

## THE MOLECULAR COMPOSITION OF DISSOLVED FREE AMINO ACIDS IN RAINWATER

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### Abstract

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Atmospheric wet deposition is now well recognized as a main source of both inorganic and organic nitrogen for both marine and terrestrial environment. Until now more than 80% of dissolved organic nitrogen fraction of rainwater has remain uncharacterized. In this present study the free dissolved amino acids were determined in the urban samples using amino acids analyzer. Rainwater samples were collected from Irbid and the Hashemite University (HU) campus during winter 2015-2016. Dissolved free amino acids were determined in each samples. The concentrations of TFAA for whole Irbid and HU samples were  $0.078 \pm 0.0083$  and  $0.080 \pm 0.0065$  respectively, these concentrations are significantly similar to those previously described data for marine and terrestrial rainwater and aerosol samples collected from different remote areas. The results furthermore revealed that the highest proportion were phenylalanine and isoleucine in both sample sites. The results also demonstrate that the biological processes (transformation and degradation) play an important role in alteration of proteinaceous materials. As we know and back to the literature indicate to say that the description of dissolved free amino acids percentages in the rainfall and the proportion of contribution to the dissolved organic nitrogen is quiet generally uncharacterized. To our knowledge this is the first study carry on the identification of FAA in rainwater over Jordan.

*Key words:* dissolved amino acids; rainwater; amino acid analyzer; Jordan

### Introduction

It is very important to study the organic composition of precipitation because it's a part of essential fraction of the global rainwater body. The biochemical composition of dissolved organic nitrogen (DON) in precipitation has been incompletely characterized (Gorzelska et al., 1997). Various reports have showed that the organic chemical composition of aerosols was determined by a combination of factors: such as primary emission via bubble bursting from the water surface and the afterward transformation into secondary aerosol (Facchini et al., 2008a,b; Rinaldi et al., 2010).

The organic parts of rainfall has water soluble organic compounds (WSOC), which is likely composed of several classes of organic acids, amines, carbonyl compounds and amino acids (Kuznetsova et al., 2005; Saxena and Hildemann, 1996). Amino acids are abundant compounds that have active element of the organic nitrogen content of precipitation as some of them have been revealed to increase the ice nucleating power of atmospheric components (Szyrmer et al., 1997).

A few studies have showed that the aptitude of some amino acids to perform as fog condensation nuclei (Snider et al., 2010), and can also aid as a source of organic nutrients for environments (Zhang et al., 2002). Many researchers have

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extensively studied the existence of dissolved amino acids fractions in aerosols (Gorzelska and Galloway, 1990; Spitz, 1990; Milne and Zika, 1993; Saxena and Hildemann, 1996; Zhang et al., 2002; Yu et al., 2002; Wedyan and Preston, 2008; Mandalakis et al., 2010; Ge et al., 2011), in rainwater (Moppere and Zika, 1987; Mace et al., 2003a; Mace et al., 2003b; Zhang and Anastasio, 2001), fog (Zhang and Anastasio, 2001), and in dew water (Scheller, 2001). They can be also present as proteins and peptides (Kuznetsova et al., 2005; Ge et al., 2011; Moppere and Zika, 1987; Milne and Zika, 1993).

Some discharge suppliers can affect both amino acid structure of the rain and the total concentration of dissolved free amino acids in the atmosphere. Many researchers have extensively wrote that amino acid that found continental particles have been formed by algae, plants, fungi and bacteria, also it can be found in high content in soil (Milne and Zika, 1993; Scheller, 2001; Zhang and Anastasio, 2003; Mace et al., 2003a). The source of biological amino acids in both fine and coarse particles came from tobacco smoke and sewage treatment (Ge et al., 2001; Mace et al., 2003b; Leach et al., 1999).

To our knowledge, this paper is the first study that investigates the different compositions and distributions of free dissolved amino acids in rainwater at Jordan. And to assess the concentration levels of free dissolved amino acids in the rainwater instep to study their compositional and distribution changes after long-range atmospheric transport. Our investigation was carried out over two different areas at north part of Jordan.

