p < 0.05$) between the DOC concentrations in surface water of the tropical North Atlantic Ocean and of the Mauritanian shelf region. In sub-surface waters (200 - 300 m), concentrations ranged between 40.6 to 69.1 μM, with a mean of 47.8 ± 5.2 μM. The ranges of DOC concentrations in the upwelling region were from 50.9 to 91.4 μM throughout the upper 200 m of the water column, with a mean of 72.3 μM ± 10.0 in upper 40 m and 67.2 ± 10.4 μM at depths of 40 - 200 m.

The results are in agreement with the finding of the previous in the same study area. Pan et al. (in Press) reported 70 - 80 μM above 40 m, and Kahler et al. (2010) found 68.7 - 75.4 μM above 100 m in the North Atlantic subtropical gyre. In addition, the DOC concentrations observed in this study are generally in line with the literature data reported for other regions (Table 2.3). However, surface DOC concentrations reported for the North Sea by Suratman et al. (2009) are higher due to an influence of fluvial inputs which favours nutrient accumulation and phytoplankton abundance. It has been reported that phytoplankton introduce DOC to marine environments (Williams, 1995, Nagata and Kirchman, 1991, Nagata, 2000). Markedly low surface concentrations of DOC observed in the Australian sector of the Southern Ocean were due to deep mixing allowing surface water exchanges with deeper water of low DOC, high bacterial consumption, selective degradation of DOC and also absorption to particles (Ogawa et al., 1999). Little accumulation of DOC in surface water was also reported for other areas of the Southern Ocean such as the central Weddell Sea (ca. 45 - 60 μM of TOC) (Wedborg et al., 1998) and Ross Sea, Antarctica (mean DOC of 48 ± 6.0 μM in surface 50 m) (Carlson et al., 1998).

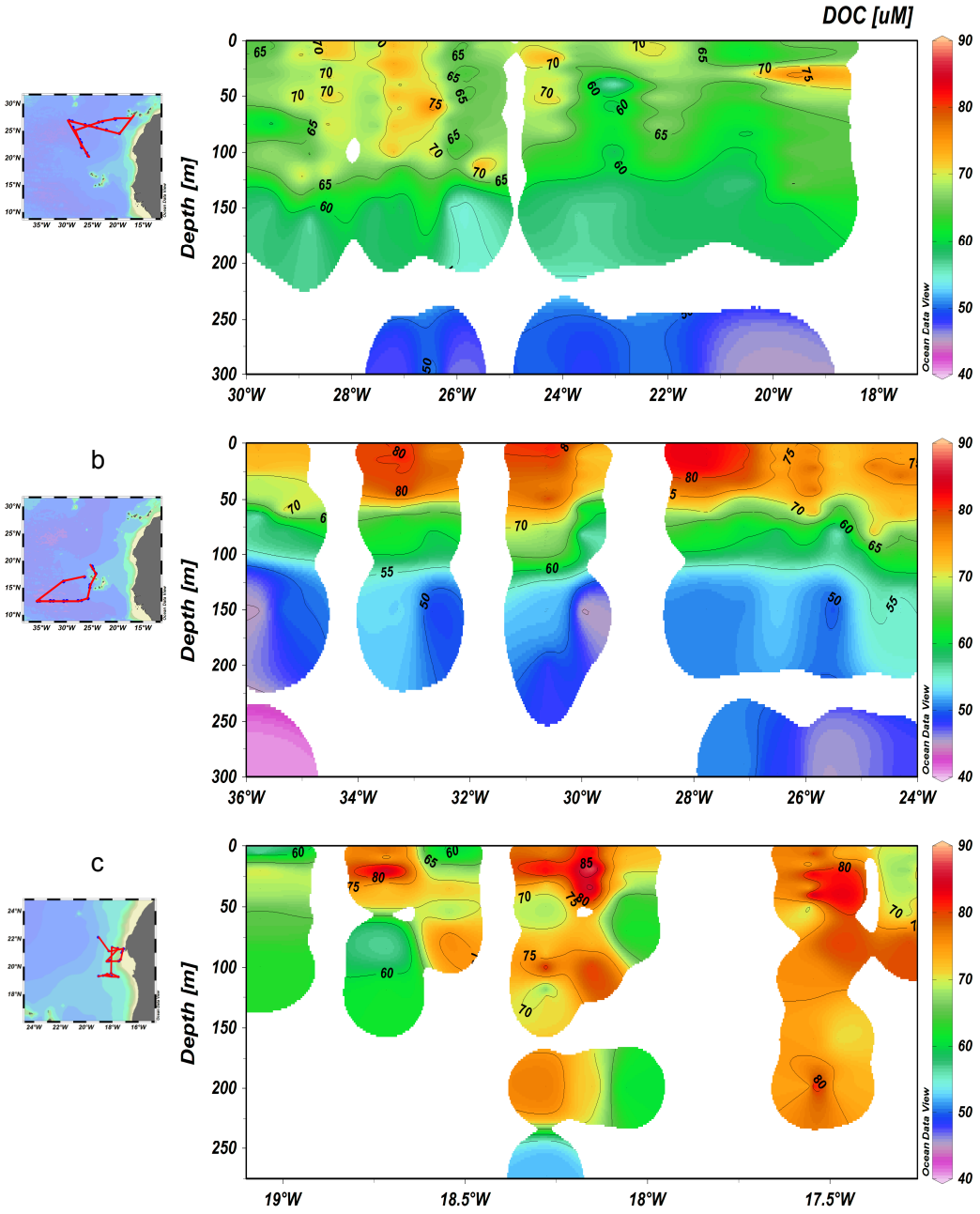

Figure 2.8 Depth contours of DOC concentrations for a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

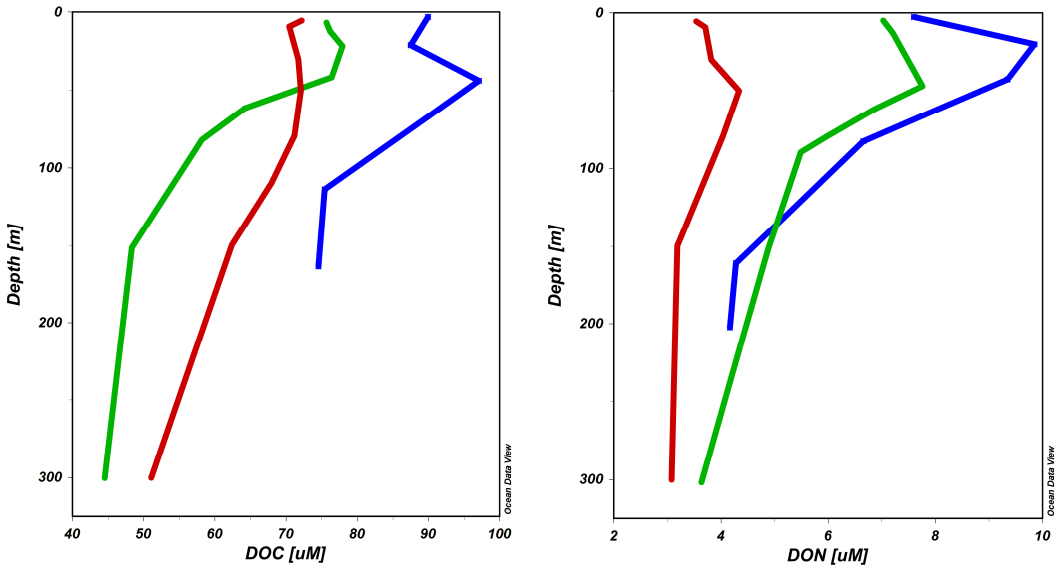

Figure 2.9 Depth profiles of DOC and DON for a) the North Atlantic subtropical gyre (red lines), b) the tropical North Atlantic Ocean (green lines) and c) the Mauritanian shelf region (blue lines).

Table 2.3 Concentrations of DOC in oceans measured by high temperature combustion technique.

Location	Depth (m)	DOC (μM)	References
Ocean-surface and sub-surface			
Middle Atlantic Bight	< 100	70 - 80	Guo et al. (1995)
Southern Ocean	< 150	45 - 55	Ogawa et al. (1999)
Western Pacific Ocean	< 100	65 - 90	Doval and Hansell (2000)
Central Indian Ocean	< 100	55 - 80	Doval and Hansell (2000)
Southern Ocean	< 100	45 - 75	Doval and Hansell (2000)
Sargasso Sea (BATS)	50 - 100	60 - 70[a]	Hansell and Carlson (2001)
Northwestern Mediterranean Sea	25 - 150	82.0 ± 5	Avril (2002)
Northwestern Sargasso Sea	5 - 10	67.6 - 69.6	Carlson et al.(2004)
Northwestern Sargasso Sea	250	51.2 - 64.2	Carlson et al.(2004)
Northern South China Sea	MLD	70 - 85	Hung et al. (2007)
North Sea (Spring)	< 20	156 - 318	Suratman et al. (2009)
North Sea (Summer)	< 20	68 - 146	Suratman et al. (2009)
North Sea (Autumn)	< 20	72 - 130	Suratman et al. (2009)
North Sea (Winter)	< 20	85 - 112	Suratman et al. (2009)
North Atlantic subtropical Gyre	0 - 100	68.7 - 75.4	Kahler et al. (2010)

(Continues)

Table 2.3 *(Continued)*

Location	Depth (m)	DOC (μM)	References
Ocean-surface and sub-surface *(Continued)*			
Equatorial Atlantic	< 40	75 - 88	Pan et al. (in Press)
Equatorial Atlantic	100	65 - 70	Pan et al. (in Press)
Equatorial Atlantic	300	50 - 55	Pan et al. (in Press)
Atlantic subtropical Gyre	< 40	70 - 80	Pan et al. (in Press)
Atlantic subtropical Gyre	100	65 - 70	Pan et al. (in Press)
Atlantic subtropical Gyre	300	50 - 55	Pan et al. (in Press)
North Atlantic subtropical Gyre	**< 40**	**52.8 - 76.3 (mean = 67.2 ± 5.6)**	**This study**
North Atlantic subtropical Gyre	**40 - 200**	**57.6 - 71.1 (mean = 64.1 ± 4.3)**	**This study**
North Atlantic subtropical Gyre	**300**	**43.3 - 56.7 (mean = 48.6 ± 3.8)**	**This study**
Tropical North Atlantic	**< 40**	**68.6 - 100.6 (mean = 77.0 ± 6.7)**	**This study**
Tropical North Atlantic	**40 - 200**	**46.1 - 67.4 (mean = 61.2 ± 4.8)**	**This study**
Tropical North Atlantic	**300**	**40.6 - 69.1 (mean = 47.8 ± 5.2)**	**This study**
Mauritanian shelf region	**< 40**	**58.9 - 91.4 (mean = 72.3 ± 10.0)**	**This study**
Mauritanian shelf region	**40 - 200**	**50.9 - 81.8 (mean = 67.1 ± 10.4)**	**This study**
Ocean-deep			
Middle Atlantic Bight	> 1000	46 - 48	Guo et al. (1995)
Southern water	> 1000	40 - 45	Ogawa et al. (1999)
Western Pacific and Central Indian	> 1000	42.3 - 43	Doval and Hansell (2000)
Sargasso Sea (BATS)	> 1000	43.6 ± 0.6[a]	Hansell and Carlson (2001)
North Atlantic (33-66°N)	> 1000	44	Kahler and Koeve (2001)
Northwestern Mediterranean Sea	> 1000	51.5 ± 2.5	Avril (2002)
Northwestern Mediterranean Sea	> 2000	50.5 ± 1.5	Avril (2002)
Northern South China Sea	> 1000	43.0 ± 3.0	Hung et al. (2007)
North Atlantic subtropical Gyre	> 1000	43 - 45	Pan et al. (in Press)

[a] reported as TOC concentrations

2.3.2.2 DON concentrations

Distributions of DON in the (sub-) tropical Atlantic Ocean are shown in Figure 2.10 and the typical depth profiles are demonstrated in Figure 2.9. DON profiles in the North Atlantic subtropical gyre showed enhanced concentrations in surface waters, ranging from 2.8 to 7.3 μM, with a mean of 4.6 ± 1.3 μM, and declining concentrations towards the pycnocline, ranging from 0.6 to 5.2 μM, with a mean of 2.4 ± 1.5 μM, at 200 - 300 m. Vertical DON profiles in the tropical North Atlantic Ocean showed different profiles, with

concentrations increasing with depth. DON concentrations showed maxima (> 15 μM) in sub-surface waters along the 12ºN longitudinal section. DON concentrations varied less with depth in the Mauritanian shelf region, with mean concentrations of 8.1 ± 2.8 μM in surface water and 5.4 ± 4.0 μM in sub-surface water at depths of 40 - 200 m. There was statistically significant difference (ANOVA, $p < 0.05$) in DON concentrations in the upper 40 m, with the highest concentration in the Mauritanian shelf region and the lowest concentration in the North Atlantic subtropical gyre.

DON concentrations in surface water observed in this study are within ranges reported by the other workers (Table 2.4). Our results are comparable with the observations of DON concentrations within the upper 100 m from the Atlantic Meridional Transect (AMT) Programme (between 10ºN - 30ºN and 15ºW - 40ºW), where concentrations of 4.9 - 5.4 μM were observed, with the elevated values near to the African upwelling zone at 18ºN (Torres-Valdes et al., 2009). Furthermore, the mean DON concentration of 4.6 ± 1.3 μM above 40 m in the North Atlantic subtropical gyre is in good agreement with the value of 4.6 ± 1.8 μM observed by Landolfi et al. (2008) in the same region. The maxima DON concentration (> 15 μM) observed in sub-surface waters along the 12ºN longitudinal section in the tropical North Atlantic Ocean coincided with the observation of Vidal et al. (1999) who reported an increase of DON concentration (ca. 11 μM) below in deeper water at 11.3ºN. In this study, mean DON concentrations were higher in surface water and lower in deeper water. This vertical distribution shows similar pattern to those observed in the other studies such as Ogawa et al. (1999) in the Southern Ocean, Vidal et al. (1999) in the Equatorial Atlantic Ocean, Sanders and Jickells (2000) in the Drake passage, Hansell and Carlson (2001) in Sargasso Sea (BATS), and Landolfi et al. (2008) in the North Atlantic Subtropical Gyre.

The contribution of DON to TDN declined with depth in all areas (Figure 2.11). DON dominated the TDN pool in the surface waters of the North Atlantic subtropical gyre and the tropical North Atlantic Ocean, accounting for more than 99% of TDN, reflecting the inorganic nutrient-limited state of these waters. The results agree with the findings of Vidal et al. (1999) and Abell et al. (2000), who reported that more than 90-98% of the TDN pool was formed by the DON fraction in the Equatorial Atlantic and the Pacific subtropical gyre. The lowest contribution of surface DON to the TDN pool was observed in the Mauritanian shelf region, where DON accounted for only 47%, as a result of the enhanced upward mixing of DIN from deep water to the surface. The finding agrees with the study of Ogawa et al. (1999), who reported a low contribution of DON to TDN (17 - 21%) in surface water of a DIN-rich upwelling area in the Southern Ocean.

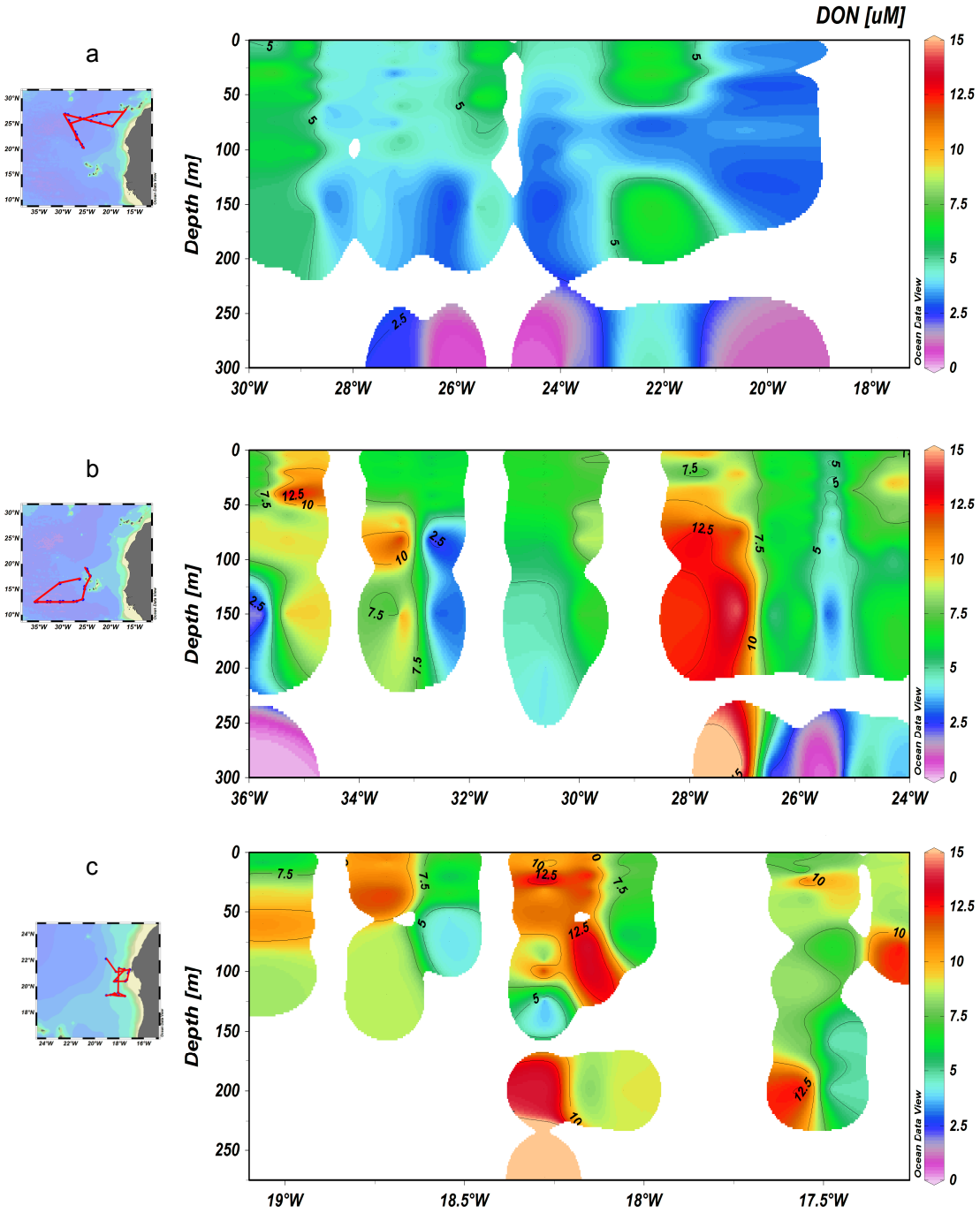

Figure 2.10 Depth contours of DON concentrations for a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

Table 2.4 Concentrations of DON and TDN in oceans measured by a high temperature combustion technique (HTC), persulfate oxidation (PO) or ultraviolet oxidation (UV).

Location	Depth (m)	DON (µM)	TDN (µM)	DON as percent of TDN	Method	References
Ocean-surface and sub-surface						
Southern Ocean	< 30	4 - 9	24 - 42	16.7 - 21.4	HTC	Ogawa et al. (1999)
Equatorial Atlantic	<100	8.2 ± 4.8[a]	n/a	> 90	PO	Vidal et al. (1999)
Pacific Subtropical Gyre	< 50	5.2 - 6.2[ab]	5.3 – 6.3	> 98	UV	Abell et al. (2000)
Drake passage	< 50	3 - 7[b]	n/a	n/a	UV	Sanders and Jickells (2000)
Drake passage	> 50	2 - 3[b]	n/a	n/a	UV	Sanders and Jickells (2000)
Sargasso Sea (BATS)	50 - 100	4.0 - 5.0[ab]	n/a	n/a	UV	Hansell and Carlson (2001)
North Atlantic (33-66ºN)	5	4.4 - 7.4[a]	n/a	n/a	HTC	Kahler and Koeve (2001)
Atlantic Ocean	0 - 200	5.6 - 6.9	n/a	n/a	UV	Varela et al. (2006)
North Atlantic subtropical Gyre	Surface	4.6 ± 1.8[b]	n/a	n/a	HTC	Landolfi et al. (2008)
North Atlantic (10-30ºN, 17-40ºW)	< 100	4.9 - 5.4	n/a	n/a	UV	Torres-Valdes et al. (2009)
North Atlantic subtropical Gyre	0 - 100	5.2 - 6.1[b]	n/a	n/a	HTC	Kahler et al. (2010)
North Atlantic subtropical Gyre	**< 40**	**2.8 - 7.3 (4.6 ± 1.3)[c]**	**2.8 - 7.3 (4.6 ± 1.3)[c]**	**99.7 - 99.9**	**HTC**	**This study**
North Atlantic subtropical Gyre	**40 - 200**	**2.9 - 6.4 (4.1 ± 1.0)[c]**	**3.3 - 6.8 (4.8 ± 1.1)[c]**	**89.0 - 94.1**	**HTC**	**This study**
North Atlantic subtropical Gyre	**300**	**0.6 - 5.2 (2.4 ± 1.5)[c]**	**8.2 – 18.5 (12.7 ± 2.4)[c]**	**6.9 - 27.8**	**HTC**	**This study**
Tropical North Atlantic	**< 40**	**3.7 - 9.3 (6.7 ± 1.4)[c]**	**3.8 - 9.3 (6.7 ± 1.4)[c]**	**98.2 - 99.9**	**HTC**	**This study**
Tropical North Atlantic	**40 - 200**	**2.3 - 15.3 (6.2 ± 3.0)[c]**	**8.3 – 36.4 (14.5 ± 7.0)[c]**	**27.9 - 42.1**	**HTC**	**This study**
Tropical North Atlantic	**300**	**0.7 - 16.6 (4.5 ± 4.7)[c]**	**24.3 – 50.0 (35.8 ± 7.4)[c]**	**2.9 - 33.1**	**HTC**	**This study**
Mauritanian shelf region	**< 40**	**4.1 - 15.1 (8.1 ± 2.8)[c]**	**8.8 – 24.5 (17.2 ± 3.9)[c]**	**47.2 - 61.5**	**HTC**	**This study**
Mauritanian shelf region	**40 - 200**	**0.14 - 15.4 (5.4 ± 4.0)[c]**	**11.5 – 34.3 (23.5 ± 6.7)[c]**	**1.2 - 44.9**	**HTC**	**This study**
Ocean-deep						
Southern Ocean	500 - 2500	2 - 4	34 - 38	5.9 - 10.5	HTC	Ogawa et al. (1999)
Equatorial Atlantic	110 - 1000	6.7 ± 4.1[a]	n/a	n/a	PO	Vidal et al. (1999)
Sargasso Sea (BATS)	> 1000	3.1 ± 0.4[b]	n/a	n/a	HTC	Hansell and Carlson (2001)
North Atlantic subtropical Gyre	> 1000	1.7 ± 0.1[b]	n/a	n/a	HTC	Landolfi et al. (2008)

[a] cited in (Berman and Bronk, 2003)
[b] reported as TON concentrations
n/a = data not available
[c] mean concentration values

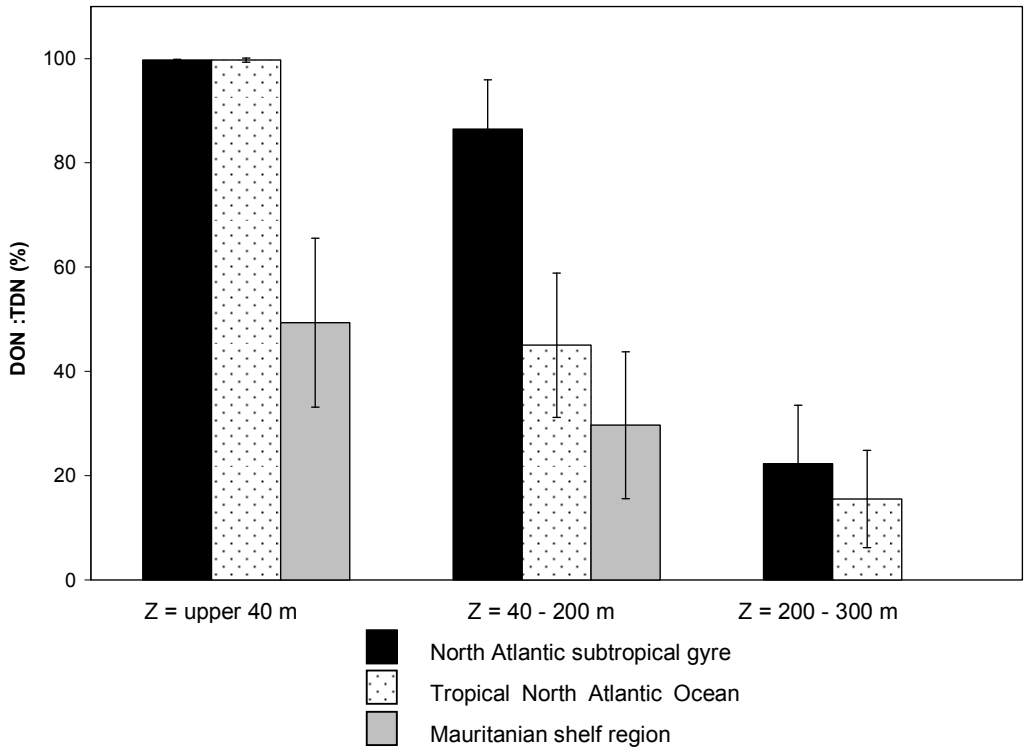

Figure 2.11 Vertical variations in the organic fraction of dissolved nitrogen contributing to total dissolved nitrogen.

2.3.2.3 Refractory fractions of DOM

DOM in deep waters is likely to be resistant to microbial degradation and consequently has turnover times of centuries to millennia (Ogawa and Tanoue, 2003, Bauer et al., 1992, Williams and Druffel, 1987). The concentrations of refractory DOM are considered to be uniform throughout the water column (Ogawa and Tanoue, 2003). Hence, the semi-labile fraction is commonly determined as the difference between surface DOM and deep water DOM (Ogawa and Tanoue, 2003, Carlson and Ducklow, 1995). In this study, there is no availability of data in deep water (below 300 m), so literature values of DOC and DON in deep water were considered. According to Hansell et al. (2009), the concentrations of DOC in the bathypelagic Atlantic Ocean ranged from 40 to 45 µM. In the Sargasso Sea at the BATS site, DOC and total organic carbon (TOC) concentrations observed below 1000 m were between 43 and 44 µM (Carlson et al., 1998, Carlson et al., 2004, Hansell and Carlson, 1998, Carlson et al., 1994). Assuming these concentrations are characteristic of a refractory DOC pool in the whole water column (i.e., 43 µM), the refractory DOC fraction observed in surface waters in this study accounted for ca. 65.4%, 57.2% and 60.9% of the bulk DOC pool in the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region, respectively. The refractory DOC fraction increased with

depth, to 82 - 90% at 200 - 300 m which is in good agreement with observations by Pan et al. (in Press).

DON concentrations in deep water at 800 m were approximately 2.5 μM in the Drake Passage (Sanders and Jickells, 2000) and 3.1 μM at depths below 1000 m in the Sargasso Sea at the BATS site (Hansell and Carlson, 2001). The contributions of DON refractory pool were estimated by assuming approximately 2.8 μM (average value of DON concentrations observed in the Drake Passage and in the Sargasso Sea) were refractory DON. The surface refractory DON fraction accounted for 60.9%, 41.9% and 34.7 % of the bulk DON pool in the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region, respectively. The refractory fraction increased up to 60 - 100% in sub-surface water. The contributions of surface refractory DON to bulk DON pool in this study were lower than the observations made during AMT reported by Robinson et al. (2006) (over 90%). It is possible that an additional semi-labile DON fraction was supplied to the study areas. One of the potential sources was atmospheric aerosols as maximum concentrations occurred during winter (Chiapello et al., 1995) which was the sampling period in this study and Cornell et al. (2003) suggested that atmospheric aerosol supplies bioavailable organic nitrogen such as urea, amines and amino acids into marine environments.

2.3.2.4 Integrated stocks

The integrated stocks of DOC and DON are depicted in Figure 2.12. The integrated stocks of DOC and DON varied with the mixed layer depths, with the maximum DOC stocks coinciding with the deepest mixed layers, in agreement with observations by Hansell and Carlson (2001) in the Sargasso Sea. The authors also reported that the amount of TOC exported to depth is determined by the deepening of the mixed layer. Therefore, this may imply that the relatively deep mixed layer in the North Atlantic subtropical gyre results in an enhanced export of DOC and DON through vertical mixing, leading to the relatively low DOC and DON concentrations in surface water.

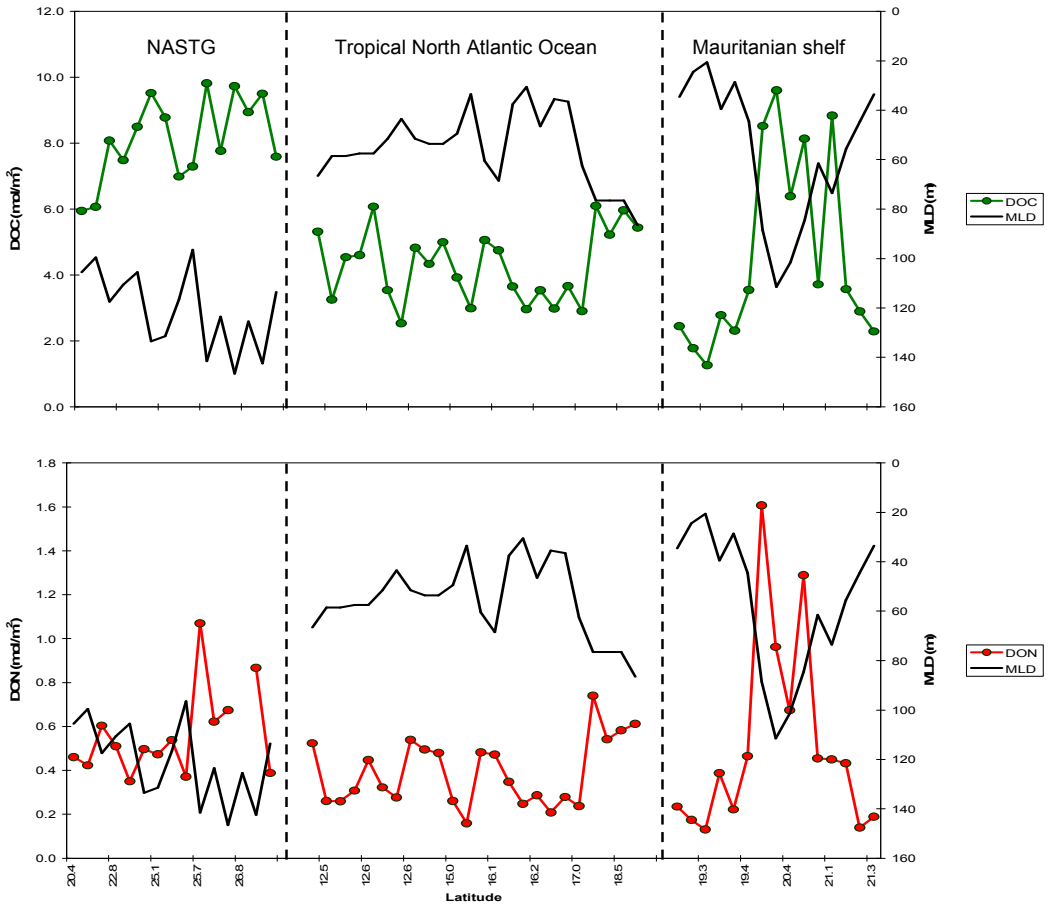

Figure 2.12 Integrated stocks (mol/m^2) of DOC and DON in the mixed layer depth (MLD; m) of the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region.

2.3.3 Organic stoichiometric ratios of bulk pool and implications

The C:N molar ratio has been used as a tool to determine the origin and processing of natural organic matter (Ogawa and Tanoue, 2003, Suratman et al., 2009). In this study, C:N molar ratios were estimated for three depth intervals (upper 40 m, 40 - 200 m and at 300 m) (Table 2.5). Figure 2.13 exhibits the relationship between DOC and DON concentrations of the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region. The mean C:N molar ratios of DOM deviated from Redfield ratio of 6.6 (Redfield, 1958), varying between 9.0 and 14.8 in surface waters (upper 40 m). There was statistically significant difference (ANOVA, $p < 0.05$) in C:N molar ratios in the upper 40 m of our study areas, with the highest ratios in the North Atlantic subtropical gyre and the lowest in the Mauritanian shelf region. The mean C:N molar ratio in the North Atlantic subtropical gyre increased to 20.3 at 300 m, which was significantly different to those at shallower depths (ANOVA, $p < 0.05$). In the tropical North Atlantic Ocean, the

mean C:N ratio in upper 40 m was 11.5, decreasing to values of 9.9 at depths of 40 - 200 m, and then increasing slightly to values of 10.5 at 300 m. However, there was no statistical difference between depths (ANOVA, $p > 0.05$). The C:N molar ratio of 9.0 was observed in the surface water of the Mauritanian shelf region and increased to 12.5 at the depth of 40 - 200 m. There was no significant difference in C:N ratios observed in upper 40 m and in sub-surface water (t-test, $p > 0.05$). The C:P molar ratios ranged from 241 to 620.5 across the study areas (Table 2.5) which were relatively high in comparison with the Redfield ratio of 106 (Redfield, 1958). The statistical analysis showed that the mean C:P molar ratio in the Mauritanian shelf region is significantly lower than those in other areas. The N:P molar ratios ranged from 26.9 to 42.5 in this study, which is enhanced relative to the Redfield ratio of 16.

Table 2.5 Stoichiometric ratios of C:N:P dissolved organic matter in the (sub-) tropical North Atlantic Ocean.

Areas	Upper 40 m	40-200 m	300 m
C:N ratios			
North Atlantic subtropical gyre	14.76	15.51	20.32
Tropical North Atlantic Ocean	11.53	9.93	10.54
Mauritanian shelf region	8.96	12.51	n/a
C:P ratios			
North Atlantic subtropical gyre	620.52[a]	n/a	n/a
Tropical North Atlantic Ocean	482.56[a]	n/a	n/a
Mauritanian shelf region	240.97[b]	n/a	n/a
N:P ratios			
North Atlantic subtropical gyre	42.44[a]	n/a	n/a
Tropical North Atlantic Ocean	41.86[a]	n/a	n/a
Mauritanian shelf region	26.90[b]	n/a	n/a

[a] DOP concentrations provided by Claire Mahaffey, University of Liverpool
[b] Estimated using the DOP value of 0.3 µM in upper 100 m off the North West African coast reported by Vidal et al. (1999)
n/a = data not available

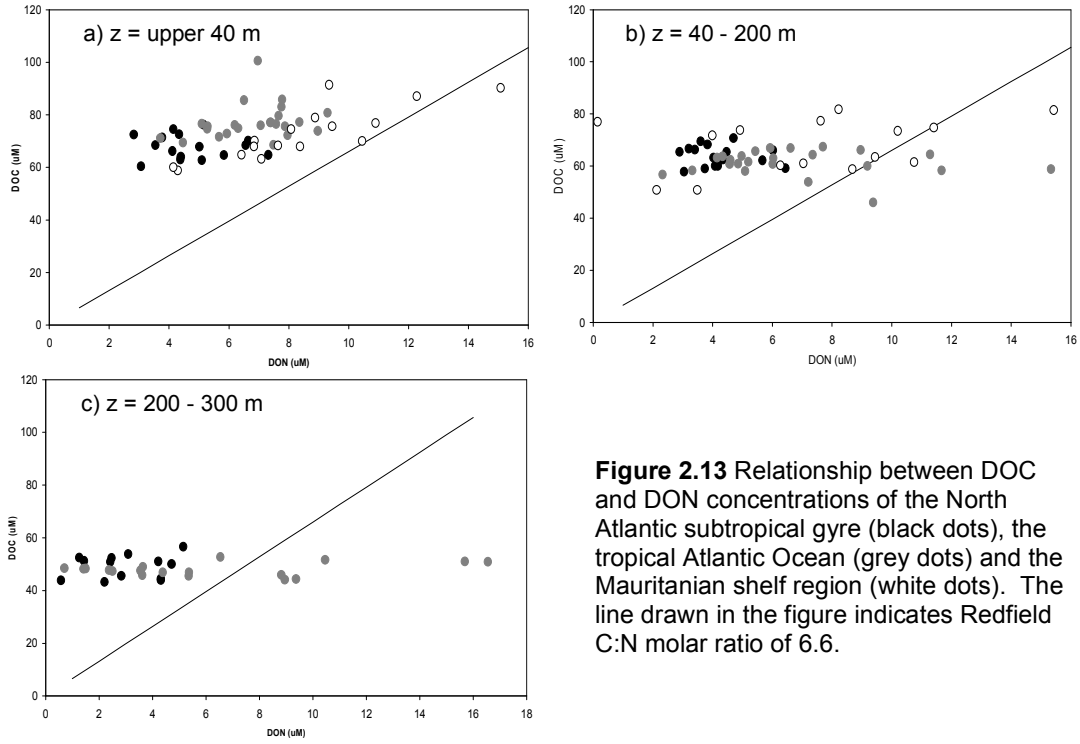

Figure 2.13 Relationship between DOC and DON concentrations of the North Atlantic subtropical gyre (black dots), the tropical Atlantic Ocean (grey dots) and the Mauritanian shelf region (white dots). The line drawn in the figure indicates Redfield C:N molar ratio of 6.6.

These results suggest that the DOM pool in the surface water was enriched in carbon relative to nitrogen and phosphorus and nitrogen relative to phosphorus compared to the Redfield ratio (106:16:1). The DOM refractory fractions reported in section 2.3.2.3 and C:N molar ratios reported in this section increased with increasing depth implying that the refractory DOM pool was enriched in carbon relative to nitrogen. The relatively low C:N molar ratio in upper water of the Mauritanian shelf region was possibly due to production of DOC by phytoplankton communities suggested by the relatively high concentration of chlorophyll *a* (Figure 2.6), and/or the high production of organic matter relative to remineralisation processes. Low C:N molar ratios have also been observed after spring bloom events in the Sargasso Sea and in the Antarctic (Carlson et al., 1998, Carlson et al., 2000). Moreover, relatively high C:N ratios observed in deep waters in this study indicate the relatively rapid decomposition of organic nitrogen during downward transport (Carlson et al., 2000, Ogawa et al., 1999). Relatively high C:P and N:P molar ratios compared to Redfield ratios of 106:1 and 16:1, respectively, were possibly a result of preferential remineralization of DOP relative to DOC and DON (Loh and Bauer, 2000). The other plausible explanation was an addition of DON generated via nitrogen fixation, particularly during periods of dissolved inorganic nutrient shortage, leading to high N:P ratios (Cavender-Bares et al., 2001).

