© by PSP Volume 27 – No. 5A/2018 pages 3506-3511 Fresenius Environmental Bulletin

EFFECTS OF DIFFERENT DRYING METHODS ON THE DETERMINATION OF NITROGEN IN SEDIMENT

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ABSTRACT

Drying methods of sediment samples are commonly divided into natural air-drying, low-temperature drying and freeze drying, the processing efficiency and time-consuming of three treatment methods are very different. In this paper, sediments were sampled from Taihu Lake, and dried under natural air, low temperature or freeze, then analyzed using the same method for the determination of total nitrogen (TN), ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N). Statistical analysis of the results showed significant differences between sample treatments relating to the mean TN, NH_4^+-N, NO_3^--N concentration. Referring to dry samples, the average content of samples using freeze-dried method and air-dried method, were 1.07, 1.33, 0.91 and 1.01, 1.23, 0.68 for TN, NH_4^+-N, NO_3^--N, respectively. NO_3^--N content of dry samples was the highest. The ANOVA showed that there was no significant difference about TN, NH_4^+-N and NO_3^--N among the three methods. However, there were significant differences when two methods were compared. The result of LSD showed that there were significant differences between Frozen-dried Method (B) and Dried Method (A) with p=0.039; and between Frozen-dried Method (B) and Air-dried Method (C) with p=0.043 for TN. In addition, the correlation analysis results showed a significant correlation at the level of $P < 0.01$, suggesting that the effects of different pretreatment methods on TN, NH_4^+-N and NO_3^--N extraction are holistic, not due to changes in individual samples. Compared with air-drying, low-temperature drying, freeze-drying process can truly indicate the nitrogen composition of sediments, it is an ideal way of sample drying.

KEYWORDS:
Drying methods, TN, NH_4^+-N, NO_3^--N, Sediments

INTRODUCTION

The eutrophication of aquatic systems is an environmental problem of major concern, not only in industrialised countries but in the developing world as well [1]. And excessive nitrogen loading, which is one of the most active elements in the biogeochemical cycling processes, has been regarded as one of the most important factors causing eutrophication of lakes [2]. The traditional views show that when nitrogen deficiency occurs, lake ecosystems can obtain nitrogen from the atmosphere through biological nitrogen fixation to meet their nitrogen needs, so according to this assumption, the main limiting factor of the primary productivity of water bodies is phosphorus availability. However, recent research [3, 4] has shown that nitrogen fixation does not meet ecosystem demands, and nitrogen or nitrogen and phosphorus limitation are generally accepted. Furthermore, eutrophic lakes often exhibit nitrogen limitation, meaning that they are sensitive to additional nitrogen inputs. Internal loading is an important source of nitrogen pollution [2], since they retain or release nitrogen depending on the sediment phase to which it is associated and on the environmental conditions. So the sediments play a vital role in N cycle, which involves physical, chemical and biological processes [5].

However, not all forms of nitrogen are released into the water, where they could contribute to eutrophication, from the deposition phase [6]. And it may be more efficiently evaluated on the basis of different N forms.

For this reason, it is necessary to know not only the total amount of nitrogen in the sediment, but the main nitrogen forms including ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in sediment. Thus, nitrogen experimental accuracy is essential [7], and before the experiment, the samples need pretreatment. The method most commonly used for the sediment preparation are Vacuum Freeze Drying method, Natural Drying method and Temperature Drying method. Some scholars use fresh samples to experiment directly.

Different treatment methods lead to changes in

soil/sediment properties [8]. Digestion methods for determination of total phosphorus in river sediments have been compared [9]. Sediment temperature has been found that there are impacts on NH_4^+ turnover in intertidal sediments [10]. But the pretreatment methods on the impact and causes of various nitrogen forms from the sediments, have no unified conclusions.

The study of the accurte method for the determination of N in the sediment is, therefore, a key step in understanding the N content in rivers and lakes. It could be also of prime importance to know the N content on a large scale for water management quickly, and make measures in restoration of water bodies in time.

The overall objective of the present study was to compare the three methods including stoving at 60 ℃, natural withering and vacuum freeze drying were investigated and set up a rapid method for determining the contents of TN and various N forms in the sediment cores sampled. In addition, the relationships between various N forms were also investigated.

MATERIALS AND METHODS

Materials. The following analytical reagent-grade chemicals: Potassium persurfate ($K_2S_2O_8$), Sodium hydroxide (NaOH), hydrochloric acid (HCl), Potassium chloride (KCl), Sodium salicylate ($C_7H_5NaO_3$), Sodium citrate ($Na_3C_6H_5O_7$), Dichloro cyanuric acid sodium ($C_3O_3N_3C_{l2}Na$), Sediment standard sample (GSD–22) were analytical grade from Nanjing Chemical Reagent Factory (Nanjing, China) were used.