## Materials and Methods

### Sample collection

The rainwater samples were collected from two locations (Irbid (32.5570° N, 35.8479° E) and the Hashemite University (HU) campus (32.1029° N, 36.1811° E)) of Jordan during the winter 2015-2016 period (Figure 1). The first location (Irbid) was selected since it is situated in a way that is physically separated from the main station area by a distance, to decrease ultimate pollution from the research station as much as possible. The sample collection was set to 5 days, but the real sampling time diverse, subject to rain and wind events. Due to these events the actual sampling volumes varied from between 50 to 100 ml each. Field blanks were collected once per week by placing 100 mL of deionized water into the rainwater collector overnight and then collecting the water in the same way as a rainwater sample. The full set of chemical analyses was also done on the field blanks to assess possible contamination from the collector or from sample handling.



Fig. 1. Location of the Irbid and HU sampling sites

### Sample processing

To avoid contamination in our experiment we used cleaned glasswares, which include filters. The glasswares were equipped by washing in alkaline detergent, placing in 1N HCl overnight followed by rinsing with organic free water obtained with a distilled water and then heating in oven at 110°C for 10 to 12 h. The samples were filtrated through 0.45 µm cellulose acetate filters then kept at 5 °C until analysis to recognize the amino acids in rainwater samples. All samples were run in duplicates.

The samples were derivatized with o-phthalaldehyde (OPA) and then analyzed by amino acids analyzer with fluorescence detection in order to determine the total amino acids content. A laboratory procedural blank were also analyzed.

## Results and Discussion

### Dissolved free amino acids determination in Irbid and Zarka areas

Twelve amino acids were examined in the rainwater samples collected at The Hashemite University Camp near international highway and Irbid city. 50 samples were collected between 2 November 2015 and 3 March 2016 (Figure 2).

The mean concentrations and range of amino acids (DFAA, DHAA and TDAA), calculated from the sum of their

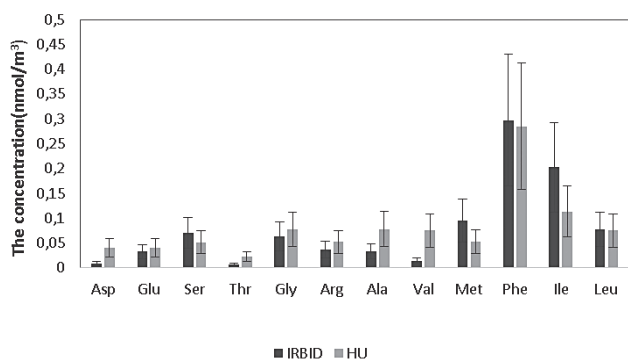


Fig. 2. DFAA profile at Irbid and HU samples

distributions in all rainwater samples for the whole data set are shown in Table 1. The mean concentrations of TFAA for whole Irbid and HU samples were  $0.078 \pm 0.0083$  and  $0.080 \pm 0.0065$  respectively, these concentrations are significantly similar to those previously described data for marine and terrestrial rainwater and aerosol samples collected from different remote areas. Matsumoto and Uematsu reported the average concentrations a round  $0.0107 \text{ nmol/m}^3$  for marine samples (Matsumoto and Uematsu, 2005). Wedyan and Preston demonstrated the average DFAA concentration of  $0.2 \text{ nmol/m}^3$  in samples collected from the Atlantic Ocean this evidence that the marine may affected the concentrations of DFAA (Wedyan and Preston, 2008). While Barbaro and his colleague reported a mean of  $0.334 \text{ nmol/m}^3$ , in Venice Lagoon (Italy) (Barbaro et al., 2008), Mandalakkis determined the average in Finokalia (Greece) of  $0.172 \text{ nmol/m}^3$  (Mandalakkis et al., 2011) and Mace and his team found ranges of  $0.015\text{-}0.160 \text{ nmol/m}^3$  in Tasmania (Australia) and  $0.03\text{-}0.8 \text{ nmol/m}^3$  in Erdemli (Turkey) (Mace et al., 2003b; Mace et al., 2003c). While Scalabrin reported the mean total DFAA concentration was  $0.00107 \text{ nmol/m}^3$  (Scalabrin et al., 2012) and Barbaro and his team found that the concentration of DFAA was  $0.011 \text{ nmol/m}^3$  (Barbaro et al., 2015).