2.3.4 Contribution of DOC to water column respiration

AOU is a parameter indicating the oxygen utilised by biochemical processes during organic carbon mineralization (Avril, 2002). AOU can be calculated as the difference in concentration between the observed oxygen and its equilibrium saturation concentration (Garcia et al., 2006). Spatial variations of AOU profiles in this study are presented in Figure 2.14. AOU values were close to zero in surface waters. The maximum AOU was approximately 212 µmol/kg, observed in sub-surface water at a station near Cape Verde Island. The dynamics of oxygen in the water column are driven by multiple factors such as heterotrophic bacterial respiration, air-sea gas exchange and photosynthesis. In order to eliminate oxygen affected by air-sea gas exchange and photosynthetic process, only oxygen values existed below the isopycnal layer σ-t 26.2 for the North Atlantic subtropical gyre, σ-t 25.6 for the tropical North Atlantic Ocean and σ-t 26.5 for the Mauritanian shelf region were taken into account. Statistical analysis shows that AOU in water masses isolated from the atmosphere showed a significant correlation with density (Table 2.6).

To understand the contribution of DOC flux to oxygen consumption, the relationship between DOC and AOU in water masses isolated from the atmosphere was investigated. Aristegui et al. (2002) assumed that 1.0 mol of oxygen contributes to the degradation of 0.69 moles of organic carbon (Respiratory quotient or respiratory coefficient = -0.69). The best fit least squares regression equations between DOC and AOU were depicted in Figure 2.15. For the regressions of DOC versus AOU below the thermocline in the upper 200 m, no statistically significant correlation was found at the 95% significance level in sub-surface water at the Mauritanian shelf region ($p > 0.05$). However, a strong significant relationship ($p < 0.05$) was present in the tropical North Atlantic Ocean. The percentage of AOU ascribed to DOC respiration was 14.1%. A weak, but significant, relationship ($p < 0.05$) was found in the North Atlantic subtropical gyre, with 16.5 % of oxygen consumption related to DOC degradation.

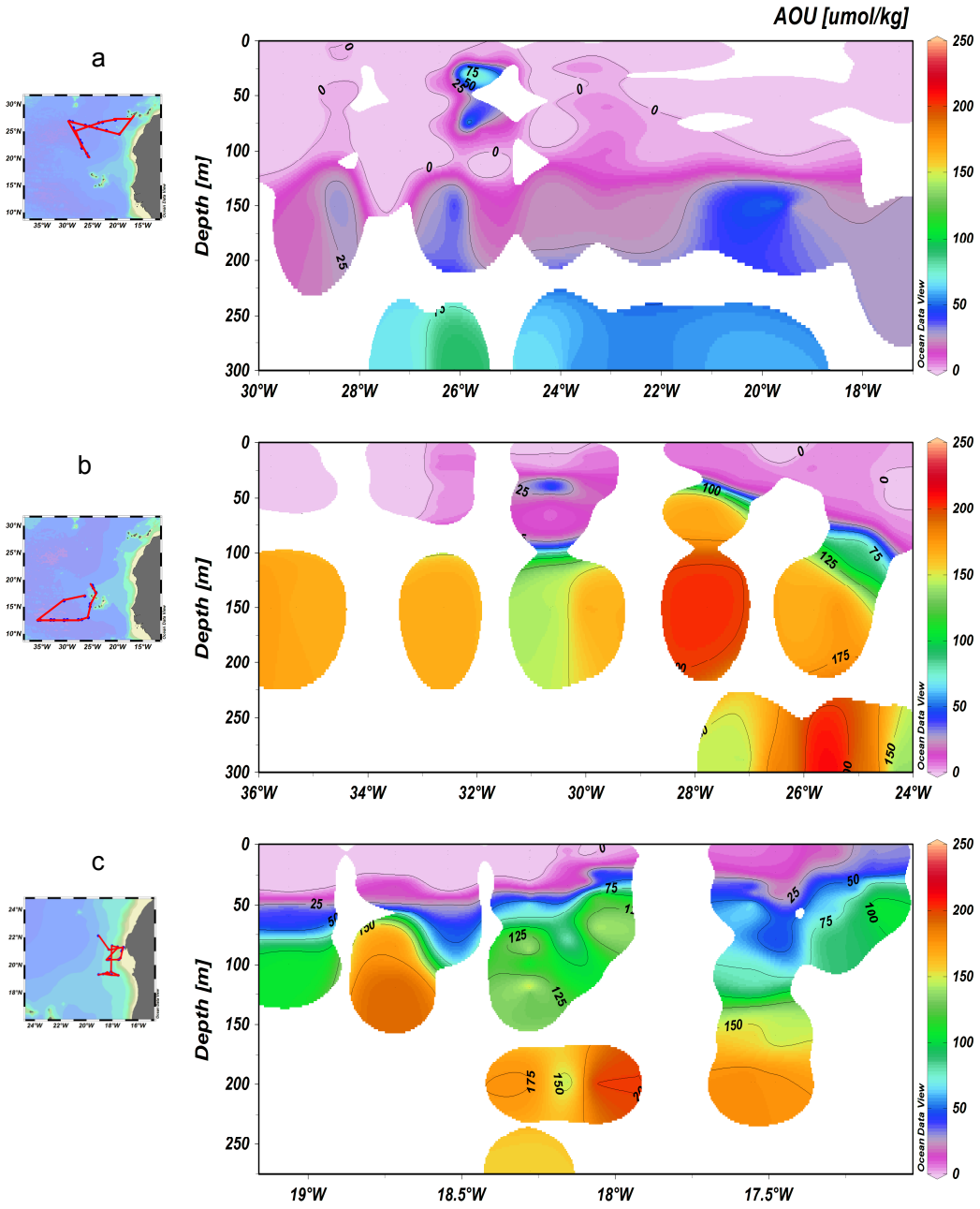

Figure 2.14 Vertical AOU gradients of a) the North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

Table 2.6 Correlations between AOU and density in the water mass isolated from atmosphere.

Variables	*n*	Confident level at 95 %	Correlation coefficient (r)
North Atlantic subtropical gyre	29	0.001	0.604*
Tropical North Atlantic Ocean	34	0.000	0.729*
Mauritanian shelf region	32	0.000	0.727*

* Correlation is significant at the 0.05 confident level.

On the whole, DOC accounted for a small contribution to AOU, which indicates that the bulk of respiration, particularly in the (sub-) tropical North Atlantic Ocean, was supported by the flux of POC. The contribution of DOC to water column respiration varies between regions with a relatively small contribution observed in most studies as indicated in Table 2.7. The small contribution of DOC respiration to AOU reflects that a large proportion of DOC is remineralized in surface waters (Hung et al., 2007). In the tropical North Atlantic Ocean, the relatively low contribution of DOC oxidation to AOU coincides with the extensive oxygen minimum zone in this area which is maintained by a high particulate organic carbon loading as a result of enhanced productivity resulting from nutrient supply through equatorial upwelling. The fraction of AOU derived from DOC degradation in this study appears to be within the ranges reported for the tropical North Atlantic Ocean (Pan et al., in Press) of approximately 10 - 30%, and for the Equatorial Atlantic (Thomas et al., 1995) of approximately 10 - 20%. The results indicate that only a relatively small fraction of AOU would be due to DOC oxidation, in agreement with previous studies (Hansell et al., 1993, Guo et al., 1994, Thomas et al., 1995). The insignificant correlation between DOC and AOU in the Mauritanian shelf region was possibly due to the high particle export (Fischer et al., 2009). Unfortunately, there was no data available on downward POC fluxes to investigate this hypothesis. In addition, the significant correlation between density and AOU and no correlation between DOC and AOU were evidenced in the Mauritanian shelf region reflecting that the distribution of AOU was related to mixing of water masses rather than *in situ* DOC oxidation. The result agrees well with the observations of Hansell et al. (1993) who concluded that mixing was the main mechanism controlling the distribution of AOU as there was a correlation between salinity and AOU but no correlation between DOC and AOU.

Table 2.7 Correlations between AOU and density in the water mass isolated from atmosphere.

Regions	*n*	DOC/AOU	Contribution of DOC to AOU [a] (%)	References
Global ocean	5371	-0.058 AOU (R^2 = 0.69)	8.4	Aristegui et al. (2002)
Indian Ocean				
North western Indian Ocean		-0.062 (full water depth)	9.0	Kumar et al. (1990)
Central Indian Ocean	68	-0.13 [b] (R^2 = 0.44) (between σ-t 26.75-27.0)	18.8	Doval and Hansell (2000)
Pacific ocean				
Western North Pacific Ocean		-0.23 (upper 500 m)	33.3	Ogura (1970)[cb]
Equatorial Pacific Ocean	41	-0.163 (R^2 = 0.49) (near surface water)	23.2	Martin and Fitzwater (1992)
Equatorial Pacific Ocean	117	-0.074 [b] (R^2 = 0.78) (40-400 m interval)	10.7	Peltzer and Hayward (1996)
Santa Monica basin		-0.068 [b] (R^2 = 0.26) (full water depth)	9.9	Hansell et al. (1993)
South Pacific Ocean	84	-0.23 [b] (R^2 = 0.45) (between σ-t 26.75-27.0)	33.3	Doval and Hansell (2000)
North-western Mediterranean Sea	21	-0.31(R^2 = 0.92)	44.9	Avril (2002)
Northern South China Sea		-0.0851 to -0.2311 (R^2 = 0.68) (mixing layer and ~ 1000 m interval)	12.3 - 33.5	Hung et al. (2007)
Gulf of Mexico	25	-0.15 (R^2 = 0.90) (upper 1500 m)	21.7	Guo et al. (1994)
Atlantic Ocean				
Equatorial Atlantic Ocean		-0.09 (April) (R^2 = 0.74) to -0.14(November) (R^2 = 0.40) (sub-surface data)	13.0 20.3	Thomas et al. (1995)
North Atlantic Ocean		-0.43 [b] (upper 3000 m)	62.3	Debaar et al. (1993) [d]
North Atlantic Subtropical Gyre		-0.07 (R^2 = 0.05) to -0.22 (R^2 = 0.60) (σ-t 26 - 27)	10.1 - 31.9	Pan et al. (in Press)
North Atlantic subtropical gyre	***24***	***-0.114 (R^2 = 0.28)***	***16.5***	***This study***
Tropical North Atlantic Ocean	***46***	***-0.097 (R^2 = 0.66)***	***14.1***	***This study***
Mauritanian shelf region	***32***	***insignificant***	***-***	***This study***

[a] calculated by assuming respiratory coefficient = -0.69
[b] TOC/AOU
[c] ratio reported from Doval and Hansell (2000)
[d] ratio reported from Thomas et al. (1995)

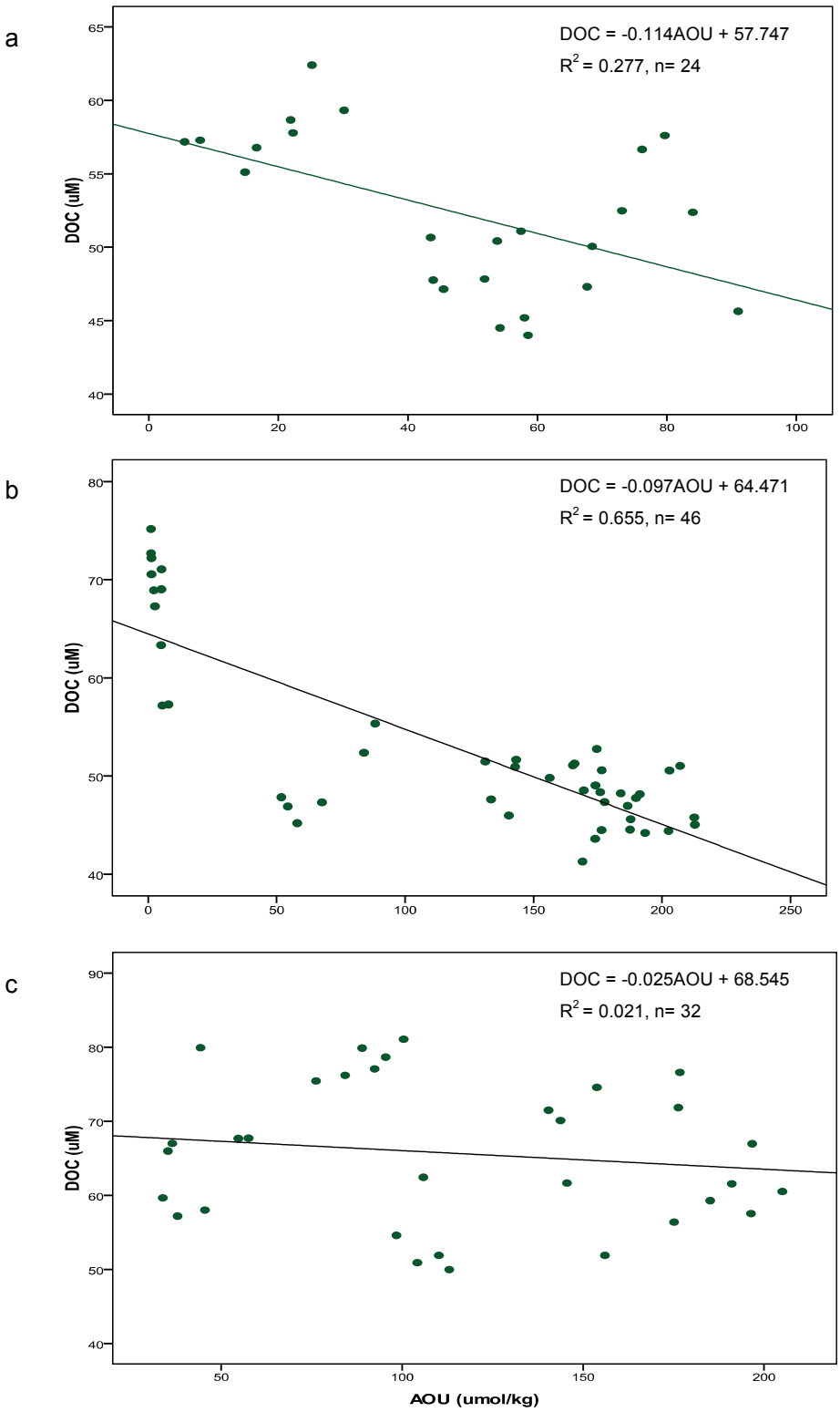

Figure 2.15 Correlations between DOC and AOU a) North Atlantic subtropical gyre, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region. The plots only show data points isolated from atmosphere and above 300 metres depth.

2.3.5 Environmental controls on DOC and DON distribution

In order to understand the influence of physical and biogeochemical processes on DOC and DON distributions in this study, a statistical correlation assessment was carried out. Table 2.8 summarizes the correlations between DOC and DON with density, chlorophyll *a*, bacterial abundance and DIN. The results indicate that DOC and DON did not show statistically significant correlations with the number of bacterial cells or chlorophyll *a* in the study areas. In addition, there was no relationship between DOC and DON with density and DIN in the Mauritanian shelf region. By contrast, DOC was inversely correlated with density, showing strong relationships in the North Atlantic subtropical gyre ($R^2 = 0.55$, $p < 0.05$, n= 154) and the tropical North Atlantic Ocean ($R^2 = 0.79$, $p < 0.05$, n =196). There were weak inverse correlations between DON and density in the North Atlantic subtropical gyre ($R^2 = 0.23$, $p < 0.05$, n = 125) and the tropical North Atlantic Ocean ($R^2 = 0.034$, $p < 0.05$, n = 203). Statistical correlation analysis demonstrated strong relationships between DOC and DIN in the North Atlantic subtropical gyre ($R^2 = 0.56$, $p < 0.05$, n = 142) and in the tropical North Atlantic Ocean ($R^2 = 0.77$, $p < 0.05$, n = 190), whereas relative weak correlations between DON and DIN were observed in both areas.

The results show that DOC and DON in the North Atlantic subtropical gyre and the tropical North Atlantic Ocean were significantly controlled by vertical and horizontal mixing. The mean DOC and DON concentrations in the tropical North Atlantic Ocean were higher than those in the North Atlantic subtropical gyre, which fits well with the observations of Roussenov et al. (2006) who suggested that the deeper mixed layer in the North Atlantic subtropical gyre favours a greater downward dilution. In addition, the enhanced DOC in surface water observed in the tropical North Atlantic Ocean was likely to be a result of the highly stratified water supporting an accumulation of DOC in the upper ocean (Hansell, 2002). The accumulation of DOC in the upper stratified water is a general pattern observed in other areas such as the Arctic Ocean (Dittmar and Kattner, 2003) and the North-western Mediterranean Sea (Avril, 2002). Another possible explanation is that only 14-17% of DOC was removed by microbial degradation (Table 2.7), with low chlorophyll *a* concentrations (Figure 2.6a and b) which suggest that mixing of water masses is the dominant mechanism controlling DOC distributions.

Table 2.8 Correlations between DOC and DON with density chlorophyll *a* bacteria cells and DIN.

Variables		*n*	Confident level at 95 %	Correlation coefficient (r)
North Atlantic subtropical gyre				
DOC	Density	154	0.000	-0.744*
	Chlorophyll *a*	111	0.714	-0.035
	Bacteria cells[a]	17	0.096	-0.417
	DIN	142	0.000	-0.748*
DON	Density	125	0.000	-0.479*
	Chlorophyll *a*	93	0.142	-0.153
	Bacteria cells[a]	15	0.315	-0.279
	DIN	125	0.000	-0.567*
Tropical North Atlantic Ocean				
DOC	Density	196	0.000	-0.889*
	Chlorophyll *a*	105	0.131	-0.148
	Bacteria cells[a]	21	0.597	-0.123
	DIN	190	0.000	-0.879*
DON	Density	196	0.008	-0.185*
	Chlorophyll *a*	114	0.528	0.060
	Bacteria cells[a]	21	0.107	0.362
	DIN	202	0.017	-0.167*
Mauritanian shelf region				
DOC	Density	88	0.605	-0.055
	Chlorophyll *a*	44	0.063	-0.281
	Bacteria cells	16	0.478	-0.191
	DIN	84	0.117	0.172
DON	Density	87	0.410	0.089
	Chlorophyll *a*	44	0.065	-0.281
	Bacteria cells[b]	16	0.478	-0.191
	DIN	83	0.746	-0.036

[a] data were obtained from underway sampling onboard the RRS Discovery (D326).
[b] data were obtained from CTD sampling onboard the RRS Discovery (D338). Only bacterial abundance in the surface water was included in the statistic analysis.
* correlation is significant at the 0.05 confident level.

DOM is primarily supplied by biological production (Ogawa and Tanoue, 2003, Hansell et al., 2009). DOM was released by phytoplankton biomass (Suratman et al., 2009, Nagata, 2000) during their exponential growth phase observed during axenic experiments (Suratman et al., 2008). According to chlorophyll *a* profiles, the North Atlantic subtropical gyre (Figure 2.6a) and the tropical North Atlantic Ocean (Figure 2.6b) were low productivity areas (chlorophyll *a* < 0.25 mg/m^3) (Varela et al., 2005). The absence of any

statistical correlation between DOC and DON with chlorophyll *a* values observed in this study is in line with many other observations in oligotrophic marine environments (Menzel and Ryther, 1970, Hansell and Carlson, 2002).

The distribution of DOC and DON can also depend on the nutrient status in the water column. Phytoplankton introduce DOM into the water column via uptake of essential nutrients and trace elements such as nitrate, phosphate and iron (Koeve, 2001). Sanders et al. (2005) estimated averagely 140 mol/m^2 of nitrogen were converted from nitrate to TON in surface waters of the Irminger basin. According to Williams (1995), the accumulation of DOC can be related to a shortage of inorganic nutrients. In addition, Varela et al. (2005) found that DON release rates of phytoplankton showed a positive relationship with nitrate uptake in the Central Atlantic Ocean (Eastern Canary Coastal Province and Eastern tropical Atlantic Province) where high values of nitrate uptake exist in sub-surface water. Nitrogen uptake information is not available for our study so the concentrations of DIN, predominantly in the form of nitrate, were considered. The correlations between DOC and DON with DIN in this study revealed statistically significant, inverse correlation in the North Atlantic subtropical gyre and the tropical North Atlantic Ocean (Table 2.7), possibly reflecting a conversion of dissolved organic nutrient to inorganic nutrient pools by phytoplankton communities. On the other hand, the inverse correlation may be a consequence of the inorganic nutrient deficient status of the water. By comparison, there was no such a correlation found in the Mauritanian shelf region where nutrient-enriched water masses are mixed upwards to form a productive region.

The occurrence of elevated DON concentrations in sub-surface water in the tropical North Atlantic Ocean (Figure 2.10) suggests a physical influence of turbulent upward fluxes of DON from below the thermocline, which were calculated at the Guinea Dome (11.3ºN) to be 5 - 10 times greater than downward fluxes of DON (Vidal et al., 1999). Despite the fact that there was no statistical correlation between DOC and DON with chlorophyll *a* or the number of bacterial cells, some biological mechanisms can be postulated to explain this observation. Firstly, enhanced DON concentrations were associated with enhanced nitrogen fixation rates (Figure 2.16) and a high abundance of *Trichodesmium* cells (Figure 2.17). According to Carpenter and Romans (1991), nitrogen fixation by the diazotroph *Trichodesmium* delivers more than double the nitrogen input relative to nitrogen supplied from below the euphotic zone in the North Atlantic Ocean, which is in good agreement with Mahaffey et al. (2004) and Moore et al. (2009) who suggested that nitrogen fixation is important in the tropical North Atlantic Ocean where the aerosol-derived iron is supplied from continents. Iron is a key nutrient for the nitrogen fixers, as these organisms have a

high iron demand through their relatively high photosystem I abundance and the iron containing enzyme nitrogenase (Moore et al., 2009). Mahaffey et al. (2003) used a stable nitrogen isotope technique and found nitrogen fixation dominated in the North Atlantic subtropical gyre. Furthermore, Mulholland et al. (2003) estimated that 80 - 90% of fixed nitrogen was excreted during nitrogen fixation, while Vidal et al. (1999) reported an excess DON flux associated with an abundance of *Trichodesmium cells*, *a* nitrogen-fixing plankton. Therefore, the significant numbers of *Trichodesmium* observed in this area were likely to supply fixed nitrogen into the water column as DON. Further evidence of DON accumulation associated with *Trichodesmium* population was reported in the North Pacific Ocean (Karl et al., 1997, Karl et al., 1992). Furthermore, the accumulated organic matter in sub-surface waters along 12ºN transect was nitrogen rich, with a low C:N molar ratio ranging from 3.1 - 5.0, which is even lower than *Trichodesmium* cell C:N stoichiometry (6.3) (LaRoche and Breitbarth, 2005). C:N molar ratios in upper 300 m were uniform rather than increasing with increasing depths (Table 2.5). The N:P molar ratios presented in the upper water column were approximately 41 - 43 (Table 2.5); close to the typical N:P molar ratios of 40 to 50 reported for *Trichodesmium* cells (Letelier and Karl, 1996, Carpenter, 1983). Another plausible source of excess DON could be extracellular release during primary production (Zehr and Ward, 2002, Ogawa and Tanoue, 2003, Lapierre and Frenette, 2009).

In the Mauritanian shelf region, nevertheless, the statistical analysis showed an absence of correlation between DOC and DON and other variables (Table 2.8). In the literature there are few reports of investigations into the processes controlling DOM distributions in upwelling systems. DOM distributions in the Mauritanian shelf region were reported in relation to biogeochemical processes such as phytoplankton exudation remineralisation, zooplankton excretion (Teira et al., 2001, Dadou et al., 2001) and physical processes such as water mass subduction (Dadou et al., 2001). Suratman (2009) observed seasonal variations in the relationships between DOC and chlorophyll *a* in the North Sea which can be associated with the growth phase and species composition of phytoplankton and *in situ* nutrition conditions (Wetz and Wheeler, 2007). In addition, the influence of upwelling on organic matter distributions has been reported for many regions such as the Arabian Sea (Hansell and Peltzer, 1998, Peltzer and Hayward, 1996). Elevated DOC values were observed during periods when the upwelling system was weak or not active (e.g. El Niño), while intense upwelling *(*La Niña) tends to reduce DOC concentrations (Hansell and Peltzer, 1998, Peltzer and Hayward, 1996). Thus, the DOM concentrations in the Mauritanian shelf region are still assembly controlled through both biogeochemical and physical processes despite the fact that there was paucity of statistical proof.

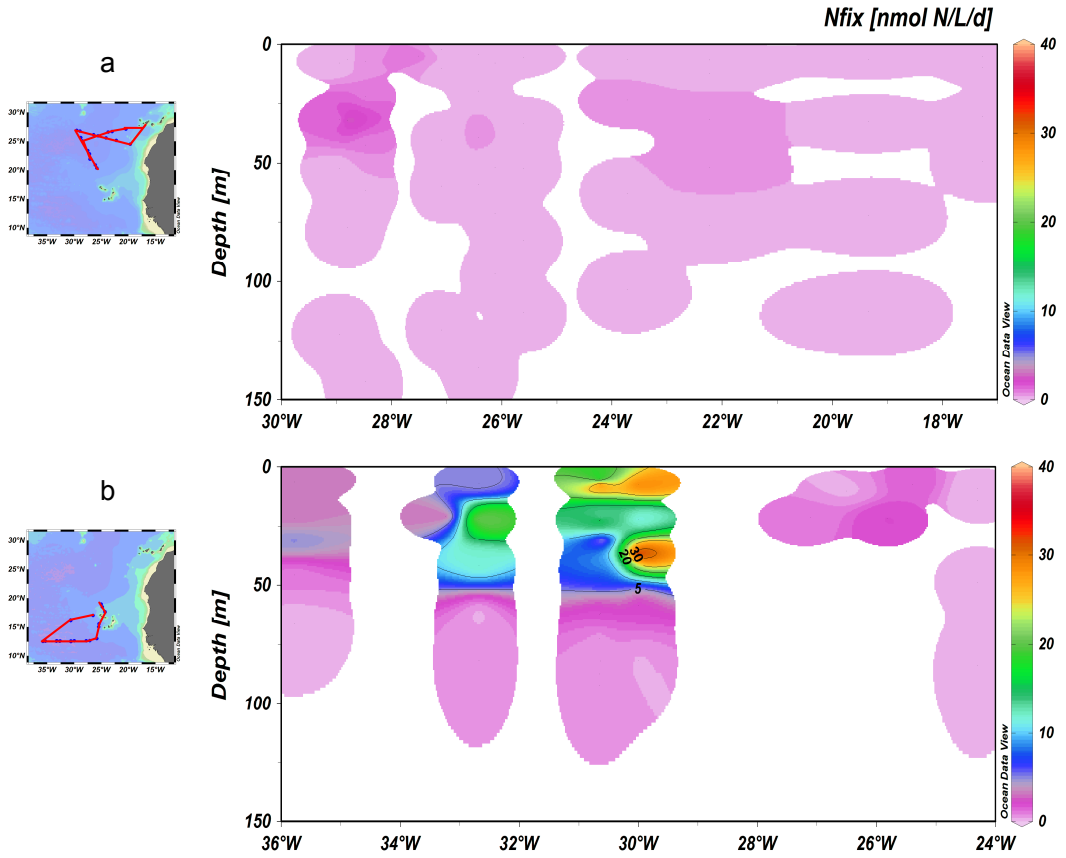

Figure 2.16 Nitrogen fixation rate of (a) the North Atlantic subtropical gyre and (b) the tropical North Atlantic Ocean.

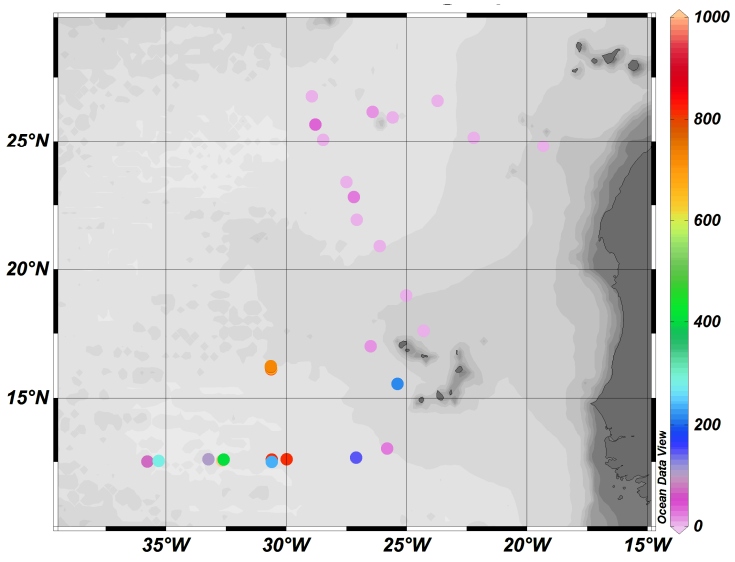

Figure 2.17 *Trichodesmium* abundance (filaments/litre) in upper water observed during the *RRS Discovery* cruise D326.

2.4 Conclusions

This study showed vertical DOC profiles that were fairly uniform over the (sub)-tropical North Atlantic with relatively high concentrations in the surface water and decreasing concentrations with depth. DON distributions showed more variability, with non-typical DON vertical profiles in the tropical North Atlantic Ocean along the 12ºN longitude section. The refractory DOC accounts for approximately 57 - 65% of total DOC and this fraction increases with increasing depth, to 85 - 92% at 200 - 300 m. The refractory DON represented 35 - 61% of the bulk DOC pool in surface water and increased to 67 - 100% in the sub-surface water at 200 - 300 m. The magnitudes of vertically integrated DOC and DON stocks coincided with the mixed layer depths. The elemental ratios of DOM in both the bulk and refractory pools were carbon-rich relative to nitrogen and phosphorus indicating an order of preferential degradation of organic matter with phosphorus > nitrogen > carbon. Only a small fraction, approximately 14 - 16%, of oxygen consumption could be accounted for by DOC oxidation in the North Atlantic subtropical gyre and the tropical North Atlantic Ocean. The insignificant correlation between DOC and AOU in the Mauritanian shelf region was a result of the high particle sinking rate in the upwelling area. The covariance of AOU and density suggests that AOU distribution in sub-surface water was dependent on mixing processes rather than *in situ* oxidation of DOC. The covariance of DOC and DON with density suggests that DOC and DON are mainly controlled by mixing processes in the North Atlantic subtropical gyre and in the tropical North Atlantic Ocean. Biogeochemical processes such as phytoplankton production and heterotrophic bacteria were likely to have a minor impact. The absence of correlation between DOC and DON with other variables (Table 2.8) in the Mauritanian shelf region is not consistent with many reports in the literature, suggesting that the DOM distributions in the Mauritanian shelf region are influenced by a complex mixture of both biogeochemical and physical processes. An air-sea interaction such as atmospheric deposition can potentially play a role in affecting distributions of DOC and DON. Atmospheric inputs play a significant role in supplying nutrients including organic matter into marine environments (Seitzinger and Sanders, 1999, Bronk et al., 2007, Berman and Bronk, 2003), particularly in this study area, which routinely receives large inputs of aerosols of terrestrial and industrial origins (Arimoto et al., 1995, Kaufman et al., 2005, Ansmann et al., 2009, Zamora et al., 2010). The study of the deposition of atmospheric organic matter is a part of this thesis. The methodology and results will be discussed in Chapter 3.

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Chapter 3

Atmospheric Organic Carbon and Organic Nitrogen Supply to the (sub-) tropical North Atlantic Ocean

Abstract

Atmospheric dry deposition is an important pathway for the delivery of organic nutrients to the surface ocean. This study aims to quantify organic nutrients (carbon, nitrogen and phosphorus) supplied by atmospheric inputs to the surface waters of the (sub-) tropical North Atlantic Ocean. This ocean region is an important recipient of dust from the Sahara and Sahel desert areas. Bulk aerosols samples were collected on the island of São Vicente (Cape Verde Islands) over a period of one year and during a dedicated cruise in the study region (D326 cruise). The aerosols were classified into 6 types associated with air mass back trajectory analysed using the HYSPLIT model and through the colour of the aerosols. The air masses transported over the (sub-) tropical North Atlantic Ocean were derived from different sources regions; namely the Sahara region, the Sahel and tropical rainforest region, Europe and America. The air masses appeared to be influenced by multiple continental sources in some periods. Air masses originating from the Saharan region (aerosol type 2) made a dominant contribution to aerosol loadings over the entire sampling periods for both land-based sampling (38.8%) and ship-based sampling (60.4 %). Leaching experiments were performed to quantify organic carbon and organic nitrogen supplied by atmospheric aerosols. The results indicated that leachable organic carbon (LOC), leachable total nitrogen (LTN) and leachable organic nitrogen (LON) were supplied by multiple source regions. Air masses transported from the Saharan region supplied the highest LOC and LTN deposition fluxes, while LON fluxes were derived predominantly from air masses of multiple origins including the Saharan and Sahel regions, Europe and North America. However, an important contribution of biomass burning to LOC, using potassium (K) as a tracer, was not found in the study. The organic N:P ratios of the aerosols transported in the air mass from all origins ranged from 117.6 - 758.6

revealing that the aerosols were enriched in organic nitrogen in relation to phosphorus. The relatively high mean organic C:N ratios of aerosols originating from the Saharan region (13.5) and from Europe and North America (12.0) implies that these aerosols were enriched in carbon relative to nitrogen. Residence times of organic nutrients in the surface ocean were estimated using dry deposition fluxes of LOC, LTN, LON and leachable phosphate (LPO) and inventory of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON) and phosphate (PO_4^{3-}) concentrations in relation to mixed layer depths. Relatively short residence times of LOC and LON were observed in the upwelling region indicating the presence of rapid biotic removal processes. This observation fits well with the enhanced abundance of heterotrophic bacteria and marine phytoplankton found in this region. In addition, abiotic processes such as phototransformation and adsorption to sinking particles are potential removal pathways for organic carbon and organic nitrogen compounds in the water column. Conversely, relatively enhanced residence times of LTN and LPO were observed in the upwelling region, reflecting the high nutrient concentrations as a result of upward transport of deep waters.

3.1 Introduction

The atmosphere is considered an important pathway for nutrient transport (Seitzinger and Sanders, 1999, Duce et al., 1991, Sarthou et al., 2003, Baker et al., 2003, Duce et al., 2008, Cape et al., 2011). Atmospheric aerosols can be generated by many sources such as vehicle emissions (de Leeuw et al., 2003, Mahowald et al., 2005); biomass burning (Mace et al., 2003a, Duan et al., 2004, Zheng et al., 2005, Ansmann et al., 2009); industrial activities (Hartmann et al., 2008, Lewandowska et al., 2010); agriculture activities (Dragosits et al., 1998, Mace et al., 2003b) and deserts (Markaki et al., 2003, Hartmann et al., 2008, Schepanski et al., 2009, Baker et al., 2010, Lesworth et al., 2010). Atmospheric aerosols from anthropogenic sources contribute to regional environmental issues because of their relatively small size spectrum and consequent involvement in long-range aerosol transport. Examples of such anthropogenic aerosols include the Indo-Asian Haze which is transported from Asia over the Pacific Ocean (Ramanathan et al., 2001) and the biomass burning smoke from western African which is transported as far as South America (Ansmann et al., 2009).

A number of early studies have focussed on geochemical signatures and mineralogical characteristics of aerosols. The depositions of aerosol-derived inorganic nutrients and trace elements have been investigated in a range of studies due to their role in the primary productivity of marine organisms (Violaki et al., 2010, Krishnamurthy et al., 2010). However, the deposition of organic nutrients and consequent influences on the marine ecosystem are insufficiently understood, particularly in the (sub-) tropical North Atlantic Ocean which not only receives atmospheric aerosols from the Saharan and Sahel regions but also atmospheric pollution emitted by anthropogenic sources in North America, Europe and Africa (Arimoto et al., 1995, Kaufman et al., 2005, Ansmann et al., 2009, Zamora et al., 2010). There is evidence that the growth and productivity of phytoplankton and heterotrophic bacteria is not determined solely by inorganic nutrients. The utilisation of organic nutrients as alternative nitrogen and phosphorus sources has been widely reported (Seitzinger and Sanders, 1999, Berman and Bronk, 2003, Bronk et al., 2007). Atmospheric aerosols supply significant amounts of bioavailable organic carbon and organic nitrogen to marine organisms (Cornell et al., 1995, Seitzinger and Sanders, 1999, Peierls and Paerl, 1997, Duarte et al., 2006) and form a significant source of fixed nitrogen to marine systems (Zhang et al., 2002). Aerosol-derived organic matter consequently stimulates marine heterotrophic bacteria and phytoplankton productivity (Paerl et al., 1990, Seitzinger and Sanders, 1999, Duarte et al., 2006, Lewandowska et al., 2010) and therefore also impacts the carbon cycle (Pulido-Villena et al., 2008).

Aerosols can be present in the atmosphere as primary and secondary aerosols. Primary aerosols are defined as particles that are emitted directly into atmosphere during combustion processes such as gasoline combustion and biomass and field agricultural fires (Duan et al., 2004). Aerosols originating from natural sources such as plant spores, pollen, leaf fragments or soil organic matter and mineral dust are also categorised as primary aerosols (Fermo et al., 2006, Baker et al., 2006). Secondary aerosols are defined as aerosols originated from atmospheric chemical conversion processed, such as gas-to-particle conversion, condensation, and other physical and chemical processes (Fermo et al., 2006).

Components contained in aerosols can be used as tracers to identify their origins (Trapp et al., 2010). For instance, silicon (Si) (Baker et al., 2006) and aluminium (Al) are dominant in desert type aerosol and have been used as proxies for mineral dust from the Saharan desert (Guieu et al., 2010, Herut et al., 2002). Phosphate can be derived from a variety of sources including mineral dust, biomass burning and fossil fuel combustion (Mahowald et al., 2008, Baker et al., 2010). Recently, Trapp et al. (2010) reported that manganese (Mn) forms an excellent proxy for dust from the Sahara. Nitrogen oxides (NO_x) emitted during combustion processes are the dominant source of nitrate in aerosols (Aneja et al., 2001), whilst ammonium is associated with emissions from biomass burning, marine sources and agricultural activities (Baker et al., 2006). Non-sea salt sulphate is predominantly associated with fossil fuel combustion but is also an indicator of biomass burning (Gabriel et al., 2002, Baker et al., 2010). In addition, potassium (K) can be used as indicators of biomass burning activities (Duan et al., 2004). Vanadium (V) and nickel (Ni) are enriched in aerosols produced by urban, petrochemical sources, electrical power plants and ships (Divita et al., 1996, Vitolo et al., 2000, Wang et al., 2006, Navarro et al., 2007, Seggiani et al., 2007, Querol et al., 2007). It has been widely reported that fuel oils are enriched in vanadium (Chen and Duce, 1983). Nickel reflects a contribution from smelters and incinerators (Cempel and Nikel, 2006, Trapp et al., 2010). Cadmium (Cd) is emitted from the manufacturing of non-ferric metal, incineration of solid municipal waste, and possibly from construction in marine environments with corrosion resistant substances (Guieu et al., 2010, Trapp et al., 2010).

Organic carbon occurs in both primary aerosols as biogenic aerosol (Lewandowska et al., 2010) and aerosols formed during combustion processes (Andreae et al., 1998, Liousse et al., 1996, Zheng et al., 2005), agricultural activities and biomass burning (Baker et al., 2006, Baker et al., 2010, Duan et al., 2004) or in secondary aerosol particles after oxidation and gas-to-particle transformation of volatile organic compounds (VOC) (Fermo

et al., 2006). Organic carbon has been observed in aerosols collected along the coastal region of Baltic Sea, which is adjacent to agricultural and industrial areas (Lewandowska et al., 2010). Biomass burning also contributes to atmospheric organic carbon in many regions (Duan et al., 2004, Zheng et al., 2005, Graham et al., 2003). Atmospheric carbonaceous compounds include sugars and sugar alcohols, fatty acids, anhydrosugars and short-carbon carboxylic acids (Graham et al., 2003).