All tubes, including glass tubes, centrifuge tubes and test tubes, were cleaned three times with tap water prior to use. Impurities were removed with a clean brush used only for this study. Next, the tubes were washed once using deionised water before being immersed into an ultrasonic bath filled with deionised water for 10-15 minutes.

Sample collection. The near-shore area around GongHu Bay in Taihu Lake was selected as the sampling and the samples were collected from 7 sampling sites including 58 samples. All samples for nutrients were preserved well and brought back to the laboratory and stored in the dark at 0–4°C.

Chemical analysis of the sediment. The sediment samples were divided into three parts and named after A, B and C. The A samples were placed in the oven at 60℃ for drying. The B samples were processed by the freeze-drying. And the C samples were air-dried treated in nature. Then all samples were milled by agate mortar and sieved by the 100 mesh screen after removing plant debris, rubble and other impurities.

The oxidizing reagent was prepared by dissolving 40 g $K_2S_2O_8$ and 15 g NaOH in 1L of distilled water [11]. The reagent was analytical grade. The oxidizing reagent was prepared daily. A 10 ml oxidizing solution was added to each sample which was then autoclaved for 1 h at 123℃. This mixture was cooled to room temperature for the determination of total nitrogen. Then add 2ml hydrochloric acid (1:9) in the colorimetric tube, and use the high water content to 50 ml. Absorbance values were measured at wavelengths 220 nm and 275 nm.

Ammonium and nitrate nitrogen were measured with an auto-analyzer 3. A 0.5000g sample was added with 50mL Potassium chloride (KCl) in the 100ml centrifuge tube. And then oscillation for 12 hours, extract supernatant after centrifugation.

Data quality control and analysis. Relative standard deviations, 95% confidence intervals and analysis of variance (ANOVA) were determined according to standard procedures. These statistical analyses were used to evaluate accuracy and precision of the methods.

RESULTS AND DISCUSSION

Analysis of water and sediment samples. While collecting sediment samples, the pH, dissolved oxygen and other water quality indicators were also measured in overlying water (Table 1).

TABLE 1
Physical and chemical properties

Sampling Points	Wind Velocity/ m/s	Water Depth/ m	pH	Water Temperature/ °C	Eh/mv	DO/mg/L	SD/m	Conductivity/ μs/cm
P-1	2.4	1.0	7.26	29.3	96.5	0.90	0.02	392
P-2	3.6	1.4	7.89	29.2	78.0	6.03	0.20	386
P-3	2.4	1.8	8.30	29.1	66.2	7.90	0.18	383
P-4	4.7	1.9	8.45	29.4	67.3	8.13	0.23	386
P-5	1.9	1.1	7.73	29.4	66.2	4.98	0.18	389
P-6	1.9	1.6	8.21	29.2	45.6	7.47	0.18	385
P-7	2.8	1.7	8.37	29.0	55.5	7.92	0.16	385

The total nitrogen (TN) were determined photometrically by UV-Vis Analyst (UV-6100, Shanghai, China), the ammonium (NH_4^+-N), nitrate nitrogen (NO_3^--N) by auto-analyzer (auto-analyzer 3, Germany) with precision of <5–10%. The results were shown in Table 2.

The Table 2 indicated that the orders of the mean concentrations of TN and the N forms, which were pretreated with different ways are as follows: $\mathbf{C}_{TN}$ (Frozen-dried Method) > $\mathbf{C}_{TN}$ (Air-dried Method) > $\mathbf{C}_{TN}$ (Dried Method), $\mathbf{C}_{NH4+-N}$ (Frozen-dried Method) > $\mathbf{C}_{NH4+-N}$ (Air-dried Method) > $\mathbf{C}_{NH4+-N}$ (Dried Method), $\mathbf{C}_{NO3--N}$ (Dried Method) > $\mathbf{C}_{NO3--N}$ (Frozen-dried Method) > $\mathbf{C}_{NO3--N}$ (Air-dried Method).

However, the ANOVA, which is applied when only one grouping variable is present in the dataset, showed that there was no significant difference about TN, NH_4^+-N and NO_3^--N between the three methods. However, there were significant differences when two methods were compared. The result of LSD, that is one of method which could analyze a plurality of average to pairwise comparison, showed that there were significant differences between Frozen-dried Method (B) and Dried Method (A) with p=0.039; and between Frozen-dried Method (B) and Air-dried Method (C) with p=0.043 for TN. In addition, the differences being in NH_4^+-N and NO_3^--N were also significant, comparing two methods (Table 3).