Table 1

The mean concentrations and ranges of DFAA, DHAA and TAA (= DFAA+DHAA) in rainwater samples ( $\text{nmol/m}^3$ )

	Concentration		
	DFAA	DHAA	TAA
IRBID			
Range	0.0070-0.29	0.022-0.28	0.029-0.53
Mean $\pm$ St Err	$0.078 \pm 0.0083$	$0.08 \pm 0.0065$	$0.15 \pm 0.013$
HU			
Range	0.022-0.29	0.0093-0.40	0.043-0.63
Mean $\pm$ St Err	$0.080 \pm 0.0065$	$0.092 \pm 0.01$	$0.16 \pm 0.015$

The mean total concentration of dissolved hydrolyzed amino acids (DHAA) fraction detected in this report was less than the former reports e.g. Wedyan and Preston who found the mean concentration was  $0.01 \text{ nmol/m}^3$  (Wdyan and Preston, 2008) and Yamashita and Tanoue was  $0.25 \text{ } \mu\text{mol/m}^3$  (Yamashita and Tanoue, 2004). The concentrations of individual amino acids ranged from  $0.0070\text{-}0.29 \text{ nmol/m}^3$  for Irbid sample and  $0.022\text{-}0.29 \text{ nmol/m}^3$  HU samples. The profiles of the DFAA for both Irbid and HU samples are shown in Figure 2.

The concentrations' proportions (%) of main amino acids within the DFAA of both samples are presented in Figure 3. As can be seen from the both Figures 2 and 3, the concentrations of the DFAA were highly variable between sample sites.

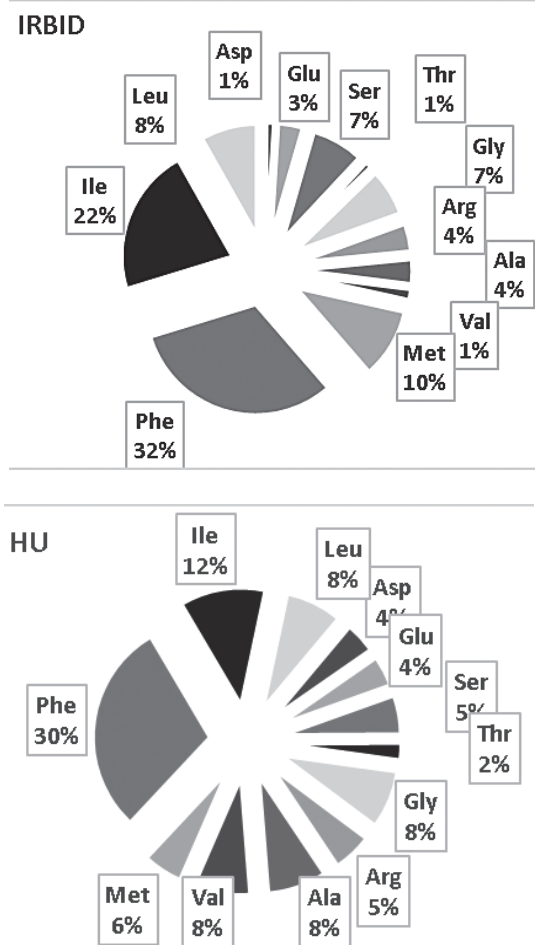


Fig. 3. The percentage (%) of dominant amino acids within the DFAA of both samples Irbid and HU