Organic nitrogen in aerosol can be generated from several sources. Lesworth et al. (2010) reported water soluble-organic nitrogen in air masses from desert regions and also from anthropogenic sources. Anthropogenic atmospheric organic nitrogen sources are considered to be more importance than natural sources (Shi et al., 2010, Violaki et al., 2010). Deposition of anthropogenic organic nitrogen to the surface ocean for the year 2000 was reported to be 80% of deposited atmospheric organic nitrogen. The deposition rate is predicted to slightly increase up to 82.6% by 2030 with more deposition to open ocean regions (Duce et al., 2008). Moreover, organic nitrogen was also found in biogenic aerosols such as pollen spores and plant debris (Violaki et al., 2010). The determination of specific organic nitrogen species in aerosols is challenging and a limited number of studies have reported specific organic nitrogen compounds in aerosols. Organic nitrogen species identified in atmospheric aerosols can be related to their origin and atmospheric chemical re-processes, and include amines and amino acids (Zhang et al., 2002, Cornell et al., 2003, Mace et al., 2003a), urea (Cornell et al., 2001, Cornell et al., 2003, Shi et al., 2010), peroxyacetyl nitrate, methyl cyanide and N-substituted polycyclic aromatic hydrocarbons (Anastasio and McGregor, 2000).

The aim of this study is to present LOC and LON concentrations in atmospheric aerosols transported over the (sub-) tropical North Atlantic Ocean, an area which receives significant atmospheric dust from continents. This study also aims to identify the sources of the aerosols and assess the potential effects of dust on bacteria and phytoplankton communities.

3.2 Methods

3.2.1 Aerosol sample collection

Bulk aerosol land-based sampling was performed from a 30 m high observatory tower on the island of São Vicente, Cape Verde (16°51'49 N, 24°52'02 W) (Figure 3.1). Due to its relatively close proximity to African coast (approximately 485 nautical miles), the island of São Vicente is an ideal sampling site. The island is situated within the main transport route of North African dust over the tropical North Atlantic Ocean and year-round receives significant amounts of African dust, with periodic intense dust storms. The sampling tower is on the coast, facing the predominant north-easterly trade winds observed in this region; consequently contamination risk derived from island sources is negligible.

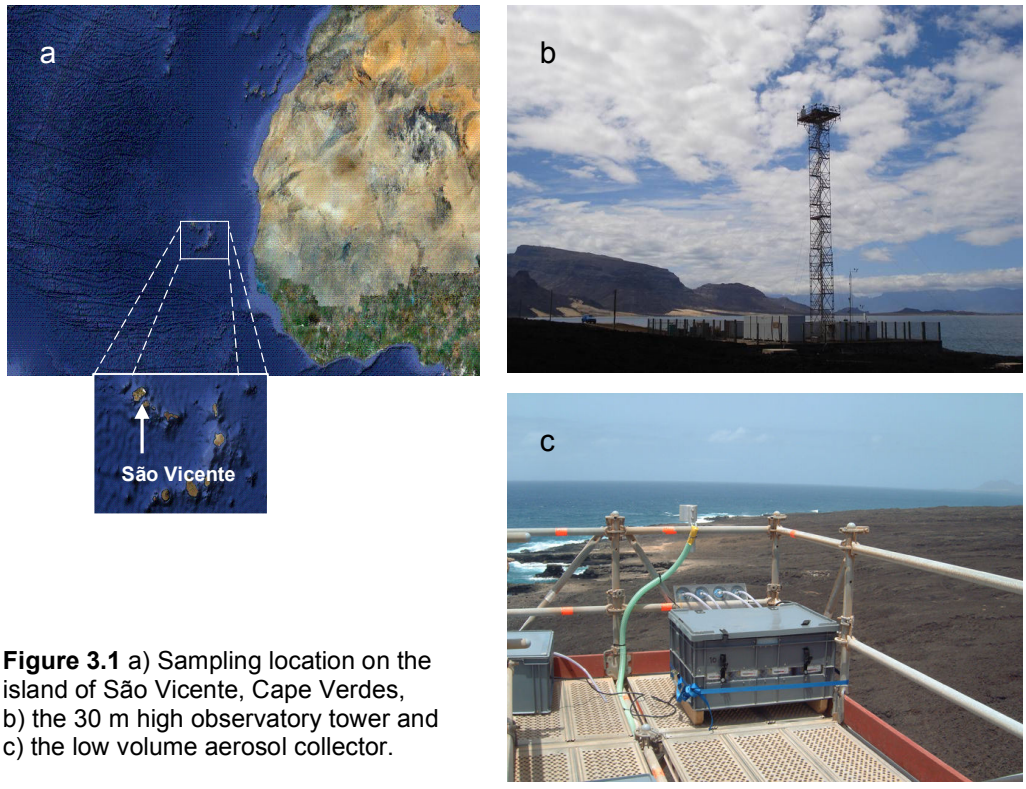

Figure 3.1 a) Sampling location on the island of São Vicente, Cape Verdes, b) the 30 m high observatory tower and c) the low volume aerosol collector.

The sampling period was in the period between July 2007 and June 2008. Aerosol samples were collected (in duplicate) on acid washed, 47 mm, 0.4 µm polypropylene filters (Sterlitech Inc, USA) using a low-volume aerosol collection system pumping at a rate of ~30 litres of air per minute. Mass flow meters were used to measure the volume of air pumped over the filters. The filters were collected and replaced every 48 to 72 hours. Ship-based sampling was performed onboard the *RRS Discovery* (D326 cruise) in the period between January and February 2008; the sampling approach at sea was similar to the land-base aerosol sampling. However, the collection periods were slightly shorter and

varied between 8 and 40 hours depending on dust loads. The sampler was located on the deck above the bridge. The contamination from ship exhaust was prevented by cutting off air flow to the pumps when the wind speed and direction was outside specified limits (< ± 90º from the bow; < 2 m/s) (Patey, 2010). Collected filters containing atmospheric aerosols and procedural blank filters were stored at -20ºC prior to leaching experiments and analysis. Procedural blanks were obtained during the sampling exercises to evaluate contamination through sample handling by deploying cleaned filters in the system without running the pumps; these filters were immediately removed from the system and stored in a similar manner to the aerosol filters.

3.2.2 Aerosol leaching experiments

Leaching experiments were carried out in a class -100 laminar flow hood. The filtration unit (Millipore, Polysulphone) was acid-washed using 10% hydrochloric acid and subsequently rinsed with deionised water (MilliQ, Millipore; > 18.2 MΩ.cm^{-1}) to remove inorganic and organic impurities from the apparatus. Leaching experiments for LOC and LON in deionised water were performed by quickly passing 100 mL of deionised water through the whole dust-laden 47 mm polypropylene filter (Buck et al., 2006).
The filtrate was subsequently transferred into a pre-combusted glass ampoule. The filtrate was acidified to pH 2 using 50% v/v hydrochloric (final concentration was 0.1% v/v) before flame-sealing with a butane/propane burner and storage in a refrigerator at 4ºC until analysis. The fraction of organic carbon and organic nitrogen measured following this procedure will be referred to as the leachable fraction. The sequential leaching experiment was performed to test the efficiency of the leaching procedure for aerosol-derived organic carbon and organic nitrogen by passing a second 100 ml of deionised water through the selected filters. The result shows that the majority of LOC (> 90 %) and LON (> 98 %) are extracted in the first 100 mL of leachate. The procedure blanks were negligible in all filters. Filter blanks were obtained by passing 100 mL of deionised water through non-deployed clean polypropylene filters. The filter blanks for the experiment were 4.91 ± 6.9 µM for LOC and 0.68 ± 0.9 µM for LTN. The filter blanks (n = 18) accounted on average for ~ 28 % and ~ 2.3 % of mean LOC and LTN obtained in aerosol filters, respectively. The relatively large contribution of LOC in the blank can be attributed to the filter material. All data presented in this study have been blank-corrected. Negative values observed in the data set may be due to errors associated with the three nitrogenous analyses (ammonium, nitrite + nitrate and total dissolved nitrogen) (Baker et al., 2010) or the contribution from filter blanks. The standard error of ammonium, nitrite + nitrate and total dissolved nitrogen measurements was approximately 1.31 µM. The negative values were not included in the calculations. The reproducibility of the analyses was ascertained by

analysing replicate aerosol filters collected over the same sampling period. Duplicates of deionised water leaching analyses were conducted on eight pairs of aerosol filters. The replicate pairs had a variation (relative standard deviation) of ± 0.8% to ± 27.7% with an average of 10.4% for LOC. The precision from LTN replicates varied from ± 0.05% to ± 22.98% with an average of 6.9%.

Chemical characteristics of aerosols have been reported by different researchers in different ways. Arithmetic means were typically used in many studies (Shi et al., 2010, Violaki et al., 2010, Mace et al., 2003a, Trapp et al., 2010, Koulouri et al., 2008, Karthikeyan et al., 2009, Sarthou et al., 2003, Arimoto et al., 1995, Pio et al., 2008, Ying et al., 2007, Lewandowska et al., 2010, Zhang et al., 2002). Lesworth et al. (2010) and Baker et al. (2010) reported the median concentrations of elements in aerosol, while the geometric mean was used by Herut et al. (2002), Kocak et al. (2004; 2007). The atmospheric concentrations are reported in this study as geometric mean (hereafter mean) owing to the log-normality of the dataset and to avoid undue influence from high concentration tails by using arithmetic means (Herut et al., 2000). The log-normality of the dataset was verified by applying the Kolmogorov-Smirnov test (Kocak et al., 2004) at 99% confidence level. Figure 3.2 demonstrates the log-normal distributions of the aerosol population for LOC and LON. Leaching experiments were also performed on selected samples using low nutrient surface seawater (collected on the D326 cruise) as a leaching solution in order to investigate the relative importance of the matrix on the leaching process.

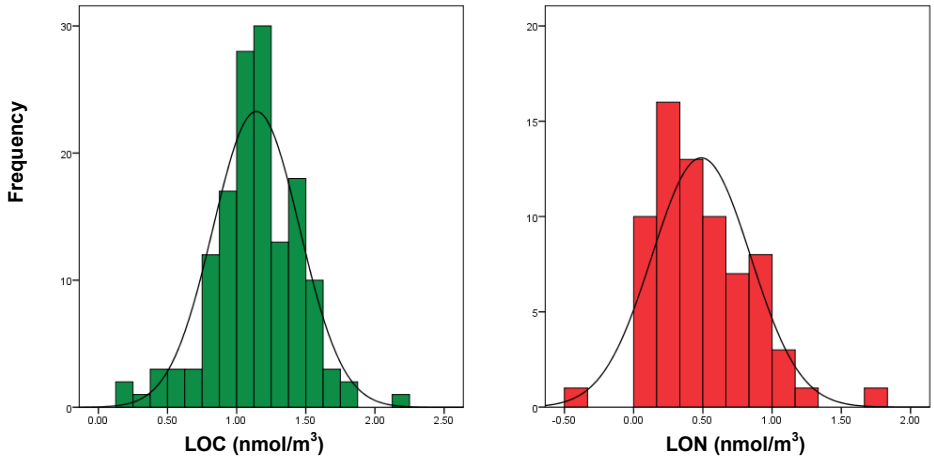

Figure 3.2 Population frequency histogram of LOC and LON log-transformed atmospheric concentrations and their expected normal distributions (black lines) at Cape Verde.

3.2.3 Chemical analysis

3.2.3.1 Leached organic carbon and nitrogen measurements

Analyses for organic carbon and nitrogen leached by deionised water and seawater were undertaken using a high temperature combustion technique (Badr et al., 2003). The analyses were undertaken using a Shimadzu TOC-V CPH/CPN instrument. Principally, a sample is injected into the total carbon combustion tube containing a catalyst (Pt at 720 ºC) with a ceramic wool layer on top. The purpose of the ceramic wool layer is to prevent built up of salt on the Pt catalyst. During the oxidation process, non-purgeable carbon contained in the sample is converted to carbon dioxide (CO_2), and inorganic and organic nitrogen compounds are converted to NO (Pan et al., 2005). The sample combustion products from the combustion tube are subsequently carried by a carrier gas to an electronic dehumidifier and halogen scrubber before delivery to a non-dispersive infrared gas analyser (NDIR) where the CO_2 is detected. Subsequently, excited nitrogen dioxide (NO_2) species are produced by the reaction between the NO and ozone, and detected in the chemiluminescence detector (NCD). A summary of instrumental conditions is presented in Table 3.1.

Table 3.1 Instrumental conditions of the Shimadzu TOC-V CPH/CPN instrument.

Parameters	Conditions
Combustion temperature	720 ºC
Carrier gas	Oxygen (ultra pure, 99.999%)
Carrier gas flow rate	150 ml/min
Sample sparge time	2:30 mins
Minimum number of injections	3
Maximum number of injections	5
Number of washes	2
Standard deviation maximum	0.100
CV maximum	2.00%
Injection volume	100 µL

Calibration of the instrument was performed using a potassium hydrogen phthalate (KHP) and glycine mixture. The deep Sargasso seawater CRM obtained from Hansell laboratory, University of Miami, was used to assess the performance of the HTC technique. The results showed that the analyses of the deep Sargasso seawater for average carbon and nitrogen were 42.3 $\pm$ 3.6 µM and 33.9 $\pm$ 1.5 µM, respectively, which are in good agreement with the certified values (41-44 µM for C and 32.25 - 33.75 µM for N, Batch 9). Carbon contamination typically comes from the catalyst (Spyres et al., 2000, Sharp et al., 1995), therefore regularly cleaning and conditioning of the catalyst minimises contamination (Alvarez-Salgado and Miller, 1998, Wiebinga and de Baar, 1998). Fresh

deionised water was used as a system blank to evaluate the system contamination. For this, fresh deionised water was acidified and analysed as a sample. The system blanks in this study were 0.6 µM for carbon and 0.3 µM for nitrogen. LON was derived as the difference between LTN and leachable inorganic nitrogen (ammonium and nitrite + nitrate) according to the equation (1):

$$LON = LTN – (NH_4 + NO_2 + NO_3) \quad (1)$$

3.2.3.2 Relevant data

Additional relevant data regarding the leaching experiments, namely concentrations of inorganic nitrogenous species, aluminium, sulphate, phosphate, potassium, vanadium, cadmium and nickel were obtained from Patey (2010). The methodologies for the determinations are depicted in the Table 3.2. This data was obtained from the same samples from which the organic carbon and organic nitrogen data was obtained. The oceanographic data related to D338 cruise was obtained from the British Oceanographic Data Centre.

Table 3.2 List of relevant parameters and analytical approaches.

Parameters	Analytical approaches
Nitrate + Nitrite	Nutrient autoanalyser (segmented flow analysis + colourimetric chemistry)
Phosphate	Nutrient autoanalyser (segmented flow analysis + colourimetric chemistry)
Ammonium	OPA fluorescence
Aluminum	ICP-MS Analysis
Sulphate	Ion Chromatography
Potassium	ICP-MS Analysis
Vanadium	ICP-MS Analysis
Cadmium	ICP-MS Analysis
Nickel	ICP-MS Analysis

3.2.4 Atmospheric transport and aerosol type classification

Air mass origin and transport pathways are key factors controlling concentrations of aerosols collected at sea and on sampling towers (Baker et al., 2006). The air mass back trajectories during the sampling period were calculated to identify the potential air mass origins. Five-day air back trajectory analysis was conducted using the HYSPLIT (Hybrid Single particle Lagrangian Integrated Model) (Draxler and Rolph, 2010) at 12 hours intervals. Owing to the possibility of gravitational settling or mixing of aerosols between altitudes, six arrival altitudes were chosen; 30 m, 750 m, 1500 m, 2000 m, 2500 m and 3000 m. The air masses measured at 750 m and above are indicators of long-range transport of aerosol, while the air mass measured at 30 m is indicating local ground boundary winds. The aerosol type classifications were carried out using a modification of the classification method by Hess et al. (1998) and Lewandowska et al. (2010). The dominant air mass origins of individual samples obtained from HYSPLIT were used for classification of their aerosol types. Since the Cape Verde Islands are in the North Atlantic Ocean, aerosols collected on the island of São Vicente were always affected by either an oceanic environment or shared effects of an oceanic and a continental environment. Furthermore, some filters were likely to collect aerosol influenced by multiple continental sources. In addition, the classification of aerosol type took account of the fact that the colour of mineral dust is typically yellow to brown (Baker et al., 2010). Thus, any sample with a typical mineral colouration was classified as being derived from the Saharan region. The regional sources were divided into 6 aerosol types as described in Table 3.3; two of which represent aerosol transported from multiple sources (M2/3 and M2/3/4). Ship-collected aerosols were classified in a similar manner to the land-collected aerosols. The representative 5-day air back trajectories calculated for each aerosol type and numbers of observations are depicted in Figure 3.3. The accuracy of the air-mass trajectory calculations was evaluated visually inspecting filters for which the calculated 5-day air-mass back-trajectories indicated an aerosol type 2. Eleven out of 45 filters, corresponding to 24.5% of the filters, did not present the typical colour of mineral dust, suggesting that the air-mass back-trajectories were not reliable for these samples. These results are in agreement with Stohl (1998) who suggested that back trajectory analysis carries an uncertainty of about 20-30%.

Table 3.3 Regional origin classification of aerosols collected on the island of São Vicente, Cape Verdes. Numbers of samples in each aerosol type are given in parentheses.

Influences	Aerosol characteristics	Aerosol types	Descriptions	Regions crossed by backward trajectories
Maritime	Ocean	1	Representing air masses transported over unpolluted marine environments or over regions with very low anthropogenic pollution pressures. The aerosols consist mostly of sea-salt particles.	Atlantic ocean
Maritime-continental	Desert	2	Representing air masses containing mineral aerosol or desert dust.	North and Northwest Africa (Saharan region)
	Desert/polluted	3	Representing air masses transported over the area located below 15 ºN which encounter pollution from biomass burning.	Sahel region, Tropical Savannah region and Tropical Rainforest region
	Polluted	4	Representing air masses transported over the continents generating high loading of contaminants from industrial sources.	Europe and/or America
	Desert/polluted	M2/3	Representing mixed air masses transported over the African continent	African continent
	Desert/polluted	M2/3/4	Representing mixed air masses transported over the African continent and European/North American continents	African continent and European/North American continents

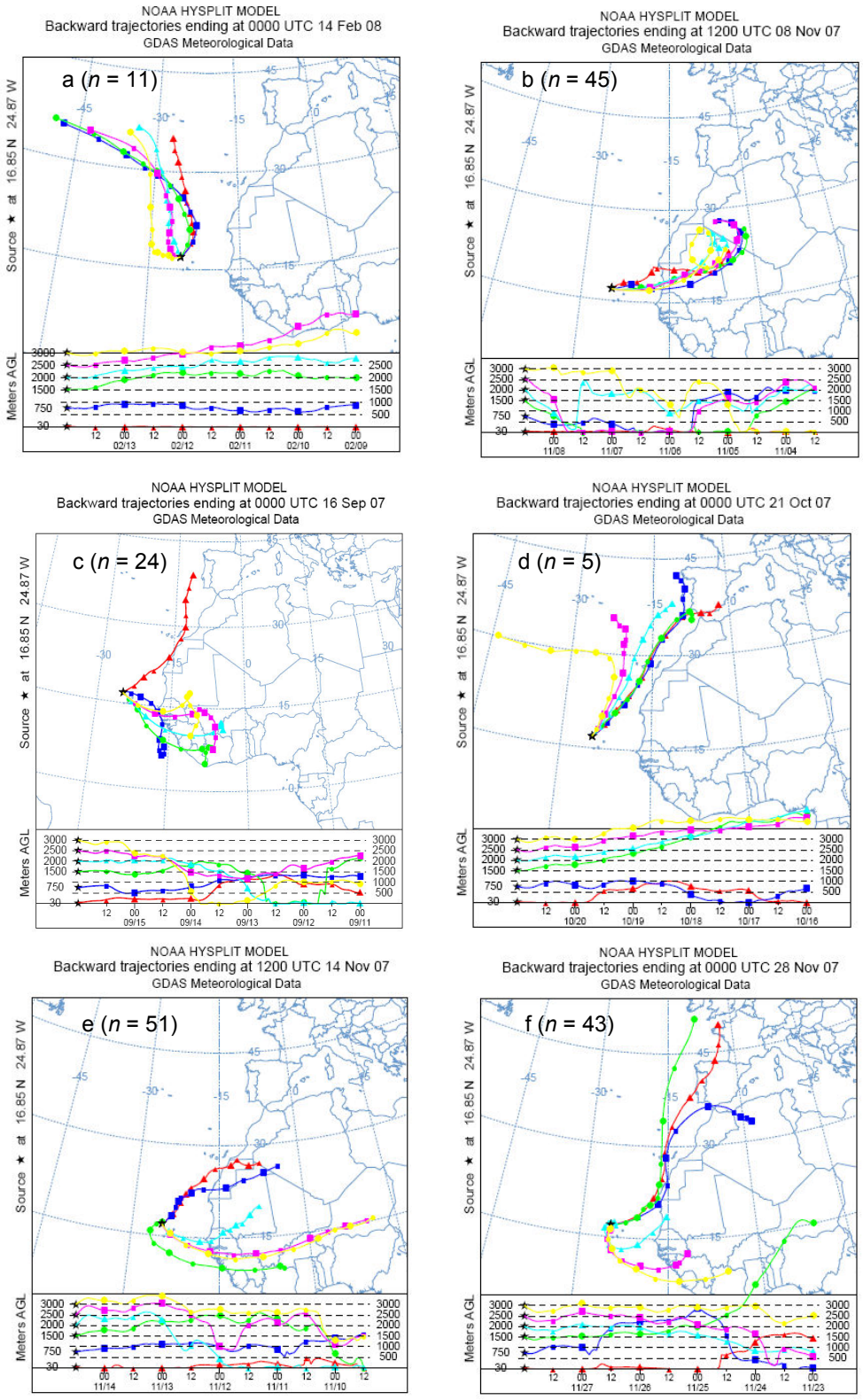

Figure 3.3 Representative five-day air back trajectory plots and number of observations of a) aerosol type 1, b) aerosol type 2, c) aerosol type 3, d) aerosol type 4, e) aerosol type M2/3, and f) aerosol M2/3/4. The back trajectory plots show atmospheric levels for air masses arriving at 30 m (red lines), 750 m (blue lines), 1500 m (green lines), 2000 m (light blue lines), 2500 m (pink lines) and 3000 m (yellow lines) in the island of São Vicente, Cape Verde.

3.2.5 Dry deposition fluxes estimation

The dry deposition flux (*F*) is defined using the equation (2) given by Baker et al. (2010):

$$F = (C_c V_c + C_f V_f) \quad (2)$$

Where:

C is the atmospheric aerosol mean concentration (μmol/m^3),

V is the deposition velocity (m/s),

f is the fine mode fraction,

c is the coarse mode fraction.

The deposition velocity in the equation varies according to aerosol particle size. Owing to an absence of deposition rate measurements in this study, a deposition velocity of 0.02 m/s was used for coarse-mode aerosol and 0.001 m/s for fine-mode aerosol as derived based from experimental and model results for mineral aerosol transported from land to the ocean over a distance of less than 1000 km (Duce et al., 1991). As size-segregated aerosol data was unavailable, the deposition fluxes were determined using relevant data from other studies. The fluxes of organic carbon deposition were estimated using the size distribution data observed by Koulouri et al. (2008) who determined that approximately two-thirds (62%) of organic carbon was found in fine-mode aerosol (<1.3 μm diameter size fraction) in the Eastern Mediterranean, a region which is strongly influenced by mineral dust transports from North Africa. For organic nitrogen, Spokes et al. (2000) assumed that organic nitrogen was distributed equally across the aerosol size spectrum. The fluxes of organic nitrogen deposition were estimated by assuming 46 % and 54 % of organic nitrogen distributed in coarse-mode (>1.0 μm) and fine-mode (<1.0 μm) aerosol, respectively (Lesworth et al., 2010). These values agreed with the study of Violaki et al. (2010), who reported a contribution of 49% of the coarse mode aerosol to the total loading. However, it should be noted that organic nitrogen can be present in a bimodal size distribution reflecting various aerosol origins. For instance, Cornell et al. (2001) observed a small proportion (20%) of organic nitrogen present in coarse-mode aerosol (>1 um diameter size fraction) in 'clean' samples from Hawaii. On the other hand, 72% of organic nitrogen existed in the coarse-mode aerosol at Cape Grim, Tasmania (Mace et al., 2003b). The deposition flux of total nitrogen was defined as an aggregate of nitrogen species in a sample.

3.2.6 Residence time estimation

According to Croot et al. (2004), the residence time (T) of a aerosol derived chemical compound in the ocean is defined using the equation (3):

$$T = M/F_d \quad (3)$$

Where:

M is the inventory (μmol/m^2),

F_d is the dry deposition flux per day (μmol/m^2/d).

We will assume that atmospheric depositions determine the residence time of water column LOC, LTN and LON. The inventories of DOC, TDN and DON were estimated in relation to the mixed layer depths of individual sampling stations during D326 and D338 cruises.

3.2.7 Statistical analysis

Common factor analysis with Varimax rotation was performed in order to specify the probable sources of LOC and LON in aerosols. The data matrix contained seventeen variables including nitrate, non-sea salt sulphate, potassium, manganese, aluminium, silicon, phosphate, ammonium, sodium, magnesium, chloride, vanadium, nickel, cadmium, LTN, LOC and LON. The factor analysis was undertaken using SPSS software. The factors that have eigenvalue greater than 1 were retained by the factor analysis. To obtain only factor loadings that accounted for greater 5% of the variance of the given variable, according to Simcik et al. (1999), only the factor loadings with absolute values greater that 0.23 were taken into account.

3.3 Results and discussion

3.3.1 Aerosol origins

The HYSPLIT analysis and the coloration of aerosol filters demonstrated that air masses transported over the (sub-) tropical North Atlantic were derived from multiple sources. The findings are depicted in Figure 3.4 and indicate that the air masses observed during the land-based sampling corresponded to all types of aerosols with the contribution of 3.3%, 38.8%, 12.6%, 2.5%, 22.1% and 20.8% for aerosol type 1, aerosol type 2, aerosol type 3, aerosol type 4, aerosol type M2/3 and aerosol type M2/3/4,respectively. During ship-based sampling, there were 3 types of aerosols that made an important contribution to the aerosol loading: namely, aerosol type aerosol type 1 (20.8%), aerosol type 2 (60.4%) and aerosol type M2/3/4 (18.8%). The general characteristics of the land-based collected aerosol in each aerosol type are depicted in Figure 3.5. The filters defined as aerosol type 1 were mostly clean. However, some of them contain a small amount of grey particles which could be due to the contribution of polluted air masses from continents. Aerosol type 2 contained brown particles which is a typical colour of mineral dust from Saharan region. The other types of aerosol showed a greyish colour.

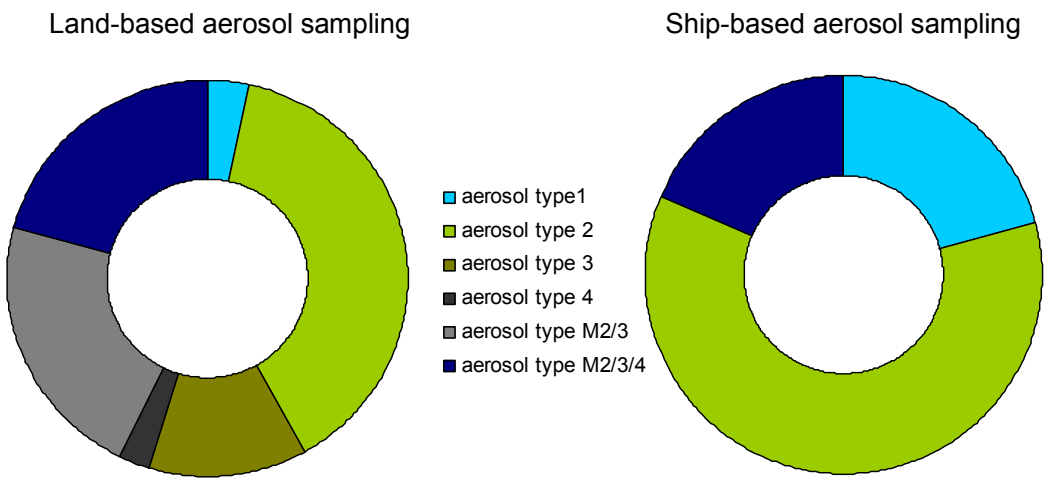

Figure 3.4 Contribution of the number of days which each aerosol type was observed to the entire sampling period.

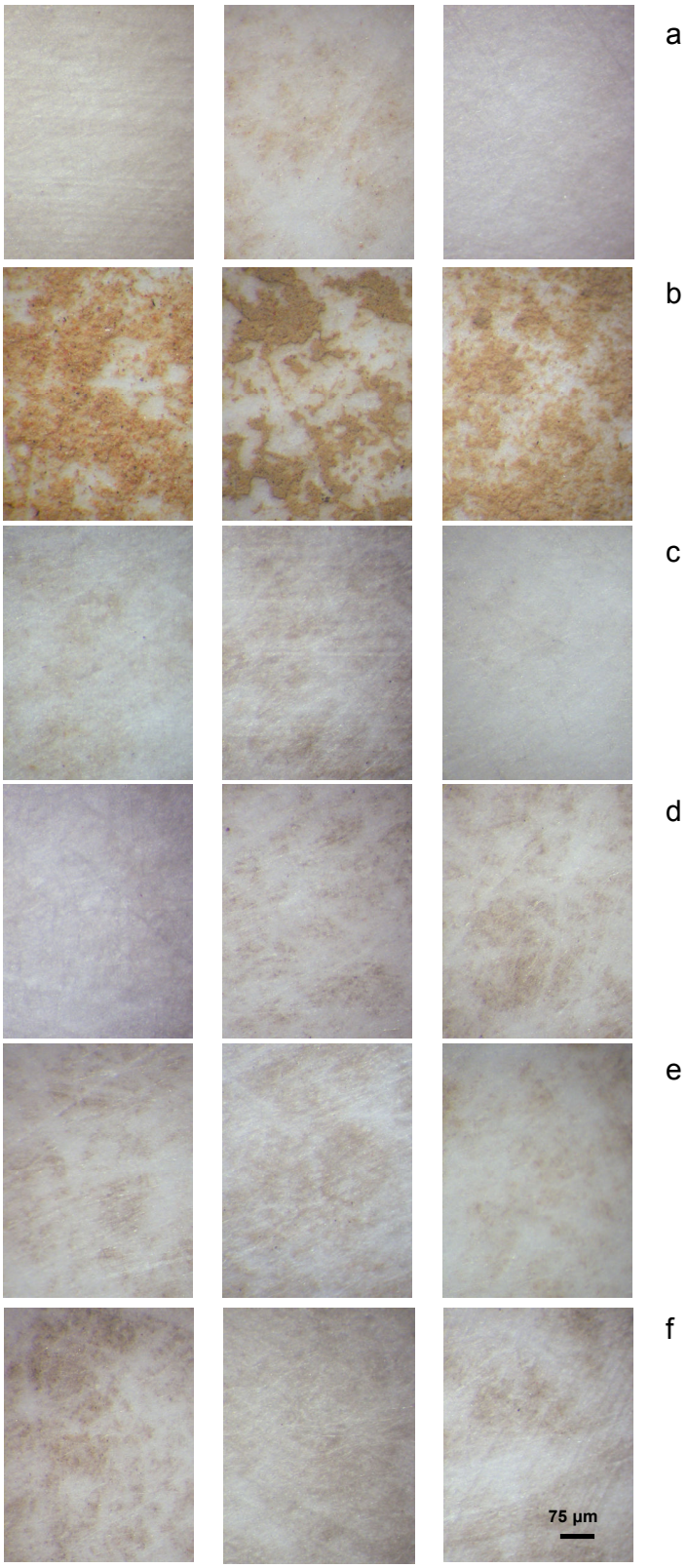

Figure 3.5 The colours of selected aerosol filters of a) aerosol type 1, b) aerosol type 2, c) aerosol type 3, d) aerosol type 4, e) aerosol type M2/3 and f) aerosol type M2/3/4. The filters were photographed using a stereomicroscope on a scale of 1 mm on the paper = 15 µm on the filter.

3.3.2 Aerosol leaching

3.3.2.1 Aqueous leaching of samples

For twenty-two pairs of aerosol samples collected on the island of São Vicente, a comparison was made between seawater and deionised leaching under consistent experimental conditions. The correlations between organic fractions leached using deionised water and seawater were weak (Figure 3.6a and b). The correlations for the total and inorganic nitrogen species were stronger and statistically significant at 0.05 confident level. A smaller fraction of total nitrogen and inorganic nitrogen species were leached from aerosols when using seawater rather than in deionised water (ca. 40-60 % lower) (Figure 3.6c, d and e). There are several factors controlling leachability of elements contained in aerosols including the nature of the aerosols (crustal or anthropogenic), leaching solutions and size fraction of the aerosol (Guieu et al., 1997, Colin et al., 1990). Published leaching experiments have reported important differences in protocols and findings. Some workers (Jickells and Spokes, 2001, Ridame and Guieu, 2002, Chen et al., 2006) found that only small quantities of aerosol - derived iron are released in seawater leaching experiments. In contrast, Aguilar-Islas et al. (2010) found equal or higher iron dissolution when using seawater as leaching solution which was most likely due to the higher ionic strength of seawater. Aerosol-derived phosphorus shows higher dissolution in deionised water than in seawater (Ridame and Guieu, 2002, Cao et al., 2006, Chen et al., 2006). In contrast to the finding in this study, Chen et al. (2006) suggested that aerosol inorganic nitrogen shows similar solubility in deionised water as in seawater. Despite the fact that the leachability of LOC was slightly higher in deionised water (Figure 3.6a) and the leachability of LON was slightly higher in seawater (Figure 3.6 b), there is no data regarding the solubility of organic carbon and organic nitrogen in seawater available in the literature. In this study, therefore, the differences between seawater and deionised water leaching efficiencies have not been taken into account, and we only report the deionised water leaches. Further studies on the effects of leach solution chemistry are required. However, it has been documented that aerosol properties (chemical / mineral composition, history and size distribution) are likely to exert more control on the observed solubility than the leaching protocol used (Aguilar-Islas et al., 2010, Anderson et al., 2010).

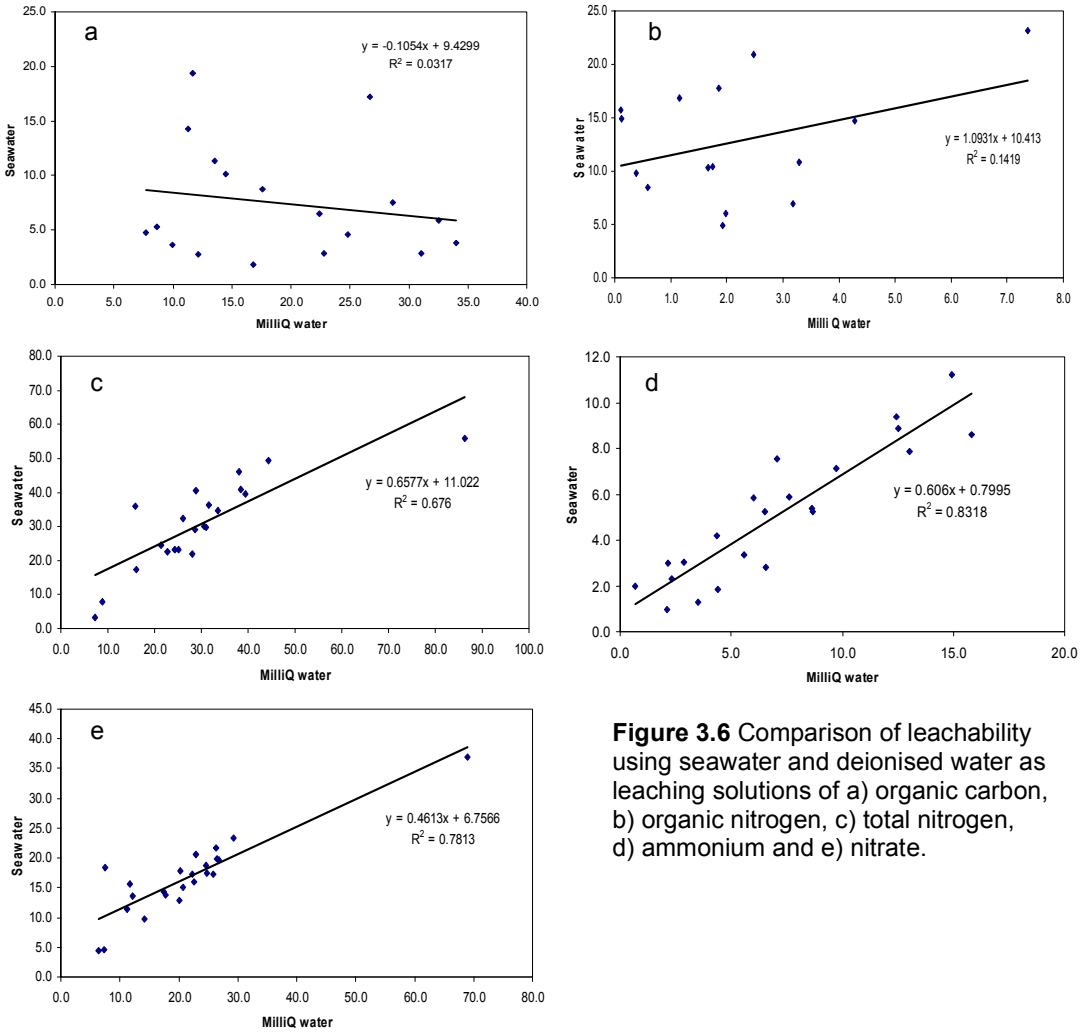

Figure 3.6 Comparison of leachability using seawater and deionised water as leaching solutions of a) organic carbon, b) organic nitrogen, c) total nitrogen, d) ammonium and e) nitrate.