TABLE 2
Statistical analysis of the total nitrogen and N forms in sediments under different pretreatments

Sampling Point	Pretreatment	TN(mg/kg)	NH_4^+-N(mg/kg)	NO_3^--N(mg/kg)
P-1	A	1841.549	162.7958	1.52011
	B	1890.054	234.2503	1.71274
	C	1856.79	244.9166	1.86638
P-2	A	2007.249	132.8642	15.02784
	B	2126.964	195.8098	8.52806
	C	1976.849	172.7665	8.36952
P-3	A	2285.103	62.92915	10.26417
	B	2488.876	90.5791	8.65854
	C	2360.929	108.0978	7.09977
P-4	A	1566.007	63.74968	7.70989
	B	1709.261	58.45656	7.81069
	C	1617.832	54.61581	7.58889
P-5	A	1614.045	76.48835	5.36472
	B	2006.602	95.58828	4.26936
	C	1726.275	71.00223	3.294
P-6	A	2614.293	162.7553	2.64813
	B	2771.752	262.5496	2.07523
	C	2572.958	157.5721	1.90022
P-7	A	1950.587	86.09086	4.9242
	B	2028.248	74.85979	2.80224
	C	1949.785	81.47754	0.85036

* A: Dried Method, B: Frozen-dried Method, C: Air-dried Method.

TABLE 3
LSD method for multiple comparisons

LSD	(I)Pretreatment	(J)Pretreatment	The difference of average value (I-J)	SDE	Sig.	95% confidence interval	
						upper limit	lower limit
TN	A	B	-148.80*	71.05	0.039	-290.02	-7.57
		C	-2.84		0.968	-144.06	138.39
	B	A	148.80*		0.039	7.57	290.02
		C	145.96*		0.043	4.74	287.18
	C	A	-2.84		0.968	-144.06	138.39
		B	-145.96*		0.043	-287.18	-4.74
NH_4^+-N	A	B	-42.31*	20.42	0.041	-82.88	-1.73
		C	-35.13		0.089	-75.71	5.45
	B	A	42.31*		0.041	1.73	82.88
		C	7.17		0.726	-33.41	47.75
	C	A	35.13		0.089	-5.45	75.71
		B	-7.17		0.726	-47.75	33.41
NO_3^--N	A	B	1.40*	0.44	0.002	0.53	2.27
		C	1.12*		0.012	0.25	1.98
	B	A	-1.40*		0.002	-2.27	-0.53
		C	-0.28		0.521	-1.15	0.59
	C	A	1.12*		0.012	0.25	1.98
		B	0.28		0.521	-0.59	1.15

*: $p < 0.05$.

Referring to dry samples, the average content of samples using freeze-dried method and air-dried method, were 1.07, 1.33, 0.91 and 1.01, 1.23, 0.68 for TN, NH_4^+-N, NO_3^--N, respectively (Fig. 1). The most obvious chang is NH_4^+-N, comparing freeze-dried method and dried method, and the difference of NO_3^--N is also obvious, comparing air-dried method and dried method.

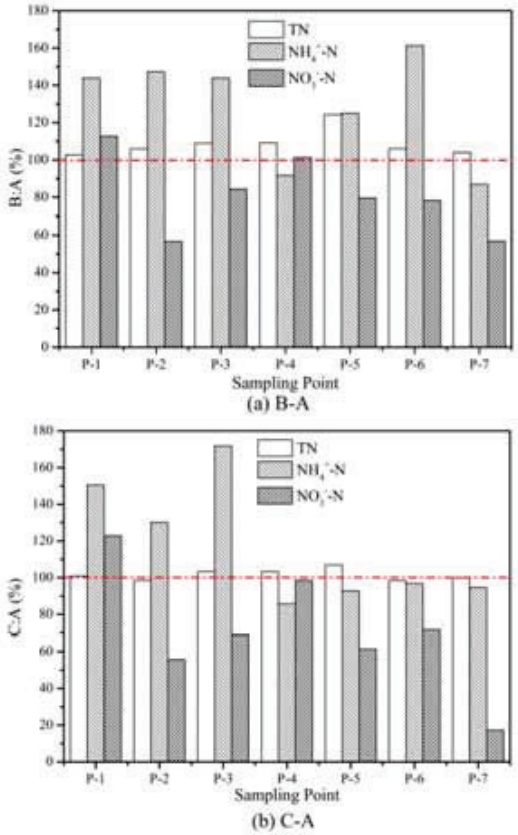

FIGURE 1
Deposit ratio of various nitrogen forms content under different pretreatment methods

TABLE 4
The Correlation analysis of sediment same indicators under different pretreatments

Pairwise comparison	TN	NH_4^+-N	NO_3^--N
Dried Method--Frozen-dried Method	0.949**	0.913**	0.596**
Dried Method--Air-dried Method	0.960**	0.899**	0.900**
Frozen-dried Method--Air-dried Method	0.968**	0.915**	0.548**

**: $p < 0.01$.

Correlation analysis. The correlation analysis results of total nitrogen and nitrogen forms content in different pretreated methods were shown in Table 4. It shows a significant correlation at the level of $P < 0.01$, suggesting that the effects of different pretreatment methods on TN, ammonia nitrogen, and nitrate nitrogen extraction are holistic, not due to changes in individual samples.