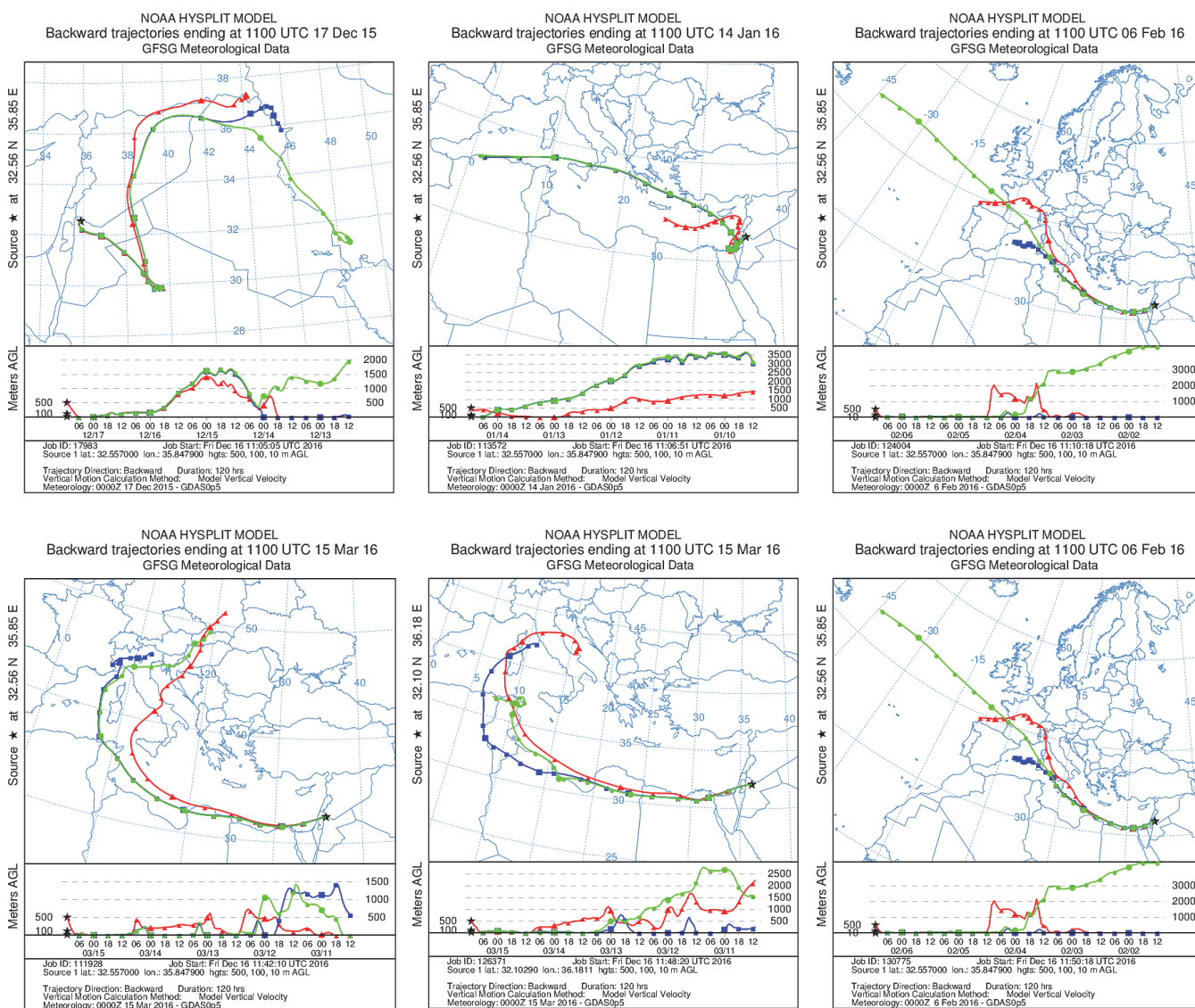


Fig.4. 5-Days backward trajectories analyses during the sampling collection periods (winter 2015-2016)

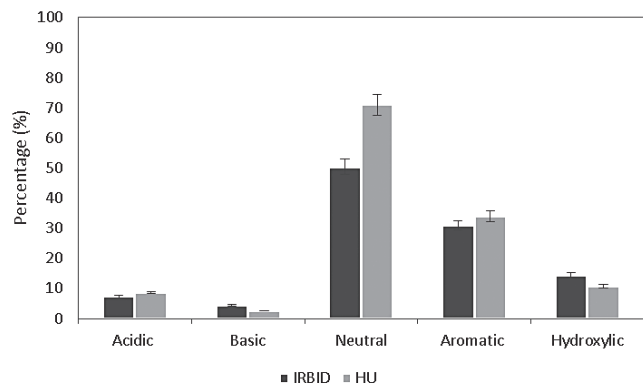
This is of course not surprising because of diversity of the origin of the sample sources and this can be concluded from the air mass back trajectories Figure 4 represent a component of terrestrially marine derived organic matter. The analysis of all 5-days backward trajectories collected during sampling periods revealed both marine and land sources. For this it suggest that the biotic materials affected the concentrations and distributions of total free amino acids in these samples. This suggests that the contribution of amino acids to the sites is both qualitatively and quantitatively different.