3.3.2.2 Leached organic carbon and leached organic nitrogen

The land-collected aerosols filters (150 samples) were obtained over a period of 366 days between July 2007 and June 2008, while the ship-collected aerosols filters (29 samples) were obtained over 24 days between January and February 2008. A total of 9 out of 150 samples from the land-based sampling were affected by rain events, however the LOC and LON concentration of those samples were not statistically different from non-rain affected samples (t-test, $p > 0.05$). Table 3.4 summarizes the LOC, LTN and LON concentrations in aerosol samples according to aerosol type. The LOC concentrations were in the range 7.7 to 18.4 nmol/m^3 for the land-based sampling and in the range 8.8 - 30.0 nmol/m^3 for the ship-based sampling. The LTN concentrations ranged from 12.2 nmol/m^3 to 30.3 nmol/m^3 for land-based sampling and from 2.3 nmol/m^3 to 17.2 nmol/m^3 for the ship-based sampling. Relatively low concentrations of LOC and LTN were found in aerosol type 1 which represents ocean aerosols. However, there were samples in this

category that contained elevated LOC and LTN concentrations obtained during both land-based sampling and ship-based sampling. According to the HYSPLIT analysis, those samples were likely influenced by air masses with North American anthropogenic emissions (Li et al., 2002, Derwent et al., 2004). Relatively high values of LOC were observed in aerosol type 2 of land-based and ship-based collected aerosols. In Table 3.5, the mean concentrations of LOC observed in this study are compared to some other studies (Graham et al., 2003, Pio et al., 2008, Lewandowska et al., 2010). The results show relatively lower concentrations due to the fact that the other studies took place in different regions with different atmospheric organic carbon sources. For instance, high concentrations of organic carbon in Amazonian aerosols were associated with biogenic particles (e.g. yeasts, fungal spores and fern spores) (Graham et al., 2003), while Pio et al. (2008) suggested that the high observed concentration of organic carbon in Aveiro region was a result of summer forest fires. In addition, anthropogenic activities such as a ship-building, oil-refining, phosphorus and sulphate processing were responsible for the elevated concentration of atmospheric organic carbon observed in the Polish coastal zone (Lewandowska et al., 2010). Furthermore, the sampling site in this study is located on an oceanic island with limited industrialisation, minimizing the influence of industrial continental sources. However, with the findings by other workers together with this study we can conclude that both crustal sources and anthropogenic sources were significant sources of LOC.

Table 3.4 Mean concentrations of LOC, LTN and LON, number of observations (n) and total sampling days of aerosols collected on the island of São Vicente[a] and onboard D326.

Aerosol types	*n*	Number of days observed	*Concentrations (nmol/m^3)*		
			LOC	*LTN*	*LON*
Land-collected aerosols					
1	6	12	7.69 ± 12.86 (5)	12.15 ± 10.92 (6)	1.31 ± 1.70 (2)
2	55	142	18.44 ± 10.26 (54)	29.78 ± 11.75 (55)	1.15 ± 2.57 (38)
3	24	46	11.99 ± 14.67 (22)	26.50 ± 14.83 (24)	1.46 ± 2.73 (13)
4	4	9	14.91 ± 5.47 (4)	30.32 ± 13.69(4)	1.05 ± 1.80 (4)
M2/3	31	81	9.37 ± 6.37 (31)	23.96± 11.87(30)	1.46 ± 2.41 (21)
M2/3/4	30	76	13.32 ± 12.31 (30)	25.64 ± 13.14(30)	2.46 ± 5.58 (18)

(Continues)

Table 3.4 (*Continued)*

Aerosol types	*n*	Number of days observed	*Concentrations (nmol/m^3)*		
			LOC	*LTN*	*LON*
Ship-collected aerosols					
1	5	5	8.84 ± 10.97 (4)	2.34 ± 4.37 (5)	-
2	20	14.5	29.99 ± 19.03 (18)	16.26 ± 11.62 (20)	4.47 ± 3.17 (6)
3	-	-	-	-	-
4	-	-	-	-	-
M2/3	-	-	-	-	-
M2/3/4	4	4.5	16.30 ± 8.02 (4)	17.18 ± 10.50 (4)	3.91 ± 6.70 (2)

[a] number of samples with positive values in each determination are given in parentheses.

Table 3.5 LOC concentrations in atmospheric aerosols reported in literature.

Location	Sampling period	Mean concentration	*n*	References
Brazil, Amazon region (fine fraction, < 2.5 μm)	July 2001	1130 ng/m^3 (~ 93.3 nmol/m^3)	6	Graham et al. (2003)
Brazil, Amazon region (coarse fraction, > 2.5 μm)	July 2001	2260 ng/m^3 (~ 188.3 nmol/m^3)	6	Graham et al. (2003)
Aveiro region, Portugal (Baseline periods)	Summer 2003	2.6 μg/m^3 (~ 216.7 nmol/m^3)	n/a	Pio et al. (2008)
Aveiro region, Portugal (intense forest fire period)	Summer 2003	6.4 μg/m^3 (~ 533.3 nmol/m^3)	n/a	Pio et al. (2008)
Gdynia Poland	September 2007	4.97 ± 2.46 μg/m^3 (~ 414.2 nmol/m^3)	20	Lewandowska et al. (2010)
Land-based collected aerosol	**July 2007-June 2008**	**13.51 ± 11.32 nmol/m^3**	**150**	**This study**
Ship-based collected aerosol	**Jan-Feb 2008**	**22.62 ± 18.95 nmol/m^3**	**29**	**This study**

n/a = data not available

As a result of relatively large errors involved with the three nitrogenous analyses, there is limited data available for LON in aerosol type 1 (n = 2 for land-based collected aerosol and n=0 for ship-based collected aerosol) and consequently this ocean aerosol is under-represented. However, the mean concentrations of LON in aerosols type 1 was 1.3 nmol/m^3 which is in the range observed in Hawaii and Cape Grim, 0.93-3.3 nmol/m^3 (Table 3.6) (Cornell et al., 2001, Mace et al., 2003b). LON in land-based collected aerosols varied between 1.05 and 2.46 nmol/m^3, while LON in ship-based collected aerosols varied between 3.91 and 4.47 nmol/m^3. The mean concentration of LON in the land-based aerosol samples in this study was generally lower than those reported in the other regions, which can be due to various reasons. Firstly, the values reported for this study were derived from mean concentrations observed over a year and were highly

variable over time, whereas the other studies collected samples during a particular period of relatively strong dust loading. Shi et al. (2010) observed high water-soluble organic nitrogen concentrations in aerosols during dust storms. Water-soluble organic nitrogen concentrations in $PM_{2.5}$ in northern California were relatively high in winter when more than 80% of annual precipitation events take place compared to the rest of the year (Zhang et al., 2002). Therefore, to allow comparisons with the other studies, LON concentrations in ship-based collected aerosols in Table 3.4 coinciding to dust events period were compared to other studies carried out in the North Atlantic and Mediterranean region. The LON value of 4.31 nmol/m^3 in this study is lower than those values observed by Mace et al. (2003c) and Lesworth et al. (2010) by factors of 6.7 and 2.2, respectively. However, Mace et al. (2003c) undertook land based-aerosol sampling on the Mediterranean coast where the air masses had an opportunity to mix with air masses transported over local agricultural and industrial sources (Kubilay and Saydam, 1995) , which provides a probable explanation of the high values. In addition, the variation of LON concentrations can be linked to air mass source origins. The concentrations of water soluble organic nitrogen shown in Table 3.6 were observed in different regions influenced by aerosol transport from various sources. Enhanced concentrations of organic nitrogen in water-soluble form were observed in the Amazon basin and Singapore and were related to heavy biomass burning emissions (Mace et al., 2003a, Karthikeyan et al., 2009), while the aerosols in Eastern Atlantic Ocean, Hawaii, Gulf of Aqaba and China sea were related to emissions from anthropogenic sources. Another explanation is that other workers mostly report the concentration as an arithmetic means (Shi et al., 2010, Violaki et al., 2010, Mace et al., 2003a, Trapp et al., 2010, Koulouri et al., 2008, Karthikeyan et al., 2009, Sarthou et al., 2003, Arimoto et al., 1995, Pio et al., 2008, Ying et al., 2007, Lewandowska et al., 2010, Zhang et al., 2002) which are affected by the high concentration tails (Herut et al., 2000) leading to an increased mean concentration.

The contribution of LON to LTN in this study was estimated based on the slope of the relationship of leachable inorganic nitrogen (LIN) to LTN in order to avoid the relative large accumulative error in the LON measurements. The results demonstrated that LTN is present predominantly as LIN, in agreement with Baker et al. (2006). The contribution of LIN to LTN was 65 - 89 % (Figure 3.7), implying that LON approximately represented 11 - 35 % of LTN, which agrees with many other studies (Table 3.6). In general, ship-collected aerosols have higher mean LOC and LON concentrations than the land-collected aerosols and this can be explained by the contribution of the major dust events that occurred during D326 cruise.

Table 3.6 LON concentrations in atmospheric aerosols reported in literature.

Location	Sampling period	Mean concentration	*n*	DON as percent of TDN	References
Mace Head, Eastern Atlantic Ocean	June 1996	44.0 nmolN/m^3	n/a	33.8	Spokes et al. (2000)
Mace Head, Eastern Atlantic Ocean	May 1997	130.0 nmolN/m^3	n/a	40.9	Spokes et al. (2000)
Oahu, Hawaii (trade wind sample)	July-August 1998	3.3 nmolN/m^3	13	31	Cornell et al. (2001)
Oahu, Hawaii (dirty sample)	July-August 1998	28.5 nmolN/m^3	3	64	Cornell et al. (2001)
Northern California[a]	August 1997-July 1998	18.9 nmolN/m^3	41	20	Zhang et al. (2002)
Amazon, Brazil (wet season)	March - May 1999	3.5 nmolN/m^3	27	45	Mace et al. (2003a)
Amazon, Brazil (dry season)	September - October 1999	61.0 nmolN/m^3	37	43	Mace et al. (2003a)
Erdemli, Turkey	March –May 2000	29.0 $\pm$ 42.0 nmolN/m^3	n/a	26$\pm$ 28.0	Mace et al. (2003c)
Cape Grim,Tasmania[b]	November – December 2000	3.60 $\pm$ 5.7 nmol/m^3	13	21	Mace et al. (2003b)
Cape Grim,Tasmania[c]	November – December 2000	0.93 $\pm$ 0.95 nmol/m^3	2	25	Mace et al. (2003b)
East China Sea	September – October 2002	16.0 $\pm$ 19 nmol/m^3	n/a	10	Nakamura et al. (2006)
Western North Pacific	March 2004	54.0 $\pm$ 36 nmol/m^3	n/a	24	Nakamura et al. (2006)
Singapore	March – April 2007	0.60 $\pm$ 0.38 ug/m^3 (~42 nmol/m^3)	7	30	Karthikeyan et al. (2009)
South China Sea	April 2005	65.0 $\pm$ 20 nmol/m^3	n/a	34	Shi et al. (2010)
Yellow Sea	Mar 2005, April 2006	132 nmol/m^3	n/a	17	Shi et al. (2010)
Qingdao	Mar-Apr 2006	180.0 $\pm$ 126 nmol/m^3	n/a	20	Shi et al. (2010)
Atlantic Ocean	Oct – Nov 2002 Sep – Oct 2004 Oct - Nov 2005	9.8 $\pm$ 10 nmol/m^3	40	25	Lesworth et al. (2010)
Gulf of Abaqa, Israel	August 2003 – September 2005	8.0 $\pm$ 5 nmol/m^3	137	10.3	Ying et al. (2007)
Land-based collected aerosol	**July 2007-June 2008**	**1.58 $\pm$ 3.25 nmol/m^3**	**150**	**11**	**This study**
Ship-based collected aerosol	**Jan-Feb 2008**	**4.32 $\pm$ 3.70 nmol/m^3**	**29**	**35**	**This study**

[a] sampling only fine mode fraction aerosol ($PM_{2.5}$)
[b] samples collected during non-baseline condition, defines as wind direction flow toward Cape Grim Baseline Air pollution.
[c] samples collected during baseline condition, defines condensation nucleus counts $\leq$ 600 CN/cm^3 and winds direction between 198° and 280°.
n/a = data not available
[d] reported as annual volume weighted concentration of DON

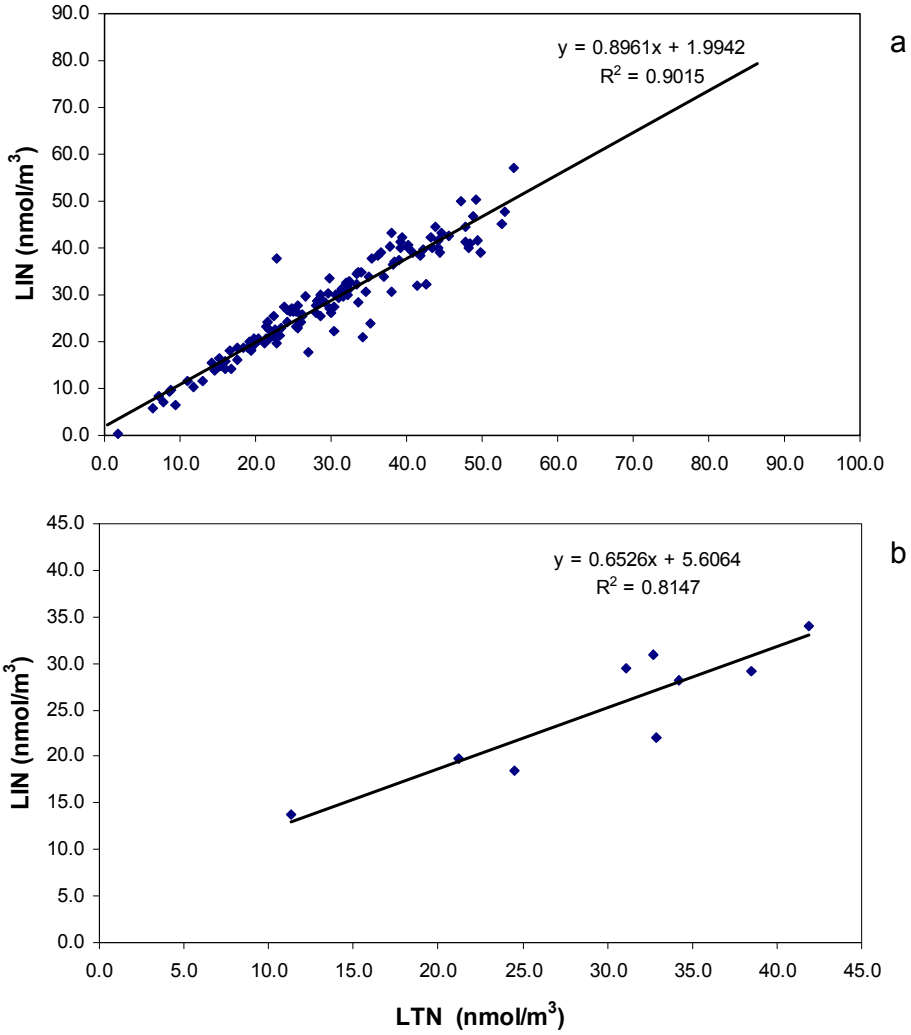

Figure 3.7 Contribution of LIN to LTN concentrations of a) land-based collected aerosol and b) ship-based collected aerosol.

3.3.3 Relative composition of aerosols

In Figure 3.8 we present the results for tracers of mineral dust (aluminium, silicon, manganese) (Losno et al., 1991, Herut et al., 2002, Baker et al., 2006, Baker et al., 2010, Guieu et al., 2010, Trapp et al., 2010) and indicators of anthropogenic sources (nitrate, ammonium, nickel, and vanadium) (Trapp et al., 2010, Aneja et al., 2001, Baker et al., 2010, Cempel and Nikel, 2006) and of mixed mineral dust and anthropogenic sources (phosphate) (Mahowald et al., 2008, Baker et al., 2010) obtained from the samples also analysed for LOC, LTN and LON. The loading of the key tracers in each aerosol type was in good agreement with to those reported in other studies suggesting that the classification of the six aerosol types used for this study is representative. For instance, aerosol type 2 had elevated concentrations of aluminium, silicon, manganese and phosphate which represent the air mass travelling from Saharan region (Figure 3.8a, b, c

and d). The air masses transported from polluted continents, aerosol type 3, 4, M2/3 and M2/3/4, showed relatively high mean concentrations of nitrate, ammonium, nickel, and vanadium (Figure 3.8e, f, g and h). Furthermore, significant phosphate concentrations (Figure 3.8d) were observed in the other types of aerosol which is in agreement with Mahowald et al. (2008) and Baker et al. (2010), who suggested that atmospheric phosphate was generated from multiple sources.

In Figure 3.9, the LOC, LTN and LON from the land-based sampling are shown as box and whisker plots of concentration distributions in the different aerosol types. All type of aerosols contained LOC with the relative high concentrations in aerosol type 2. The presence of organic compounds in this aerosol type can be associated with the existence of microorganisms. Griffin et al. (2007) and Schlesinger (2006) reported relationships between African dust loading and culturable airborne microorganisms (bacteria and fungal). However, further research is needed in order to prove this hypothesis. Aerosol type 2 was also possibly contaminated by aerosols transported over industrialised areas according to nickel and vanadium concentrations which were similar for maritime-continental influenced aerosols (Figure 3.8g and h). This finding agreed with the observations by Baker et al (2006) suggesting Saharan dust aerosols collected during Atlantic Meridional Transect programme were mixed with anthropogenic components. Moreover, Lelieveld et al. (2002) and Kallos et al. (2007) also concluded that anthropogenic polluted air masses from Europe and Euro-Mediterranean were transported towards the North Africa and subsequently mix with desert dust laden air masses from the Saharan region. The presence of organic carbon in mineral dust was likely a result of reactions of chemical compounds from anthropogenic origins on the surface of mineral dusts and interactions of semi-volatile compounds with mineral dusts (Aymoz et al., 2004, Putaud et al., 2004, Falkovich et al., 2004, Koulouri et al., 2008).

In Figure 3.9, the concentrations of LON in all aerosol types were not clearly differentiated. Although aerosol type 4 (Europe and North America) was a weaker source of LON, a relatively high value was observed in aerosol type M2/3/4 which was linked to mixed air masses from several sources. Elevated LTN observed in marine-continental influenced aerosols implies that LIN formed the major contribution to LTN, which represents an influence of anthropogenic emissions. This finding agreed with those by Lesworth et al. (2010) who reported that enhanced water-soluble organic nitrogen in the air masses transported over the Saharan region associated with air masses from terrestrial anthropogenic emissions from Europe due to elevated nitrate, ammonium and non-sea salt sulphate. Therefore, the occurrences of LOC, LTN and LON in the various

aerosol types (Figure 3.9) indicated clearly that they were supplied by multiple natural and anthropogenic sources.

In addition, the varimax-rotated factor analysis was performed to further investigate the LOC and LON sources. The result of varimax-rotated factor analysis for the land-based collected aerosol is presented in Table 3.7. The first four factors that have an eigen value greater than 1 cover 82.9% of the variability in the data. The first factor accounts for 35.7% of the total variance with high loading of vanadium, nickel, ammonium and cadmium. This factor is assigned to represent an anthropogenic source, possibly from industrial sources including oil refineries, incineration of waste and intensive farming. Factor 2 is highly correlated with sodium, magnesium and chloride and explains 23.9% of the total variance in the system. This factor can be identified as a marine source. Factor 3 explains 15.4% of the total variance in the system. This factor is highly loaded on nitrate and moderately loaded on non-sea salt sulphate and manganese. Nitrate and non-sea salt sulphate are predominantly associated with fossil fuel combustion (Gabriel et al., 2002, Baker et al., 2010). Hence, this factor can be attributed to anthropogenic sources, including fuel combustion. Finally, factor 4 explains an additional 7.8% of the total variance. This factor consists of high loading for aluminium, silicon and phosphate which are proxies for mineral dust so this factor can be identified as a crustal source. The loading of LOC in factor 3 is 0.727, while it is 0.373 for factor 4, indicating that LOC in the aerosols originated predominantly from anthropogenic sources (combustion sources) and crustal sources which agrees with the finding in Figure 3.9a and is also in agreement with other reported work (Druffel et al., 1998, Schlesinger et al., 2006, Griffin et al., 2007, Lewandowska et al., 2010). The presence of low loading for LON in factor 2 (0.347) and factor 4 (0.499) implies that LON originated mainly from marine environments and crustal sources. This result contradicts the observation by Lesworth et al. (2010) who found that an anthropogenic origin can be a possible source of aerosol-derived LON. However, it should be noted that only 41% of the variation in LON is explained by those four factors according to the communalities as a result of a limited number of LON data points.

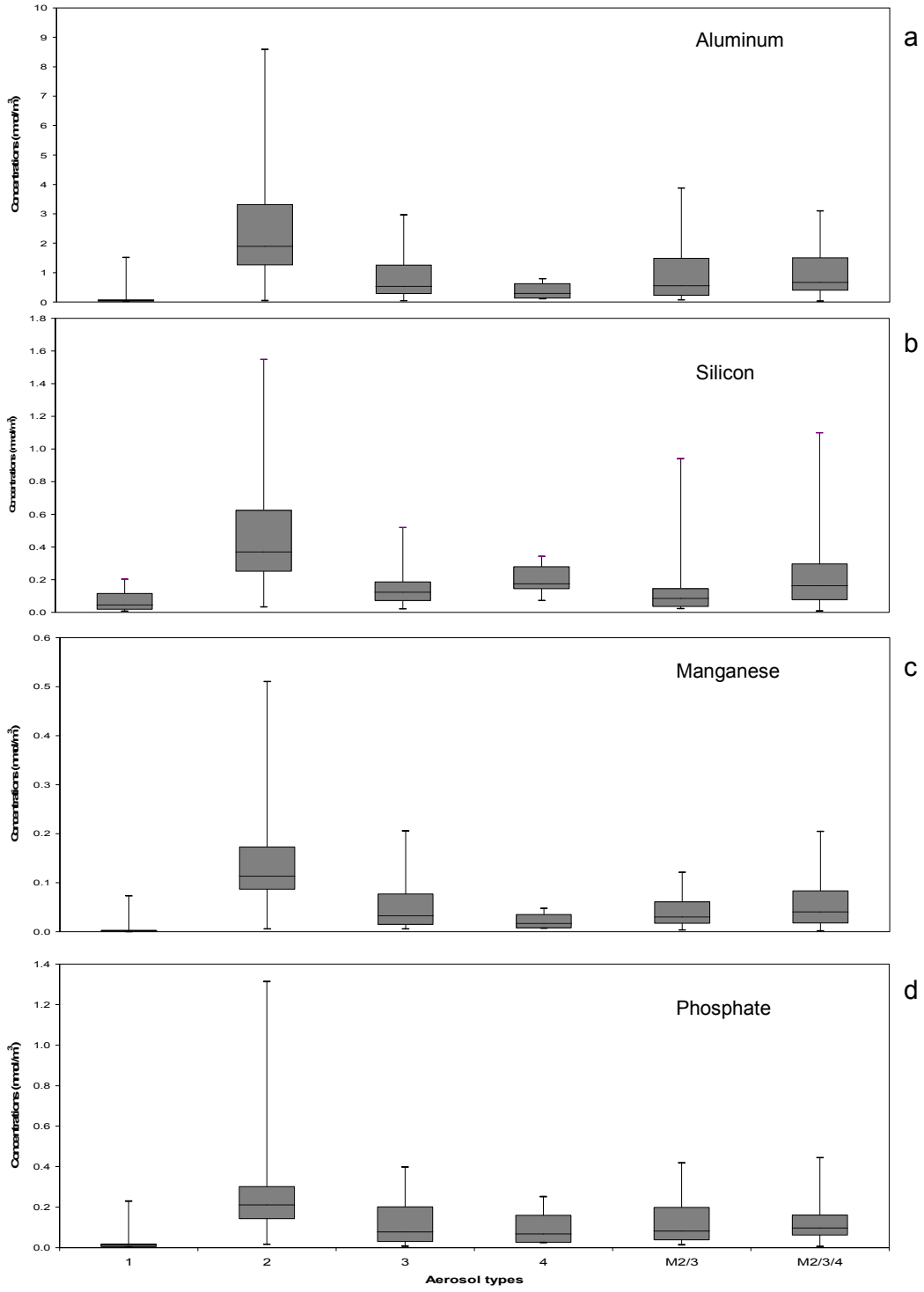

Figure 3.8 Box and whisker plots showing ranges of concentrations of a) aluminium, b) silicon, c) manganese, d) phosphate, e) nitrate, f) ammonium, g) nickel, and h) vanadium in bulk aerosols according to aerosol type. Aerosol types (1= ocean aerosol, 2 = desert aerosol, 3 = desert/polluted aerosol, 4 = polluted aerosol, M2/3 = desert/polluted aerosol from Africa and M2/3/4 = desert/polluted aerosol from Africa, Europe and America) are characterised in Table 3.3. The bottom and the top edge of each box are located at the sample 25 and 75 percentiles. The horizontal lines in a box represent mean concentrations of the data set.

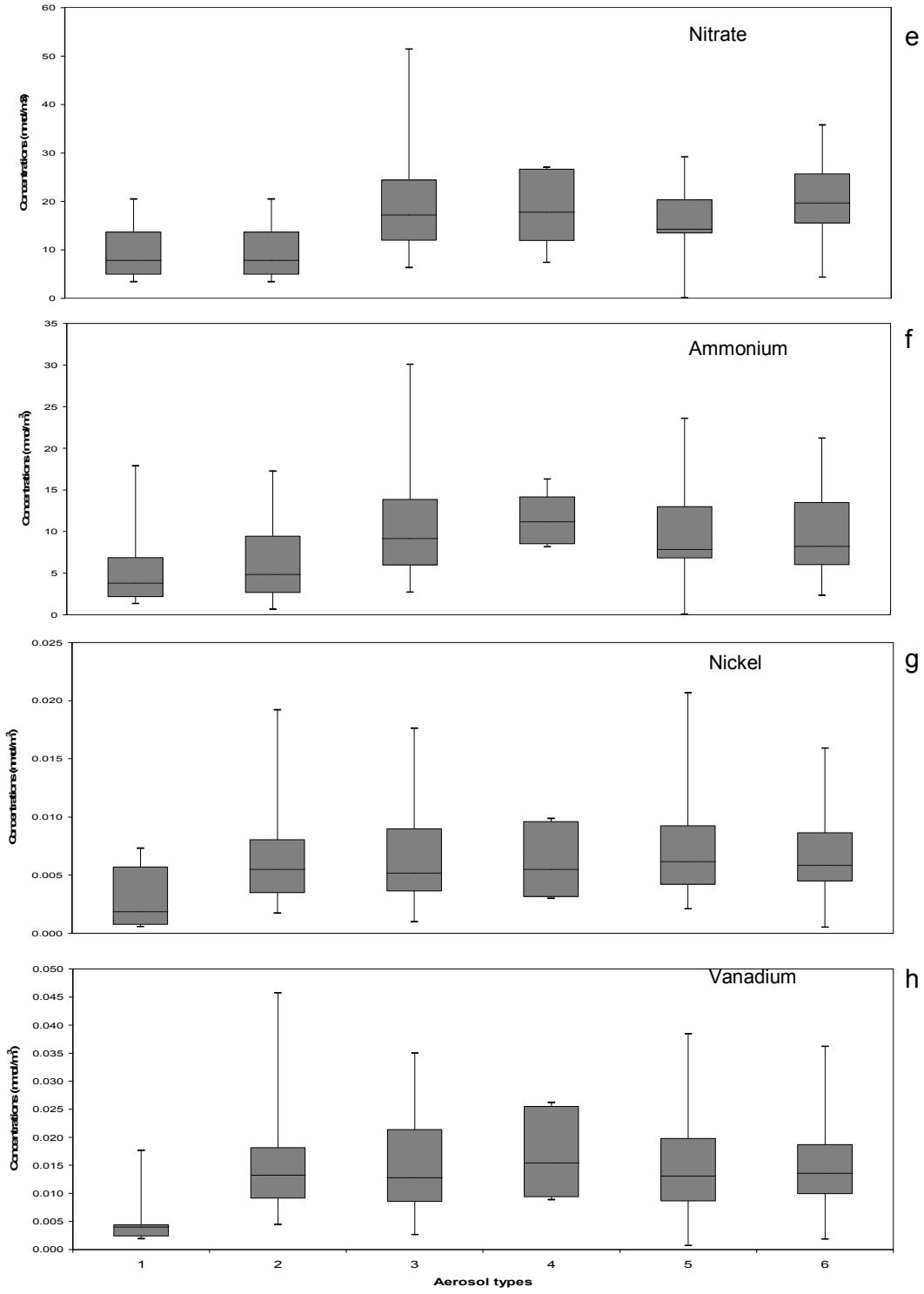

Figure 3.8 *(Continued)*

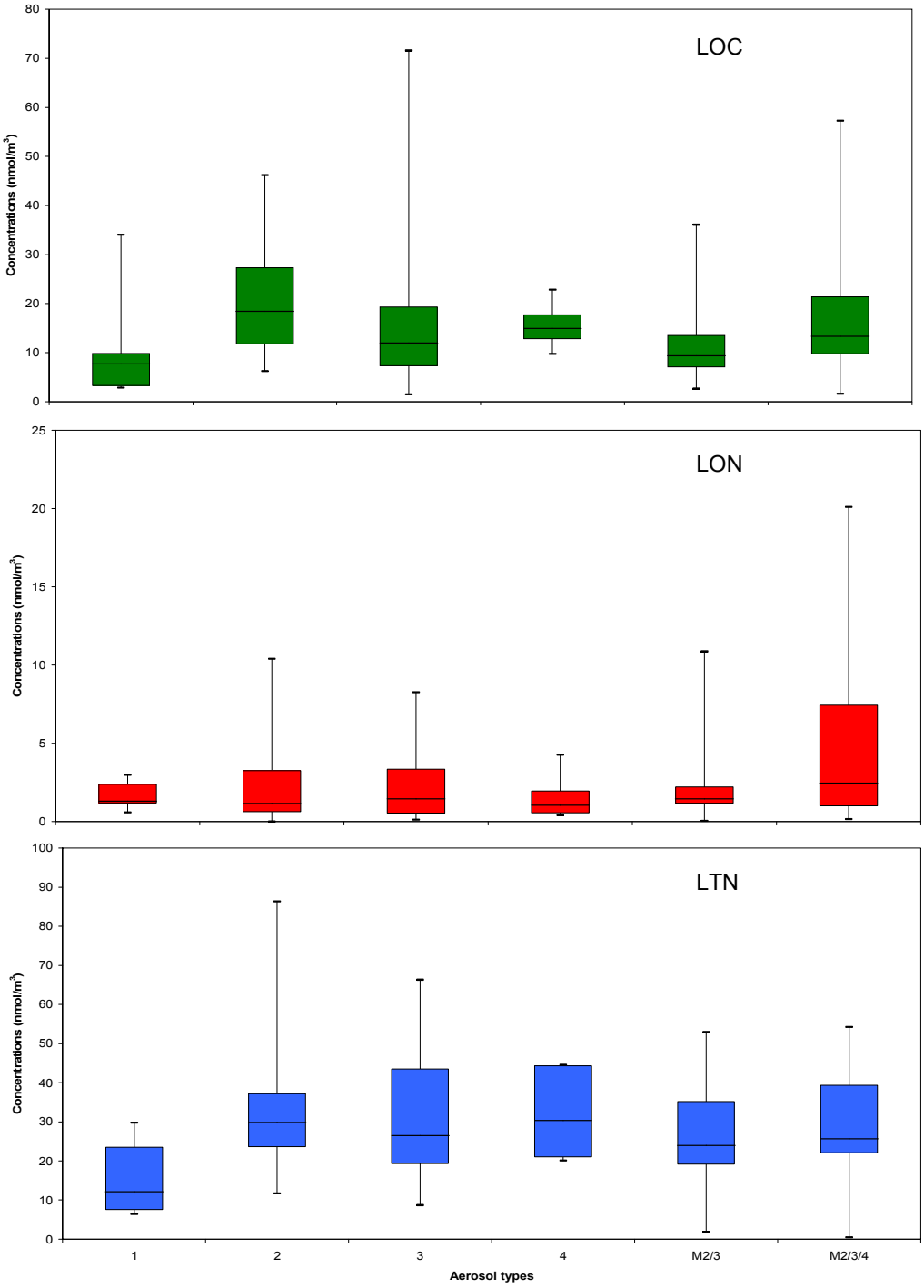

Figure 3.9 Box and whisker plots showing range of concentrations of organic carbon, total nitrogen and organic nitrogen leached from land-collected aerosols according to aerosol type. Aerosol types (1 = ocean aerosol, 2 = desert aerosol, 3 = desert/polluted aerosol, 4 = polluted aerosol, M2/3 = desert/polluted aerosol from Africa and M2/3/4 = desert/polluted aerosol from Africa, Europe and America) are characterised in Table 3.3. The bottom and the top edge of each box are located at the sample 25 and 75 percentiles. The horizontal lines in a box represent mean concentrations of the data set.

Table 3.7 Varimax-rotated factor component matrixes for LOC and LON and relevant variables in aerosol from Cape Verde atmosphere.

	Factor			
	1	**2**	**3**	**4**
V	.932	-	-	-
Ni	.906	.241	-	-
Ammonium	.867	-	-.240	-.239
Cd	.855	-	-	-
Cl	-	.960	.-	-
Na	-	.949	-	-
Mg	.254	.931	-	-
Nitrate	.304	-	.904	-
Mn	-	-	.798	.405
LOC	**-**	**-**	**.727**	**.373**
nssSO4	.595	-	.622	-
Al	-	-	-	.918
Si	-.249	-	.267	.818
PO4	.378	-	.272	.690
LON	**-**	**.347**	**-**	**.499**
Eigenvalue	5.355	3.587	2.316	1.173
% of Variance	35.70	23.91	15.44	7.82
Cumulative %	35.70	59.62	75.06	82.88
Probable source	Anthropogenic (petrochemical processes)	Marine	Anthropogenic (Combustion)	Crustal

3.3.4 Seasonal variability of aerosol LOC, LTN and LON concentrations

The general idea of the sources of the bulk aerosols has been reported in section 3.3.3. In this section, the Cape Verde aerosol concentration data are plotted starting from the beginning of the sampling period in July 2007 to June 2008 in order to investigate the seasonal variability and to identify origins of the aerosol at specific periods. The concentrations of LOC, LTN, LON and other key ions in land-based collected aerosols varied depending on the season (Figure 3.8). Statistical tests were performed using ANOVA analysis to determine variance of those components. LOC concentrations were statistically higher in summer (mean = 21.1 nmol/m^3) than in the other seasons ($p < 0.05$) (Figure 3.10a), while no statistical difference ($p > 0.05$) were observed for LTN and LON over the year (Figure 3.10b and c). Phosphate, which is an indicator of mineral dust, biomass burning and fossil fuel combustion (Mahowald et al., 2008, Baker et al., 2010) was relatively high in autumn (mean = 0.22 nmol/m^3) and winter (0.15 nmol/m^3) (Figure 3.10d). The proxies of mineral dust (Guieu et al., 2010, Herut et al., 2002, Baker et al., 2006, Losno et al., 1991, Trapp et al., 2010) were relatively high in summer (silicate; mean = 0.35 nmol/m^3) (Figure 3.10f) and winter (aluminium; mean = 1.43 nmol/m^3, silicate; mean = 0.28 nmol/m^3 and manganese; mean = 0.08 nmol/m^3) (Figure 3.10e, f and g). Vanadium, sulphate and non-seasalt sulphate, which reflect polluted air masses (Chen and Duce, 1983, Gabriel et al., 2002, Querol et al., 2007, Trapp et al., 2010, Baker et al., 2010, Cempel and Nikel, 2006), were statistically higher in summer and autumn compared with other seasons (Figure 3.10h, i and j). Nitrate showed a similar trend to LON and LTN (Figure 3.10k), while ammonium was statistically lower in winter (Figure 3.10l).

Summer and autumn were characterised by a stronger anthropogenic sources with relatively higher concentrations of elements that represent anthropogenic contamination. Elevated concentrations of vanadium, non sea-salt sulphate and ammonium indicate a possible influence from combustion processes, petrochemical sources and agricultural activities. Saharan dust was also a potential source of LOC and LON because elevated concentrations of silicate were observed in summer. However, in summer Saharan dust transport predominantly occurs at high altitude, favouring long-range transport and deposition over the western tropical North Atlantic (Carlson and Prospero, 1972, Prospero and Carlson, 1972) and yielding lower dust loadings in the vicinity of the Cape Verde Islands. It has been reported that organic carbon, phosphate and ammonium can be derived from biomass burning (Baker et al., 2006, Baker et al., 2010, Duan et al., 2004). However, biomass burning takes place primarily in southern Africa in the period between June and October (Sinha et al., 2004, Roberts et al., 2009) rather than in the northern

Africa, suggesting that phosphate and ammonium were not produced by biomass burning in that period. In winter, maximum dust concentrations occurred as a result of low-altitude dust transport in addition to the long-range transport that takes place at high altitude (Chiapello et al., 1995). The low-altitude dust transport favours the dust deposition in the eastern Atlantic Ocean where our study site is located. LOC and LON were related to the Saharan region as relatively high concentrations of mineral dust tracers were observed. Anthropogenic sources provided a lower contribution to LOC and LON. In addition, LOC and LON were potentially influenced by biomass burning as it primarily occurs between November and February in the Sahel region (Roberts et al., 2009, Cooke et al., 1996) which is in the transport pathway to the observatory tower on the island of São Vicente, Cape Verde. The contribution of biomass burning to LOC will be discussed in section 3.3.5. Spring seemed to be mainly characterized by combustion and agricultural influences according to nitrate and ammonium profiles. The contributions of Saharan dust and petrochemical activity were low as a result of relatively low aluminium, silicate, manganese and vanadium concentrations. However, further studies including aerosol size segregation may be necessary to differentiate natural and anthropogenic origins (Koulouri et al., 2008, Violaki et al., 2010).

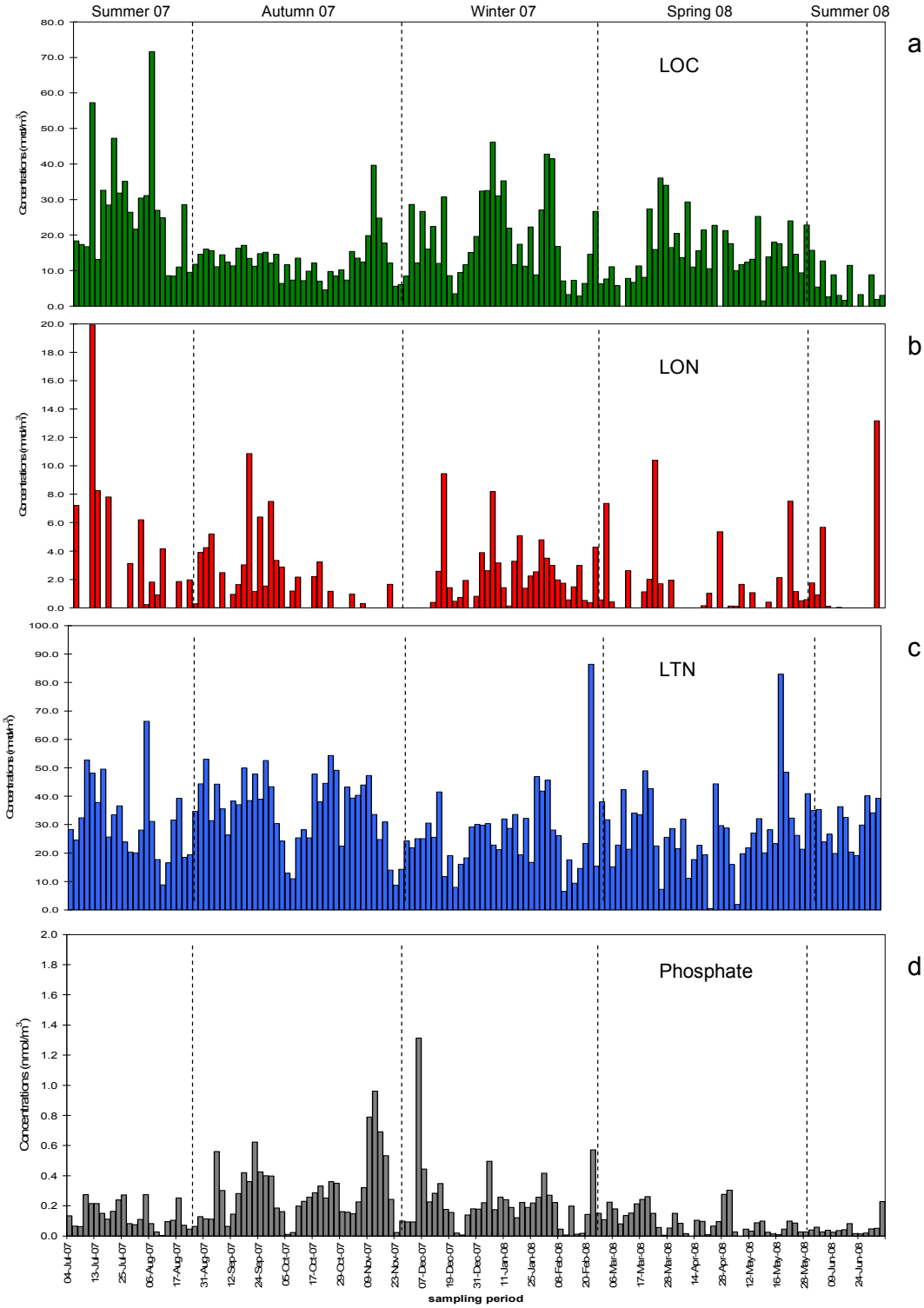

Figure 3.10 Seasonal variations in concentrations of a) LOC, b) LON, c) LTN, d) phosphate, e) aluminium, f) silicate, g) manganese, h) vanadium, i) sulphate, j) non-sea salt sulphate, k) nitrate and l) ammonium in land-based collected aerosols.