The results showed that there were different impacts for the partitioning patterns of TN, NO_3^--N, NH_4^+-N by the three methods, and the effect of the three methods on partitioning pattern of TN (<24 %) was found lowest among the nitrogen of TN, NH_4^+-N and NO_3^--N, and NH_4^+-N the highest (up to 71.78 %), referring to dry samples. It has been reported that the rates of ammonia and nitrite oxidation are strongly influenced by the nature of nitrifying cultures and a variety of environmental factors, including substrate concentration, dissolved oxygen (DO), temperature, pH and so on [12]. In the study, the main difference of three pretreatments was temperature, which were 60 ºC, -70 ºC and 30 ºC for Dried Method, Frozen-dried Method and Air-dried Method, correspondingly. So the high temperature can promote nitrification: $NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- + Energy$; $NO_2^- + 0.5O_2 \rightarrow NO_3^- + Energy$.

It has been studyed by Kim that both ammonia oxidation rate and nitrite oxidation rate increased significantly with the increase in temperature from 10 to 30ºC [12]. Frozen-dried Method can remove more than 95% of the water in the samples, thus the samples can be stored at room temperature or high temperature for a long time.

Until now, oxidation of ammonium has only been known to proceed under aerobic conditions. Recently, we observed that NH_4^+ was disappearing from a denitrifying fluidized bed reactor treating effluent from a methanogenic reactor. Both nitrate and ammonium consumption increased with concomitant gas production. A maximum ammonium removal rate of 0.4 kg N m^{-3} d^{-1} (1.2 mM/h) was observed. The evidence for this anaerobic ammonium oxidation was based on nitrogen and redox balances in continuous-flow experiments. It was shown that for the oxidation of 5 mol ammonium, 3 mol nitrate were required, resulting in the formation of 4 mol dinitrogen gas. Subsequent batch experiments confirmed that the NH_4^+ conversion was nitrate dependent. It was concluded that anaerobic ammonium oxidation is a new process in which ammonium is oxidized with nitrate serving as the electron acceptor under anaerobic conditions, producing dinitrogen gas. This biological process has been given the name 'Anammox' (anaerobic ammonium oxidation), and has been patented [13, 14].

In the anaerobic environment, Anammox with nitrite as electron acceptor, carbon dioxide as the carbon source, converts ammonia nitrogen into nitrogen and organic synthesis. There is the following formula of Chemometrics:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \xrightarrow{\text{anaerobic condition}} 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$ [15].

According to our monitoring data, the lack of nitrite nitrogen in the water column limits the further

transformation of ammonium nitrogen by this approach when black bloom occurred. The concentration of ammonium nitrogen continues to rise which accumulates a lot in the water column. Ammonium nitrogen can only be transformed in a small range of oxygen environment at the water-air interface.

CONCLUSIONS

Statistically significant differences were found between the drying methods (natural air-drying, low-temperature drying and freeze drying) used for nitrogen analyses in sediments. Referring to dry samples, the average content of samples using freeze-dried method and air-dried method, were 1.07, 1.33, 0.91 and 1.01, 1.23, 0.68 for TN, NH_4^+-N, NO_3^--N, respectively. NO_3^--N content of dry samples was the highest. According to the ANOVA, there were significant differences between Frozen-dried Method and Dried Method with p=0.039; and between Frozen-dried Method and Air-dried Method with p=0.043 for TN. In addition, the differences being in NH_4^+-N and NO_3^--N were also significant.

Compared with air-drying, low-temperature drying, freeze-drying process can truly indicate the nitrogen composition of sediments, it is an ideal way of sample drying.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (41573061), the National Major Science and Technology Program for Water Pollution Control and Treatment (2012zx07101-008-02), the Comprehensive Management of Water Environment of Taihu Scientific Research Project of Jangsu Province (JSZC-G2014-212) and the China Scholarship Council (CSC) under the State Scholarship Fund (File No. 201706860028).

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Received: **16.11.2017**
Accepted: **06.02.2018**

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