The results of the current study are similar as mentioned before to previous studies done in early 1990s (Gorzelska

and Galloway, 1990). Zhang and Anastasio found that total concentration of free and combined ranged from 1.25 to 3.65 nmol/m<sup>3</sup> in atmosphere fine particles and from 1.62 to 5.88 nmol/m<sup>3</sup> in for water (Zhang and Anastasio, 2003). In comparison, previously reported free amino acids values in rainwater are significant (Gorzelska et al., 1994; Wedyan, 2005). In our data rainfall concentration provided from a scavenging factor of 500 in the range 4.8 to 457 μM. A few other studies have studied the concentrations of amino acids in other forms of precipitation including particles, dew waters and fog drops (Gorzelska and Galloway, 1990; Zhang and Anastasio, 2003; Gorzelska et al., 1994; Wedyan, 2005; Yan et al., 2015; Barbaro et al., 2015).

### *Amino Acids classes within the samples*

By back to the literature, the amino acids were divided in to functional groupings according to their chemical properties following to this role the amino acids were classified in to acidic, neutral, basic, and aromatic and hydroxyl amino acids (Figure 5).



**Fig. 5 Average mole percentage (%) of grouped DFAA in Irbid and HU rainwater samples. Average  $\pm$  standard error**

Neutral amino acids were the most abundant in the DFAA samples in both areas that comprised up to 50% and 71% for Irbid and HU respectively. In order of abundance, these were followed by aromatic amino acids and hydroxyl amino acids. These results are similar to those previously found in different ecosystem (Wedyan, 2005; Dittmar and Kattner, 2003) where neutral compound represented 50-70% of TDAA.

### *Distribution of Amino Acids*

Looking to the distribution of the DFAA (Figure 2) with the air mass back trajectories (Figure 4) give us a margin to suggest that amino acids in rainwaters collected at both study areas can have two possible sources. First one comes from biological sea spray particles (Matsumoto and Uematsu, 2005; Wedyan and Perston, 2008), or from soil dust (Mace et al., 2003b) that are transportable for a many days by atmosphere. The second one is that amino acids had marine sources and may be underwent many chemical alterations. Our results propose that amino acids were existing in the particles over the surface of the Mediterranean sea from bubble bursting processes, and air mass also passed over the land where they persisted for several times before deposit (Barbaro et al., 2015; Wedyan, 2014).

A number of common features can be identified in the distributions of the individual amino acids in the different

sample sets. For example phenylalanine was the dominant amino acids in our samples. The leucine and isoleucine were also presented in a notable amount (Figure 3). These results generally support the idea that amino acids distributions by themselves cannot be used to determine the origin of organic matter in the environment because the proteins in organism are too similar in composition (Dittmar et al., 2001). For example glycine, valine, aspartic acid, glutamic acid, serine and alanine can also often be related to bacterial sources (Nagata et al., 2003). Salway demonstrated that arginine formed by organism during the urea cycle (Salway, 1999). All of these an evident that the rainwater contains a dissolved biological materials and it might be persistent and transported through the atmosphere. In future, we are going to understand the dissolved organic nitrogen within the rainwater samples by measurement all fractions of amino acids (combined and particulates). And also investigate the input of total amino acids N in rainwater it will be possible to better understand to influence of organism and local or long range sources.

## **Conclusion**

This work investigates that concentration of dissolved free amino acids (DFAA) and structures in rainwater samples collected from two sites. The results provide significant evidence that the DFAA found in the rainwater already come from both marine and land and the biological processes (transformation and degradation) play an important role in alteration of proteinaceous materials. As we know and back to the literature lead to say that the characterization of total dissolved amino acids fractions in the rainwater and the proportion of contribution to the dissolved organic nitrogen is still largely uncharacterized. Consequently, there is an opportunity for further research on this point.

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