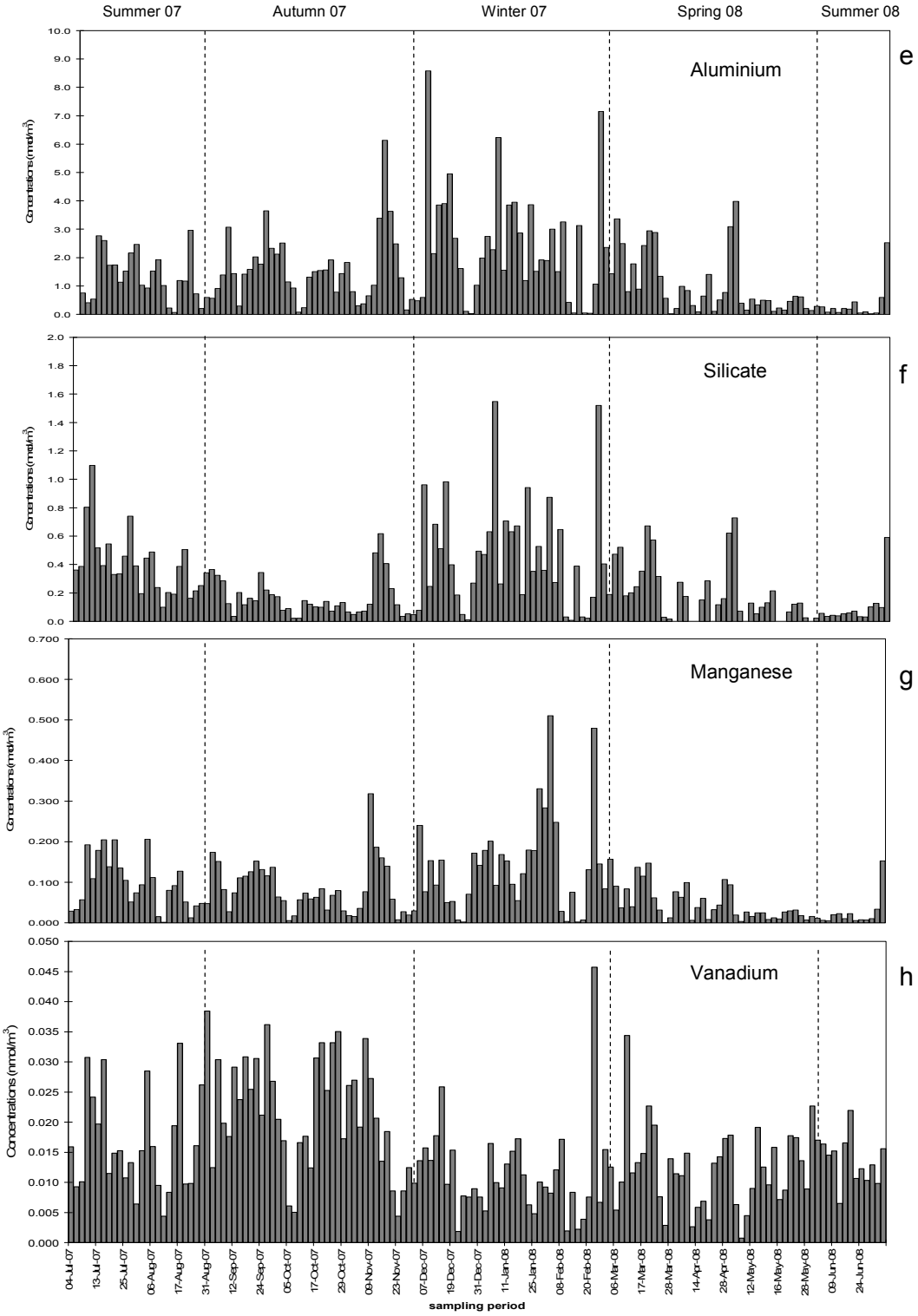

Figure 3.10 *(Continued)*

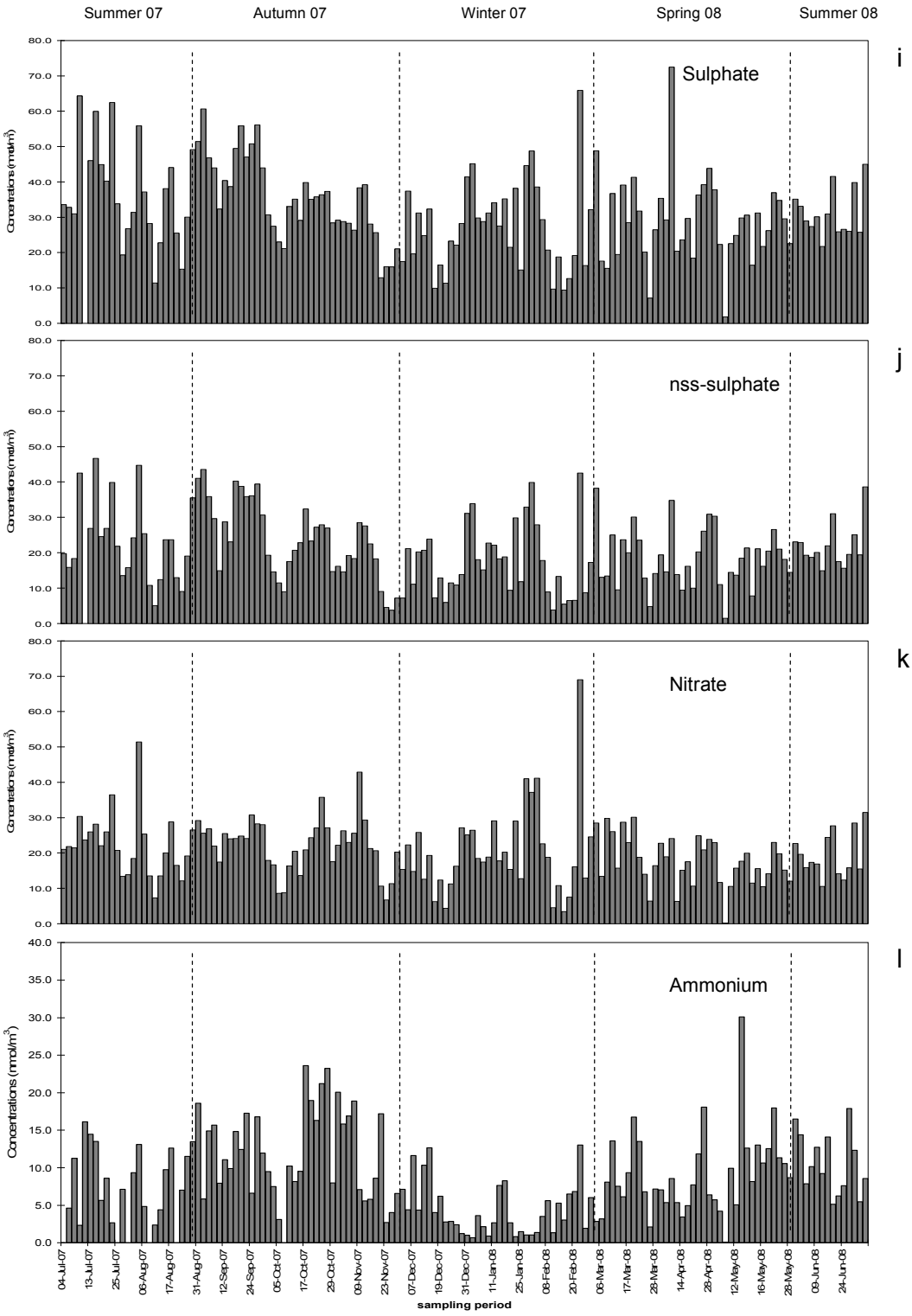

Figure 3.10 *(Continued)*

3.3.5 LOC and biomass burning

Cooke et al. (1996) and Roberts et al. (2009) reported that biomass burning occurs in the Sahel region in the period between November and February, agreeing with the MODIS Rapid Response System Global Fire Maps. The fire maps in Figure 3.11 (http://rapidfire.sci.gsfc.nasa.gov/firemaps) indicate the occurrence of biomass burning during the study period from October 2007 to March 2008.

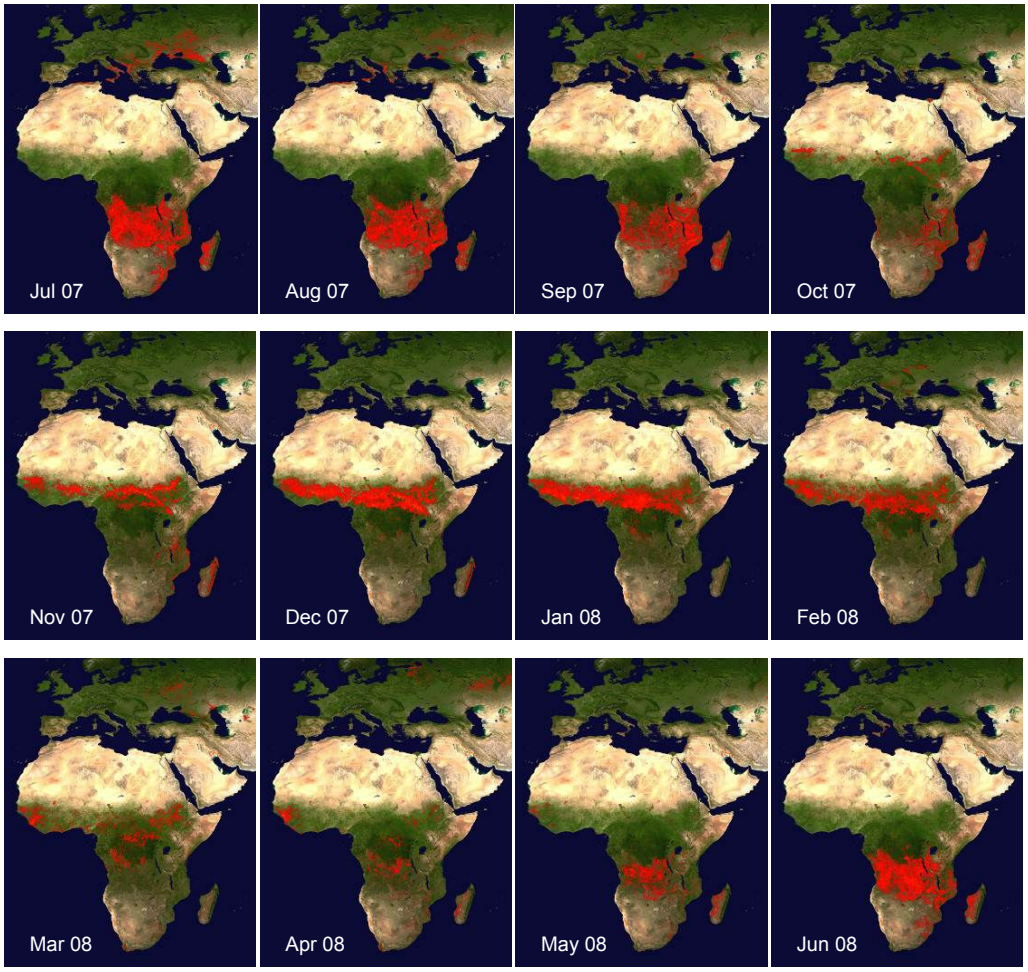

Figure 3.11 Fire maps in Africa between July 2007 and June 2008. This map is produced by MODIS Rapid Response System Global Fire Maps.

Potassium is a major electrolyte contained in plant cytoplasm (Duan et al., 2004). The main input of K to agriculture in Africa is derived from soil reserves and approximately 60% of K output in African cultivated land is contained in crop residues (Sheldrick and Lingard, 2004), therefore, K is typically emitted after the combustion of plant matter (Duan et al., 2004, Pio et al., 2008). In addition, biomass burning aerosols form primarily as carbonaceous compounds, mostly in the form of organic carbon. Considering the regression correlation of organic carbon and K, Duan et al. (2004) and Zheng (2005)

confirmed that biomass burning is an important source of organic carbon in Beijing. However, it should be noted that K is also one of the major elements in seawater and in crustal sources (Pio et al., 2007). With K data available from the Sao Vicente aerosol collection, statistically significant correlations between LOC and K concentrations were observed (Figure 3.12), in good agreement with the findings of Duan et al. (2004) and Zheng (2005). Mean K concentrations were 4.97 nmol/m^3 during the main burning season (n = 15) and 3.7 nmol/m^3 in other periods (n = 21). Mean LOC concentrations were 20.5 nmol/m^3 and 4.5 nmol/m^3 for the biomass burning season (n = 15) and other periods (n = 19), respectively (Figure 3.13).

In an attempt to assess the contribution of biomass burning to LOC derived from the leaching experiment, K related to biomass burning (K_{BB}) was first estimated using the approach proposed by Pio et al. (2008). K provided by terrestrial sources was removed from non sea-salt K using the crustal K/Ca ratio, 0.12 observed over Europe (Pio et al., 2007). K related to biomass burning (K_{BB}) is defined using equation (4):

$$K_{BB} = K^+ - (0.036 \times Na) - [0.12 \times (Ca_{nss} - Ca_{BB})] \quad (4)$$

Where :

K^+ is the total soluble potassium in the aerosol sample,
Na is the total soluble aerosol sodium,
Ca_{nss} is non sea-salt aerosol calcium,
Ca_{BB} is calcium related to biomass burning.

The result of K_{BB} estimation showed negative values demonstrating that aerosols collected during biomass burning season were not likely impacted by biomass burning from Sahel region. In addition, the results also indicated no contribution of biomass burning to LOC over the (sub-) tropical North Atlantic Ocean. In other words, LOC in aerosol samples came from other sources as organic carbon can be derived from several combustion processes (Liousse et al., 1996, Andreae et al., 1998, Zheng et al., 2005).

It is possible that (sub-) tropical Atlantic Ocean is not strongly affected by deposition of biomass burning aerosols originated from the Sahel region. The smoke was likely transported over the equatorial Atlantic (Cooke et al., 1996, Quinn et al., 2001), which agrees with findings that biomass burning aerosols from western Africa were observed over the Central Amazon basin in February 2008 (Ansmann et al., 2009). However, the

data collected in January 2008 may not be wholly representative of the biomass burning season for the Sahel region.

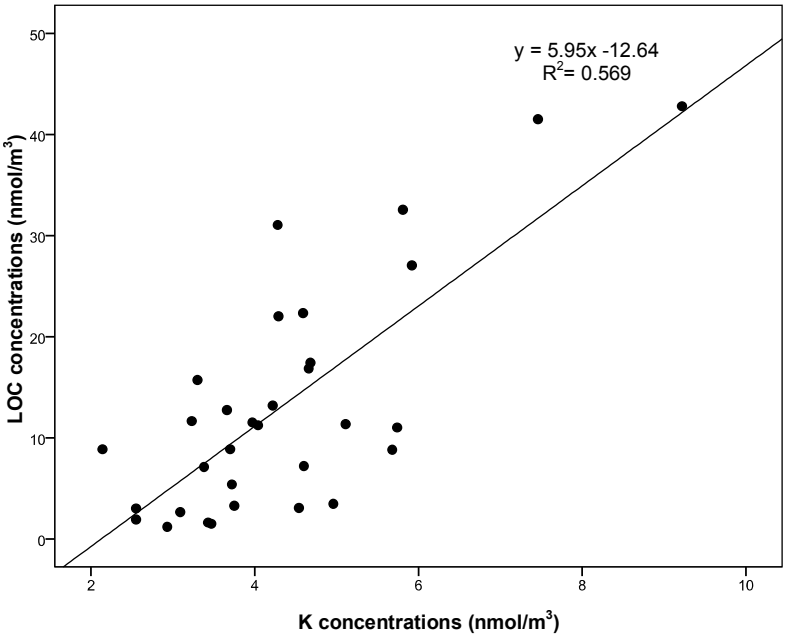

Figure 3.12 Correlation between LOC and K concentrations in land-based collected aerosols.

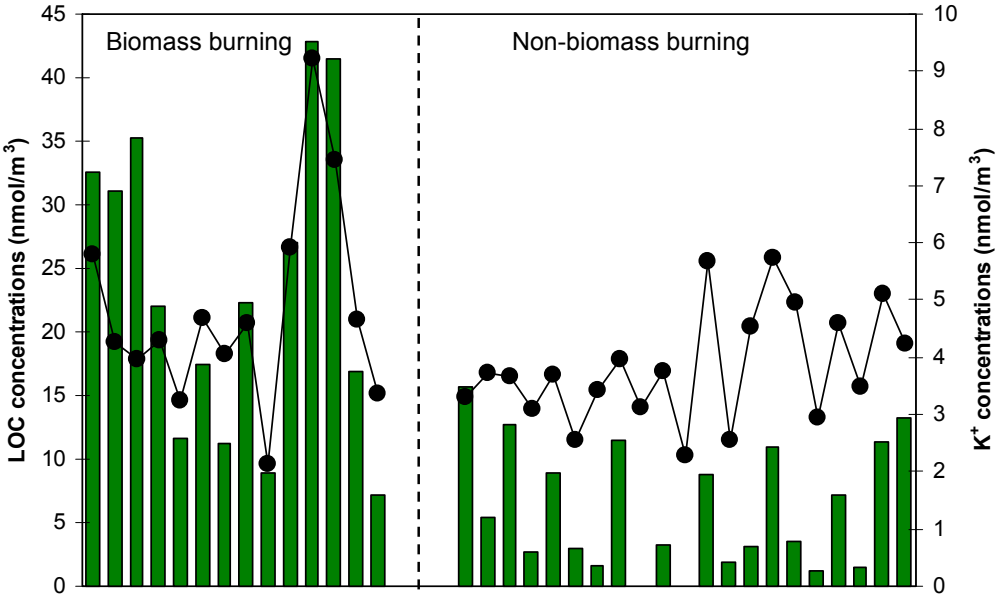

Figure 3.13 Variability of LOC and K concentrations in aerosol samples obtained at Cape Verde within biomass and non-biomass burning seasons. Bars – LOC, lines – K.

3.3.6 Atmospheric dry deposition flux of LOC and LON

The dry deposition fluxes were calculated using the deposition velocity (m/s) and the atmospheric aerosol concentration (μmol/m^3). The dry annual deposition fluxes were determined from land-based sampling (section 3.3.1), using the full sampling period (366 days). In addition, the ship-collected samples were used, but flux results were affected by major dust events leading to overestimation bias in the deposition fluxes. There were significant variations in aerosol concentrations and the number of days observed with different aerosol types (Table 3.4). Thus, the mean dry deposition flux of each source (F_i) was weighted in relation to the number of days the specific aerosol type were observed (D_i) as reported in Table 3.4. The calculations of the LOC, LTN and LON dry deposition fluxes for each aerosol type is presented in Table 3.8 suggesting strong differences between the aerosol types for LOC, LTN and LON.

Table 3.8 Dry annual deposition fluxes of LOC, LTN and LON and the contribution of LON fluxes to LTN fluxes.

Source Region	Dry deposition flux (FD)(μmol/m^2)			LON as percentage of LTN
	LOC	*LTN*[a]	*LON*	
1	65.57	174.87	13.26	7.58
2	1859.52	5228.95	137.51	2.63
3	391.75	1427.55	56.68	3.97
4	95.32	290.07	7.96	2.74
M2/3	538.77	2105.00	99.24	4.71
M2/3/4	719.22	2690.19	157.27	5.85

[a] LTN deposition flux is defined as a summation of nitrate, ammonium and LON deposition flux. The dry deposition flux of nitrate and ammonium were estimated by assuming 90% and 14%, respectively, of each species presenting on coarse mode fraction (Baker et al., 2010).

The results showed that aerosol type 2 (Saharan region) contributed most to the dry deposition fluxes of LOC and LTN to (sub-) tropical North Atlantic Ocean while dry deposition flux of LON was highest for aerosol type M2/3/4. However, dry deposition flux of LON was relatively high in aerosol type 2 as well. These observations indicate that the Saharan region can be a significant source of organic matter. However, relatively high LOC and LTN fluxes were also observed in the aerosol type M2/3 and M2/3/4 suggesting that LOC, LTN and LON were derived from multiple sources which fitted well with the data depicted in Figure 3.8 and 3.8. Dry annual deposition fluxes (F_{an}) were derived as an aggregation of the annual deposition flux of each source region; $F_{an} = \sum F_i D_i$. The results indicate that the dry annual fluxes in the (sub-) tropical North Atlantic Ocean of LOC, LTN

and LON were approximately 3.67 mmol/m^2, 11.90 mmol/m^2 and 0.47 mmol/m^2, respectively (Table 3.9).

Table 3.9 Comparison of organic carbon and nitrogenous species deposition fluxes in atmospheric aerosol reported in the literature.

Location	Sampling period	Average deposition flux	*n*	LON as percent of LTN	References
LOC					
North East Subtropical Atlantic Open Ocean *(21° N)*	May – June 2003	146 mmol/m^2/year	n/a		Duarte et al. (2006)
North East Subtropical Atlantic Open Ocean *(26° N)*	May – June 2003	1277.5 mmol/m^2/year	n/a		Duarte et al. (2006)
Southern Baltic sea, Gdynia	September – October 2007	98.6 mmol/m^2/year	20		Lewandowska et al. (2010)
Cape Verde	**July 2007-June 2008**	**3.67 mmol/m^2/year**	146		**This study**
LTN					
North East Subtropical Atlantic Open Ocean *(21° N)*	May – June 2003	49.28 mmol/m^2/year	n/a		Duarte et al. (2006)
North East Subtropical Atlantic Open Ocean *(26° N)*	May – June 2003	48.18 mmol/m^2/year	n/a		Duarte et al. (2006)
Singapore	March - April 2007	4.98 kg/ha/year (35.6 mmol/m^2/year)	9		Karthikeyan et al. (2009)
Eastern Mediterranean	2003-2006	45.1mmol/m^2/year	108		Violaki et al. (2010)
Cape Verde	**July 2007-June 2008**	**11.9 mmol/m^2/year**	**99**		**This study**
LON					
Gulf of Aqaba, Israel	2003 - 2005	n/a	137	14[a]	Chen et al. (2007)
Singapore	March - April 2007	1.5 kg/ha/year (~10.7 mmol/m^2/year)	9	30	Karthikeyan et al. (2009)
Eastern Mediterranean	2003 - 2006	17.4 mmol/m^2/year	108	38.6	Violaki et al. (2010)
Mediterranean	2001 - 2003	n/a	118	32	Markaki et al. (2010)
Cape Verde	**July 2007 - June 2008**	**0.47 mmol/m^2/year**	**99**	**4.96**	**This study**

[a] estimated from the contribution of soluble inorganic to total soluble nitrogen
n/a = data not available

As seen in Table 3.9, the deposition fluxes observed in this study are generally lower than those reported by the other workers. The atmospheric deposition is dependent on atmospheric aerosol concentration, deposition velocity and aerosol size distribution (Koulouri et al., 2008, Baker et al., 2010, Lesworth et al., 2010). Baker et al. (2010) confirmed that uncertainty in deposition velocities leads to uncertainty in dry input estimation. Duarte et al. (2006) estimated organic carbon and total nitrogen deposition

fluxes using the depositional velocity measured in situ for large particle sizes, which may result in overestimation of the fine-mode fraction. Lewandowska et al. (2010) estimated deposition flux by taking calculated local wind speed for the study area into account while the deposition velocity used in this study obtained from the experimental and model result studied by Duce et al. (1991). Furthermore, the atmospheric fluxes for this study were estimated based on yearly mean value, whereas the others estimated fluxes based on short-term measurements. The atmospheric concentrations in this study are expected to be lower than those in aerosol sampled in a particular period such as a strong dust transport season. Thus, a lower atmospheric concentration corresponds to a lower deposition flux. For example, the deposition flux of LOC reported by Lewandowska et al. (2010) is relatively high, mainly due to a strong contribution from anthropogenic sources such as shipyards, refineries and chemical production plants. In addition, high LON deposition fluxes reported by Karthikeyan et al. (2009) (~ 10.7 mmol/m^2/year) as a consequence of high atmospheric concentrations (~ 42 nmol/m^3, in Table 3.5) were measured during a one-month sample collection period. As a result of the lower atmospheric concentrations observed in this study, LON deposition fluxes account for only a relatively small fraction of the LTN deposition fluxes (Table 3.8), ranging between 2.74% and 7.58% with the mean of 4.96%, suggesting that the inorganic nitrogenous (nitrate and ammonium) species dominate dry deposition fluxes of nitrogen. However, LON concentrations are obtained by subtracting the sum of inorganic nitrogen from LTN and are subject to large errors derived from summing the uncertainties in each nitrogen analysis (Cornell et al., 2003, Baker et al., 2010).

3.3.7 Influence of atmospheric deposition on marine bacteria and phytoplankton communities

In general, the use of Redfield-type nutrient ratios (Redfield, 1958) takes into account only inorganic nutrient fractions due to the uncertainty in the bioavailability of organic fractions (Markaki et al., 2010). However, organic C:N ratios can be used to explain the influence of aerosol inputs on the biological carbon pump as organic carbon and organic nitrogen are key nutrients for bacterial and phytoplankton communities. Anderson (1992) indicated bacterial carbon-limited growth using dissolved organic nitrogen utilisation and dissolved organic matter C:N ratios. Organic C:N ratios were used as tracers of food quality, food assimilation and nitrogen excretion for heterotrophic bacteria. With the threshold organic C:N ratio below 10.2:1, ammonium will be excreted by bacteria. Conversely ammonium will be utilised as a growth substrate when the C:N ratio is greater than 10.2:1. In order to quantify possible influences of atmospheric aerosols on the marine nutritional balance, C:N ratios of aerosol derived-organic compounds were estimated based on calculated dry

deposition fluxes (Table 3.8). Organic C:N ratio of aerosols collected on the island of São Vicente ranged from 4.6-13.5 (Table 3.10). The results suggest that the aerosols were nitrogen depleted relative to Redfield ratios, with the exception of aerosol type 1, 3, M2/3 and M2/3/4.

Table 3.10 Molar ratio for organic dry deposition fluxes.

Aerosol types	C : N	N : P[a]
1	4.95	758.59
2	13.52	117.61
3	6.91	270.73
4	11.98	319.69
M2/3	5.43	216.12
M2/3/4	4.57	245.70

[a] owing to an absence of organic phosphorus, N:P ratio was defined as the dry deposition flux ratio of LTN to leachable PO^4. The fluxes of phosphate were estimated by assuming 85 % of phosphate presented in coarse fractions (Markaki et al., 2003).

Arrigo (2005) observed optimal molar N:P ratios for phytoplankton during growth processes ranging from 8 to 45 depending on nutrient conditions. In this study, molar ratio of N:P dry deposition flux were estimated in order to consider the influence of atmospheric deposition on phytoplankton communities. However, there was no available dissolved organic phosphorus data, therefore molar ratio of leachable TN to phosphate were considered. The dry deposition flux ratio for N:P in this study ranged from 117.6 to 758.6 (Table 3.10). In agreement with the previous study (Baker et al., 2010), the ratios diverged strongly from the optimal phytoplankton requirements reported by Arrigo (2005), indicating that the aerosols collected on the island of São Vicente were relatively nitrogen-enriched and phosphorus-deficient. This phosphorus-depleted condition can be further linked to carbon cycling as heterotrophic bacteria show reduced DOC mineralisation and assimilation rates into cells when exposed to phosphorus-limited conditions (Thingstad et al., 1997, Pulido-Villena et al., 2008). As a result, excess DOC will feasibly be exported from the surface to deep ocean through vertical mixing (Tanaka and Rassoulzadegan, 2002) or sinking following particle absorption (Nagata and Kirchman, 1996, Druffel et al., 1998, Karakas et al., 2009).

3.3.8 Residence times for LOC, LTN and LON

Oceanic residence times reflect the average duration over which compounds remain in a part of the water column. The residence time is governed by several factors such as inputs, solubility, advection, diffusion and scavenging and other removal processes (Croot et al., 2004, Jickells, 1999, Sarthou et al., 2003). To date, a few studies have reported residence times of trace metals (Fe, Al and Mn) in dust-affected ocean regions, such as the equatorial Atlantic (Croot et al., 2004), the eastern Atlantic ocean (Sarthou et al., 2003) and the Sargasso Sea (Jickells, 1999). The residence times of aerosol derived-organic carbon and aerosol derived-nitrogen in this area have not yet been reported. In this study, the residence times were derived using atmospheric dry deposition fluxes and inventories. The atmospheric dry deposition fluxes of LOC, LTN and LON were obtained from section 3.3.6, while the atmospheric dry deposition flux of leachable phosphate (LPO) was estimated following Markaki et al. (2003). The inventories of DOC, TDN and DON were calculated using observed mixed layer depths and concentrations in seawater samples collected onboard the *RRS Discovery* on cruise D326 in the period between January and February 2008 and on cruise D338 in the period between April and May 2009. The sampling stations on cruise D326 were in the (sub-) tropical North Atlantic Ocean, between 12ºN - 27ºN and 17ºW - 36ºW. On cruise D338, the sampling stations covered the Mauritanian shelf region between 19ºN - 22ºN and 17ºW - 19ºW. The ranges of mixed layer depths were approximately 100 - 150 metres in the North Atlantic subtropical Gyre and approximately 30 - 90 metres in the tropical North Atlantic Ocean, while the Mauritanian shelf region had approximately 20 - 110 metres mixed layer depths. Mixed layer depths were estimated based on an isothermal mixed layer depth of 0.5ºC (ILD 0.5) according to Hooker et al. (2000), defined as the shallowest water depth at which the temperature differs from the surface layer by more than 0.5ºC. The inventories of these three areas are presented in Table 3.11.

In general, the turnover time of an organic compound increases with an increasing of biological resistance (Ogawa and Tanoue, 2003). Hansell et al. (2009) stated that the removal time scale of semi-labile DOM fraction ranged from a year to decades. The residence times of LOC, LTN, LON and LPO relative to atmospheric depositions are presented in Table 3.12 suggesting that LOC, LTN, LON and LPO are semi-labile which is consistent with the conclusions of Duarte et al. (2006) who suggested that the terrestrial aerosol derived-organic carbon is semi-refractory, according to 4-day incubation experiments. Of the 3 study areas, the North Atlantic subtropical Gyre had longest residence times for LOC and LON, whereas the Mauritania shelf region showed relatively long residence times for LTN and phosphate.

Table 3.11 Inventories of DOC, TDN, DON and phosphate (PO_3^{4-}) in the (sub-) tropical North East Atlantic ocean (µmol/m^2). Mixed layer depths used from observations in the various regions.

	North Atlantic subtropical gyre	Tropical North Atlantic Ocean	Mauritanian shelf region
DOC	7.96 x10^3	4.16 x10^3	3.75 x10^3
DON	0.52 x 10^3	0.36 x 10^3	0.39 x 10^3
TDN	9.11 x10^3	9.70 x10^3	16.27 x10^3
PO_4^{3-}	0.06 x10^3	0.11 x10^3	0.54 x10^3

Table 3.12 Estimated residence times of LOC and LOC in the mixed layer depth of the (sub-) tropical North Atlantic Ocean relative to atmospheric inputs.

Location	Atmospheric deposition flux (µmol/m^2/d)	Inventories in mixed layer depth (µmol/m^2)	Residence times (days)
LOC			
North Atlantic subtropical gyre	10.03[a]	7.96 x10^3	793.62
Tropical North Atlantic Ocean		4.16 x10^3	414.76
Mauritanian shelf region		3.75 x10^3	373.88
LTN			
North Atlantic subtropical gyre	32.56.[a]	9.11 x10^3	279.67
Tropical North Atlantic Ocean		9.70 x10^3	297.94
Mauritanian shelf region		16.27 x10^3	499.72
LON			
North Atlantic subtropical gyre	1.26[a]	0.52 x 10^3	406.25
Tropical North Atlantic Ocean		0.36 x 10^3	281.25
Mauritanian shelf region		0.39 x 10^3	304.69
LPO			
North Atlantic subtropical gyre	0.20[a]	0.06 x10^3	297.75
Tropical North Atlantic Ocean		0.11 x10^3	568.65
Mauritanian shelf region		0.54 x10^3	2698.30

[a] estimated from divided dry annual deposition fluxes in Table 3.8 by 366 days

The residence times for LOC and LON reflected biotic and abiotic processes in oceanic environments. The short residence times reflect a removal process through biotic consumption (Carlson, 2002) and also can be potentially related to abiotic removal processes via photo-transformation (Moran and Zepp, 1997). For instance, the relative short residence times in Mauritanian shelf region are likely related to the corresponding high abundance of heterotrophic bacteria (Figure 3.14) and phytoplankton (Figure 3.15), which are recognized as the predominant consumers of DOM in marine environments (Berman and Bronk, 2003, Hansell and Carlson, 2001). Photo-transformation processes form one of the abiotic removal pathways for DOM, inducing degradation of DOM to CO_2 or intermediate organic breakdown products (Moran and Zepp, 1997, Berman and Bronk, 2003, Bronk, 2002). The shallower mixed layer depths observed in the tropical North Atlantic Ocean and the Mauritanian shelf region possibly aid photo-transformation processes. Sorption onto sinking particles and transport to deep sea can be another removal pathway of DOM (Nagata and Kirchman, 1996, Druffel et al., 1998, Karakas et al., 2009). It has been reported that high particle sinking rate in the Mauritanian upwelling region is consistent with the lateral transport of total organic carbon fluxes (Fischer et al., 2009). Total organic carbon fluxes to deep waters of 0.29 Tg per year were estimated in the upwelling region (Fischer and Karakas, 2009). The relatively high DOC and DON inventories in the North Atlantic subtropical Gyre water contributed to the longer residence time calculated. The relatively long residence times for LTN and LPO in the Mauritanian shelf regions are as a result of relatively high inventory TDN and PO_4^{3-}. High TDN inventory in this area with moderate DON inventory indicates that DIN, particularly nitrate, dominates TDN. The Mauritanian shelf region is known as an intense upwelling region (Schafstall et al., 2010) where surface water generally enriches with nutrients (DIN and phosphate) mixed upward from deep water . This can be confirmed by the shallow nitraclines observed in Chapter 2 and with enhanced surface concentrations of chlorophyll *a* depicted in Figure 3.15. The enhanced residence times for TDN and P are hence a consequence of the omission of the upwelling supply of these compounds in our calculations.

However, estimates of residence times were upper limits since atmospheric deposition was the only factor taken into account and other fluxes (e.g. photosynthesis, advection and diffusion) were not considered. For instance, a DOC flux of 10^4 μmol/m^2/d (120 mg C/m^2/d) was generated from biological and physiological processes during an incubation experiment conducted in the North East Atlantic subtropical gyre (Teira et al., 2003). This flux is significantly higher than the atmospheric organic carbon flux (10.03 μmol/m^2/d) observed in this study by a factor of approximately 1000. In addition, according to Charria

et al. (2008), the DON meridional input estimated using a 3-D modeling approach was 106 µmol/m^2/d (0.039 mol N/m^2/yr), which is 85 times higher than the atmospheric organic nitrogen flux (10.03 µmol/m^2/d) observed in this study.

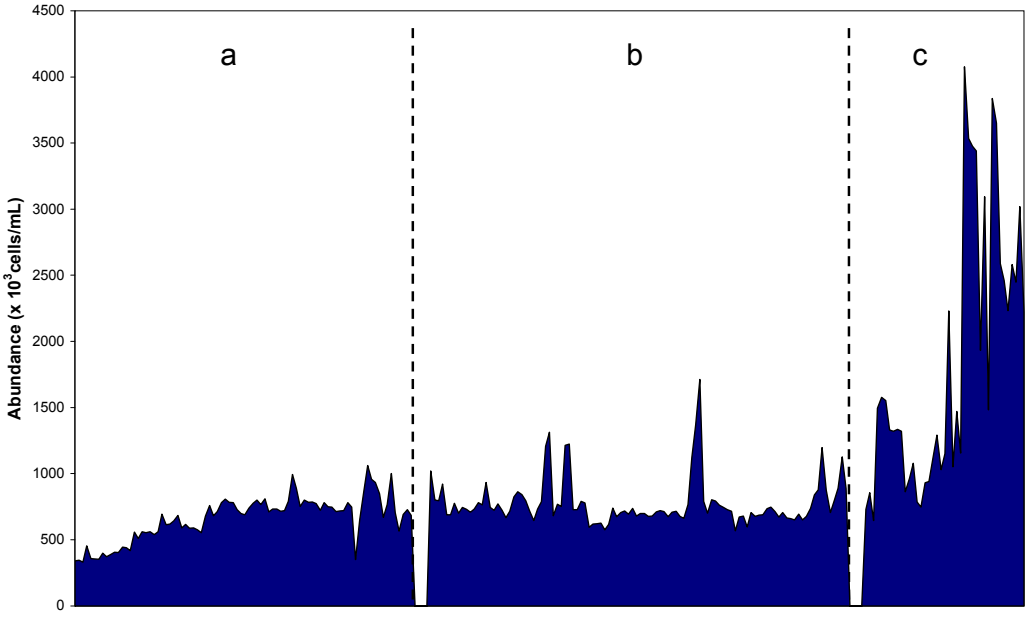

Figure 3.14 Underway data of Synechococcus abundance in a) the North Atlantic subtropical Ocean, b) the tropical North Atlantic Ocean and c) the Mauritanian shelf region.

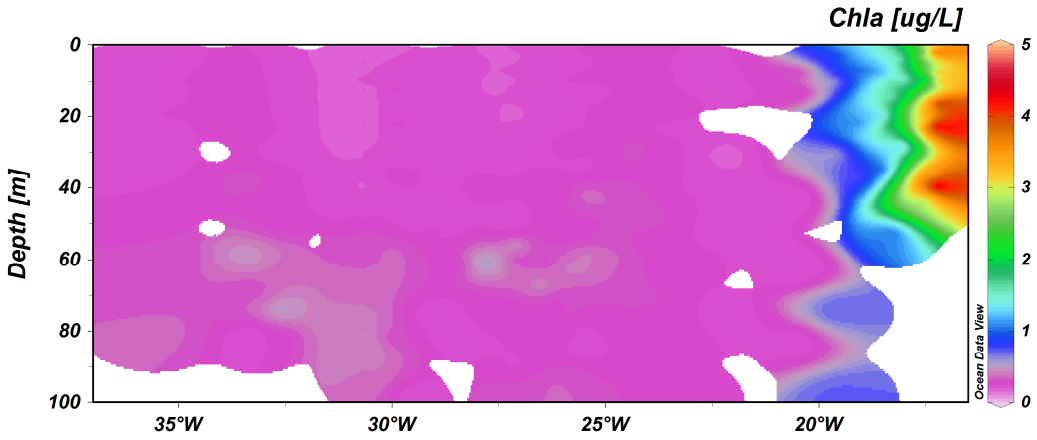

Figure 3.15 Chlorophyll *a* concentrations in the (sub-) tropical North Atlantic Ocean and the Mauritanian shelf region.

3.4 Conclusion

Leachable aerosol-derived organic carbon, total nitrogen and organic nitrogen were quantified in leaching experiments using deionised water. Aerosols transported over the (sub-) tropical North Atlantic Ocean in the period between July 2007 and June 2008 were predominantly derived from the Saharan and Sahel regions and industrial continental regions (Europe and North America). Air masses were mixed in the study region for parts of the sampling period. Six aerosol types were classified in this study and formed reasonably representations of different air mass origins indicated by key components as reported in previous studies. The concentrations of LOC, LTN and LON collected during D326 cruise (ship-based sampling) were higher than those collected on the island of São Vicente, Cape Verde (land-based sampling) owing to the effect of major dust events that took place during the ship-based sampling. Varimax-rotated factor analysis has descried four components (sources) in the land-based aerosol namely crustal, marine and two different anthropogenic sources which explains 82.9% of the variability in the data. The anthropogenic sources contributed 51.1 %, marine contribute 23.9 % and crustal 7.8% of total variance. The first anthropogenic source contained a high loading of vanadium, nickel, ammonia and cadmium and can be related to petrochemical industries and incineration of waste. Another anthropogenic source was likely associated with fossil fuel combustion due to high factor loading for nitrate and non-sea-salt sulphate. The air masses from the Saharan region (aerosol type 2) were dominant throughout the sampling period and were responsible for the highest dry deposition flux of LOC and LTN. The mean fluxes of LOC and LTN were 3.67 mmol/m^2/year and 11.9 mmol/m^2/year, respectively. The highest flux of LON was observed in air masses originating from multiple sources (aerosol type M2/3/4) with a mean of 0.47 mmol/m^2/year. The deposition flux of LON represents approximately 5% of the total nitrogen flux indicating that inorganic nitrogen was the main nitrogen species deposited in the (sub-) tropical North Atlantic Ocean. The atmospheric concentrations and deposition fluxes observed in this study are generally lower than those reported by the other workers due to the timescale and season of the sampling period, an origin of atmospheric aerosol and reporting of an atmospheric value (arithmetic mean, geometric mean or median). Although biomass burning regularly occurs in Sahel region during winter, the (sub-) tropical North Atlantic Ocean may not be the main deposition region for biomass burning aerosols according to potassium values. Therefore, it can be concluded that there was no contribution of biomass burning to LOC measured in this study. However, further studies are needed as this conclusion was made based on limited potassium data. The role of organic carbon and organic nitrogen leached from aerosols to biological cycle were observed through molar ratios. Molar C:N ratios varied between 4.57 to 13.52. Aerosols in air masses transported from the Saharan region

and from industrial continents (Europe and North America) contained excess nitrogen relative to carbon when compared with Redfield ratios. Aerosols in air masses transported from all sources were nitrogen-enriched relative to phosphorus. An abundance of marine heterotrophic bacteria and phytoplankton resulted in relatively short residence times of LOC and LON in the Mauritanian shelf region. Abiotic processes such as photo-transformation and sorption onto sinking particle possibly played a part in removing organic matter in this region. Conversely, the accumulation of nutrients (nitrate and phosphate) brought upward from deep water in the Mauritanian shelf region lead to the relatively long residence times of LTN and LPO.

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Chapter 4

Application of 6- aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reagent to reversed-phase high performance liquid chromatographic (RP-HPLC) determination of amino acids in seawater and aerosol extract solutions

Abstract

A pre-column derivatisation procedure with 6-aminoquinolyl-*N*-hydroxysuccunimidyl Carbamate (AQC) was optimized to determine amino acid concentrations. The analytical system consisted of binary reversed-phase high performance liquid chromatography (RP-HPLC) pumps with a fluorescence detector (excitation wavelengths at 250 nm and emission wavelengths at 395 nm), and a reversed-phase column C18 (AccQ Tag^{TM}, Waters Corp) (150 x 3.9 mm, particle size 4 µm). Mobile phase A was 140 mM sodium acetate with 17 mM triethylamine containing 1 ppm of ethylenediaminetetraacetic acid (EDTA) and 0.1 % sodium azide (Cohen, 2000). Mobile phase B was 60% Acetonitrile (ACN) in UV-irradiated deionised water (v/v). Mobile phase A was set to pH 5.05. The method allowed the optimum separation of 14 free amino acids (aspartic acid, glutamic acid, glycine, arginine, threonine, alanine, proline, valine, methionine, ornithine, lysine, isoleucine, leucine and phenylalanine) and three pairs of amino acids (asparagine/serine, glutamine/histidine and cystine/tyrosine). A high variability in peak area was observed so the addition of potential internal standards L-2-aminoadipic acid (Aada), 2-aminopimelic acid (Apma), L-aminobutyric acid (L-aba) and Methyl Leucine (MLeu) was examined as a mechanism for correcting for day to day variability in the experimental analysis.

A vapour-phase hydrolysis method combined with AQC derivatisation was applied for the determination of dissolved hydrolysable amino acids (DHAA). Method detection limits (MDL) for dissolved free amino acids (DFAA) ranged from 1.34 nM for Leucine to 17.69 nM for Alanine. MDL for DHAA ranged from 6.00 nM for Ornithine to 74.16 nM for Threonine. Instrument detection limits (IDL) were lower than MDL for both DFAA and DHAA.

The application of the optimized pre-column derivatisation procedure to open ocean samples and aerosol leaching solutions are reported. Samples from water column profiles collected in the (sub-) tropical North Atlantic Ocean and the Mauritanian shelf region were analysed for DFAA and DHAA concentrations. Leaching solutions of bulk aerosols influenced by the air masses transported over Saharan and Sahel regions were examined for the atmospheric concentrations of leachable free amino acids (LFAA) and leachable hydrolysable amino acids (LHAA). The concentrations of DFAA and DHAA in seawaters were below the limits of detection of the optimized method (< 68.1 nM and < 660.5 nM, respectively). Aerosol leaching solutions contained LFAA < 0.054 nmol/m^3 and LHAA < 0.64 nmol/m^3.

4.1 Introduction

In the marine environment, dissolved amino acids are a preferential source of nitrogenous growth substrate to ammonium as use of ammonium demands an additional large number of carbon atoms for incorporation into biomass (Anderson, 1992). Amino acids exist as compounds in both the low molecular weight dissolved organic nitrogen (DON) pool (DFAA) and the high molecular weight DON pool (dissolved combined amino acids, DCAA) (Berman and Bronk, 2003). DFAA are important compounds for supporting bacterial growth and can be good indicators of organic matter degradation (Simon, 1991, Cowie and Hedges, 1994, Berman and Bronk, 2003, Chen et al., 2004). DFAA are a preferential nitrogen source for phytoplankton and bacterial utilisation (Middelboe et al., 1995, Chen et al., 2004). DCAA can support up to 10 - 50% of carbon and 40 - 100% of nitrogen requirements for marine bacteria (Kroer et al., 1994) although some amino acids have also been reported to be biologically recalcitrant (Keil and Kirchman, 1991).

DFAA and DCAA can originate from both allochthonous and autochthonous sources. Allochthonous inputs include terrestrial runoff and atmospheric deposition (Mace et al., 2003a, 2003b, 2003c, Chen et al., 2004, Jennerjahn et al., 2004, Hawkins et al., 2006, Shi et al., 2010). Release of autochthonous amino acids may come directly from living phytoplankton; bacteria via active release of exo-enzymes; zooplankton via faecal pellet dissolution, excretion and sloppy feeding; viral lysis; or solubilization of organic particles (Rosenstock and Simon, 1993, Long and Azam, 1996, Grossart and Simon, 1998, Rosenstock and Simon, 2001, Bronk, 2002, Berman and Bronk, 2003). Due to the coupling between uptake and release, amino acids generally are present in marine environments at nanomolar concentrations (Fuhrman, 1990, Wing et al., 1990, Mulholland et al., 2003). Therefore, accurate and highly sensitive amino acid analyses are required in order to understand the dynamics of DFAA biogeochemical processes in seawater.

RP-HPLC combined with pre-column derivatisation with fluorescent tags and detection by fluorescence has been widely used for amino acids analysis (Roth, 1971, Benson and Hare, 1975, Lindroth and Mopper, 1979). The advantages of the procedure include a reduction in analysis time and high sensitivity (Cohen and Michaud, 1993, Hernandez-Orte et al., 2003, Bosch et al., 2006). For derivatisation, Ortho-phthalaldehyde (OPA) and AQC have both been cited as appropriate agents for the analysis of amino acids in seawater without interference problems due to salt (Cohen and Michaud, 1993, Bosch et al., 2006). By creating strongly fluorescing derivatives with amino acids in aqueous solution, OPA, in combination with 2-mercaptoethanol, has been frequently used for measuring amino acids both as a post-column and pre-column reagent. However, OPA

forms unstable derivatives and does not react with secondary amino acids (Bidlingmeyer et al., 1984, Jorgensen and Jensen, 1997, Cohen, 2000, Bosch et al., 2006) which can result in underestimation of the total concentrations of amino acids. Recently, AQC has been reported as an alternative pre-column derivatising agent for amino acid analysis. AQC reacts rapidly with both primary and secondary amino acids and, furthermore yields stable derivatives (at room temperature stable for a week) which are highly fluorescent (Cohen and Michaud, 1993, Cohen and Deantonis, 1994, Hernandez-Orte et al., 2003). The other notable advantages of using AQC include quantitative and linear reactions (vanWandelen and Cohen, 1997, Cohen and Deantonis, 1994) and chromatographic consistency (Strydom and Cohen, 1994). In addition, no effect of contaminant generated from the glassware on the quality of AQC during hydrolysis process has been observed (Strydom and Cohen, 1994). AQC has been widely used for determination of amino acids in feed grains (Cohen and Deantonis, 1994), infant foods (Bosch et al., 2006), food and beverages (e.g. cheeses, beer and vinegars) (Kabelova et al., 2009, Kabelova et al., 2008, Li et al., 2007, Callejon et al., 2008) and also seawater (Jorgensen and Jensen, 1997). However, a lower sensitivity for amino acids eluting with early retention times has been reported for AQC derivatisation of DHAA in seawater. Therefore, there is a clear need for higher sensitivity amino acid analyses.

The performance of a chromatographic method can be controlled through a combination of chromatographic conditions (such as mixing gradient steepness, column oven temperature and mobile phase pH) (Cohen and Deantonis, 1994, Snyder and Dolan, 2007). In this study, we present method optimization and application of AQC pre-column derivatisation for the quantification of dissolved amino acids in seawater. The aims of this study are: 1) to optimize methodology to determine DFAA and DHAA concentrations in low amino acid level samples using RP-HPLC with a binary gradient system; 2) to measure concentrations of DFAA and DHAA in the oceanic environment and in aerosol leach solutions.

4.2 Materials and methods

4.2.1 Chemicals

Individual amino acid standards including aspartic acid (Asp), asparagine (Asn), serine (Ser), glutamine (Glu), glycine (Gly), glutamic acid (Gln), histidine (His), threonine (Thr), alanine (Ala), arginine (Arg), proline (Pro), cystine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe) and L-aminobutyric acid (L-aba) were obtained from Sigma-Aldrich. L-2-aminoadipic acid (Aada), 2-aminopimelic acid (Apma) and methyl leucine (MLeu; L-α- neopentylglycine) were purchased from Fluka. Ornithine (Orn) from BDH Chemical Ltd, Poole England. Sodium acetate trihydrate (HPLC grade), ethylenediaminetetraacetic acid (EDTA), sodium azide, triethylamine (TEA), acetronitrile (ACN; HPLC grade), orthophosphate acid (analytical reagent grade) and hydrochloric acid (Sp gr 36.5%, analytical reagent grade) were supplied by Fisher Scientific. AccQ•Fluor Reagent kit, consisting of AccQ•Fluor Reagent Powder (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, AQC); AccQ•Fluor Reagent Diluent and AccQ•Fluor borate buffer, was obtained from Waters (Milford, MA, USA). The deionised water was produced by Milli-Q system (Millipore; >18.2 MΩ cm^{-1}) and was irradiated by an ultraviolet irradiator before all applications to remove dissolved organic matter.

4.2.2 Apparatus

Analyses for amino acids were undertaken using a reversed-phase high-performance liquid chromatography (RP-HPLC) system. The system consisted of Shimadzu ($LC10AD_{vp}$) binary gradient high pressure pumps, a Shimadzu ($SCL\text{-}10A_{vp}$) system controller, a Shimadzu autosampler ($SIL\text{-}10AD_{vp}$) and a Shimadzu ($RF\text{-}10A_{xl}$) fluorescence detector. The column temperature was controlled by a Shimadzu ($C10\text{-}10AS_{vp}$) column oven. The mobile phase was degassed using a vacuum degasser (NLG Analytical). Samples were dried as part of the hydrolysis procedure using a centrifugal concentrator (Eppendorf, Model 5301) or using a freeze dryer (PowerDry LL3000, Heto). Data collection, integration, peak-identification and peak area extraction were done with a Shimadzu LC Solution software.

4.2.3 Field sampling

Seawater collection for amino acids was conducted using a stainless steel CTD rosette frame with 24 OTE (20 L) bottles. Seawater profiles were collected in the (sub-) tropical North Atlantic Ocean (from the surface down to 300 metres depth) on cruise D326 in the period between January to February 2008, and in the Mauritanian upwelling area (from

the surface down to 50 - 200 metres) on cruise D338 between April and May 2009. The samples were filtered through 26 mm, 0.2 µm sterile cellulose acetate membrane filters (Sartorius) to remove biogenic particulates. The filtrate was transferred to a polypropylene centrifuge tube supplied by Helena Bioscience Europe. Sample tubes were stored at -80 ºC until analysis.

Amino acids leached from aerosols by deionised water were obtained in experiments analogous to the LOC and LON samples described in Chapter 3. Briefly, bulk aerosol samples were collected on polypropylene filters from a 30 m high observatory tower on the island of São Vicente, Cape Verde (16°51'49 N, 24°52'02 W). The filters were stored frozen until analysis. Leaching experiments for amino acids in deionised water were performed by passing 100 mL of deionised water through the dust-laden 47 mm polypropylene filters. The filtrate was subsequently transferred into a polypropylene centrifuge tube and stored at -80 ºC until analysis.

4.2.4 Standard preparation

Stock standard solutions of individual amino acid standards and internal standards solutions were prepared in UV-irradiated deionised water at concentrations of 2.5 mM. The DFAA and DHAA standard solutions consisted of 20 and 18 individual amino acids, respectively. A stock standard 100 µM amino acid mixture solution and dilutions of stock standard solutions were prepared in UV-irradiated deionised water. Working standards and blank solutions were prepared in UV-irradiated low-nutrient surface seawater. The final concentrations of the standard solutions were between 20 nM and 200 nM for working standard solutions and 500 nM for internal standards. UV-irradiated low-nutrient surface seawater was used for blanks and for preparing a working standard as it has an equivalent ionic matrix to seawater samples, and shows similar low amino acid concentrations compared to UV-irradiated deionised water (Figure 4.1). In addition, the measured pH of low nutrient surface seawater was approximately 8.2 which favours the maximum sensitivity of the AQC derivative (Cohen and Michaud, 1993), while the pH of deionised water was approximately 5.6.

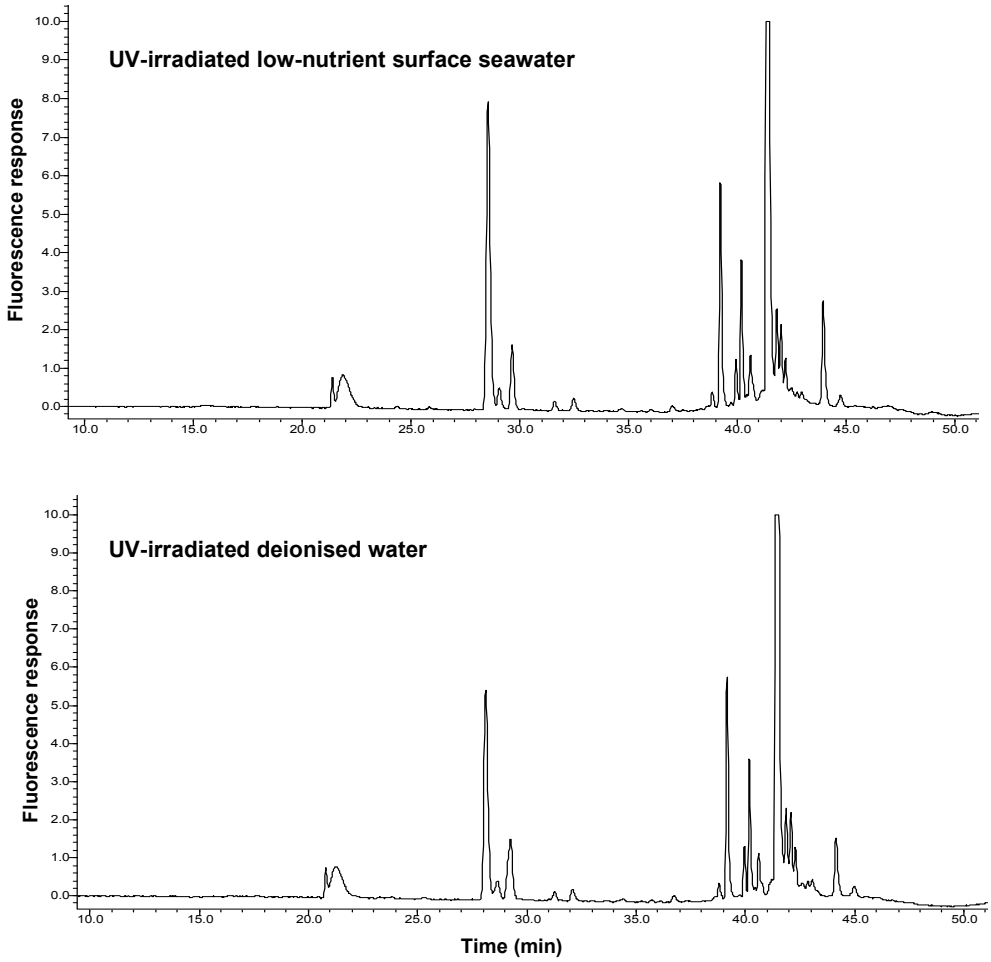

Figure 4.1 Chromatograms of AQC derivatised amino acids obtained from UV-irradiated deionised water and UV-irradiated-low nutrient surface seawater. Separations were carried out on a 150 x 3 mm reversed-phase C18 column (Hypersil, Thermo, particle size 5 µm. The column was incubated at 35ºC. Mobile phase A was set to pH 5.05. The gradient of the solvent was 0 min 100% A, 0.5 min 98% A, 15 min 93% A, 19 min 87% A, 33 min 68% A, followed by a wash with 100% B for 5 min and re-equilibration for 7 min at 100 % A.

4.2.5 Method optimization

The RP-HPLC system was as described above. Mobile phase A was 140 mM sodium acetate with 17 mM TEA containing 1 ppm of EDTA and 0.1 % sodium azide (Cohen, 2000). Mobile phase A was set to desired pH levels with 50% phosphoric acid before filtration through a 0.2 µm Whatman membrane filter, 47 mm (Fisher Scientific). Mobile phase B was 60% ACN in UV-irradiated deionised water (v/v). The mobile phases were transferred to pre-combusted (450ºC, 4-6 h) Pyrex glassware. Detection was carried out by fluorescence with excitation and emission wavelengths at 250 nm and 395 nm, respectively (Cohen and Michaud, 1993). Cohen and Deantonis (1994) suggested that changing the chromatographic conditions, such as pH of the mobile phase and the column

temperature, can improve the separation of amino acids. Chromatographic analysis was optimized on the basis of a comparison of the separation and sensitivity of AQC. Chromatograms of amino acids were obtained after separating using different reversed-phase columns packed with Octadecyl-bonded silica gel (C18). The separations were carried out with a constant HPLC solvent formulation, but with different pH levels, column oven temperatures and gradient slopes. The injection volume was varied between 250 and 500 µL.

4.2.5.1 Effect of ionic medium pH

Separations were carried out on a reversed-phase C18 column (Hypersil, Thermo Fisher Scientific) (150 x 3 mm, particle size 5 µm). The pH of mobile phase A was varied and set to pH 5.05, 5.21 and 5.50 with 50% phosphoric acid. The flow rate applied was 1.0 mL/min. The gradient of mobile phases was 0 min 100% A, 0.5 min 98% A, 15 min 93% A, 19 min 87% A, 33 min 68% A, followed by a wash with 100% B for 5 min and re-equilibration for 7 min at 100 % A. The column was maintained at 35ºC.

4.2.5.2 Effect of column incubation temperature

Different column incubation temperatures were investigated. Two experiments were employed to obtain the optimum column oven temperature.

Experiment 1: Separations were carried out on a reversed-phase C18 column (Hypersil, Thermo Fisher Scientific) (150 x 3 mm, particle size 5 µm). The flow rate applied was 1.0 mL/min. The column was maintained at 31, 35 and 39ºC. The gradient of mobile phase applied was 0 min 95% A, 0.5 min 93% A, 7 min 91.7% A, 24 min 85% A, 47 min 50% A, followed by a wash with 100% B for 4 min and re-equilibration for 4 min at 95% A. The pH of mobile phase A was set to 5.50.

Experiment 2: Separations were carried out on a reversed-phase C18 column (Econosphere, Altech) (150 x 4.6 mm, particle size 3 µm). The flow rate applied was 1.5 mL/min. The column was maintained at 35ºC and 37ºC. The gradient of mobile phase applied was 0 min 100% A, 0.5 min 98% A, 17 min 93% A, 24 min 87% A, 40 min 62% A, followed by a wash with 100% B for 5 min and re-equilibration for 7 min at 100% A. The pH of mobile phase A was set to pH 5.05.

4.2.5.3 Effect of gradient steepness

The effect of gradient steepness was tested at constant pH and temperature. Separations were carried out on a reversed-phase C18 column (AccQ Tag™, Waters Corp) (150 x 3.9 mm, particle size 4 µm) which was operated at a flow rate of 1 mL/min. Different gradient slopes were tested with the pH of mobile phase A maintained constant at 5.05 and the temperature of the column was maintained constantly at 37ºC. The gradient slopes employed can be seen in Table 4.1.

Table 4.1 Gradient table for three chromatographic conditions. Mobile phase A is described in the text and mobile phase B is 60% ACN in UV-irradiated deionised water (v/v). The total run time was 60 min for all conditions.

Gradient 1		Gradient 2		Gradient 3	
Time (min)	Mobile phase B (%)	Time (min)	Mobile phase B (%)	Time (min)	Mobile phase B (%)
0	0	0	0	0	2
0.5	2	0.5	2		
17	7	17	7	17	7
24	13	24	13	24	13
37	38	37	35	37	35
		40	100	40	100
43	100				
		45	100	45	100
48	100				
		49	0	49	2
53	0				
60	0	60	0	60	2

4.2.5.4 Preparation of amino acid hydrolysates

Hydrolysis was carried out using a vapour phase procedure as it gives higher DHAA recovery than traditional acid hydrolysis for marine waters (Keil and Kirchman, 1991). Prior to the hydrolysis process, a preliminary experiment was performed to observe the appropriated sample preparation approach. One millilitre of standard mixture solution was placed in 2.0 mL snap ring amber-vials with a large mouth (6.0 mm, SUPELCO Analytical) and prepared in three different ways. Approach 1: the standard mixture solution was frozen at -80ºC and subsequently dried using a freeze dryer (PowerDry LL3000, Heto) to dry samples before hydrolysis. Approach 2: the standard mixture solution was dried using a centrifugal concentrator (Eppendorf, Model 5301). Approach 3: the sample remained in its original status (liquid) with no treatment. The standard mixture contained in a 2 mL amber-vial was then inserted into a wide-mouth borosilicate ampoule containing 200 µL of

6 N analytical grade HCl (pre-streamed with argon) and a crystal of phenol (to eliminate oxygen). Freeze dried UV-irradiated-low nutrient surface seawater was included in the experimental sample set and served as a blank. To create a small scale oxygen free system, the wide-mouth ampoules including the headspace were flushed with an argon stream before being closed with a cover and sealed with polytetrafluoroethylene (PTFE) tape. The samples were hydrolysed in the vapour phase by placing the samples on a sand bath heated to 110 - 115ºC for 20 - 24 hours. After cooling down at room temperature, the hydrolysates were removed from the wide-mouth ampoule. The samples prepared by approach 1 and 2 were streamed with nitrogen gas for 1 hour to eliminate the excess HCl. For the liquid samples (approach 3), the excess HCl was eliminated by drying as suggested by Cohen and Deantonis (1994) using a freeze dryer (pre-frozen at -80ºC).

4.2.5.5 The application of internal standards

Four internal standards, namely L-2-aminoadipic acid (Aada), 2-aminopimelic acid (Apma), Methyl Leucine (MLeu; L-α- Neopentylglycine) and L-aminobutyric acid (L-Aba), were derivatised and analysed in order to obtain a proper internal standard for amino acid analysis in this study. For the preparation, 100 µL of standard mixture solution (1 µM) was combined with 10 µL of each internal standard solution (50 µM) and 770 µL of UV-irradiated low-nutrient surface seawater. The final concentrations of standard stock solution and each of internal standard solutions were 100 nM and 500 nM, respectively. The mixture was subsequently derivatised and analysed. The final volume of the sample after derivatisation was 1000 µL (910 µL of sample + 70 µL of borate buffer + 20 µL of derivatising agent). Chromatographic separations were carried out on a reversed-phase column C18 (AccQ Tag^{TM}, Waters Corp) (150 x 3.9 mm, particle size 4 µm). The flow rate applied was 1.0 mL/min. The pH of mobile phase A was set to pH 5.05. The column temperature was set at 37ºC. The gradient of the solvent was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 98% B for 5 min and re-equilibration for 11 min at 98% A. Four hundred µL of the derivatised standard containing 100 nM of standard mixture and 500 nM of each amino acid were injected.

4.2.6 Derivatisation procedure

To reconstitute the AccQ•Fluor reagent, 1.0 mL of AccQ•Fluor Reagent Diluent was transferred into AccQ•Fluor Reagent Powder vial. The vial was subsequently closed and mixed with a rotamixer (Hook & Tucker) for 10 seconds and heated in a sand bath at temperature 55ºC for 10 min. The reconstituted AccQ•Fluor reagent was stored in the cool dry place. In typical analysis, 10 µL of sample was buffered with 70 µL AccQ•Fluor Borate buffer. Twenty µL of AQC solution were subsequently added to initiate the derivatisation

reaction. In this study, the final volume of derivatised sample was increased to 1000 µL in order to improve sensitivity. The reaction of AQC is depicted in Figure 4.2. Typically, AQC reacts with primary and secondary amines to generate a derivatised amine within seconds. Excess reagent is swiftly hydrolysed to form 6-aminoquinoline (AMQ), N-hydroxysuccinimide (NHS) and carbon dioxide (CO_2) (Cohen, 2003b), with no further derivatisation (Cohen and Michaud, 1993, Cohen, 2003a).

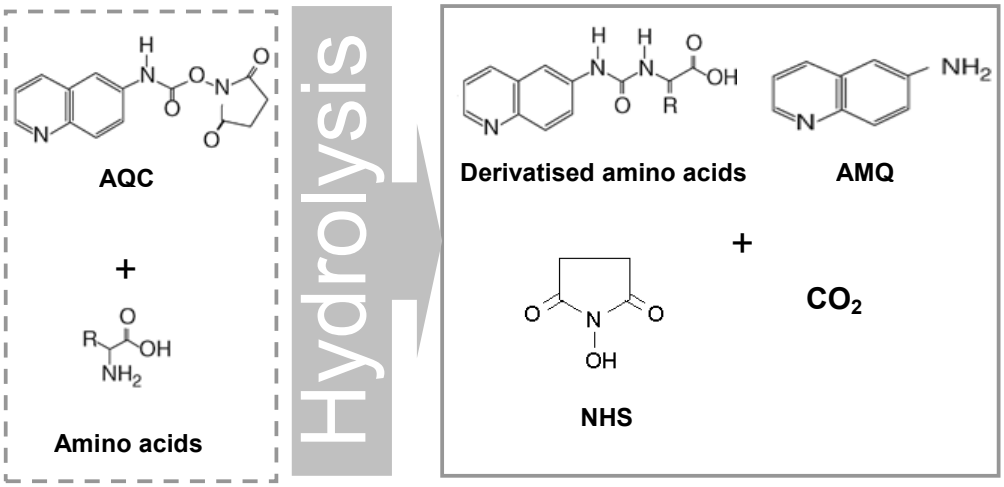

Figure 4.2 Derivatisation of amino acids with AQC.

4.2.6.1 Dissolved free amino acids

Nine hundred and ten µL of sample were transferred to a 1.5 mL snap ring clear-vials with large mouth (6.0 mm, Supelco Analytical). The sample was then buffered with 0.2 M borate buffer, at pH 8.3-8.8. The solution was stirred for about 10 s using a rotamixer. Subsequently, 20 µL of reconstituted AccQ•Fluor reagent for derivatisation was added. The vial was closed and immediately mixed by rotamixer for few seconds and left to incubate for 1 min at room temperature. The samples were then heated to 55ºC in a sand bath for 10 min and filtered before chromatographic analysis. The heating process degrades a phenolic side chain of tyrosine to form a major mono-derivatised compound (Bosch et al., 2006).

4.2.6.2 Dissolved combined amino acids

The hydrolysates were reconstituted in 980 µL of 0.2 M borate buffer at pH 8.3 - 8.8 before derivatisation. The pH range used in the study is in-line with the optimum pH for maximum AQC derivative formation reported in the literature, ranging between 8.2 - 10.0 (Cohen and Michaud, 1993). Twenty µL of reconstituted AccQ•Fluor reagent for derivatisation was added to the samples. The vial was closed and subsequently mixed in

the rotamixer for a few seconds before being left to incubate for 1 min at room temperature. The samples were then heated to 55ºC in a sand bath for 10 min and filtered before chromatographic analysis.

4.2.7 Shut-down and conditioning procedure

After the final run of a week, the column was gradually flushed and kept in 60% ACN prior to removal from the chromatographic system. In order to prevent precipitation of the acetate buffer, seizing of the pumps in ACN and contamination, the pumps and tubing were flushed with UV-irradiated deionised water, 20% concentrated nitric acid in UV-irradiated deionised, UV-irradiated deionised water and propan-2-ol. The conditioning steps can be seen in Figure 4.3.

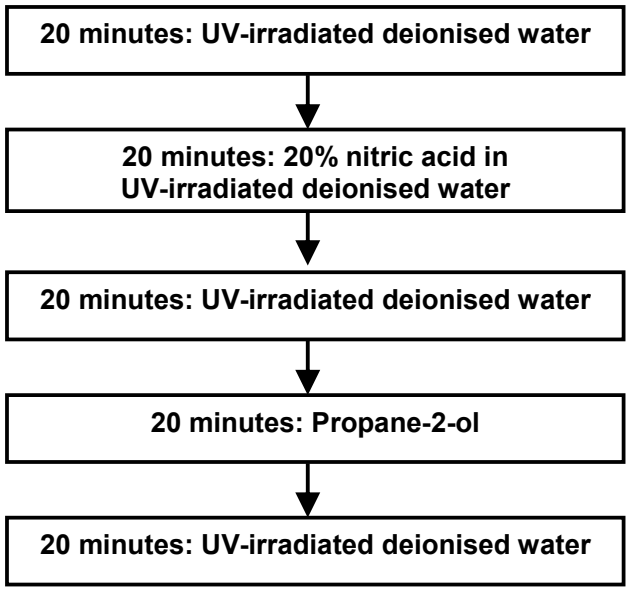

Figure 4.3 The steps of shutting down and conditioning the pump and tubing.

4.3 Results and discussion

4.3.1 Method optimization

4.3.1.1 Effect of ionic medium pH

The results showed that the pH influenced the separation of the derivatised amino acids. As can be seen in Figure 4.4, the pairs of derivatised amino acids which co-elute Asn/Ser and His/Gln were observed regardless of the pH of mobile phase A. AMQ was found to be retained longer and interfered in the determination of Asp when pH of mobile phase A was increased (Figure 4.4). In addition, at the relatively high pH levels (pH 5.21 and pH 5.50), co-elutions of Gly/Gln/His were observed. A better resolution of NH_4/Arg (peaks 9 and 10) and overall separation of the less well retained amino acids was observed when the mobile phase was set to pH 5.05 which coincides with the result in this study. Ionic medium pH of the mobile phase is known to be a crucial factor affecting the retention time and separation of AMQ and amino acids (Cohen and Deantonis, 1994, Liu et al., 1998, vanWandelen and Cohen, 1997, Hernandez-Orte et al., 2003). It has been consistently reported in other studies using AQC pre-column derivatisation that pH of mobile phase affects peak resolution causing co-elutions of peaks, particular for the peak cluster consisting of AMQ, Asp, Ser, Asn, Gly, Gln and His (vanWandelen and Cohen, 1997, Hernandez-Orte et al., 2003). In addition, Cohen (2000) reported that decreasing the mobile phase pH can improve the resolution AMQ and Asp peaks. The pH of 5.05 was selected as a working pH in this study which was also used in many other studies (Cohen and Michaud, 1993, Cohen, 2000, Jorgensen and Jensen, 1997, Bosch et al., 2006, Kabelova et al., 2009).

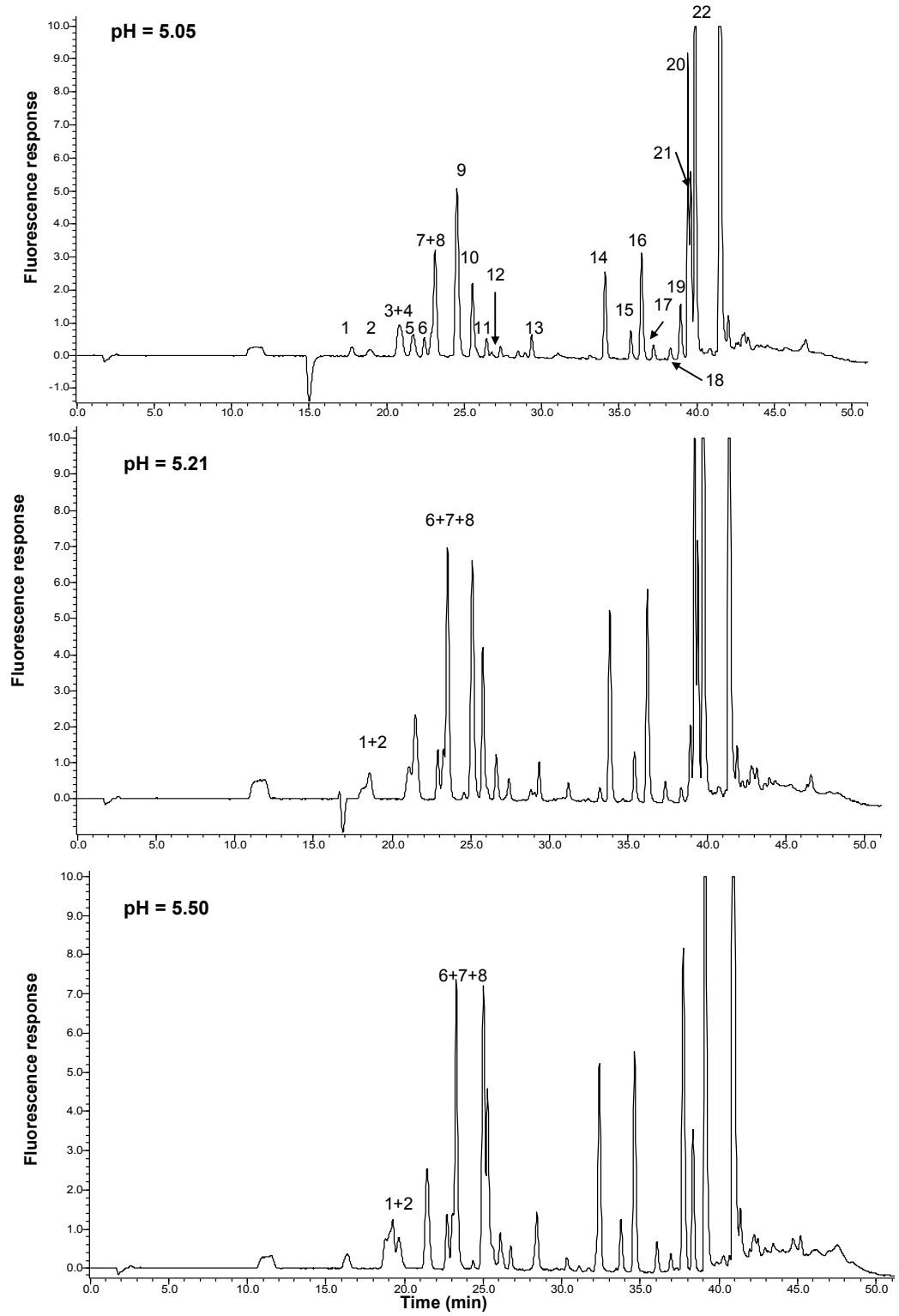

Figure 4.4 Chromatograms corresponding to the amino acids of the analysed standard mixture solution obtained with mobile phase 5.05, 5.21 and 5.50. Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe.

4.3.1.2 Effect of column oven temperature

Figure 4.5 depicts the separation of amino acids using column oven temperatures of 31, 35 and 39ºC. The retention time and separation was not observed to vary significantly within this temperature range. The pairs of derivatised amino acids which co-eluted (in this case Arg/Ala) were not resolved at any of the temperatures applied.

Figure 4.6 showed that the pair of derivatised Arg/Ala co-eluted at temperatures of 35 and 37ºC, but the resolution was better at the higher temperature. The relatively long retention time of Glu interfered with the determination of Asn, when the column temperature was maintained at 35ºC. Changes in elution order were observed and included elution of Asn/Glu after Ser when the column was at 35ºC, while separate elutions of Asn after Glu and Ser were observed at a column temperature of 37ºC. The selection of an appropriate column temperature is essential for reliable gradient methods as it influences retention and selectivity (Snyder and Dolan, 2007). Hernandez-Orte et al. (2003) set the column temperature at 34ºC to quantify amino acids present in musts and wines. However, Hernandez-Orte et al. (2003) reported the co-elution of Gly/His and NH_4^+/Ala at the selected temperature. In this study, the column temperature was maintained at 37ºC in common with the work of several other authors (Strydom and Cohen, 1994, Kabelova et al., 2009, Jorgensen and Jensen, 1997, Bosch et al., 2006).

It is not clear why the co-elution of Arg/Ala was observed in this experiment while Asn/Ser and His/Gln co-eluted in the previous experiment (the effect on ionic medium pH). However, the variation of other chromatographic conditions between these experiments - such as gradient steepness - could be responsible for the different retention times of those amino acids.

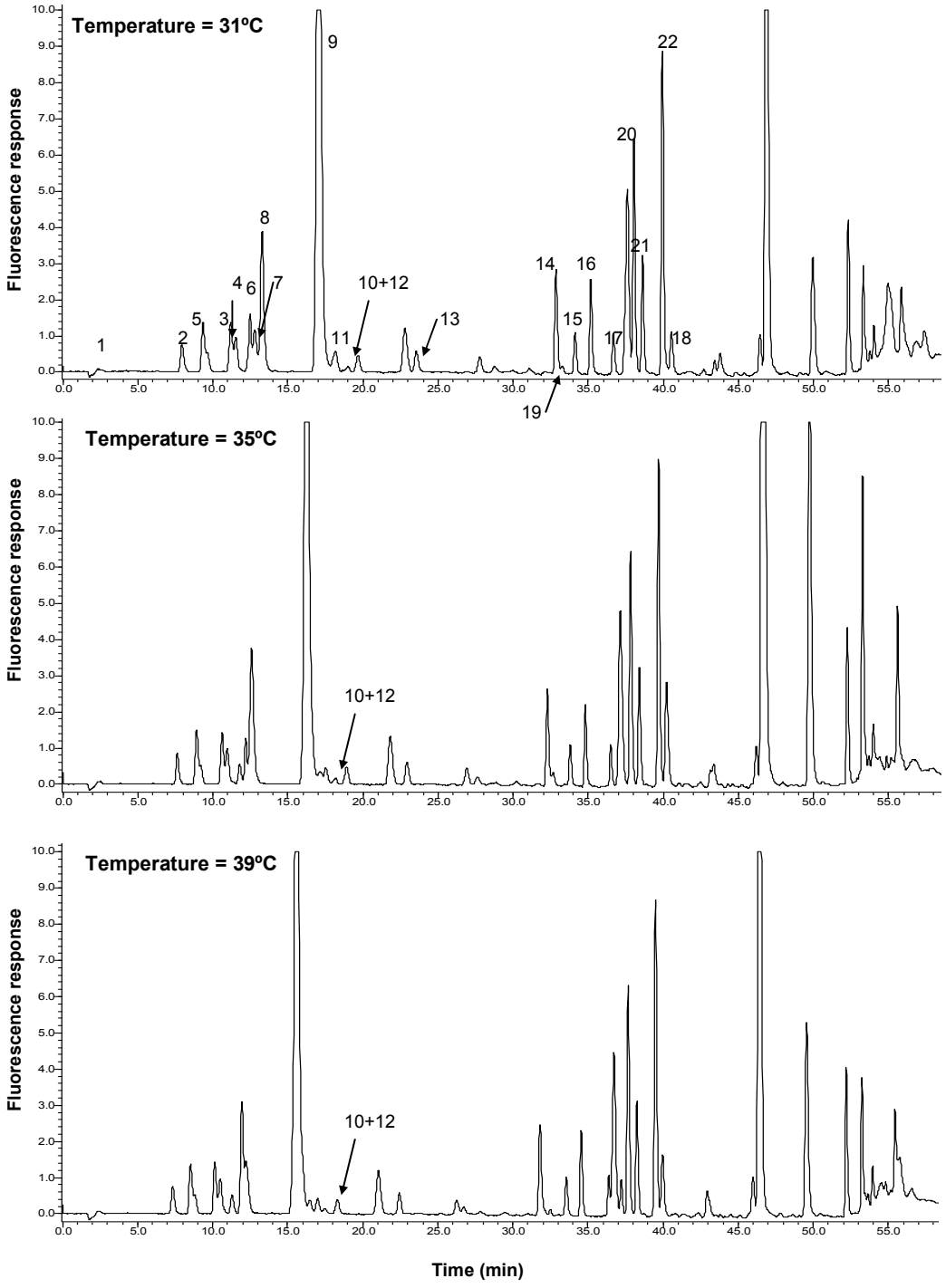

Figure 4.5 Chromatograms corresponding to the amino acids of the analysed standard mixture solution obtained with column oven temperatures of 31ºC, 35ºC and 39ºC. Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe.

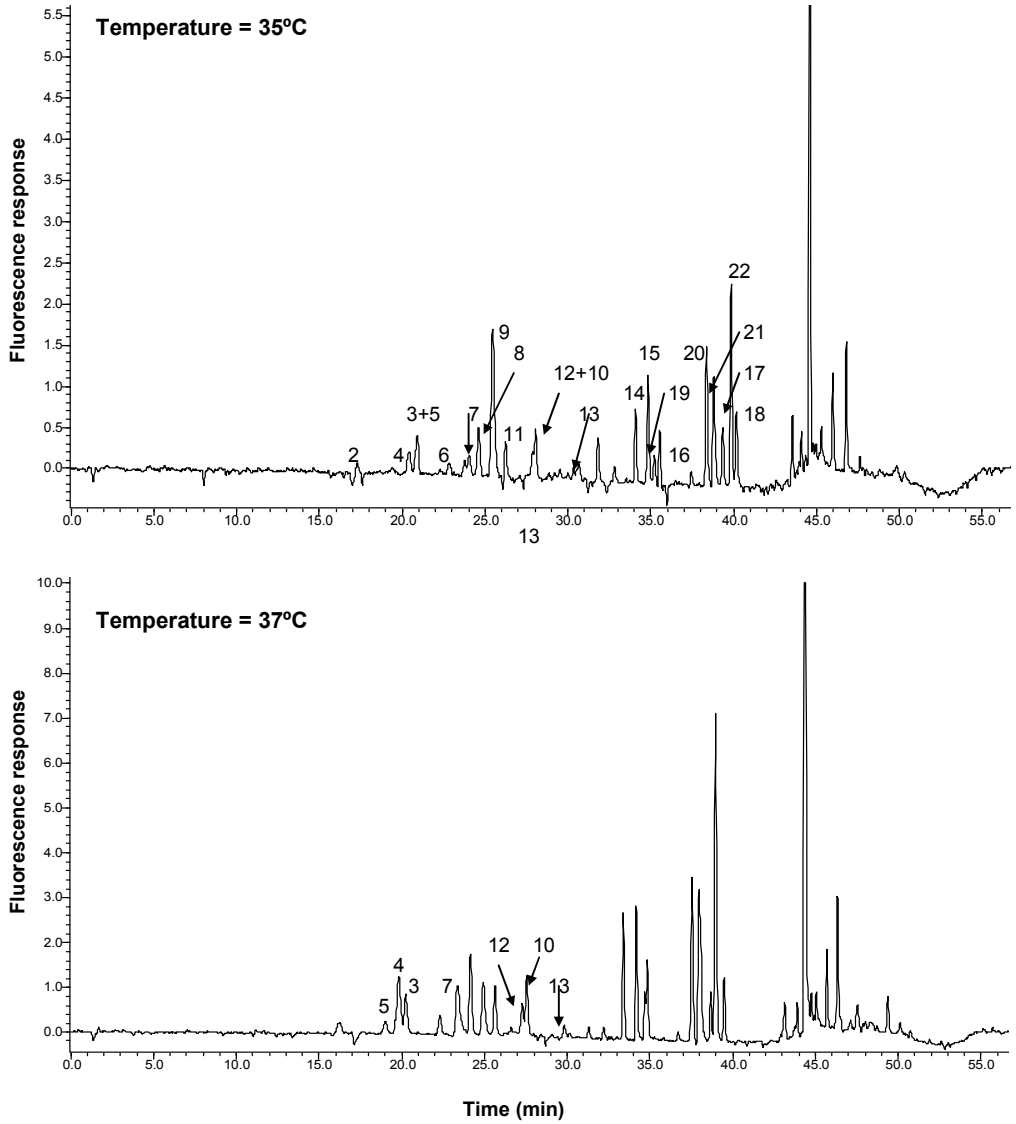

Figure 4.6 Chromatograms corresponding to the amino acids of the analysed standard mixture solution obtained with column oven temperatures of 35ºC and 37ºC. Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe.

4.3.1.3 Effect of gradient steepness

Segmented gradients were used in this experiment. All segments were linear. As indicated in Figure 4.7 the least retained amino acid derivative was Asp. The results showed that polar amino acids such as Asp, Asn and Ser eluted in <10% organic solvent. The pairs of derivatised amino acids which co-elute Asn/Ser and Gln/His were not resolved at any gradient steepness employed. Co-elution of Asn/Ser and Gln/His would not be of importance for the determination of hydrolysed amino acids, as Gln is hydrolysed to Glu and Asn to Asp. Co-elution between Tyr and Cys was observed with gradient 1 and gradient 3. However the fluorescence response for Asp was poor using gradient 2, making this gradient impractical. Furthermore, using gradient 3 allows increased resolution of the AMQ and Asp peaks. Therefore, gradient 3 was selected as the working gradient in this study.

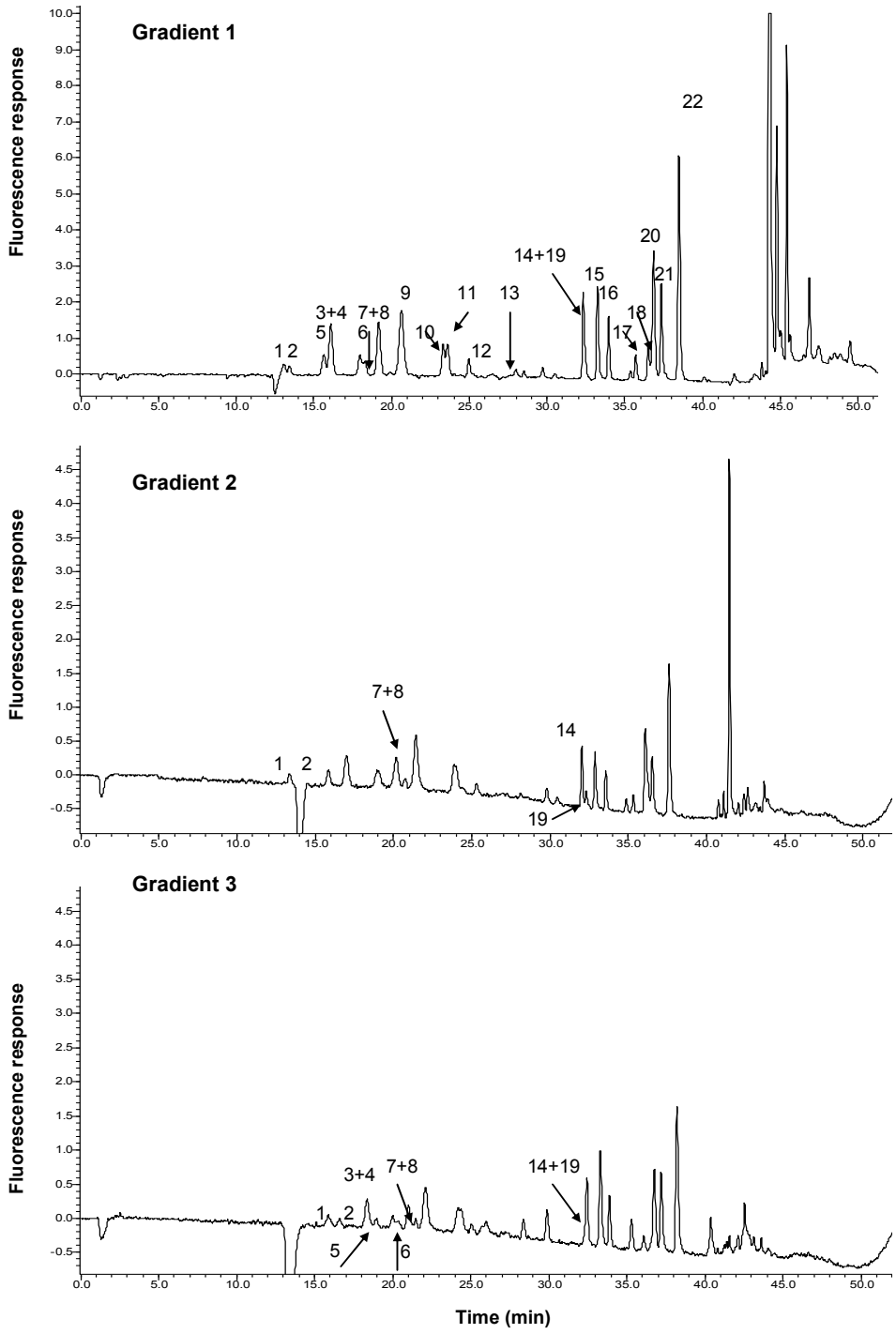

Figure 4.7 Chromatograms corresponding to the amino acids of the analysed standard mixture solution obtained with variation of gradient slopes. Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe.

4.3.1.4 Preparation of amino acid hydrolysates

Retention times of amino acids for approach 1 (the sample was dried using a freeze dryer before hydrolysis), approach 2 (the sample was dried using a centrifugal concentrator before hydrolysis) and approach 3 (the sample remained in its original status with no treatment before hydrolysis) did not differ significantly. The coefficient of variation was less than 1% for Glu, Ser, Gly, His, Thr, Ala, Arg, Tyr, Val, Met, Cys, Ile, Leu, Phe, and Lys, less than 2% for Asp and Orn and less than 3 % for Pro (Table 4.2). This indicated that the retention times of amino acid hydrolysates were mainly affected by the differences in sample preparation approaches. Figure 4.8 demonstrates the fluorescence response of samples prepared by different approaches. The fluorescence response of standard mixture hydrolysate prepared by approach 2 clearly showed a relatively lower sensitivity. The peak areas of standard mixture prepared by approach 1 were generally higher than those prepared by approach 3 by approximately 5-50%, with the exception of Gly, His, Pro and Orn (Figure 4.9). The large tailing peak of ammonia hydrolysate obscuring the Arg peak was observed in this study and also by Strydom and Cohen (1994).

Table 4.2 Retention times of amino acid standard mixture solutions prepared by the 3 different approaches. Data represent the average value of 3 replicates.

Amino acid	Average retention time	SD	%CV
Asp	17.86	0.21	1.16
Glu	21.10	0.20	0.96
Ser	21.61	0.20	0.95
Gly	24.05	0.20	0.81
His	25.99	0.21	0.82
Thr	27.28	0.22	0.81
Ala	28.88	0.21	0.73
Arg	29.23	0.20	0.69
Pro	32.98	0.77	2.32
Tyr	34.77	0.25	0.72
Val	35.69	0.28	0.78
Met	36.39	0.15	0.41
Cys	36.64	0.19	0.51
Iso	39.19	0.35	0.88
Leu	39.63	0.35	0.88
Orn	40.44	0.41	1.02
Phe	40.64	0.36	0.89
Lys	41.25	0.40	0.96

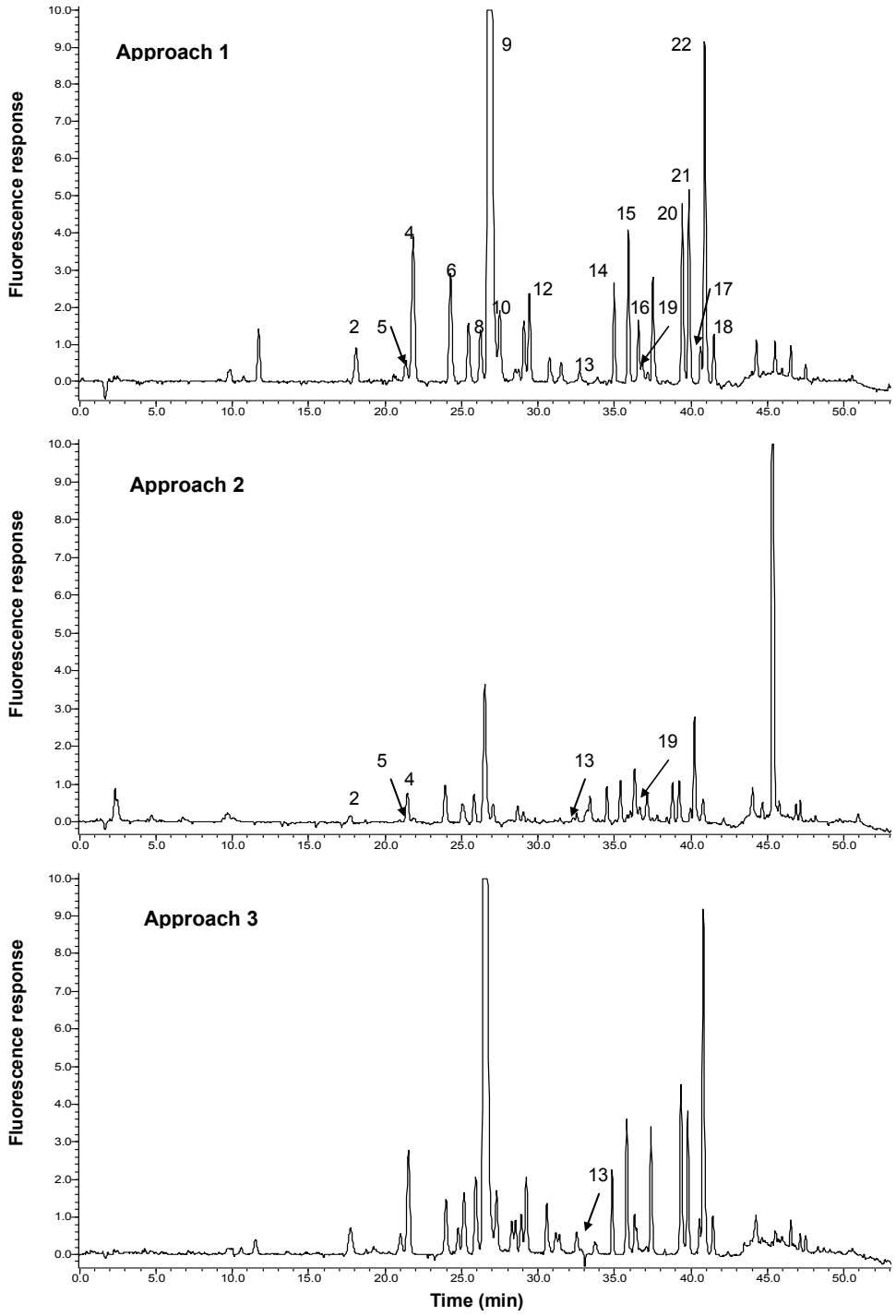

Figure 4.8 Chromatograms corresponding to the amino acids of the analysed standard mixture solution obtained with variation sample preparations. Separations were carried out on a reversed-phase column C18 (Econosphere, Altech) (150 x 4.6 mm, particle size 3 µm). The column temperature was 37ºC. Mobile phase A was titrated to pH 5.05. The flow rate applied was 1.2 mL/min. The gradient of the solvent was 0 min 100% A, 0.5 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 62% A, followed by a wash with 100% B for 5 min and re-equilibration for 7 min at 100% A. Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe.

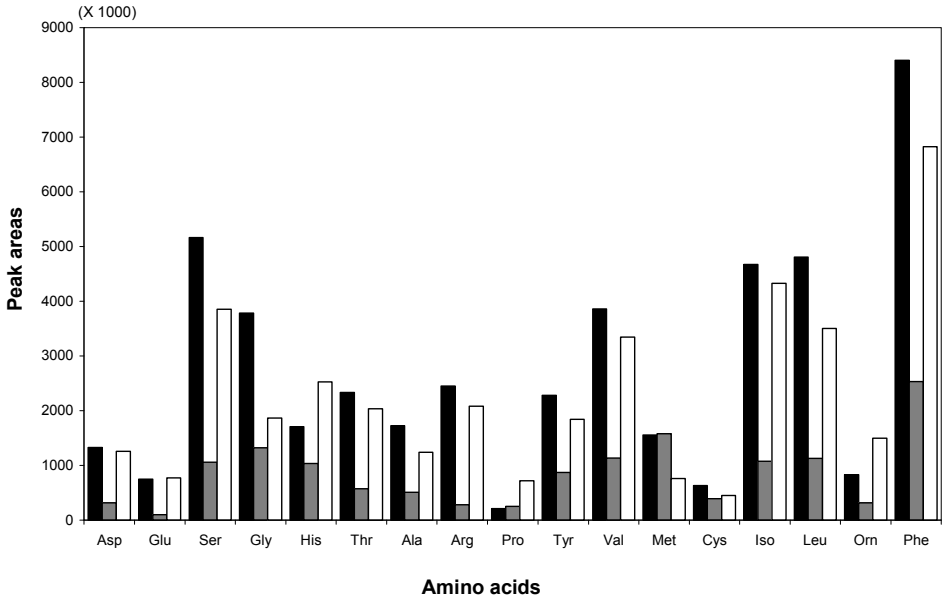

Figure 4.9 The fluorescence responses as peak areas for the standard mixture hydrolysates prepared by approach 1 (black bars), approach 2 (grey bars) and approach 3 (white bars).

4.3.1.5 Internal standards

Analysis of the internal standards Aada and Apma showed that the retention times of derivatised of Aada and Apma did not differ from Thr (23.9 $\pm$ 0.21 min) and Pro (27.8 $\pm$ 0.20 min), respectively. Analysis of MLeu produced a minor peak at 36.8 $\pm$ 0.29 min (MLeu1) and a major peak (MLeu2) at 39.2 $\pm$ 0.34 min (Figure 4.10). The retention times of Aada, Apma and MLeu1 resulted in interference with the determination of Thr, Pro and Leu, respectively. The retention time of L-aba was 29.6 $\pm$ 0.18 min and showed no interference with any other amino acid peaks in this experiment. An internal standard is commonly used for amino acid analysis for monitoring chemical and physical losses and variations during amino acid analysis. Retention times of derivatised internal standards in other studies are shown in Table 4.3. The retention time of L-aba in this study is in line with those reported by Cohen and Deantonis (1994), Bosch et al. (2006) and Kabelova (2009) with the exception of Hernandez-Orte et al. (2003) who analysed amino acids using a quaternary HPLC eluent system. Aada, Apma and L-aba are likely to be suitable as an internal standard for shorter turn-around times (less than 30 min). This finding agrees with Strydom and Cohen (1994) who suggested L-aba as an internal standard for shorter turn-around times. For the longer turn-around time, Norleucine (Nle) and Methyl Leucine are more appropriate.

Of the four internal standards, L-aba was found to be the most suitable as an internal standard as it showed no interference with standard amino acids analysed in this experiment. Additional blank and calibration standard solutions (20 - 60 nM) were analysed on the same day and under the same chromatographic conditions as a 60 nM standard solution combined with 500 nM L-aba to confirm the suitability of this compound. The results showed an appearance of unidentified peaks in blank and calibration standards (20 - 60 nM) without L-aba at the retention time of 29.08 $\pm$ 0.07 min, which was similar to the retention time of L-aba added in 60 nM standard mixtures (29.19 $\pm$ 0.01 min) (Figure 4.11). Furthermore, blank and 100 nM standard solutions alone without L-aba were analysed and the results also showed unidentified peaks at the retention time of 29.89 $\pm$ 0.01 min (Figure 4.12). Hence, this study determined that none of the potential internal standards tested were practical for amino acid analysis with the chromatographic conditions used. Time constraints meant that further exploration of suitable internal standards could not be preformed in this study. However an alternative internal standard such as Nle could be considered for future studies (Strydom and Cohen, 1994, Cohen and Deantonis, 1994).

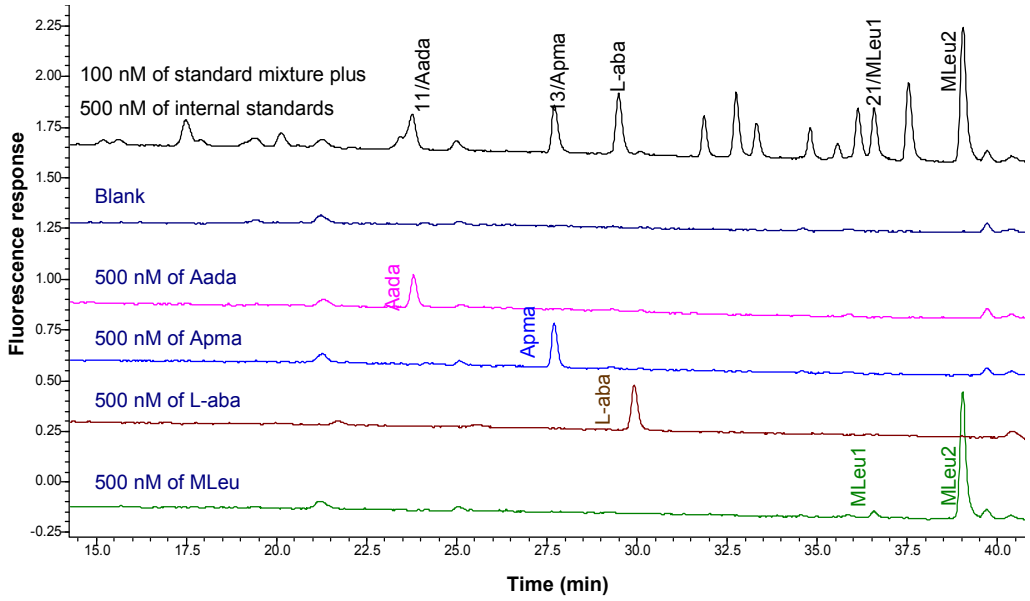

Figure 4.10 Chromatograms of amino acid standard mixture solution (black) and blank (dark blue) combined with 500 nM internal standards (Aada, Apma, L-aba and MLeu) and 500 nM individual internal standards (A-ada; pink, Apma; blue, L-aba; blown and MLeu; green). Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe. Pink= Aada. Blue = Apma. Blown = Apma. Green = MLeu.

Table 4.3 Retention time of derivatised internal standards.

Internal standards	Determination of amino acids	Retention times	References
α-aminobutyric acid (α-Aba)	grape juices and wines	48.19	Hernandez-Orte et al. (2003)
L-α-amino-n-butyric acid (Aaba)	Infant food	~29.5	Bosch et al. (2006)
α-aminobutyric acid (α-Aba)	Cheese	24.3	(Kabelova et al., 2009)
α-aminobutyric acid (Aaba)	intravenous solution	~ 25	Cohen and Deantonis (1994)
Norleucine (Nle)	amino acid standard mixture	~ 36.5 (gradient system 1) ~ 43.5 (gradient system 2)	Strydom and Cohen (1994)
Nle	feed grain	~ 38	Cohen and Deantonis (1994)
Nle	glycoprotein	~ 46	Cohen and Deantonis (1994)
Aada	**amino acid standard mixture**	**23.9 ± 0.21**	**This study**
Apma	**amino acid standard mixture**	**27.8 ± 0.20**	**This study**
L-aba	**amino acid standard mixture**	**29.6 ± 0.18**	**This study**
MLeu	**amino acid standard mixture**	**36.8 ± 0.29 (MLeu1)** **39.2 ± 0.34 (MLeu2)**	**This study**

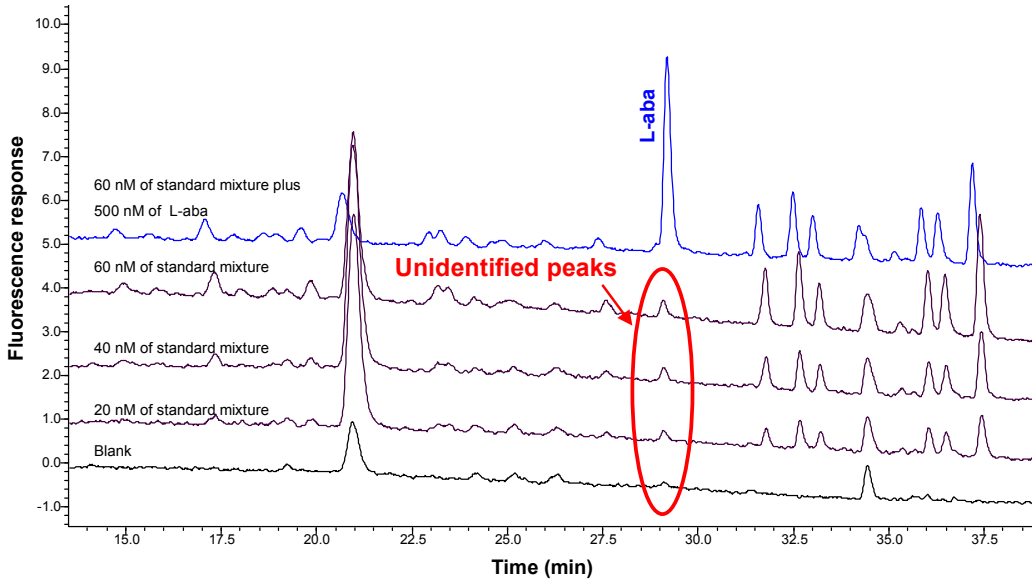

Figure 4.11 Chromatogram of amino acid standard mixture solution with/with out L-aba. Blue chromatogram represents 60 nM amino acid standard mixture solution combined plus 500 nM L-aba. Black chromatograms represent 20 - 60 nM amino acid standard mixture solution without L-aba and Blank. Separations were carried out on a reversed-phase column C18 (AccQ Tag^{TM}, Waters Corp) (150 x 3.9 mm, particle size 4 µm). The column was incubated at 37ºC. Mobile phase A was titrated to pH 5.05. The flow rate applied was 1.0 mL/min. The gradient of the solvent was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 98% B for 5 min and re-equilibration for 11 min at 98% A .

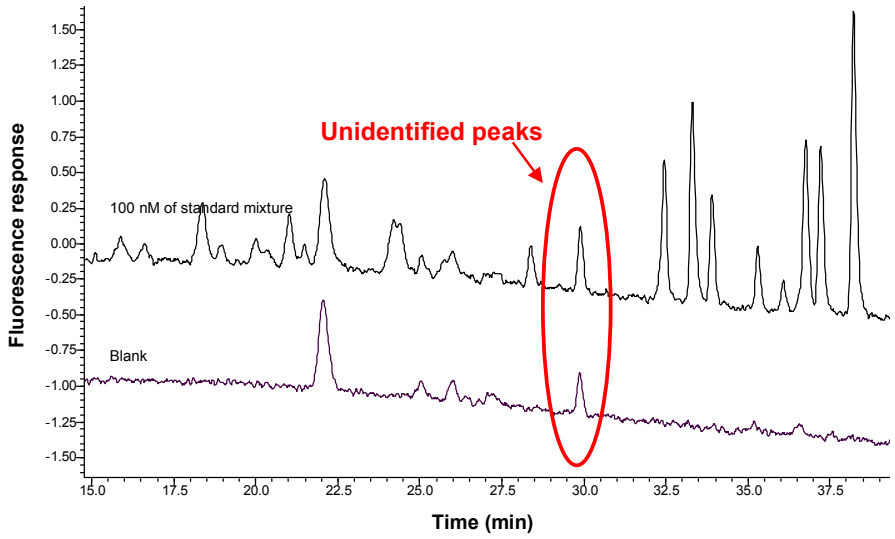

Figure 4.12 Chromatogram of 100 nM amino acid standard mixture solution and blank. Separations were carried out on a reversed-phase column C18 (AccQ Tag^{TM}, Waters Corp) (150 x 3.9 mm, particle size 4 µm).The column temperature was at 37ºC. Mobile phase A was titrated to pH 5.05. The flow rate applied was 1.0 mL/min. The gradient of the solvent was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 98% B for 5 min and re-equilibration for 11 min at 98% A.

4.3.2 Analytical Reproducibility

Reproducibility of the analyses was examined through the calculation of the coefficient of variation. Twenty identical standard samples were derivatised and analysed over 3 weeks. The final sample concentration was 60 nM, the total derivatisation volume was 1 mL, and 400 µL was injected for analysis. Data presented in Table 4.4 shows coefficients of variation of retention time and peak area for each amino acid. Average coefficients of variation for retention time were in the range from 0.60 to 1.47%. The coefficients of variation for the retention time of the hydrophobic amino acids were less variable. Relatively poor reproducibility of peak areas was observed for all of the amino acids with coefficients of variation ranging from 22.31 to 57.22 % (Table 4.4). The coefficients of variation for retention time and peak area obtained from this study were relatively higher than those reported in the literature.

Table 4.4 Reproducibility for peak response and retention time for 60 nM standard mixture solution (n = 20).

Amino acids	%CV (Retention time)	%CV (Area)
Asp	0.95	34.89
Ser + Asn	1.08	28.12
Glu	1.09	39.41
Gly	1.05	28.31
His+Glu	1.10	25.33
Arg	0.88	26.07
Thr	0.84	30.28
Ala	1.47	37.97
Pro	0.72	22.31
Cys + Tyr	0.60	24.36
Val	0.60	27.16
Met	0.60	27.21
Orn	0.70	57.22
Lys	0.65	41.00
Iso	0.66	29.96
Leu	0.67	30.49
Phe	0.69	26.20
Average	0.85	31.55

The poor reproducibility illustrates the need for the use of an appropriate internal standard to compensate for the variance caused by, for example, solvent or derivatisation reagent ages and changes in sample pH (Salter, 2007). In addition, insufficient analytical reproducibility can be connected to poor control of experimental conditions, malfunctioning or poorly performing equipment, changes in column performance with time, insufficient column equilibration between gradient runs, differences in equipment dwell volume when using different HPLC systems and batch-to-batch differences in columns (Snyder and Dolan, 2007). It is worth noting that mechanical problems with the HPLC pumps, which happened occasionally, may have contributed towards the high variability of the analyses.

4.3.3 Linearity and detection limits

Linearity of fluorescent response was tested by the analysis of serial dilutions of the amino acids standard mixture ranging from 20 nM to 100 nM for DFAA and ranging from 50 nM to 200 nM for DHAA, without addition of an internal standard. Linearity of the data was calculated by examining the correlation coefficient of the linear regression line for a plot of the response versus concentration of amino acid. The results in Table 4.5 demonstrate that the correlation coefficients (r) for DFAA were higher than 0.986 with the exception of Asp (0.9826), His/Gln (0.985), Ala (0.9649), Lys (0.9690) and Phe (0.9785). The correlation coefficients for the linear response with DHAA were mostly higher than 0.991 with the exception of Gly (0.9866), His (0.9899), and Pro (0.9889) (Table 4.6). In general, correlation coefficients were relatively low compare to those reported in the literature (Cohen and Michaud, 1993, Hernandez-Orte et al., 2003) which were calibrated using an internal standard. However, the correlation coefficients of Asp observed in this study were better than that reported by Salter (2007) before methyl-threonine correction (r = 0.8420). Low correlation coefficients reflect the high variability of the analyses. The working amino acids standard mixture in this study, ranging from 20 nM to 200 nM, was prepared from the stock standard solutions of individual amino acid 2.5 mM. Serial dilutions of the initial concentrations possibly resulted in high variability of the analysis which coincides with Chase and Hoel (1975) who reported an increasing of variability of particle concentration estimation caused by serial dilutions.

The method detection limit (MDL), was defined as the sample standard deviation of the lowest concentration multiplied by three. The instrument detection limit (IDL) was estimated on a basis of signal to noise ratio (S/N). The results showed a slight difference between the MDL and IDL of the derivatised DFAA standard mixture (Table 4.5). The MDL and IDL for derivatised DFAA standard mixture were 68.1 nM and 61.1 nM, respectively. However, approximately 2 times difference between the MDL (660.5 nM) and IDL (347.4 nM) for derivatised DHAA standard mixture was observed (Table 4.6). The MDL is generally higher than IDL since the MDL represents the detection limit of the method and involves all analytical steps including the determination step, while IDL shows the smallest concentration of a substance that an instrument can measure (Patnaik, 2004). Therefore, MDL was used as the detection limit of DFAA and DHAA in this study as high variability in the analyses was observed. The high variability of the analysis resulted in poorer linearity and a higher detection limit of DFAA and DHAA could be improved by the use of a suitable internal standard.

Table 4.5 Detection limits for derivatised DFAA standard mixture and correlation coefficients for linearity.

Amino acids	*R*	MDL (nM)	IDL (nM)
Asp	0.9826	9.42	31.38
Ser+Asn	0.9899	6.74	6.84
Glu	0.9862	3.36	21.79
Gly	0.9979	5.65	17.75
His + Gln	0.9721	15.33	9.51
Arg	0.9873	3.51	10.74
Thr	0.9894	1.39	12.54
Ala	0.9649	17.69	35.06
Pro	0.9917	13.98	11.99
Cys + Tyr	0.9860	14.03	3.86
Val	0.9898	8.62	3.12
Met	0.9875	7.82	5.74
Orn	0.9883	7.47	13.04
Lys	0.9690	15.87	25.01
Iso	0.9904	6.75	4.11
Leu	0.9876	1.34	4.16
Phe	0.9785	6.19	2.04
Total free amino acids		**68.10**	**61.10**

Table 4.6 Detection limits for derivatised DHAA standard mixture and correlation coefficients for response linearity.

Amino acids	*R*	MDL (nM)	IDL (nM)
Asp	0.9938	17.18	44.88
Ser	0.9936	49.68	21.57
Glu	0.9978	16.88	38.72
Gly	0.9866	59.11	44.30
His	0.9899	52.03	23.09
Arg	0.9935	48.06	13.75
Thr	0.9929	74.16	15.92
Ala	0.9928	61.55	50.28
Pro	0.9889	40.06	18.33
Cys+Tyr	0.9943	34.28	6.29
Val	0.9979	28.48	4.29
Met	0.9991	50.86	8.17
Orn	0.9914	6.00	14.74
Lys	0.9991	15.18	29.05
Ile	0.9979	26.14	5.50
Leu	0.9956	34.13	5.47
Phe	0.9954	46.76	3.02
Total hydrolysable amino acids		**660.53**	**347.39**

4.3.4 Recovery efficiencies

A comparison of chromatograms of the 18 amino acid standard mixture (Asp, Ser, Glu, Gly, His, Arg, Thr, Ala, Pro, Tyr, Val, Met, Orn, Lys, Cys, Ile, Leu, Phe) before and after hydrolysis were conducted in order to calculate the percent recovery of the analysis. The recovery efficiencies are shown in Table 4.7. The chromatograms relating to the hydrolysates of amino acid standard mixtures derivatised at pH 8.3 show higher recoveries than the chromatograms relating to the amino acid standard mixture derived without hydrolysis with the exception of Met, Orn and Lys. Relatively higher recoveries of the hydrolysates of amino acid standard mixture derivatised at pH 8.8 were observed for Ser, Gly, Ala and Pro compared to the non-hydrolysed standard mixture. The peak areas of derivatised amino acids are depicted in Figure 4.13. Co-elution of Cys/Tyr was observed in the non-hydrolysed sample but Tyr completely separates from Cys after hydrolysis (Figure 4.14). Therefore, the recovery efficiencies of Cys and Tyr were not calculated. The results of recovery efficiencies were not in agreement with other studies.

There are several causes that can influence the recovery efficiencies of amino acid hydrolysis such as the presence of salt in a sample (Strydom and Cohen, 1994) and the hydrolysis temperature (Salter, 2007). The variation of hydrolysate pH can possibly be critical although the samples were set to the optimum pH range (8.2-10.0). Another possible reason is the co-elution of other breakdown products with AQC after hydrolysis (Cohen and Michaud, 1993).

Table 4.7 Recovery efficiencies of 18 amino acids standard mixture before and after hydrolysis.

Amino acids	Peak area (before hydrolysis)	Hydrolysate pH 8.3		Hydrolysate pH 8.8	
		Peak area	% recovery	Peak area	% recovery
Asp	128806	286264	222.24	92786	72.04
Ser	384243	1104853	287.54	565178	147.09
Glu	239168	505215	211.24	236130	98.73
Gly	145876	4497730	3083.26	1404288	962.66
His	347979	565896	162.62	317838	91.34
Arg	426538	1264560	296.47	426681	100.03
Thr	649521	721471	111.08	346213	53.30
Ala	208517	238842	114.54	248270	119.06
Pro	292101	832834	285.12	544144	186.29
Cys+Tyr	1181027				
Cys		1326656		4082126	
Tyr		1111721		556844	
Val	1557582	1769211	113.59	887565	56.98
Met	1021291	893173	87.46	416439	40.78
Orn	502341	317535	63.21	342918	68.26
Lys	221397	140597	63.50	208948	94.38
Ile	1285611	2415689	187.90	1155385	89.87
Leu	1284194	1996852	155.49	904365	70.42
Phe	2481555	5148549	207.47	2281355	91.93

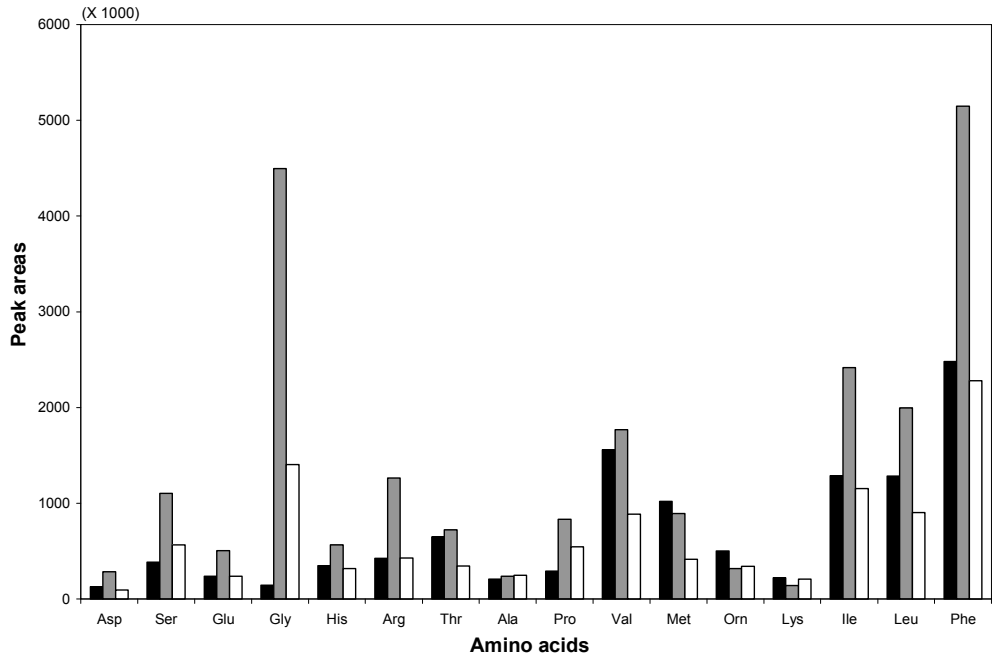

Figure 4.13 The fluorescence response (peak area) on analysis for amino acid standard mixture (black bars), hydrolysate of amino acid standard mixture derivatised at pH 8.3 (grey bars) and hydrolysate of amino acid standard mixture derivatised at pH 8.8 (white bars).

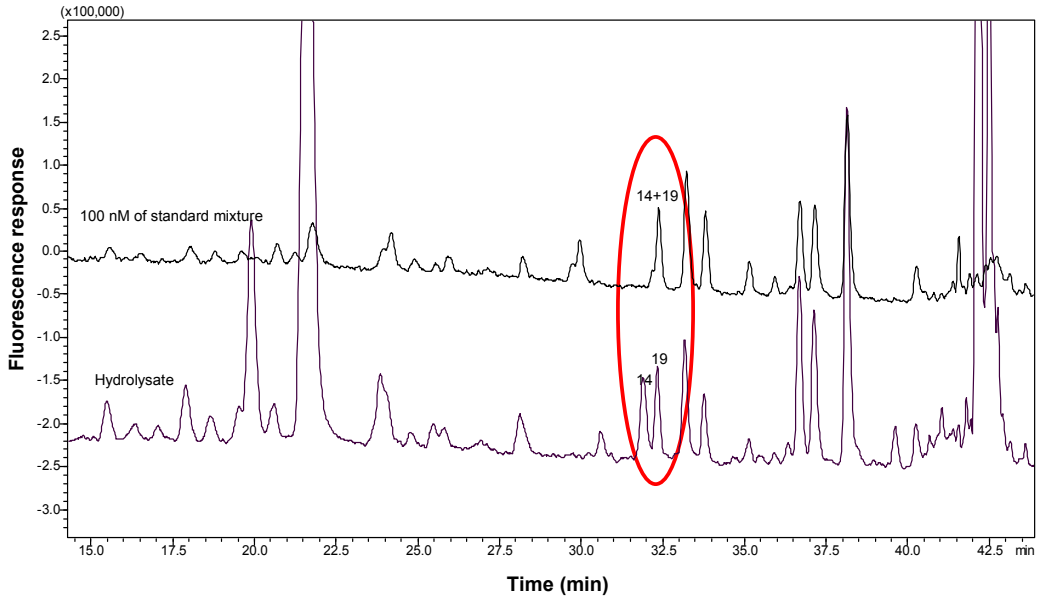

Figure 4.14 Chromatogram of 100 nM amino acid standard mixture solution with and without hydrolysis. Separations were carried out on a reversed-phase column C18 (AccQ Tag^{TM}, Waters Corp) (150 x 3.9 mm, particle size 4 µm). The column temperature was 37ºC. Mobile phase A was set to pH 5.05. The flow rate applied was 1.0 mL/min. The gradient of the solvent was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 98% B for 5 min and re-equilibration for 11 min at 98% A.

4.3.5 Application of the RP-HPLC method to the determination of DFAA and DCAA in seawater and aerosol leach solution

The analysis of seawater and aerosol leaching solutions was carried out using the following conditions: the column temperature was set to 37ºC; mobile phase A at pH 5.05; mobile phase B: 60% ACN in UV-irradiated deionised water (v/v). The gradient of the solvent was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 100% B for 5 min and re-equilibration for 11 min at 98% A. Separations were carried out on a reversed-phase column C18 (AccQ Tag^{TM}, Waters Corp) (150 x 3.9 mm, particle size 4 µm). Flow rate was 1 mL/min. Acquisition time was 60 min. Detection was carried out using fluorescence with excitation and emission wavelengths at 250 nm and 395 nm, respectively. Hydrolysis was carried out using vapour phase hydrolysis which requires 24 hours at a temperature of 110-115 ºC. The hydrolysis time can be reduced to 1 hour by increasing temperature to 150ºC (Cohen, 2000). However, Salter (2007) suggested that a high temperature and short duration hydrolysis method resulted in very inconsistent fluorescence responses. Alternatively, hydrolysis using microwave allows for a further decrease of the hydrolysis time to 20 min (Jorgensen and Jensen, 1997). The chromatography for derivatised amino acid standard mixture is shown in Figure 4.15.

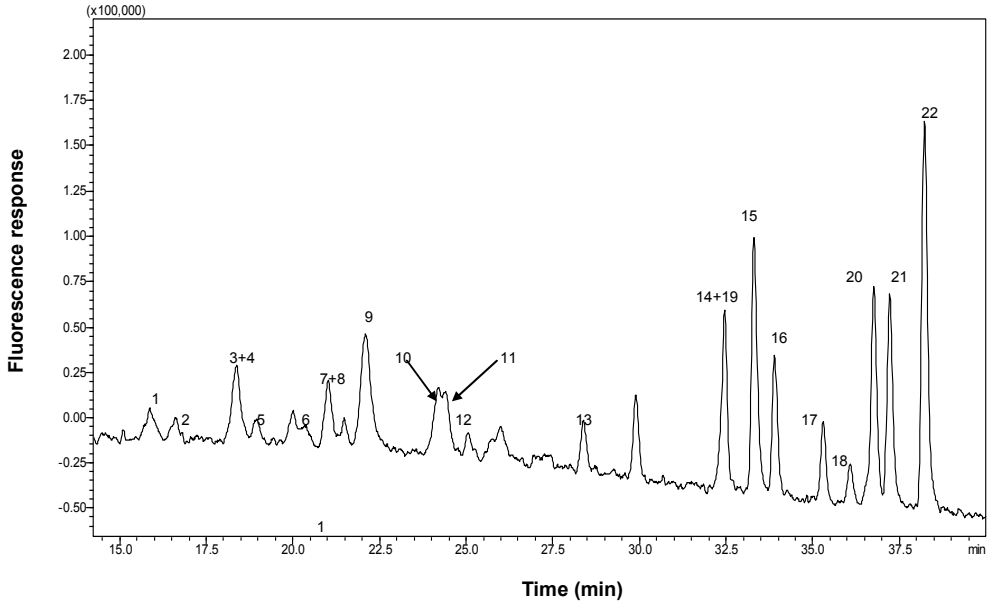

Figure 4.15 Chromatogram of 100 nM standard mixture solution analysed by the optimized method. Peaks: 1 = AMQ; 2 = Asp; 3 = Asn; 4 =Ser; 5 = Glu; 6 = Gly; 7 = Gln; 8 = His; 9 = NH_4; 10 = Arg; 11 = Thr; 12 = Ala; 13 = Pro; 14 = Tyr; 15 = Val, 16 = Met; 17 = Orn; 18 = Lys; 19 = Cys; 20 = Ile; 21 = Leu; 22 = Phe.

Sub-surface waters of the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region at depth between 20-35 m were analysed for DFAA and DHAA. The depth between 20 - 35 m was chosen as the depth where DOC concentrations showed a maximum as reported in Chapter 2. The fluorescence response for DFAA and DHAA were below the detection limits of 0.07 μM and 0.66 μM, respectively. In Table 4.8, DFAA concentrations reported in the literature were up to 3.5 μM (Clark et al., 1972, Tada et al., 1998, Kuznetsova and Lee, 2002, Chen et al., 2004, Wedyan and Preston, 2008, Yang et al., 2009). DHAA concentrations in oceanic environment range between 0.20 μM to 22.8 μM (Benner, 2002, Lee and Bada, 1977, Druffel et al., 1992a, Yamashita and Tanoue, 2003, Wedyan and Preston, 2008).

Table 4.8 Concentrations of dissolved amino acids in oceans.

Study areas	DFAA (μM)	DCAA (μM)	DHAA (μM)	Methods	References
Southern California coastal water (surface water)	1.0	n/a	n/a	Thin-layer chromatography	Clark et al. (1972)
Delaware Bay	n/a	0.2-10	n/a	Liquid phase hydrolysis with OPA derivatisation	Keil and Kirchman (1991)
Pacific Ocean	n/a	0.2-0.4	n/a	Liquid phase hydrolysis with OPA derivatisation	Keil and Kirchman (1991)
North Atlantic Ocean (upper 50 m)	~ 0.05	n/a	n/a	OPA derivatisation	Kirchman et al. (1994) *in* Bronk (2002)
Estuarine water	n/a	2.45	n/a	Microwave phase hydrolysis with AQC derivatisation	Jorgensen and Jensen (1997)
Seto Inland Sea, Japan	Up to 1.87	n/a	n/a	Modified Fluormetric	Tada et al. (1998)
Main Channel, New York	0.02 - 3.5	0.12 -1.94	n/a	Vapour phase hydrolysis with OPA derivatisation	Kuznetsova and Lee (2002)
Surface ocean (<100 m)			0.2 - 0.5	Acid hydrolysis with OPA derivatisation	Lee and Bada (1977) and Druffel et al (1992b) *in* Benner (2002)
Ise bay	n/a	n/a	0.29 - 1.70	Acid hydrolysis with OPA derivatisation	Yamashita and Tanoue (2003)
Coastal area (surface water)	n/a	n/a	0.26 - 0.83	Acid hydrolysis with OPA derivatisation	Yamashita and Tanoue (2003)
Northwestern Pacific (surface water)	n/a	n/a	0.26 - 0.37	Acid hydrolysis with OPA derivatisation	Yamashita and Tanoue (2003)
Paerl River Estuary	0.15 - 1.10	1.1 - 4.0	0.41 - 12.6	Vapour phase hydrolysis with AQC derivatisation	Chen et al. (2004)
Atlantic Ocean (surface waters)	0.7 - 2.9	n/a	5.0 – 22.8	Acid hydrolysis with OPA/IBLC derivatisation	Wedyan and Preston (2008)
Yellow sea China (sub-surface layer)	0.57	n/a	n/a	OPA derivatisation	Yang et al. (2009)
Yellow sea China (surface microlayer)	0.94	n/a	n/a	OPA derivatisation	Yang et al. (2009)
North Atlantic subtropical gyre	**< 0.07**	**n/a**	**< 0.66**	**Vapour phase hydrolysis with AQC derivatisation**	**This study**
Tropical North Atlantic Ocean	**< 0.07**	**n/a**	**< 0.66**	**Vapour phase hydrolysis with AQC derivatisation**	**This study**
Mauritanian shelf Region	**< 0.07**	**n/a**	**< 0.66**	**Vapour phase hydrolysis with AQC derivatisation**	**This study**

n/a = data not available

The DFAA and DHAA concentrations observed in the study deviate from those in the literature owing to the physical and biogeochemical processes controlling amino acids in our study region. The physical and biogeochemical factors governing dissolved organic matter in the (sub-) tropical North Atlantic Ocean and the Mauritanian shelf region have been discussed in Chapter 2. Moreover, the amino acid concentration data reported in the literature may be either underestimated or overestimated. The amino acid analysis using derivatisation with OPA reacts with only primary amines, while AQC reacts with both primary and secondary amines (Jorgensen and Jensen, 1997, Bosch et al., 2006). Therefore, the amino acid concentrations reported in the literature using OPA as a derivatising agent is likely to underestimate the actual amino acid concentrations. On the other hand, contamination possibly leads to an overestimation. Armstrong et al. (2001) suggested that the analysis of amino acids requires specialized sample pre-treatment, handling and an ultraclean experimental environment because even slight contamination of any sample with dust or aerosol particles can influence the results of amino acid analysis. In this study, contamination was minimised in several ways such as irradiating deionised water and seawater, combusting the mobile phase glassware, filtering mobile phase A and setting up system and column conditioning procedures. Hubberten et al. (1994, 1995) observed that hydrophilic amino acids appear to correlate with chlorophyll *a*, while no correlation was found between hydrophobic amino acids and chlorophyll *a* suggesting that amino acids are preferentially utilised by marine plankton. However, in this study, the undetectable amino acid concentrations prevent comparison with other parameters.

LFAA and LHAA were measured in the bulk aerosol filters collected at São Vicente, Cape Verde Islands. The bulk aerosol filters were classified into 6 types and leachable organic nitrogen concentrations contained in the aerosol filters were analysed as described in Chapter 3. LFAA in land-based sampling aerosol filters which represent aerosols transported from Saharan and Sahel regions from January to February 2008 were determined. The results indicated that mean LFAA concentration was < 0.05 nmol/m^3 accounting for < 2.6% of LON, indicating that LFAA was generally a minor component of LON in the study region. The contributions of LFAA to LON were in the range reported in the literature (Table 4.9). Mean LHAA concentration was < 0.64 nmol/m^3. The relatively high LHAA in Davis, California is possibly due to the different emission sources. Air masses in Davis were influenced by emissions from agriculture, urban centres and transport (Zhang et al., 2002), while air masses over the Cape Verde islands during sampling were transported from the Saharan and Sahel regions. Concentrations of

nitrogen contained in individual amino acids possibly reflect a source of LFAA. According to Glavin et al. (2001), biological organisms produce Arg, an amino acid containing 4 nitrogen atoms, during the urea cycle. Biological organisms such as bacteria contain high concentrations of Gly, Pro and Val (Nagata et al., 2001, Glavin et al., 2001). Mace et al. (2003c) observed that Gly, Arg, Pro and Val contributed approximately 75% of the total amino N in aerosols which were indicative of biological organisms. Furthermore, Asp, Glu, Ser, Gly, Ala, and Val are dominant amino acids accounting for 70% of total amino N in *Escherichia coli* (Salway, 1999). The presence of Met in Amazon aerosols can reflect the decomposition of protein during burning or a gas phase reaction (Mace et al., 2003a). In this study, the concentrations of individual amino acids were below limits of detection, hence a conclusion on the source of amino acids can not be drawn. However, the relationship between airborne microorganisms and African dust loading has been observed in several studies (Schlesinger et al., 2006, Griffin et al., 2007, 2001, Wedyan, 2008).

Table 4.9 Mean leachable organic nitrogen and leachable amino acids in aerosols reported in the literature.

Study sites	Concentrations (nmol/m^3)			%LFAA of LON	References
	LON	LFAA	LHAA		
North Atlantic Ocean	1.4 - 5.0	0.003 - 1.61	n/a	2 - 17[a]	Gorzelska and Galloway (1990)
Davis, California	18.9	0.58	3.23	4.0	Zhang et al. (2002)
Tasmania	3.6	0.1	n/a	~ 3	Mace et al. (2003b)
Eastern Mediterranean	29	0.37	n/a	1.3	Mace et al. (2003b)
Amazon Basin (dry season)	61	0.21	n/a	0.83	Mace et al. (2003a)
Amazon Basin (wet season)	3.5	0.11	n/a	1.8	Mace et al. (2003a)
Atlantic Ocean	n/a	0.003 – 0.1	0.02 – 0.4	n/a	Wedyan and Preston (2008)
Qingdao	180	1.56	n/a	0.94	Shi et al. (2010)
Yellow sea	87 - 204	0.87 - 1.00	n/a	0.60 - 2.12	Shi et al. (2010)
Cape Verde Islands (February 2008)	**2.4**	**< 0.054 (n = 3)**	**< 0.64 (n = 16)**	**< 2.25**	**This study**

[a] estimated by Gorzelska and Galloway (1990) based on concentrations of organic carbon reported for Bermuda aerosol (Hoffman and Duce, 1974) and the C:N ratio of sea-spray aerosol for the North Atlantic (Gershey, 1983)

Since it was not possible to detect amino acids in the samples, additional approaches were conducted to improve sensitivity of the chromatography response. Firstly, a seawater sample was extracted by OASIS^{TM} HLB sample extraction products supplied by Waters Corp. The sample extraction protocol using the OASIS^{TM} HLB sample extraction products is depicted in Figure 4.16. In this experiment, the eluted liquid was evaporated in a nitrogen stream and reconstituted in 910 µL of UV-irradiated-low nutrient surface seawater before the derivatisation process. The liquid derived from the washing step was also collected and derivatised. The results revealed that amino acids partially eluted during the washing step (Figure 4.17) because methanol is one of the most common organic solvents used as an organic modifier for RP-HPLC technique. Therefore, some amino acids contained in the sample were lost leading to an underestimation of the amino acid concentration.

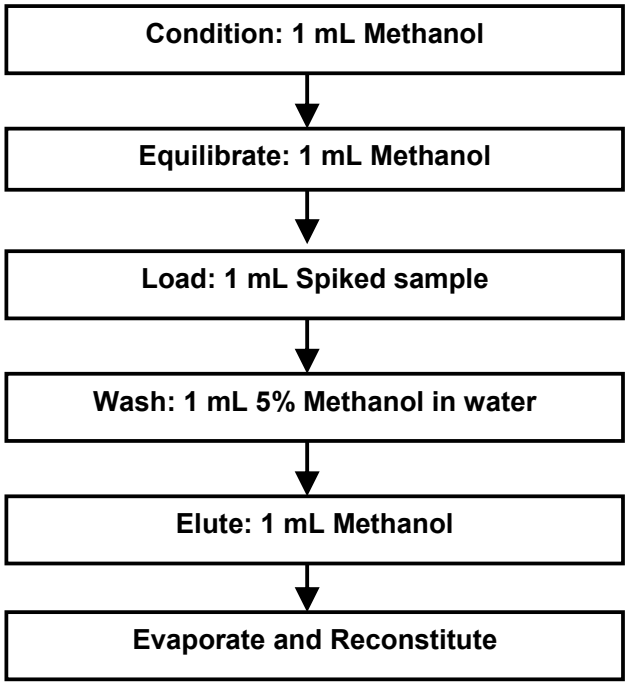

Figure 4.16 The sample extraction protocol using the OASIS^{TM} HLB sample extraction products.

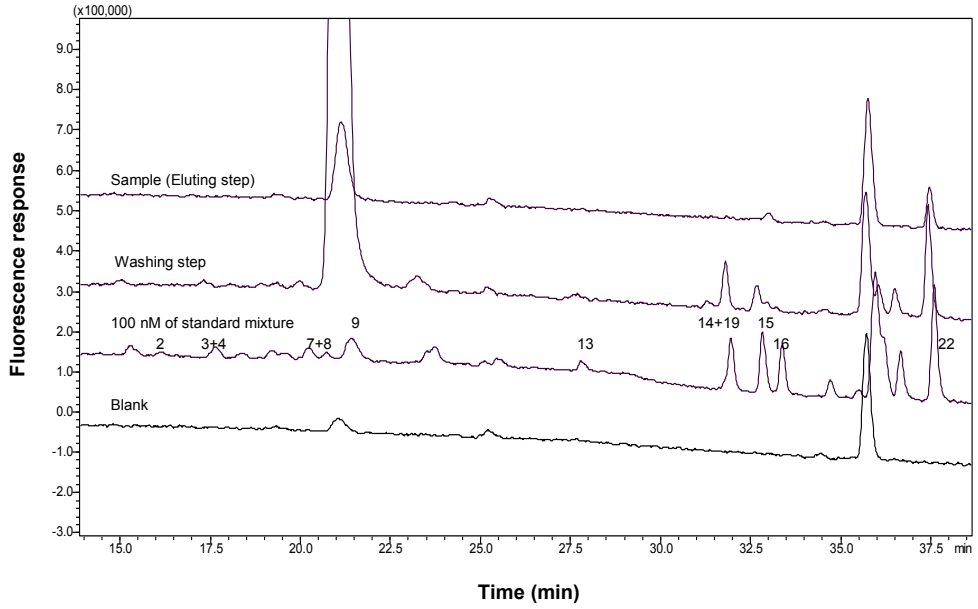

Figure 4.17 Chromatogram of 100 nM amino acid standard mixture solution, blank, liquid derived from the washing step of a sample and the eluted sample.

Secondly, 5 mL of seawater sample were transferred to a 7 mL screw top clear vial supplied by SUPELCO Analytical. The samples were dried in the freeze dryer. The dried samples were subsequently reconstituted in UV-irradiated low-nutrient surface seawater and derivatised. The final volume of derivatised sample was 1000 µL. The samples were analysed using the chromatographic conditions described above. Although salt does not interfere with the AQC derivatising reaction (Cohen and Michaud, 1993, Bosch et al., 2006), the high salt content in the concentrated seawater samples possibly affected the performance of the HPLC analysis by blocking the system. Consequently back pressure increased and leaks were observed during analysis, probably as a result of the precipitation of salt. High back pressure and system leaks caused precipitation or dust collecting in the column is a common occurrence with HPLC analysis, especially RP-HPLC (Hanai, 1999).

4.4 Conclusion

RP-HPLC combined with AQC pre-column derivatisation was used to quantify dissolved amino acid concentrations present in seawater and aerosol leach solutions. Chromatographic conditions such as temperature, mobile phase pH and elution gradient were optimized using a binary gradient system. Different column oven temperatures (31, 35, 37 and 39ºC), ionic medium pH (pH 5.05 - 5.50) and gradient steepness were tested. The analysis of seawaters and leaching solutions of aerosol was carried out in the optimized condition: pH of mobile phase A = 5.05, column oven temperature = 37 ºC, flow rate = 1.0 mL/min and the solvent gradient was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 98% B for 5 min and re-equilibration for 11 min at 98% A. The detection was carried out on fluorescence with excitation at 250 nm and emission wavelengths at 395 nm. The appearance of Pro confirms that AQC reacts with primary and secondary amino acids. Hydrolysis was conducted using vapour phase hydrolysis with long duration (24 hours) at temperature 110 - 115 ºC. Chromatogram response showed relatively high sensitivity when the samples were dried using a freeze dryer prior to hydrolysis process. L-aba, Aada, Apma and MLeu were not suitable as internal standards because they co-eluted with amino acid peaks and one unidentified peak. The coefficients of variation for the retention time ranging from 0.60% (Cys/Try, Met and Met) to 1.47% (Ala). The coefficients of variation for the peak areas ranged from 22.31% (Pro) to 57.22% (Orn). The linear responses (correlation coefficient, r) for DFAA were higher than 0.986 with the exception of Asp (0.9826), His/Gln (0.985), Ala (0.9649), Lys (0.9690) and Phe (0.9785). The linear responses for DHAA were mostly higher than 0.991 with the exception of Gly (0.9866), His (0.9899), and Pro (0.9889). The optimized method allows the optimum separation of 14 DFAA and three pairs of DFAA (Asn/Ser, Gln/His and Cys/Tyr). However, during hydrolysis the amine group of Asn and Gln are de-aminated to yield Asp and Glu and ammonium. Therefore, the co-elution of Asn/Ser and Gln/His are resolved on hydrolysis.

Method detection limits (MDL) were 68.1 nM for DFAA and 660.5 nM for DHAA. Instrumental detection limits (IDL) were 61.1 nM for DFAA and 347.4 nM for DHAA. The amino acid concentrations of seawater collected from the North Atlantic subtropical gyre, the tropical North Atlantic Ocean and the Mauritanian shelf region in addition to aerosol leach solutions prepared from samples collected at São Vicente, Cape Verde Islands were all below the detection limit. Increasing the concentration of the samples using a sample extraction product containing methanol during washing process was tested. The results showed that the method is not suitable for amino acid pre-concentration as amino acids were lost during the washing step. Increasing the concentration of the samples by

increasing the original volume of sample was also examined. The high sample volume (4 - 5 times the original volume) was dried, then reconstituted to 1 mL during derivatisation and analysis. This approach caused mechanical problems (high back pressure and leaking) as a result of salt precipitation.

This study represents a significant step forwards in the use of RP-HPLC combined with AQC pre-column derivatisation for amino acid analysis at low concentrations such as found in oligotrophic seawater and aerosol leach solutions. The method of derivatisation of amino acids with AQC used needs further work to improve the sensitivity, reproducibility, linearity and to find a suitable internal standard.

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Chapter 5

Conclusions

5.1 Overall conclusions

5.1.1 The North Atlantic subtropical gyre

The North Atlantic subtropical gyre had relatively deep pycnoclines, nitraclines and low chlorophyll *a* concentrations (< 0.5 μg/L), indicating that this area was non-productive. DOC showed elevated concentrations in the upper 40 m, with a mean of 67.2 μM and 65.4% of the bulk DOC pool was refractory fraction. DOC concentration decreased to 48.6 μM in sub-surface waters (200 - 300 m) in which the refractory fraction accounted for 90.6% of the bulk DOC pool. The contribution of DOC to oxygen consumption accounted for 16.5% in the sub-surface waters where the water masses are isolated from the atmosphere. The bulk of respiration was supported by a flux of particulate organic carbon with a small contribution of DOC to oxygen consumption. Distributions of DON in the North Atlantic subtropical gyre showed enhanced concentrations in surface waters, with a mean of 4.6 μM, and declining concentration towards deep water, with a mean of 2.4 μM. The refractory DON in surface waters accounted for 60.9%, while the refractory DON in sub-surface waters increased to 100%. The high contributions of DON to TDN were observed in surface waters of the North Atlantic subtropical gyre (> 99%), indicating the inorganic nutrient-limited state of the area. DFAA and DHAA concentrations were below the detection limit of 0.07 μM and 0.66 μM, respectively. The relatively deep mixed layer (100 - 150 m) in this area favoured an enhanced DOC and DON stocks. In comparison with the Redfield ratio, the elemental ratios of DOM were carbon-rich relative to nitrogen and phosphorus. Considering the correlation between DOC and DON with other variables (density, chlorophyll *a*, bacteria cells and DIN) together with the contributions of DOC to bulk respiration and the integrated DOC and DON stocks, physical processes such as vertical and horizontal water mixing appear to be the predominant factors controlling DOM distributions in this area. The residence times of LOC and LON relative to atmospheric depositions in this area were approximately 794 and 407 days, respectively, suggesting that LOC and LON were semi-labile fractions as they had removal time scales ranging from one year to several decades (Hansell et al., 2009).

5.1.2 The tropical North Atlantic Ocean

The tropical North Atlantic Ocean showed a relatively strongly stratified water column with a mixed layer depth of approximately 30 - 90 m. Chlorophyll *a* profiles were similar to those observed in the North Atlantic subtropical gyre and indicated that this study area was of low productivity, with chlorophyll *a* concentrations of less than 0.25 mg/m^3 (Varela et al., 2005). Vertical DOC profiles also showed a similar pattern to those observed in the North Atlantic subtropical gyre with elevated concentrations in the surface waters and decreasing concentrations with depth. Mean DOC concentration was 77.0 μM in surface waters and the refractory fraction represented 57.2% of the bulk DOC pool. The mean DOC concentration was 47.8 μM in sub-surface waters (200 - 300 m) and the refractory fraction accounted for 92.0%. The relatively enhanced DOC concentrations in surface waters in this area were the result of water stratification which acts as a barrier to DOC exchange between the surface and deeper water. A relatively low contribution of DOC degradation to oxygen consumption (14.1%) was observed, which related to an extensive oxygen minimum zone maintained by a high particulate organic carbon loading. Non-typical vertical DON profiles were present with enhanced concentrations in sub-surface waters. The maximum DON concentration was present in the sub-surface waters along the 12ºN longitudinal section which is in agreement with the DON concentrations reported by Vidal et al. (1999). The occurrence of enhanced DON concentrations in sub-surface waters suggests the presence of an upward DON turbulent flux from below the thermocline Vidal et al. (1999). Important biological processes in this area include nitrogen fixation (Mahaffey et al., 2004, Moore et al., 2009) and could be responsible for the elevated DON concentrations in sub-surface waters. The DON concentrations in surface waters accounted for more than 99% of the bulk TDN pool, reflecting the inorganic nutrient-limited state of the area which resulted in low chlorophyll *a* concentrations. Mean concentration of DFAA was less than 0.07 μM, while mean concentration of DHAA was less than 0.66 μM. The stoichiometric patterns (C:N:P) indicated that DOM pool was enriched in carbon relative to nitrogen and phosphorus and nitrogen relative to phosphorus. The LOC and LON of atmospheric depositions in this area were predominantly semi-labile fractions, with residence times of approximately 415 and 298 days, respectively.

5.1.3 The Mauritanian shelf region

The Mauritanian shelf region showed a strong upward movement of water masses resulting in the relatively shallow pycnocline and nitracline that were observed. Distributions of DOC in the Mauritanian shelf region were fairly uniform and similar to the distribution of DOC observed in the North Atlantic subtropical gyre and the tropical North Atlantic Ocean, with relatively high concentrations in the surface waters and decreasing with depth. The mean concentration of DOC was 72.3 μM in surface waters, of which 60.9% was the refractory fraction. The mean concentration was 67.2 μM at a depth of 40 - 200 m where 84.8% of the DOC bulk was in the refractory fraction. Distributions of DON showed similar profiles to the North Atlantic subtropical gyre. Enhanced DON concentrations were observed in surface waters, with a mean of 8.1 μM. The DON concentrations decreased in deeper water, with a mean concentration of 5.4 μM. The refractory DON fraction was 34.7% of the bulk DON pool in surface waters and increasing to 52.15% in sub-surface waters. A relatively small contribution of DON to the TDN pool (47%) was present in surface waters due to the upward mixing of DIN from deep water. Concentrations of DFAA and DHAA were below the limit of detection. Organic stoichiometric ratios of the bulk DOM pool (C:N:P) in surface waters showed similar patterns to those in the North Atlantic subtropical gyre and the tropical North Atlantic Ocean, showing an order of preferential degradation of organic matter with phosphorus>nitrogen>carbon. Although there was no statistical proof of environmental controls on DOC and DON distributions, there were a few reports in the literature suggesting that the distributions of DOC and DON in this area are controlled by a complex mixture of biogeochemical processes and physical processes (Teira et al., 2001, Dadou et al., 2001). Relatively short residence times of LOC and LON was attributed to a large abundance of marine heterotrophic bacteria and phytoplankton and also abiotic processes such as photo-tranformation and sorption onto sinking particle. The accumulation of nitrate and phosphate in seawater was responsible for the relatively long residence times of LTN and LPO.

5.1.4 Deposition of atmospheric organic matter in the (sub-) tropical North Atlantic Ocean

Aerosols transported in the air masses over the (sub-) tropical North Atlantic Ocean were predominantly derived from the Sahara and Sahel regions and industrial continental regions (Europe and North America). The aerosols were sources of organic carbon and nitrogen. The air masses from the Saharan region were dominant throughout the sampling period and were responsible for the highest dry deposition flux of LOC with a mean of 3.67 mmol/m^2/year, and LTN with a mean of 11.9 mmol/m^2/year. The highest flux of LON

was observed in air masses originating from multiple sources (Sahara region, Sahel region and industrial continental regions) with a mean of 0.47 mmol/m^2/year. The deposition flux of LON represents approximately 5% of the total nitrogen flux indicating that inorganic nitrogen was the main nitrogen species deposited in the (sub-) tropical North Atlantic Ocean. There was no contribution of biomass burning to LOC observed in this study. Aerosols in air masses transported from the Saharan region and from industrial continents (Europe and North America) contained excess nitrogen relative to carbon when compared with Redfield ratios. Aerosols in air masses transported from all sources were nitrogen-enriched relative to phosphorus.

5.1.5 Optimization of DFAA and DHAA analysis

The method for DFAA and DHAA analysis in samples with low amino acid concentrations was optimized using RP-HPLC combined with AQC pre-column derivatisation. The optimized conditions were: pH of mobile phase A = 5.05, flow rate = 1.0 mL/min, column oven temperature = 37 ºC, and the solvent gradient was 0 min 98% A, 17 min 93% A, 24 min 87% A, 37 min 65% A, followed by a wash with 98% B for 5 min and re-equilibration for 11 min at 98% A. The detection was carried out on fluorescence detector with excitation at 250 nm and emission measured at 395 nm. The optimized method can quantify both primary and secondary amino acids. Hydrolysis was conducted using vapour phase hydrolysis with long duration (24 hours) at a temperature of 110-115 ºC. Chromatograms showed relatively high sensitivity when the samples were dried using a freeze dryer prior to the hydrolysis step. Aaba, Aada, Apma and MLeu were not suitable as internal standards for this optimized method because they co-eluted with amino acid peaks and one unidentified peak. The optimized method allows the optimum separation of 14 DFAA and three pairs of DFAA (Asn/Ser, Gln/His and Cys/Tyr). However, during hydrolysis the amine group of Asn and Gln are de-aminated to yield Asp and Glu and ammonium. Therefore, the co-elution of Asn/Ser and Gln/His are resolved by hydrolysis. Method detection limits (MDL) were 0.07 µM for DFAA and 0.66 µM for DHAA.

5.2 Future work

5.2.1 Fluxes of DOC and DON

The results in this study showed that atmospheric aerosols supply organic carbon and organic nitrogen to surface waters. To calculate the contribution of atmospheric dry deposition to DOM pools in the (sub-) tropical North Atlantic Ocean, vertical water column DOC and DON fluxes are required to allow comparison with the atmospheric deposition fluxes observed in this study. According to Vidal et al. (1999), the turbulent flux of DOM can be calculated using the following equation:

$$F_N = K_\rho(z)(\partial[N]/\partial z)$$

Where:

$K_\rho(z)$ is the vertical turbulent diffusion (m^2/s),

$\partial[N]/\partial z$ is the gradient in the measured nutrient concentration across the thermocline ($mmol/m^{-4}$).

5.2.2 Refractory fractions of DOC and DON

The distributions of DOM concentration in the deep ocean vary depending on the location of the study site (Hansell, 2002). In this study, literature values of deep-water DOC and DON concentrations were used to estimate the refractory fractions owing to no availability data in the deep water below 300 m. Thus, additional works on deep-water sample collection in the study area are required in order to improve understanding of the continuum of biological lability.

5.2.3 Contribution of biomass burning

In this study, the conclusion that biomass burning did not make a contribution to LOC in aerosols transport over the (sub-) tropical North Atlantic Ocean was reached based on limited available potassium data. Therefore, long-term measurements of potassium in atmospheric aerosols will improve understanding of the contribution of biomass burning to aerosol organic carbon concentrations in these areas.

5.2.4 Global organic aerosol deposition

The result in this study shows that the aerosols transported in the air masses over the (sub-) tropical North Atlantic Ocean were sources of organic carbon and nitrogen. Hence, the atmospheric organic carbon and organic nitrogen in other oceanic regions should be

further investigated to extend our understanding of the atmospheric deposition of organic carbon and organic nitrogen in the global scale.

5.2.5 Aerosol size distributions

In this study, literature data on size-segregated aerosol were used in conjunction with measurements of total aerosol concentrations to estimate fluxes of organic carbon and organic nitrogen. Furthermore, Cape et al. (2011) suggested that the size segregation of aerosols can provide additional information on the original sources and transformation processes occurring during aerosol transport. Therefore, the determination of aerosol size distributions is required to improve the accuracy of flux estimations and of aerosol source identifications.

5.2.6 Organic compounds of the aerosol

Graham et al. (2003) reported that organic compounds can be used as specific tracers of the origin sources in the Amazonian aerosols. For instance, anhydrosugars (levoglycosan, mannosan, galactosan) are specific tracers for biomass burning, while dicarboxylic and hydroxyacids are associated with photochemical production of a secondary aerosol. Therefore, the further study on the characterisation of the organic compounds contained in the aerosols will enable us to improve the aerosol origin identification.

5.2.7 Optimization of DFAA and DHAA analysis

The optimized chromatographic conditions arrived at in this work are only suitable for the analysis of amino acid concentrations greater than 0.07 μM for DFAA and 0.66 μM for DHAA. In order to determine DFAA and DHAA concentrations in low amino acid level samples and also quantify the concentrations of individual amino acids, further improvements in method sensitivity are needed. In addition, a suitable internal standard is required to minimize day to day variability of the analysis and to improve the linearity and reproducibility of the method. Moreover, the efficient sample preconcentration technique is another requirement to increase the concentration of the sample to a level compatible with the optimized method.

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